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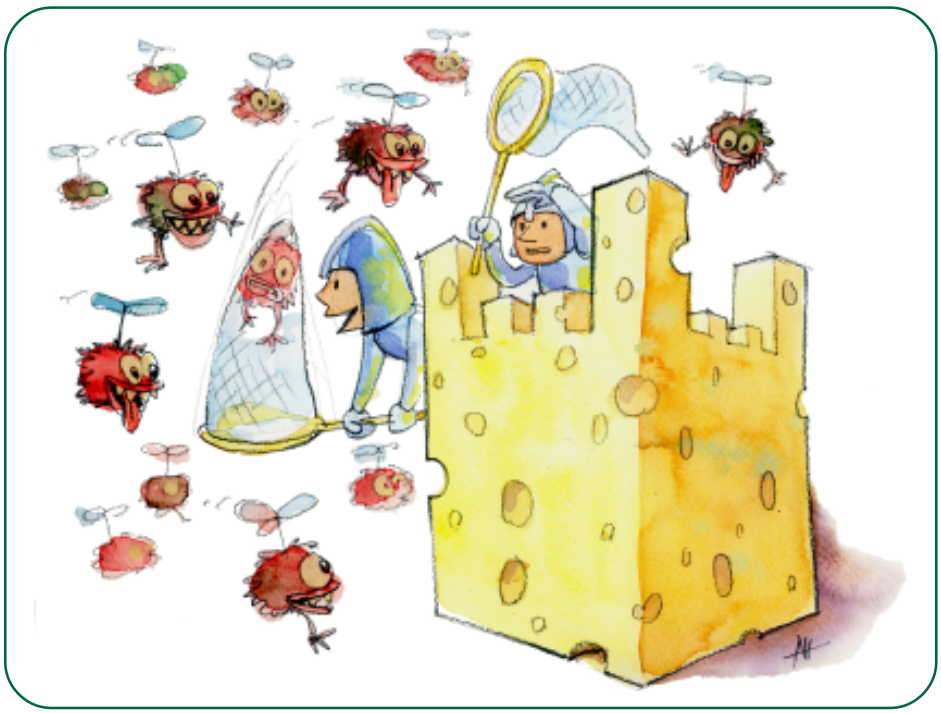

DairyNET

Hygiene Control in Nordic Dairies

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DAIRYNET – HYGIENE CONTROL IN NORDIC DAIRIES

Gun Wirtanen & Satu Salo (eds.)
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ABSTRACT

The research work for the third Nordic dairy hygiene project P00027 DairyNET – Hygiene control in dairy environment, which has been funded by the Nordic Innovation Centre (formerly the Nordic Industrial Fund), has focused on hygiene and quality issues relating to raw milk, process surfaces, process waters, air and final products. The work was carried out in the Nordic dairies Arla Foods, Milko, Nordurmjolk, Norrmejerier, Skåne mejerier, TINE and Valio Ltd together with the research organisations BioCentrum-DTU, the Danish Technological Institute (DTI), the Icelandic Fisheries Laboratories (IFL), the Royal Institute of Technology (KTH), MATFORSK, SIK, the University of Akureyri and VTT Biotechnology as well as the following suppliers of chemicals, reagents and equipment: Finnsonic Oy, JohnsonDiversey, Lagafors Fabriks AB, Mjöll, TetraPak Nordic Processing and Orion Diagnostica Oy from May 2001 to October 2004. Docent Gun Wirtanen, VTT Biotechnology, co-ordinated the project. Mr. Oddur Gunnarsson was the senior advisor at the Nordic Innovation Centre for this project. The various studies were reported in the Nordic co-operation project DairyNET in order to achieve major synergy benefits through openminded discussions at the project meetings. New procedures in hygiene based on the project findings have been implemented in processing at the dairies. The results of the five national projects and of the dairy network can be summarised as follows:

- Chemical residues of the cleaning agents or disinfectants left on the surface are examples of the new process hygiene indicators to be measured. as a of the Microbiological testing results showing no growth can lead to misinterpreting process hygiene, especially if only the total microbial load is measured. The **1st synergy task** concerning chemical-residue testing in the food processing area was chosen because normally no tests are run to check chemical residues left on the surfaces after rinsing. The results of the luminescent bacteria light inhibition method show that this method can be

used to measure residues both in liquids and on surfaces. The aim of the **2nd synergy task** was to establish a reference method for cleaning open production equipment. Hot spots or critical points are detectable by using UV light. This work showed that the places with known hygienic design problems could be visualised. Visualising these known "hot spots" is important because it enables us to pinpoint problematic places on more complex devices/machines, where special attention is needed during the specified cleaning procedure. Weather all fungi found in the cheesery air contaminate the cheese or not is also the subject of discussion? In the **3rd synergy task** a selective medium Creatine sucrose dichloran agar (CREAD) together with a general agar was used for the isolation of airborne moulds to find out if CREAD agar can be used for the rapid detection of harmful mould growth in cheeseries. Moulds growing on CREAD agar are species found on cheese.

- According to Norwegian regulations, the bacterial count level in **raw milk** before pasteurisation should not exceed 300,000 per ml. Milk from the farm should contain less than 100,000 per ml. The main factors affecting the bacterial number are the hygiene, age and storage history of the milk. The aim of this project was to investigate the hygiene at pre-pasteurisation sites in dairies in order, to find any correlation between cleaning, hygienic conditions and raw milk quality before processing. Five Norwegian dairies were visited in order to inspect the cleaning and processing routines and results from quality control. In this survey the results showed that the dairies using traditional cleaning routines obtained the best hygienic level. One reason for this was probably the organic contamination of the disinfecting solutions. None of the dairies could prove a connection between the microbial level in raw milk from the farm and in dairy silos and the level in the pasteurised product.

- ***Listeria monocytogenes*** is an important foodborne pathogen and its source, **contamination routes** and detection are crucial in the food industry. The present investigation was directed towards the occurrence and distribution of *L. monocytogenes* in the whole distribution line at an Icelandic dairy. More than 1400 samples were collected over the year in order to obtain possible seasonal variation. No positive samples of *L. monocytogenes* were found in the products (n = 200) and in the dairy process line (n = 250). In the milk collected during the winter, thirteen (13) samples were found to be positive

and none of the samples taken in the summertime were positive (n in both series 459). These results indicate there is a seasonal variation in the presence of *L. monocytogenes* in raw milk from this area. For the traceability of *L. monocytogenes*, further samples were collected from all milking cows (n = 20) from one of the infected farms, together with 30 additional samples from the shed environment and the silage. Results show that one cow and eight samples in the shed environment were positive for *L. Monocytogenes;* the isolates from the same farm were all the same ribogroup and pulsotype.

- In the Swedish project **air, water and cheese at four cheese production plants** were analysed for Gram-negative bacteria, yeast and mould. The results demonstrate the possibility of waterborne contamination in processing plants due to insufficient hygiene and maintenance of water sources e.g. hoses, nozzles and taps. The microbes most frequently retrieved from the water sources were bacteria e.g. *Acinteobacter* spp. and *Pseudomonas* spp.*,* yeasts e.g. *Candida* spp. and *Rhodotorula* spp. as well as moulds e.g. *Phoma* spp., *Fusarium* spp. and *Acremonium* spp. *Penicillium* spp. were rarely isolated from water. Air sampling may be used for the identification of positions in the processing line critical for contamination. The few samples of cheese analysed indicate air as the primary source for moulds contaminating cheese, and water as an additional source for *Geotrichum* spp. and *Trichoderma* spp.

- A bioaerosol can be defined as a suspension containing microscopic solid or liquid particles as well as microbes. There are a number of ways in which biological and microbiologial material may be made airborne, e.g. by human movement, sneezing or coughing. Fungal bioaerosols have been observed to be highly dependent on weather conditions. As part of the Swedish contribution to the Nordic DairyNET project, a **hygiene survey** was carried out on **airborne microbial particles** and the total particle burden in the air at four cheese plants located in different parts of Sweden. The microbiological monitoring was performed with three air sampling devices using different media for assessing the growth of bacteria, yeast and moulds. In general, the test results indicate that a more controlled environment is eligible. To improve the efficacy of the microbiological monitoring an appropriate combination of media and monitoring devices should be used. In a Finnish study the air quality at **Herajoki dairy** was examined. Background

information for this study was collected by familiarisation with the air-filtration systems and the history of the building at Herajoki dairy. In this study, the seasonal variation and the construction work at the dairy have to be taken into account when the results are examined. The air quality seemed to be microbiologically good, according to the references available.

- In another case study at Herajoki dairy the purpose was to **optimise the CIP cleaning and check the purity of CIP-waters**. It was shown that the ATP method provides an instrument for quick testing of the water cleanliness. Optimisation of the CIP cleaning will bring savings in water, chemicals and time for the dairy. The **hygiene of returnable plastic crates** cleaned in a washing tunnel was also determined at the Herajoki plant. The results showed that the bottom parts of the crates were the dirtiest and had the worst cleaning results. Design of Experiment (DOE, Taguchi method) was used to optimise the cleaning procedure based on ultrasonic washing. The ultrasonic cleaning method is applicable for cleaning returnable plastic crates and it stands comparison with the washing tunnel method, as was shown using artificially soiled crate pieces in pilot-scale studies. In this work the suitability of three cultivation methods i.e. conventional culturing, Petrifilm and **DryCult** for monitoring the total bacterial load was also verified in the cleaning of returnable plastic crates. The DryCult® TPC is a practical choice if the tests have to be carried out on-site.

- Plate heat exchangers (PHE) are widely used in the dairy industry for heating, cooling and regenerative duties, both for utilities and product. The risk of recontamination is related to leakage through the plate. Sometimes fatigue cracks, wear or corrosion occur and a hole appears through the plate. Some years ago double-walled plate heat exchangers, in which the plates are just pressed together by means of screws in the PHE frame, appeared on the market for use at dairies. Tests at a German institute were carried out to check if leakage appears, when one layer of the double-wall plate is pierced. These results showed that a hole in one of the layers of the double wall caused a leakage detectable by visual control. However, the experience of using double-wall heat exchangers in dairy applications is quite limited.

- The production of cheese is partly open, which provides plenty of opportunities for contamination when the product is being processed. **Equipment** is an important source of **microbial contamination**. Critical spots were found under the conveyor belts and on the rollers. The results showed that the final rinsing water in the cleaning machine used for cheese moulds was contaminated with microbes. In order to prevent contamination, procedures for inspecting and cleaning the cheese-mould cleaning machine must be established. The cleanliness assessment should involve the following: a definition of critical areas/spots; daily checking by an independent person not part of the cleaning group; documentation; auditing the cleaning effect on a regular basis; action taken when deviations are found; and feedback to the cleaning group.

- Disinfection is required in food plant operations where wet surfaces provide favourable conditions for the growth of microbes. The use of effective disinfectants minimises contamination of the product, enhances shelf-life and reduces the risk of foodborne illness. Prolonged exposure of the surfaces to disinfectants enhances the microbicidal effect but exaggerated exposure often leads to an increase in the resistance against the disinfectant used. In the Icelandic project the **minimum inhibitory concentration** was determined on 140 isolates obtained from a dairy environment with the use of 4 disinfectants. The strains isolated and characterised showed in general that the more tolerant strains had a two to threefold decrease in generation time with increasing disinfectant concentrations, whereas a smaller decease was observed for the sensitive strains. In the Norwegian project studies were also focused on the occurrence of **resistant microbes** in the food industry. Questions whether misuse of disinfectants have imposed selective pressure and contributed to the emergence of disinfectant-resistant microbes in process environments have been asked. Microbes have survived chemical treatment, such as g fogging disinfection, and disinfection in footbaths in dairies. Bacteria were isolated from about 75% of the footbaths tested, and none of the disinfectants used totally prevented bacterial survival. *Serratia marcescens* isolated from footbaths containing Tego were resistant to the recommended in-use concentration. *Methylobacterium* sp., *Rhodococcus erythropolis* and *Rhodotorula mucilaginosa,* which were isolated from the process environment, also showed resistance to several disinfectants.

Detailed advice and directions concerning the hygienic design of equipment is the topic of numerous guidelines and harmonised European standards. The five poor hygienic design cases (a static fluid bed, a DuoSafe PHE, a cheese slicer, a bag filler and a plastic bottle filler) show that practical knowledge of processing and process conditions is needed to be able to perform hygienic engineering. However, to this day studies on the hygienic construction and maintenance of entire process plants are scarce. The **integrated approach in hygienic engineering** is the result of a systematic approach to hygienic plant construction from single parts and connections across process lines to meet the hygienic demands of entire process plants. The system builds on the proper definition and identification of the types of possible problems related to each level in the hierarchy. This approach will be published in the forthcoming European Hygienic Engineering and Design Group (EHEDG) guideline.

Preface

The requirements for improved hygiene are beingraised due to the development of the food industry, including prolonged shelf life, centralised production and long-distance transportation, automated cleaning systems, reduced cleaning time and demands for environmentally friendly cleaning agents. The development of detection and identification methods for assessing microbial and organic soil on surfaces, in the air, on packaging and in raw materials as well as in products is needed to ensure that the results obtained are reliable and repeatable. The intention is to minimise the risk of spoiled foodstuffs caused by pathogens or other harmful microbes. From an industrial point of view it is important to maintain and extend the interactive contacts between persons dealing with safety and environmental questions in Nordic dairies. Three subsequent Nordic 3-year projects in dairy hygiene, Sanitation in dairies – Sanitering i mejeri; Evaluation of cleaning agents and disinfectants for use in dairies: methods and mechanisms (DairyNI); and DairyNET – Hygiene control in dairies, have been carried out from 1994 to 2004.

The research work for the DairyNET-project (P00027) was carried out mainly at the following Nordic research institutes: the Danish Technological Institute (DTI), the Icelandic Fisheries Laboratories (IFL), Matforsk, SIK and VTT Biotechnology. The dairies involved in the project were Arla Foods from Sweden and Denmark, Milko, Norrmejerier and Skåne mejerier from Sweden, Nordurmjolk from Iceland, TINE from Norway and Valio Ltd from Finland. The six other enterprises were Finnsonic Oy, JohnsonDiversey, Lagafors Fabriks AB, Mjöll, Orion Daignostica Oy and Tetra Pak Nordic Processing. University personnel from BioCentrum-DTU, the Royal Institute of Technology (KTH) and University of Akureyri were also involved in this research project. The senior advisor at the Nordic Innovation Centre (NICe) previously the Nordic Industrial Fund involved in the project was Oddur Gunnarsson. Dr Gun Wirtanen from VTT Biotechnology co-ordinated the project. Funding from the national technology agencies in Denmark, Finland, Iceland and Sweden as well as industrial and research partners was also available.

The project group and the steering group met eight times during the project. The project was carried out in accordance with Nordic synergy and national project plans. The aims of the NICe-funded DairyNET project were to extend the

Nordic dairy hygiene platform and to provide reliable methods and procedures for improved hygiene to be used at process scale. The achievements in this network project have been summarised in this book, which has been reviewed by Prof. Raivo Vokk and Dr. Tina Veskus at Tallinn Technical University. Our special thanks are due to Antti Huovinen, who has painted the picture for the book cover. A vocabulary for the abbreviations used in this book is given in Appendix 1. The subjects covered HACCP systems, a checklist for hygiene control in dairies, milk quality through the whole process line, air quality in dairies, contamination routes for *Listeria*, the quality of process waters, the efficiency of CIP procedures, hygiene in supply systems, ultrasound cleaning procedures, microbial adhesion and biofilm formation, bacterial resistance to disinfectants, hygienic design and integrated hygiene systems.

Contents

1. INTRODUCTION TO THE PROJECT

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VTT Biotechnology, Espoo, Finland

1.1 BUILDING BLOCKS IN NORDIC DAIRY HYGIENE CO-OPERATION

The chain of co-operation projects between Nordic dairies and research institutes funded by the Nordic Industrial Fund has been built-up by three individual 3-year projects: Sanitation in dairies – Sanitering i mejeri; DairyNI – Evaluation of cleaning agents and disinfectants for use in dairies: methods and mechanisms; and DairyNET – Hygiene control in dairies. VTT Biotechnology has been the coordinator of these projects and the project partners taking part in the subsequent projects have increased during the project series. During the long project chain; solutions to one case have raised more questions and led to new research studies and applications.

1.1.1 Sanitation in dairies project

The first Nordic co-operation project, Sanitation in dairies – Sanitering i mejeri, started in 1994. The Sanitation in dairies – Sanitering i mejeri project P93156 was part of the NORDFOOD programme. The project partners were from Norway, Sweden and Finland and they comprised two dairies, TINE and Valio, and three research institutes; Matforsk, SIK and VTT Biotechnology. The purpose of the Sanitation in dairies project was to harmonize the procedures used for testing sanitation agents in Nordic dairies. Furthermore, the efficiency of cleaning agents and disinfectants used in dairies was tested under controlled circumstances to enable a comparison of the agents. The main results and conclusions made in the Sanitation in dairies project are reported in a book written by Wirtanen *et al.* (1997). The specific topics of the project were:

- the testing of disinfectants in suspensions and on surfaces on a laboratory scale,
- the evaluation of monitoring methods for measuring the cleaning efficiency of surfaces in open and closed systems,
- the use of metabolic indicators in monitoring the viability of microbes,

- the use of detergent solutions for the release of microbial cells from surfaces using both swabbing and spraying and
- the testing of disinfection on an industrial scale.

1.1.2 DairyNI project

The second Nordic co-operation project was DairyNI – Evaluation of cleaning agents and disinfectants for use in dairies: methods and mechanisms (P96049). This project was part of Nordfood2 programme and it started in April 1997 and lasted until January 2000. The participants came from Norway, Iceland, Sweden and Finland and they represented three dairies, TINE, Arla and Valio, one technochemical company, Suomen Unilever Oy DiverseyLever and three research institutes, Matforsk, SIK and VTT Biotechnology as well as the universities of Helsinki and Reykjavik. The experiments in this project focused on monitoring sanitation methods in open and closed systems e.g. fogging, ozonation, footbath hygiene, the cleaning of cheese moulds and yoghurt pasteurises, the development of testing procedures for measuring disinfectant efficacy, microbial resistance phenomena against disinfectants, life cycle assessment and an evaluation procedure for the functionality of cleaning procedures. The main results and conclusions made in the DairyNI – Evaluation of cleaning agents and disinfectants for use in dairies: methods and mechanisms (P96049) project are reported in a book written by Wirtanen *et al.* (2002a). The specific topics of the project were:

- method development for the evaluation of microbial resistance against disinfectants
- evaluation of efficiency of fogging and footbath disinfection
- development of methods to study cost, usage and effects on environment
- evaluation of cleaning chemicals on spore-contaminated surfaces in flow systems
- evaluation of the efficiency of disinfectants on spores
- evaluation of methods for the practical measurement of process hygiene
- development of testing methods and optimisation of environmentally less harmful cleaning chemicals
- testing of efficiency of cleaning agents and disinfectants with and without soil
- microbial evaluation of cleaning procedures on a pilot scale.

1.1.3 DairyNET project

The third Nordic co-operation project is DairyNET – Hygiene control in dairies (Figure 1.1). This network project started at May 2001 and ended in October 2004. The aim of this project was to provide reliable methods for validating and improving cleaning and hygiene on a process scale at dairies by applying new knowledge and by using available laboratory and pilot-scale equipment in the experimental set-ups. From an industrial point of view it is important to maintain and extend the interactive contacts between persons dealing with safety and environmental questions in Nordic dairies. The work is performed in independent national research projects, Nordic synergy tasks and networking within project meetings (Figure 1.2). A summary of activities is given in Appendix 2.

A list of publications, oral and poster presentations and theses carried out in the project is given in Appendix 3. The summary of the project was translated into Swedish (Appendix 4), Finnish (Appendix 5) and Icelandic (Appendix 6).

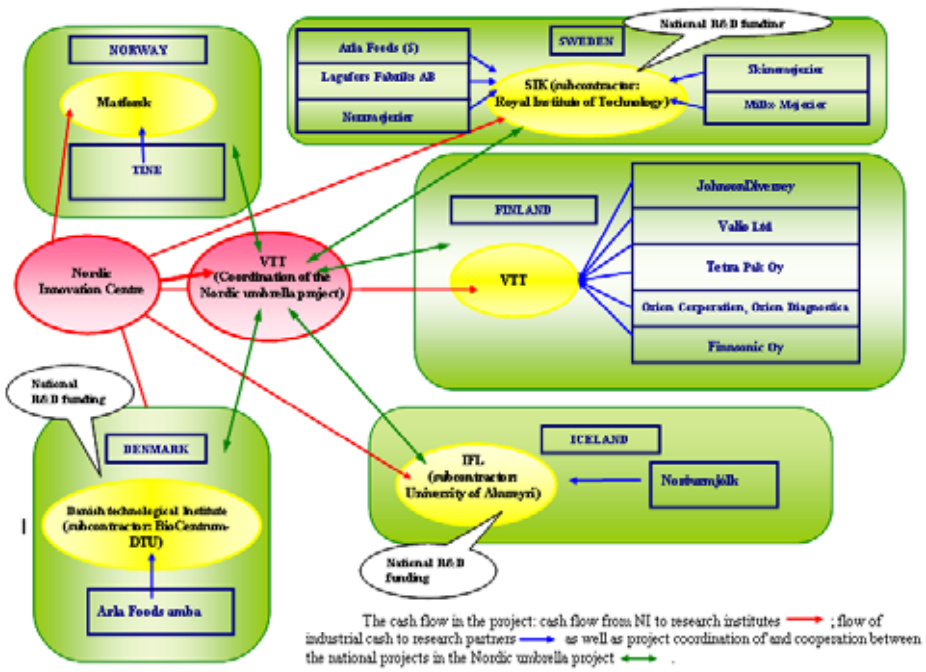

Figure 1.1. The partners in the Nordic dairy hygiene network.

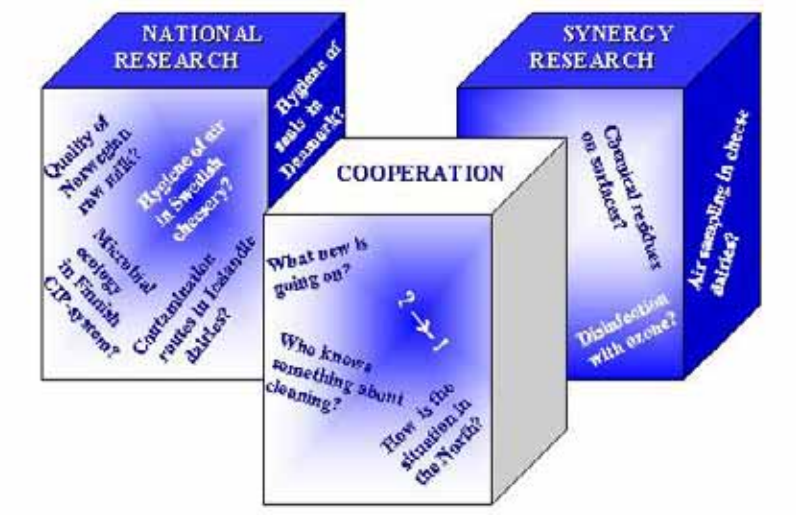

Figure 1.2. Building blocks of the tasks in the Nordic DairyNET project.

1.2 PROJECT STRUCTURE OF THE DAIRYNET-PROJECT

1.2.1 National projects

The Nordic network project is based on research studies performed in five national projects. The national technology agencies and/or industry fund these national projects. The results obtained and plans for further research work in the independent national projects are presented at Nordic network project meetings. Examples of the work performed in the national projects have been reported at both the 34th and the 35th R^3-Symposia held in Turku and Elsinore (Wirtanen & Salo, 2003; Friis, 2004). The activities in the national projects within the DairyNET project were to develop procedures and methods for checking the cleaning efficiency on a pilot scale. The aim was to transfer improved procedures and methods into dairy processes. The subjects were:

- milk quality through the whole process line (N)
- air quality in various types of dairies (FIN, SE)
- contamination routes for *Listeria* spp. (IS)
- HACCP systems (DK)
- checklist for the hygiene control in cheese dairies (SE)
- quality of process waters (FIN, SE)
- efficiency of CIP procedures (DK, FIN)

- hygiene in supply systems (IS)
- ultrasound cleaning of milk crates (FIN)
- microbial adhesion and biofilm formation (N, SE)
- bacterial resistance to disinfectants (IS, DK, N, FIN)
- hygienic design and integrated hygiene systems (DK).

1.2.2 Synergy projects

The aim of this part of the project is to familiarise all partners with the new methods, which can be used on a process scale. This part is performed applying new knowledge and using available laboratory and pilot-scale equipment in the experimental set-ups. The organiser of each synergy task prepared instructions, sent material to the other participants in addition to collecting and reporting the results. The three synergy tasks were performed similarly in all Nordic countries:

- detecting detergent and disinfectant residues,
- soil detecting with an ultraviolet light lamp and
- detection of airborne fungi.

1.2.3 Co-operation and networking

The various forms of communication between the project partners consist of personal contacts at national and Nordic project meetings, dairy personnel meetings and the confidential homepage provided by NICe. From an industrial point of view it is important to maintain and extend interactive contacts between people dealing with safety and environmental questions in Nordic dairies alongside the hygiene research. Research scientists also need interactive communication with other researchers and representatives from industry. The Nordic project meetings are co-ordinated by VTT Biotechnology. There have been eight DairyNET project meetings. The project partners have visited TINE in Oslo, SIK in Gothenburg, IFL and Norđurmjólk in Akureyri, the Biotechnological Institute in Kolding, JohnsonDiversey in Turku, Skånemejerier in Malmö and Valio Ltd in Herajoki. National research studies are reported and presented during these project meetings. The dairy representatives involved in this project have met in meetings arranged in conjunction with the project meetings. The closing seminar of the DairyNET project was held at Svartå Slott in Karis.

2. DAIRY HYGIENE AND RAW MILK QUALITY

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The milk from healthy cows that are free from infection is essentially sterile, but may become contaminated during milking and storage at the dairy farm, or transportation and storage at the processing site. The microbial load of raw milk will depend on the age of the milk, hygiene (which affect the level of contamination and the characteristics of the microbial contamination flora) and factors affecting growth, such as storage temperature. Since raw milk is stored at 2–4^{o}C, the microbial spoilage flora is dominated by psychrotrophic organisms.

2.1 MICROBIAL QUALITY OF RAW MILK QUALITY

An investigation of the microbial flora of raw milk from three dairies in Norway and Sweden (Eneroth *et al.,* 1998) showed that Gram-negative bacteria dominated. Sampling milk from the silo tank and the tube between the float hopper and pasteuriser showed that *Pseudomonas* spp. were present in 100%, *Enterobacteriaceae* spp. in about 60% and *Aeromonas* spp. in 50% of the samples taken. Gram-positive bacteria were detected in 4% of the samples taken from the silo tank, but in none of the samples from the pasteuriser. It has been shown in earlier studies that refrigerated raw milk was exclusively spoiled by Gram-negative bacteria, the majority of which were *Pseudomonas fluorescens* biovar I, *P. fragi*, *P. lundensis* and *P. fluorescens* biovar III. Minor groups in the raw milk included *Enterobacteriaceae* spp., and *Acinetobacter* spp. In 65% of the cases pasteurised milk was spoiled by essentially the same Gram-negative spoilage organisms occurring in raw milk. Bacterial tracking by ribotyping has indicated that Gram-negative bacteria from raw milk may contaminate milk after pasteurisation (Ralyea *et al.,* 1998).

Pasteurised milk may also be spoiled by *Bacillus* spp. which may survive heat treatment or recontaminate the milk after heat treatment. *Bacillus* spores may survive both heat and chemical disinfection (Faille *et al.,* 2001), and

investigations of the adherence of bacteria to various milk contact sites indicated that biofilm development may occur on gaskets in spite of cleaning-in-place procedures (Austin & Bergeron, 1995). However, the results of (Lin *et al.,* 1998) showed that *B. cereus* spores in raw milk are the major source of *B. cereus* in pasteurised milk and that post-pasteurisation contamination along the milk processing line is possibly a minor source.

The effect of storage time and temperature on microbial levels in milk is well-documented and relatively easy to measure and control. However, the influence of hygiene on the quality of raw milk is less predictable. The hygienic level of the raw milk side is difficult to determine and, as opposed to pasteurised milk, it is not possible to use the level of microbes in raw milk as an indirect measure of hygiene. Measuring hygiene can be performed by visual inspection, preferably under UV-light. Most of the processing equipment is not easily accessible and endoscopes in piping can be used, but this method is expensive and laborious. Microbial sampling may also be performed, but as with visual inspection, large areas are not accessible for sampling. In addition, only small areas can be sampled and the results are not easy to interpret. The correlation between the fouling and the microbial load is not clear.

The aim of this project was to investigate the hygiene in the raw milk side of dairies, to find critical points during the processing line that may contribute to contamination of critical raw milk and compare visual inspection with microbial sampling.

2.2 Visual Control and Microbial Sampling at the Dairies Visited

Five Norwegian dairies were visited. Routines for milk receiving, cleaning, and quality control were examined by verbal communication with the quality manager and other relevant employees, visual inspection and examining written documentation.

Tank trucks, air separators, piping, valves and silo tanks (through man-hole covers) in the raw milk side of the dairy were inspected using UV-light (Labino 135 TrAc Pack spotlight, >50 000 mW/cm^2). Microbial samples were taken from the processing line after cleaning and disinfection. From the results in Dairy 1,

common sampling points were chosen to enable comparisons between the dairies. However, additional samples were occasionally taken in the other dairies at sites with visible fouling. The surfaces were swabbed with a cotton swab moistened in peptone water. The area of the surface was not standardised as most of the samples were taken inside piping, valves and tanks. Further analysis of the samples varied between the dairies:

Dairy 1 and 2: The cotton swabs were streaked on Plate Count Agar (PCA). Samples of 0.1 ml of residual water at the bottom of silo tanks and used disinfectant/hot water were spread on PCA. The plates were incubated at room-temperature (approx. 20°C) during transportation to the institute and further incubation at 20°C at the institute. Mono-cultures of bacteria isolated from colonies with a different appearance on PCA were made by serial inoculation on PCA and visual inspection. The pure isolates were stored on glycerol (20%) at -80°C.

Dairy 3, 4 and 5: The swabs and samples of 0.1 ml of residual water at the bottom of silo tanks and used disinfectant/hot water were inoculated in tubes containing 10 ml of UHT milk and incubated at 7°C at the dairies. After three days (four days for dairy 4) and ten days of incubation, the samples were taken for an assessment of CFU on PCA and the number of spore forming bacteria. Samples of 0.1 ml were taken from the milk and spread on PCA. Serial dilutions were made and 0.1 ml of each dilution spread on PCA. The plates were incubated at 20°C and the number of CFUs counted after 3 and 6 days of incubation. In order to determine the number of spore-formers, 1 ml of samples of inoculated UHT-milk were heated at 80°C for 10 min before 0.5 ml was spread of on PCA. The plates were stored at 4°C after counting and sent by mail to MATFORSK. Mono-cultures of bacteria isolated from colonies with a different appearance on PCA were made by serial inoculation on PCA and visual inspection. The pure isolates were stored on glycerol (20%) at -80°C.

2.2.1 Identification and characterisation of microbes

The identification of microbes from Dairy 1 was performed using conventional methods, such as Gram-stain, oxidase and catalase tests and hemolysis on blood agar. Motility and shape were examined in the light microscope. As a result of

these tests, isolates were grouped into five different groups of organisms as indicated in Table 2.1.

Bacterial isolates from Dairy 2 isolated from different types of surfaces were pooled, inoculated in UHT milk, heat-treated and incubated at 6°C to select for psychrotrophic spore-formers. Each isolate was also spread on *Pseudomonas* selective agar. Isolates from dairy 3, 4 and 5 were selected on the basis of their ability to grow at 6°C and survive heat-treatment as described above.

Table 2.1. Identification of strains from dairy 1 using conventional tests.

Term	Bacteria included in group	Characteristics
Pseudomonas	*Pseudomonas/Alcaligenes/ Shewanella/Flavobacterium/ Moraxella/Campylobacter Aeromonas, Vibrio*	Gram-negative, oxidase positive rod
Staphylococcus	*Staphylococcus/Micrococcus*	Gram-positive, catalase-positive cocci
Bacillus	*Bacillus (Listeria)*	Gram-positive, catalase-positive rod
Lactic acid bacteria (LAB)	*Lactobacillus/Streptococcus/ Lactococcus/Enterococcus/ Pediococcus/Leuconostoc*	Gram-positive, catalase-negative
Enterobacteriaceae	*Enterobacteriaceae/ Acinetobacter/Xantomonas*	Gram-negative, oxidase-negative rod

2.2.2 Characterisation of sporeformers

Gram-positive, catalase-negative cocci were further identified by 16s rDNA analysis. Other Gram-positive strains were stored on PCA for one week and then examined in the microscope to determine if they formed spores. Sporeformers were further identified by 16s rDNA analysis and API. To test for growth at low temperatures the dairy sporeformers, *Bacillus cereus* NVH74, *B cereus* NVH200 and three *B. pumilus* from cream, were inoculated in UHT-milk and Brain heart infusion (BHI) at 7°C and 4°C for 7 days and the number of CFU was determined by plate spreading.

The ability of spores to attach themselves to stainless steel surfaces was tested in saline. A stainless steel coupon was placed in a spore suspension of approx. 10^7 spores/ml (> 99% spores) for 2 h at 20°C. The number of attaching spores was determined by removing the spores from the coupon using an ultrasound bath and measuring CFUs by plate spreading. The efficacy of the removal method was verified by fluorescence microscopy after staining with Acridine Orange (0.02%). Resistance to heat (distilled water, 85°C, 10 min) and peracetic acid (0.3% Oxonia aktiv, 40°C, 5 min) were tested on spores attached to coupons. The number of survivors was determined by sonication of coupons and plate spreading.

2.3 Hygiene Routines in the Dairies

An overview of the cleaning and disinfection routines in each plant is shown in Table 2.2. All dairies used an alkali wash. Three dairies used heat and two peracetic acid based disinfection. Dairy 1 and 5 used the same CIP station for the raw and pasteurised side.

The internal quality control included batch controls of milk in tank trucks (antibiotics, temperature, CFU 30°C), raw milk in silo tanks (temperature, CFU 30°C). The CFU in tanks at the dairy farms was determined twice a month using Bactoscan. The visual control of tanks was performed at various frequencies from monthly to once or twice a year. The use of UV lamps for visual inspection varied.

2.4 Visual Control and Microbial Sampling

2.4.1 Control points

Sampling was performed on problem areas identified during the first dairy visit and, in addition, at points which were visually not clean under UV light. Table 2.3 shows an overview of sites usedfor visual control and sampling. Sampling sites that were tested, if possible, in all the dairies are indicated. From each site, 1–4 samples were taken.

Table 2.2. Cleaning and disinfection process in the dairies.

Dairy	Cleaning 1	Cleaning 2	Disinfection	Manufacturer
1	Alkali (MIP-FL), 1.2–1.5% at 75°C for 6 min	Nitric acid (HorolithV), 1.2–1.5% at 70°C for 6 min	Hot water 82–87°C for 3 min	Ecolab
2	Alkali (Alkaren 45), 1.1–1.2% at 65–70°C for 10 min (silo)/ 4 min (tank truck)	-	Peracetic acid (DesinfectMB), 0.2–0.3% at 20–30°C for 3 min	Novadan
3	Alkali (MIP FL), 0.9–1.0% at 65–70°C for 10 min (silo)/ 5 min (tank truck)	-	Peracetic acid (Oxonia aktiv S), 0.2–0.3% at 15–20°C for 5 min	Ecolab
4	Alkali (Climax M), 1.5–2.0% at70–75°C for 10–13 min	Nitric acid, 0.8–1.5% at 65–70°C for 10–13 min	Hot water, 83–85°C for 10–13 min	Lilleborg
5[1]	Alkali, 1.5% at 70°C for 10 min	Nitric acid, 1.5% at 65°C for 10 min	Hot water, 80–90°C for 3 min	Novadan

[1]Tank trucks and pipes with only alkali cleaning four days/week, acid cleaning one day/week and alkali plus acid once a week. Raw milk tanks with alkali plus acid five days/week.

Table 2.3. Sites for visual control and microbial sampling.

Sampling site	Common sampling site (Y/N)
Tank trucks	
Inner surface of tank	Y
Tank lid	N
Valve outlet milk, gasket	Y
Valve outlet milk, inner surface	Y
Air separators, inner surface	Y
Gasket, air separator	Y
Air separators	
Piping	N
Inner surface	Y
Lid, gasket	N
Piping	
Inner surface	N
Inner surface of valves	Y
Gaskets, valves	Y
Silotanks	
Silo tanks, welded joint	Y
Silo tanks, sensor/propeller	Y
Silo tanks, inner surface	Y
Residual water	Y
Cleaning station	
Alkali/Acid solution	N
Used disinfectant/hot water	Y

2.4.2 Visual control

By using UV light, fouling not visible in normal lamp or daylight becomes visible. Despite this, it was occasionally difficult to determine if staining was due to the surface finish, the type of steel or actually because of fouling. Fouling appeared as a dim coating which was white, and occasionally pink. On some occasions, for example on pipes connected to cleaning nozzles, around lids and on gaskets, a thick layer of fouling was found. In some silo tanks, small fluorescent particles were observed on the surfaces, but they were too small for sampling.

The degree of fouling and location of problem areas varied between the dairies. Some problem areas were observed in several dairies (such as sensors), whereas some dairies had systematic problems, e.g. welded joints. Table 2.4 shows problem areas found by visual control. Common to these points was that they were difficult to clean, due to the design or material choice.

Table 2.4. Typical problem areas in the dairies.

Problem areas	Comment
	Tank trucks
Generally	On several occasions, one or two of the tanks on the same truck was fouled, whereas the others were visibly clean. No explanation was found.
Lid and lid gasket on tank	
Cleaning nozzles	Heavy fouling was sometimes observed on the pipe connected to the nozzle
Air separator, lid and gasket	Frequently contaminated
	Piping
Gaskets	Routine changing of gaskets is important. Gaskets in dairies with bad routines were visibly contaminated and had partially deteriorated.
	Silo tanks
Welding joints Sensors	Systematic problem at one dairy
Wall under man-hole cover	Bad design at one dairy
	Cleaning
Disinfectant/ hot water	More visible contamination of disinfectant than hot water – must be checked on a regular basis

2.4.3 Microbial control

Sampling at sites with fouling was over-represented and therefore the microbial level found in this investigation does not reflect the general level in the dairies. The sampling was not quantitative as most of the points were not flat and it was not possible to standardise the sampling area. Nevertheless, an impression of the hygienic level was obtained, and the level of microbes ranged from zero colonies to full coverage of microbial growth on the agar plates.

A limited number of samples were taken using both swabbing and the contact agar plate method (Hygicult). The results indicated that more bacteria were isolated using the swab-method. The reason for this result could be that bacteria were attached to surfaces and mechanical energy was necessary to isolate bacteria. Therefore, swabbing is recommended on cleaned surfaces.

The sampling points were considered to be contaminated if bacteria were detected on the agar plates or in inoculated UHT milk. For stainless steel, the bacterial level was in general highest on surfaces with visible fouling and often no microbial contamination was found on surfaces that appeared clean. For gaskets, bacteria were isolated from both clean and fouled surface. At the dairies using heat disinfection, the number of sampling points which appeared fouled was higher than the number of points with microbial contamination. Heat disinfection may be efficient even on surfaces with a thin fouling layer. However, at the dairies using chemical disinfection, microbial contaminants were found on surfaces that appeared clean. (Figure 2.1) This indicated that the disinfection step was not efficient.

It was not possible to identify a common problem area for all dairies. Table 2.5 shows the results of the identification of bacteria from various types of sampling points in Dairy 1. The microbial flora was diverse, both on steel surfaces and rubber. With an effective cleaning programme, one would expect to find a much less diverse flora, consisting mainly of sporeformers, which will survive heat or disinfectant exposure. The cleaning solutions and hot water at Dairy 1 were visually clean. The main problem at this dairy was the fouling and microbial contaminants on welded joints. The gaskets were intact, clean and free from microbes.

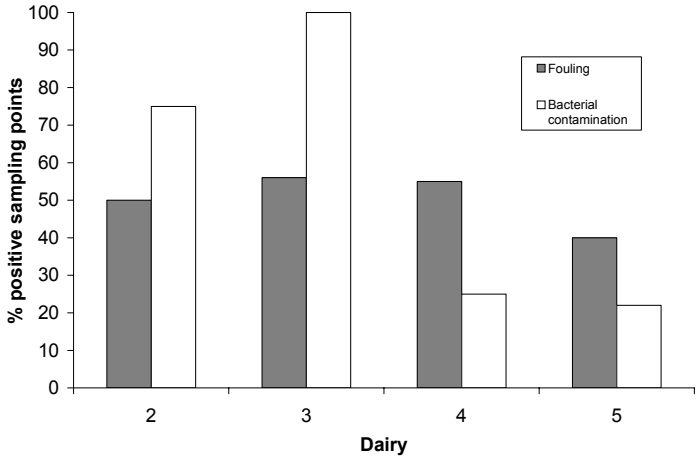

Figure 2.1. The frequency of sampling points with bacterial contamination and fouling. The numbers are based on a standardised set of sampling sites (11–13 per dairy) and 1–4 samples per site.

Table 2.5. Identity of microbes isolated at Dairy 1.

Sampling point	Material	Type of microbes[1]				
		Pseudo-monas	*Staphylo-coccus*	*Bacillus*	LAB	*Entero-bact.*
Silo, welded joints	Steel	+	+	+	-	-
Valves	Steel	-	-	-	+	-
Air separators, inside	Steel	+	+	+	+	-
Tank truck, valve, gasket	Rubber	+	+	-	-	-
Tank truck, air separator	Steel	+	-	+	-	+
Tank truck, air separator, gasket	Rubber	-	+	+	-	+

[1] microbiological/chemical tests. *Pseudomonas*: Gram-negative, oxidase-positive rods; *Staphylococcus*: Gram-positive bacteria, catalase-positive, coccoid; *Bacillus*; Gram-positive bacteria, catalase-positive rods; LAB: Gram-positive bacteria, catalase-negative; *Enterobacteriaceae*: Gram-negative bacteria, oxidase-negative rods**.**

From the inoculated milk samples from the other dairies, it was possible to determine if the isolates were psychrotrophic and sporeformers. The results are shown in Table 2.6. The microbial contamination in Dairies 2 and 3, that used chemical disinfection, was mainly due to the cleaning stations, which did not function optimally. The cleaning and disinfection solutions were soiled and contained vegetative bacteria. Vegetative bacteria should not be able to survive in user-concentrations of disinfectants, but the bactericidal activity was probably reduced due to the soiling. In addition, the gaskets were heavily contaminated with psychrotrophic vegetative bacteria (among them *Pseudomonas* spp.). Sporeforming bacteria were also found (*Bacillus* spp.), among them psychrotrophic strains in tank truck tanks, gaskets in tank truck air separators (Dairy 2) and silotanks and valves connected to silotanks (Dairy 3).

The microbial sampling in Dairy 4 and 5 showed that the level of hygiene was good. The cleaning stations functioned relatively well and gaskets were intact. Microbes were isolated from a limited number of areas with heavily soiling, such as pipes connected to a cleaning ball (psychrotrophic spores). In addition, psychrotrophic vegetative bacteria and spores were found on gaskets.

2.4.4 Further characterisation of Gram-positive bacteria

Isolates identified as *Staphylococcus* spp. were further analysed by 16s rDNA analysis. Unfortunately, only four strains were identified using this method. Two isolates from silo tanks were identified as *St. warnerii* (or *St. pasteuri*) (99%). One isolate from a disinfecting footbath was *St. cohnii* (98%). An isolate from raw milk was identified as *Kocuria varians* (99%).

Table 2.6. Overview of microbial contamination. Each point represents results from 1–4 swab samples. Psychrotrophic microbes are marked with a P and mesofile/termofile M/T.

Sampling point	Dairy 1[1]	Dairy 2[1]	Dairy 3[2]	Dairy 4[2]	Dairy 5[2]
		Silo			
Welded joints	+	(+) P		-	-
Wall	(+)		+ P	-	-
Condensed water	-	-	+ P	(+) P	
Sensor	+			(+) M/T	-
		Valves			
Gasket	-	+ P	(+) P	(+) P	-
Inside of valve	(+)	-	+ P		-
Air separators	+	(+)		-	
		Tank trucks			
Tank, inside	-	(+) P	+ P	-	-
Valve, gasket	+		+ P		(+) P
Valve, inside	(+)	-			+ M/T
Air separator	+	(+)	+ P	-	-
Air separator, gasket	+	+ P	+ P	-	
CIP station (disinfectant/hot water)		+	+ P	-	-

[1] no bacteria on PCA, (+): 1–10 CFU, +: >10 CFU

[2] <10 CFU/ml; (+): 10–100 CFU/ml; +: >100 CFU/ml after 3–5 d at 6°C

Identification of other Gram-positive isolates revealed three strains belonging to the *B. cereus*-group and two strains of *B. pumilus*. All five strains were psychrotrophic (growth at 7°C) and two of the strains belonging to the *B. cereus*-group grew at 4°C (Table 2.7). The results showed that *Bacillus* strains could adapt growth to lower temperatures, since preincubation at a low temperature gave a higher growth rate at 4°C. No differences in the growth rate between milk and BHI-broth as a growth medium were detected (Skånseng, 2003). The ability to adhere to steel surfaces seemed to be higher for *B. cereus* spores, than for *B. pumilus* spores. The strains were relatively resistant to both chemical and heat disinfection. Significant correlation between the growth rate at 7°C and the heat resistance of spores was found, and the most psychrotrophic strains were the most heat-sensitive (Skånseng, 2003).

Table 2.7. Growth characteristics of Bacillus *strains isolated from dairy.*

Strain		Sampling point	Growth 7°C, 7 d	Growth 4°C, 13 d[1]		
				UHT7	UHT	BHI
A2	*B. cereus*	Tank truck, lid, air separator	++	++	+	+
A4	*B. cereus*	Silotank, sensor	++	++	+	++
D1	*B. cereus*	Silotank, wall	++	-	-	-
A1	*B. pumilus*	Silotank, sensor	++	-	-	-
B2	*B. pumilus*	Tank truck, gasket, air separator	++	-	-	-

[1] UHT7 = bacteria were pregrown at 7°C

++: ca 2 log; +: ca 1 log; - no growth

Alkali and acid cleaning is the most important in the elimination of spores. Four of the strains were isolated either from porous material (gasket) or points that were heavily soiled. If cleaning does not function optimally (bad design, old gaskets etc), spores and vegetative bacteria will remain in the equipment. Disinfection should ideally eliminate vegetative bacteria and some spores, but in this case, neither method reached the spores as they were hidden in porous materials or in the fouled layer. One of the strains was isolated from a visually clean steel surface (silo tank) at a dairy with good hygiene. The strain had a high adherence ability and a higher resistance to heat and peracetic acid than the other strains. This strain probably had characteristics that made it able to survive in the equipment. Although the investigation is too limited to draw general conclusions, the results showed that *Bacillus cereus* present on equipment has the ability to withstand the cleaning process and grow in milk at refrigeration temperatures. To be able to assess the potential risk, the toxin production of the strains should be determined.

3. BIOAEROSOLS IN PRODUCTION AREAS

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Biological agents such as plant cells, pollen, algae, protozoa, bacteria, yeasts, mould spores and viruses originating from natural habitats can be found in the air (Brown, 1996). The great majority (approx. 73–90%) of bacterial genera in the outdoor atmosphere are Gram-positive bacteria (Lighthart, 1997). Changes in bacterial composition have been observed over time, e.g. the levels of Gram-positive bacteria spore-forming bacteria were minimum (17%) and Gram-negative bacteria maximum (22%) during the night, while during daylight Gram-positive bacteria were maximum (35%) and Gram-negative bacteria minimum (≤ 12%; Shaffer & Lighthart, 1997). Fungal bioaerosols have been observed to be highly dependent on weather conditions. The highest concentration of total colony counts appeared when the temperature was 25–30 °C, relative humidity (RH) 60–70%, and wind speed < 1 m/s (Lin & Li, 2000).

3.1 AIR AS A CARRIER OF PARTICLES

The ability of air to contain and transport living substances e.g. microbes, is, however, often forgotten. There are a number of ways in which biological and microbiological material may become airborne, e.g. through wind, rain splash and releases from animals. Almost every human and animal activity can create bioaerosols. For example, sneezing and coughing cause bacteria to become airborne. Humans shed skin and bacteria into the air, and even wearing clothes does little to stop the process (Griffiths & DeCosemo, 1994). An aerosol can be defined as a suspension of microscopic solid or liquid particles in air or gas, such as smoke, fog or mist. The size of aerosol particles is generally in the range 0.5–50 μm (Figure 3.1).

Ljungqvist and Reinmüller (1998) showed with a controlled environment that the exposure situation with a human as a contamination source gives rise to the total number of airborne particles detectable with standard methods. The ratio between viable and nonviable particles ≥ 0.5 μm in this study was estimated to be 1:10,000. In industrial processes, airborne dust particles introduce not only foreign matter into the product, but also microbial contamination (Ljungqvist & Reinmüller, 1993, 1995).

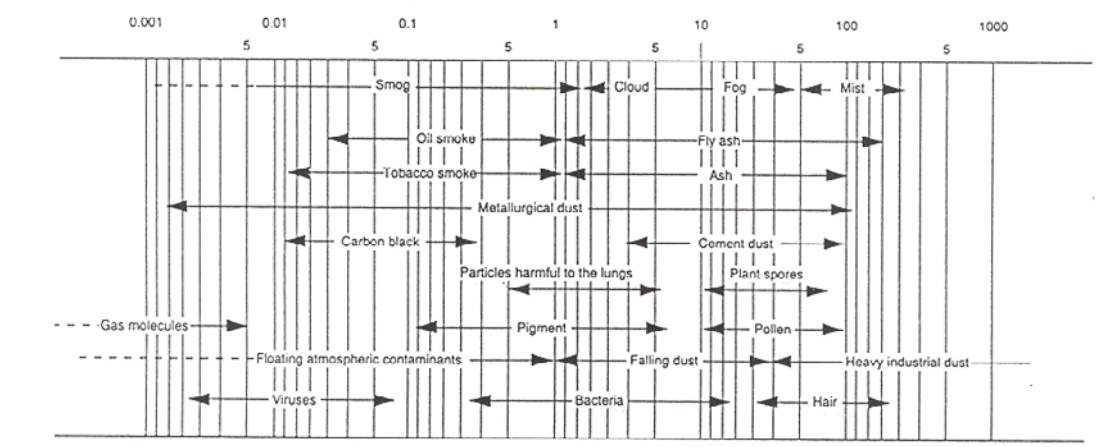

Figure 3.1. Particle size distribution (diameter as µm) of some atmospheric contaminants (Brown, 1996).

3.2 BIOAEROSOLS AND PERSONNEL

Personnel are a primary source of airborne microbes in cleanroom production areas (Schmitt, 2000). Personal hygiene must, therefore, be incorporated into the overall concept with care. The personnel must also be properly trained. Employees must fully understand the procedures and the microbiological risks inherent in inappropriate actions (Steinbeck, 1997; Schicht, 1999). It has been found that a slowly gesturing person generates approx. 500,000 particles/min and a rapidly gesturing person will generate 10 times more. Therefore, limited personnel movements and specific cleanroom garments are an essential part of strict cleanroom requirements (Schmitt, 2000). The textile of the garment itself is important; it must not be an additional source of particles released during use. The garments should protect products, manufacturing processes and/or personnel from fibres and particles released by humans from skin and garments. The main function of cleanroom garments can be divided into three classes: 1) the protection of products manufactured, 2) the protection of products as well as manufacturing processes and 3) the protection of personnel.

The cleanroom class and level of particulate contamination permissible will determine which garments should be used. It can vary from overshoes and coats to full masks and gowns (Morgan, 1992). Cleanroom garment fabrics usually act as surface filters, which means the barrier ability increases with longer test

duration and increasing particle size. The barrier ability of the fabric itself is important, and also that of the cuffs and fastenings. In addition to the barrier function of a cleanroom garment system, wear comfort is also an important criterion (Schmeer-Lioe *et al.*, 2000). There is a major need for garments that will diffuse increasingly fewer particles, especially after many washes. To achieve the best possible decontamination results, minimum residual contamination after washing in a gentle washing procedure is needed. From the operator's point of view, there is a high demand for comfort and good antimicrobial and deodorizing performance over long periods (Kuhl, 2000).

3.3 MICROBIAL VIABILITY IN THE AIR

After becoming airborne, an organism may have a very short life, its stability being influenced by RH, temperature, oxygen levels, solar and ultraviolet radiation, and chemical factors. In general, stability is lower where RH is low (Parrett & Crilly, 2000). High temperatures inactivate all pathogens, some more rapidly than others. In general, a number of different factors affecting microbes can cumulatively stress them and affect their viability (Griffiths & DeCosemo, 1994). Important factors include organism species, growth conditions under culture, growth phase, method of aerosol generation, sampling techniques and the airborne environment. Desiccation, radiation, oxygen, ozone and its reaction products as well as various pollutants can affect the viability of microbes (Griffiths & DeCosemo, 1994).

Better survival rates from the stationary rather than from the logarithmic stages have been observed for both *E. coli* and *Serratia marcescens* (Griffiths & DeCosemo, 1994). Oxygen slowly kills most airborne microbes through oxidation (Kowalski & Bahnfleth, 1998). Dehydration of microbes causes osmotic stress and may result in decreased survival. Airborne fungal contamination also correlates with air pollutants, e.g. ozone concentration (Lin & Li, 2000). For yeast cells the survival was 4 times higher under high RH (> 70%) conditions compared with low RH (20–60%). The mould spores of *Penicillium* sp. were not affected by RH (Lin & Li, 1999).

There is little information available on the survival of microbes aerosolized from food environments. Stersky *et al*. (1972) showed that *Salmonella* New Brunswick survived much longer when aerosolized from skim milk than from distilled

water. The viabilities of *Salmonella* Enteritidis and *Salmonella* Typhimurium were significantly better than those shown by aerosols of *Legionella pneumophila* and *Mycobacterium tuberculosis* studied for 2 h in air at 24 °C with 75% RH (McDermid & Lever, 1996). Yeasts are eukaryotes and are likely to be affected differently by aerosolization and sampling than bacteria. Bacterial spores survive better than the vegetative cells do.

3.4 BIOAEROSOL MONITORING

Traditionally, microbial viability is understood as the ability to divide and multiply. Only viable microbes can cause infection, while both living and dead ones, or their products, can be responsible for allergenic and toxic illnesses. Therefore, there is a major need for methods for assessing both viable microbes as well as the total number of airborne microbes (Thompson *et al.*, 1994). One basic source is the growth of microbes in a liquid medium, e.g. spilled product, rinse water or wastewater, which then becomes aerosolized (Ren & Frank, 1992). The food industry is currently following the pharmaceutical and medical industries by recognizing that microbial monitoring of air is a must in standard quality-control practices. In both the food and pharmaceutical industries, the speed with which a result needs to be obtained is a question of economics (Griffiths & DeCosemo, 1994). Many food producers now include bioaerosol monitoring as part of their Hazard Analysis Critical Control Point (HACCP) system (Parrett & Crilly, 2000):

1) to meet legal requirements in complying with guidelines which often state that air quality may have to be monitored but do not specify the methodology or the acceptable limits on use,
2) to collect epidemiological data, possibly with a view to set occupational exposure limits and
3) for scientific interest to determine how the air affects the products processed.

3.4.1 Air sampling

Aerosols exhibit complex aerodynamic behaviour resulting from a combination of physical influences that include Brownian motion, electrical gradient, gravitational field, inertial force, electromagnetic radiation, particle density, thermal gradients, hygroscopicity and humidity (Kang & Frank, 1989b).

Generally, the performance of bioaerosol sampling devices is characterized by their ability to aspirate particles into the inlet, to transmit them through the sampler's interior and to collect them on the collection surface. In the case of viable microbial sampling, the performance of samplers must fulfil the stability of microbial viability as an additional component during sampling (Thompson *et al.*, 1994).

The efficiency of a sampler in collecting a particle of a given size is related to the air velocity in the impaction nozzle. Too low a velocity in the inlet will result in failure to collect the particles of interest. Too high a velocity results in a high shear force and may cause serious damage to the microbes, thus decreasing their viable recovery. The larger the aerosol particles, the greater the overestimation or the underestimation of the aerosol concentration is likely to be (Thompson *et al.*, 1994). Contamination from the sampler, especially in clean environments, will decrease if there is a waiting period of 1–2 min after installation of and before starting the air sampler. If measurements are taken in a conventionally ventilated room, airborne contaminant counts may also vary greatly if people are moving around or if work is being performed (Meier & Zingre, 2000). The sampling time must be considered according to the process and aim of sampling. Important information from the sampling period is (Parret & Crilly, 2000):

- ⇒ location and area of the site
- ⇒ date and time of sampling
- ⇒ test temperature and moisture conditions
- ⇒ functions of the ventilation system during sampling
- ⇒ personnel in the area
- ⇒ volume of air sampled along with sampling period
- ⇒ collection media used and
- ⇒ incubation time and temperature used.

3.4.2 Air samplers

Sampler efficiency is a measure of how well the inlet of a sampler draws in particles without being affected by particle size, shape, velocity and direction (Nevalainen *et al*., 1992). The total efficiency of a sampler is determined by several factors such as the design of the inlet, collection stage and choice of collection medium, which affect the viability of the microbes collected (Henningson & Ahlberg, 1994). A summary of microbiological air samplers is given in Table 3.1.

Settle Plates

Sedimentation, also referred to as settle plate technique, has traditionally been used in the food industry. The plates are easy, inexpensive and collect particles in their original state (Kang & Frank, 1989a). However, they have serious drawbacks. The ability of settle plates to collect airborne particles is governed by the gravitational force on the particle which decreases with a velocity dependent on its mass. Hence, settle plates are biased towards collecting larger particles and are sensitive to air movement (Griffiths & DeCosemo, 1994). The method is not quantitative, and in high aerosol concentrations the uncountable numbers of colonies can be a problem (Holah *et al*., 1995a). In addition, microscope slides coated with agar can be exposed and particles counted using a microscope. This technique is only used for total particulate counts (Kang & Frank, 1989a).

Impactors and Impingers

Impaction is the most common technique for the collection of airborne viable particles (Ljungqvist & Reinmüller, 1998). In impactors the inertial forces are used to collect particles. The inertia of a particle is determined by its mass and velocity. In the collection stage of impactors, the airstream is forced to change direction, and particles with too high a level of inertia are impacted on either a solid or liquid surface. Liquid-using impactors are called impingers (Henningson & Ahlberg, 1994). Impactors with several collection stages, i.e. cascade impactors, give information on the size distribution of the aerosol (Henningson & Ahlberg, 1994). In 1958 a cascade-sieve impactor, the Andersen sampler, was developed and is now probably the best-known sampler for microbiological aerosols (Wirtanen *et al.,* 2002b). Detection of microbes relies on their ability to grow following sampling (Griffiths & DeCosemo, 1994).

There are 2 types of solid-surface impactors: slit samplers and sieve samplers. The slit sampler usually has a 0.2–1.0 mm-wide tapered slit, which produces a jet stream when the air is sampled by vacuum (Kang & Frank, 1989a; Ljungqvist & Reinmüller, 1998). The slit sampler may have a turntable for rotating the agar plate so that aerosol particles are evenly distributed on the agar surface (Kang & Frank, 1989a). The velocity of the air varies according to the slit width of the air sampler used.

Table 3.1. Methods including commercial air sampling devices used in the collection of air for microbiological sampling (Wirtanen et al., *2002b).*

Sampling principle	**Sampler**	**Collection surface**	**Comments**
cascade sieve impactor	Andersen 6-stage	agar plate	reliable results, information on size distribution, impractical in industrial use
	Andersen 2-stage	agar plate	impractical in industrial use
sieve impactor	Andersen 1-stage	agar plate	
	MicroBio MB2	contact plate	
	Surface Air System, SAS	contact plate	practical in industrial use
	MAS-100	agar plate	practical in industrial use
slit impactor	Casella MK II	agar media	
	Burkard Personal Sampler		
centrifugal airstream	cyclones	wet or dry surface	
	RCS	special agar strips	less stress to microbes than in impactors, selectivity for larger particles, practical to use
impinger	all glass impinger, AGI-30	collection fluid	Glass, efficient for collection of bacteria and yeast; impractical
	multistage liquid impinger	collection fluid	stainless steel
	BioSampler	collection fluid	more gentle and efficient (for spores) than AGI-30, impractical in industrial use
filtration	Sartorius MD8	gelatine filter	efficient for spores, decreased desiccation rate of microbes, practical in isolators and for big sampling volumes
gravitation	settle plates	agar plate	simple to use, not quantitative, unreliable
ionization	electrostatic precipitation	filter, agar media, collection fluid	Mechanically complex; collection of microorganisms on charged surfaces
temperature gradient	thermal precipitation		
specific bindings	biosensors		specific, future development; at the moment impractical in industrial use

A linear velocity of 20–50 m/s is typical, since particles with a minimum diameter of 0.5–1.0 µm do not follow the deflecting stream of air but impact against the collection surface. The smaller the size, the higher the velocity needed on impact (Ljungqvist & Reinmüller, 1998). Sieve samplers are operated by drawing air through a large number of small, evenly spaced holes drilled in a metal plate. The particles are impacted on an agar surface located below the perforated plate (Kang & Frank, 1989a). The impact velocity is dependent on the size of the perforations, distance to the impact surface and performance of the vacuum pump (Ljungqvist & Reinmüller, 1998). When the concentration of viable particles in an aerosol is high, one sieve hole may allow more than one viable particle to pass through, resulting in the formation of a single colony from 2 or more viable particles. This inaccuracy can be corrected by reducing sampling time or by using either the microscopic method or a positive-hole method for enumeration (Kang & Frank, 1989a). Normally the positive-hole correction tables are included in each commercial sieve sampler. The microbial air sampler MAS-100 (Figure 3.2) is an impactor that aspirates air either horizontally or vertically through a perforated plate with 400 holes 0.7 mm in diameter. The resulting airstream containing particles is directed onto the agar surface of a standard Petri dish (Meier & Zingre, 2000).

Figure 3.2. The MAS-100 air sampler is an impactor.

Impingement methods are highly efficient for particles greater than 1 μm when high jet velocities are used. The all-glass impinger-30 sampler is a widely used high-velocity impinger (Kang & Frank, 1989a; Griffiths & DeCosemo, 1994). The sampler operates by drawing aerosols through an inlet tube curved to simulate the nasal-passage respiratory infection potential of airborne microbes (Nevalainen *et al*., 1992). The impinger is inexpensive and simple to operate, but viability loss may occur due to the amount of shear forces involved in collection. Another limitation is that the glassware should be sterilized before each sampling (Kang & Frank, 1989a). Impingement is useful for sampling heavily contaminated air, since the liquid samples can be diluted to the appropriate level for subsequent growth culture analysis (Lin *et al*., 2000).

Centrifugal Samplers

Centrifugal samplers have a propeller that pulls air into the sampling unit and pushes the air outward to impact on a tangentially placed strip of nutrient agar set on a flexible plastic base. Particles in the incoming air may be thrown out of the airstream by centrifugal force to be caught against the peripheral surface (Ljungqvist & Reinmüller, 1998). Centrifugal samplers do not generate a high-velocity jet flow during sampling, and less stress is imposed on airborne microbes compared with impaction methods. These samplers are simple and easy to operate and can rapidly sample a high volume of air, resulting in more representative sampling (Kang & Frank, 1989a). However, they demonstrate an inherent selectivity for larger particles and, since larger particles are more likely to include viable particles, there is a tendency towards higher counts than with other types of air samplers (Ljungqvist & Reinmüller, 1998). The Reuter centrifugal sampler (RCS) is a portable hand-held instrument, much used in the biotechnology and food industry (Griffiths & DeCosemo, 1994). Air from a distance of at least 40 cm is sucked into the sampler by means of an impeller. The air enters the impeller drum concentrically from a conical sampling area. The air leaves the sampling drum in a spiral outside the cone of entering air (Kang & Frank, 1989a). An agar strip is inserted into the drum around the impeller blades. The sampler gives no indication of the size of particles (Griffiths & DeCosemo, 1994).

Several different collecting mechanisms in filtration (impaction, interception and diffusion) are active. Usually a single filter is used and all particle sizes are collected with no partitioning into size fractions (Henningson & Ahlberg, 1994). Battery-powered personal samplers with filters have long been used in the occupational hygiene field to collect respirable and inhalable dusts (Griffiths & DeCosemo, 1994). In general, the filters are inexpensive and simple to operate. The air filtration apparatus (Figure 3.3a) consists of cellulose fibre, sodium alginate, fibreglass, gelatine membrane filters (Figure 3.3b) or synthetic membrane filters mounted in an appropriate holder and connected to a vacuum source through a flow rate controller. The gelatine filter membrane is composed of gelatine foam designed to prevent vegetative microbes from being inactivated by desiccation when air is drawn through the filter (Parks *et al*., 1996). The assessment of the microbes can be carried out by directly placing the gelatine filter (Figure 3.3c) on an agar surface for incubation. The gelatine membrane is water-soluble so that it can easily be diluted for plating or be solubilized on top of a nutrient agar, resulting in bacterial colonies on the agar surface.

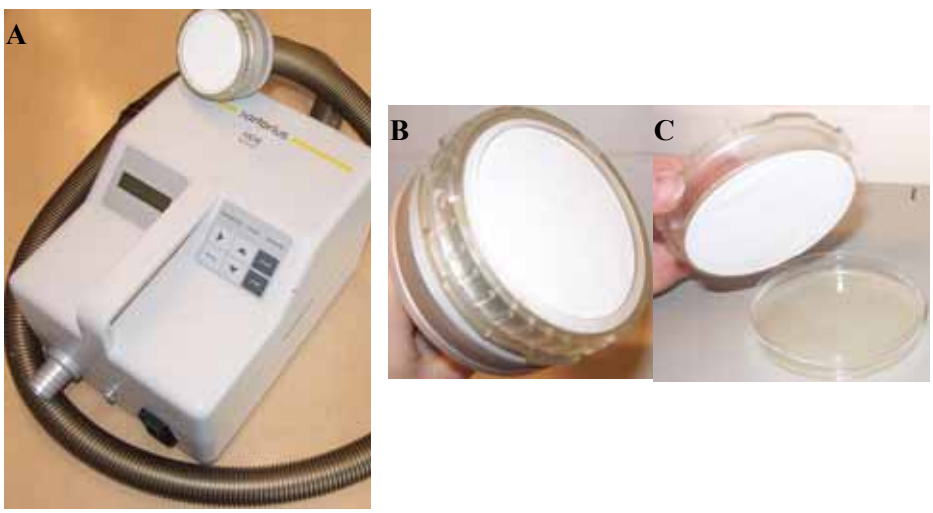

Figure 3.3. a) The MD8 equipment is based on the air filtration technique, b) a gelatine filter in the sampling holder and c) the gelatine filter is being transferred onto a Petri dish containing suitable agar.

Optical particle counters are based on laser-light scattering by a single particle and are widely used for measuring the concentration and size distribution of airborne particles. The crucial response characteristics of a particle counter include the sizing accuracy, counting efficiency and detection limits (Yoo *et al*., 1996). The detection limit of the smallest detectable particles is a crucial characteristic of the counter. Light-scattering counters are usually calibrated by means of polystyrene latex spheres (Yoo *et al*., 1996). Optical counters on the market include laser diffractometers, phase-doppler systems, intensity-deconvolution systems, and laser-particle interation system/image analysers. Other techniques used in particle counters include the electrical mobility techniques used in electrical aerosol analysers and differential mobility analysers as well as light detection and ranging (LIDAR) technology, which uses light waves in the same way that radar uses radio waves (Wirtanen *et al.,* 2002b).

3.5 BIOAEROSOL ASSAY METHODS

3.5.1 Culturing techniques

Culturing of microbes directly or through a broth on solid agar media with incubation for a certain period and at a certain temperature is the traditional method for enumerating the microbial count in the air sample. The culture technique is easy to use and requires no specialized equipment for sampling. An underestimation of the number of living cells can occur because microbes in the air may lose their ability to form colonies and still be viable, i.e. nonculturable. In cases where the airborne microbes are nonculturable, data on CFUs does not describe the actual microbial population (Heidelberg *et al.*, 1997). Epifluorescent microscopic methods for counting the total number of microbes showed that there was a wide variation in the culturability of microbial cells; the culturable amount varied from 0.02% to 10.6% (Lighthart, 1997). The culture technique is useful for direct identification of certain pathogens or spoilage microbes in the food-processing air, e.g. spoilage moulds on specific agars (Lund, 1996).

3.5.2 Fluorescence and microscopy techniques

Microscopy is a method with which the total count of microbes as well as morphological data on the microbes can be obtained. The microscopical method is relatively simple and rapid. Automatic counting and size evaluation in the assessment of airborne microbes by means of the image processing of fluorescence microscopy data reduces the analysis time (Griffiths & DeCosemo, 1994). Fluorescence microscopy can be applied in the evaluation of airborne microbes harvested e.g. on filters (Kildesø & Nielsen, 1997) and in impinger liquids (Terzieva *et al*., 1996). Technical problems related to fluorescence microscopy include low-contrast and low-light intensity, which makes automatic image processing difficult (Kildesø & Nielsen, 1997). Phase-contrast microscopy is particularly useful for counting bacterial endospores as they appear phase-bright against the darker vegetative cells (Griffiths & DeCosemo, 1994). The fluorochromes attach to particular cell components, e.g. proteins, nucleic acids, and coenzymes, which fluoresce when excited with light of a suitable wavelength (Griffiths & DeCosemo, 1994). The addition of UV fluorescence capability into aerosol counters offers a way to distinguish biological particles from most organic and inorganic particles (Seaver *et al*., 1999). Viable staining methods have been applied for the detection of viable microbes (Terzieva *et al*., 1996, Hernandez *et al.*, 1999).

3.5.3 ATP bioluminescence

Adenosine triphosphate (ATP) is present both in microbial cells and food ingredients and can be measured using the luciferase enzyme complex found in fireflies. The light output of a sample is directly proportional to the amount of ATP present. The detection limit of the method is about 10^4 cells (Wirtanen, 1995). This method is non-specific, i.e. it measures the ATP content of the microbial population in the sample as a whole (Griffiths & DeCosemo, 1994). The ATP content in airborne cells, which are stressed through assessment, can be altered by the effect of aerosolization and this can also affect the detection level of the method. Furthermore, the ATP bioluminescence method is not applicable in the detection of spores, because there is very little or no ATP in samples containing mostly spores (Wirtanen *et al.,* 1997).

3.5.4 Molecular methods

Molecular biology detection methods include polymerase chain reaction (PCR) and gene probes. The PCR analysis method permits the detection of DNA, regardless of the metabolic state of the cells. The method may, therefore, be several orders of magnitude more sensitive than culture techniques. Applications of this method for the quantification of airborne organisms are still under development. PCR-based techniques allow the detection and identification of microbes at a group or species level within a background of other microbes. The specificity, sensitivity, and reduced processing time of this technique are suitable for the detection of small amounts of target microbial cells in a sample. Specialized equipment and skilled personnel are, however, required for successful applications (Griffiths & DeCosemo, 1994; Alvarez *et al*., 1995; Maukonen *et al.,* 2003). The air samples may also contain compounds inhibitory to the amplification assay, e.g. a high concentration of nontarget DNA. In many applications, pre-enrichment of the sample is needed. Nucleic acid hybridization has been applied for the detection and identification of microbes in bioaerosols. Each hybridization format is suitable for different aerosol concentrations. It is possible to identify a fast-growing airborne organism within 24 h using the colony-hybridization technique (Alvarez *et al*., 1995; Neef *et al*., 1995).

3.6 AIR DISINFECTION IN THE FOOD INDUSTRY

3.6.1 Disinfectant fogging

Disinfectants are commonly applied as fogs in the chilled-food industry. Disinfection is an additional safeguard, not a substitute for cleaning (Holah *et al.*, 1995b). Fogging has been shown to be effective in reducing viable airborne microbes. Fogging reduces the number of viable cells effectively on upward-facing surfaces but not on vertical or downward-facing surfaces. Better spray action can be achieved by locating the spray nozzles near the target and directing the jet towards the target. The use of more aggressive disinfectants e.g. peracetic acid or aldehyde formulations can also increase the disinfection effect on vertical surfaces. Fogging performed using an active concentration of 2 mg/ml of a quarternary ammonium formulation resulted in a 4-log reduction in microbial counts on wall and floor materials as well as in the air (Burfoot *et al.,* 1999). The

concentration of chlorine in fogs must be very high e.g. 500 µg/ml to be effective, while a concentration of 10 µg/ml already causes discomfort for personnel working in the facilities. The best fogging effect was achieved using fog droplets with a diameter of 10–20 µm (Holah *et al.*, 1995b). When the droplets are smaller, the fog produced gives a uniform coverage. A fog of small droplets remains airborne for several hours, which also prevents people from working in the area. The ventilation must be effective so that the facilities are dried after fogging (Wirtanen *et al.,* 2002a).

3.6.2 Ozone disinfection

In the ozonation experiments carried out in an aerobiology cabinet, exposure of 4 µg/ml for 5–10 min reduced the number of airborne *P. aeruginosa* with 2–4 log units. A variation in the amount of ozone generated between the trials explained the differences between the results in the replicate trials. Some synergism has been found between ozonation and UV radiation in these trials. Both ozonation and UV radiation were predictable and controllable disinfection methods with 2–3-log-units reduction in airborne counts (Holah *et al.*, 1995b).

3.6.3 Ultraviolet disinfection

Shortwave UV radiation (UVC, 254 nm) has been shown to reduce the microbial load both in the air and on hard surfaces free of organic residues (Bintsis *et al.,* 2000). In general, bacterial cells are more sensitive than spores, fungi and algae, which are considerably more resistant to UV radiation (Scholte, 1996). The irradiation dose needed for the inactivation of 90% of *Bacillus subtilis* spores in a population is 120 Ws/m^2, whereas a dose of 1,320 Ws/m^2 is needed for *Aspergillus niger* mould and 3,600–6,000 Ws/m^2 for algae (Wirtanen *et al.,* 2002b). A combination of HEPA-filtered laminar air and UV radiation has been suggested for air-handling treatment in production units handling sensitive foodstuffs (Bintsis *et al.*, 2000). The microbial air quality in cold stores and egg-hatching cabinets has also been improved using UV radiation units (Bintsis *et al.,* 2000). To ensure total contamination control, UV radiation systems are used for airflow into sterile environments. The outcome of the UV radiation experiments was easier to control than that of chemical fogging. The UV radiation can reduce the airborne microbial counts by 4 log units. The effect can also be improved using 2–4 lamps and mirrors in the radiation unit. Furthermore,

the UV treatment has no residual effect (McClean, 1991, Holah *et al.,* 1995b; Bintsis *et al.*, 2000). Fungal contamination of sausages during ripening was efficiently reduced using shielded UV lamps in the salami-ripening chambers (Papa *et al.,* 1995). Bodmer (1999) showed that UVC radiation is beneficial in disinfecting the air at meat-processing plants; the treatment destroyed bacteria, yeasts and moulds, as well as viruses. During manufacture of aseptically filled dairy products, UV sterilization has been applied to both foil caps and cartons (Bintsis *et al.,* 2000). The combination of UV radiation and chemical fogging showed synergistic effects, giving lower residual counts for the combined treatment than for each treatment separately (Holah *et al.,* 1995b). Bintsis *et al.* (2000) has suggested that the use of UVC radiation technology will increase in air disinfection equipment with improved technology solutions.

4. HYGIENIC SURVEY OF AIR IN SWEDISH CHEESE PLANTS

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4.1 BIOCONTAMINATION CONTROL IN PROCESS AREAS

In the network project DairyNET – Hygiene Control in Dairies (P00027) Nordic Innovation Centre, the former Nordic Industrial Fund, studies on the air quality in various types of dairies was carried out. Experience has shown that environmental monitoring data from the food industry are very difficult to interpret and compare because technical conditions such as filter quality and number of air changes, as well as sampling devices and media, are not always defined. As a part of the Swedish contribution to the project, a hygiene survey was carried out during 2002 and 2003 on the burden of airborne particulates in four cheese plants. In the survey, particles equal to and larger than 0.5 μm and the microbial burden as colony-forming-units (CFU) were measured in conjunction with a technical comparison of the ventilation systems in the processing premises of the cheese plants. The participating dairies in this study were Arla Foods, Milko, Norrmejerier and Skånemejerier and for this investigation, four cheese plants in different parts of Sweden were chosen.

The authorities have not defined air quality for the food industry with regards to the number of viable particles. For industries needing of high quality air and cleanrooms, ISO 14644-1 (1999) 'Cleanroom and associated controlled environment – Part 1: Classification of air cleanliness' is applied. Table 4.1 illustrates the selected airborne particulate cleanliness classes for cleanrooms and clean zones. Several Good Manufacturing Practices (GMP) documents from the pharmaceutical industry, which give special requirements for clean air depending on the type of products being manufactured, are presented in chapter 4.1.1. The harmonization of the ISO 14644 and ISO 14698 standards and GMPs is not attained. ISO 14698 'Cleanroom and associated controlled environment – Biocontamination control – Part 1: General Principles and methods' gives no microbial limits but does state the following principles: formal system of

biocontamination control shall be established, implemented and maintained within cleanrooms and associated environments. This system will be used to control factors that can affect the microbial quality of the process and of the products produced.

Bulletin No 324 (IDF, 1997) from the International Dairy Federation, recommends that the dairy industry must install adequate ventilation in areas with open processing systems. It also mentions that microbially filtered air may be sufficient. The bulletin stresses the importance of zoning; a key concept in the hygienic design of dairy plants to divide the production area into risk zones according to the risks that the products are exposed to in various areas during processing. The purpose is to minimize microbial cross-contamination by e.g., restricting free movement of staff within the plant, establishing hygiene barriers, providing technical barriers such as walls, air locks, staff locks, etc., and inculcating awareness among staff of the importance of hygiene. For the red zones, i.e. high-risk zones with the strictest hygiene requirements, micro-filtrated air inlets are mentioned as building criteria. Positive pressure compared to other zones is mentioned as a flow criterion so that air will always pass from the high-risk area to rooms with lower risks.

Table 4.1. Selected airborne particulate classes for cleanrooms and clean zones according to ISO 14644-1 (1999).

ISO classification number (N)	Maximum concentration limits (particles /m^3 of air) for particles equal and larger than the considered sizes shown below (concentration limits are calculated in accordance with the equation given in ISO 14644-1)					
	0.1 µm	0.2 µm	0.3 µm	0.5 µm	1 µm	5 µm
ISO Class 1	10	2				
ISO Class 2	100	24	10	4		
ISO Class 3	1 000	237	102	35	8	
ISO Class 4	10 000	2 370	1 020	352	83	
ISO Class 5	100 000	23 700	10 200	3 520	832	29
ISO Class 6	1 000 000	237 000	102 000	35 200	8 320	293
ISO Class 7				352 000	83 200	2 930
ISO Class 8				3 520 000	832 000	29 300
ISO Class 9				35 200 000	8 320 000	293 000

Uncertainties related to the measurement process require that concentration data with no more than three significant figures be used in determining the classification level

Ostfrämjandet (Eklund *et al.,* 2001) suggests as a guideline, a concentration of not more than 10 yeasts and moulds per cubic metre (m^3) of air in cheese processing areas. Some dairies compare the zones: high risk, high care and medium care to Grade A, B and C zones in the pharmaceutical industry and apply the microbial limits given in the EU GMP.

The only recommendations elucidating 'adequate ventilation' with regard to filter quality and airflows are given for high risk zones (IDF, 1997) where micro-filtrated air (HEPA-filtered air) and overpressure are required. In the study that was performed, the filter type varied between F65 and F95. According to a multidisciplinary project on hygienic ventilation carried out in Denmark (Jensen, 2004), clear improvements were achieved when hygienic ventilation was systematically applied. The contamination frequency was reduced when e.g., HEPA filtered air was supplied over exposed products on conveyer belts. Information on filters and their efficiency is summarised in chapter 4.1.2.

The study has been supported financially by the Swedish project on milk plant hygiene in open areas and the third Nordic co-operation project DairyNET – Hygiene Control in dairies (P00027). My special thanks go to Prof. Bengt Ljungqvist (KTH) for his helpful comments during the study and to Ass. Prof. Gun Wirtanen for her critical review of the manuscript.

4.1.1 Standards and recommendations

A summary of several Good Manufacturing Practice (GMP) documents giving special requirements on airborne particles in clean air depending on the type of products being manufactured. Harmonisation of the ISO 14644 and ISO 14698 standards and GMPs is not attained. Tables 4.2–4.4 give the maximum permitted airborne particles for cleanrooms in pharmaceutical processes. Table 4.5 shows the concentrations of airborne particles in samples of outdoor air.

Table 4.2. Maximum permitted airborne particles (≥ 5 μm) per cubic metre (m^3) in pharmaceutical cleanrooms.

EU GMP Annex 1			ISO 14644-1	
Grade	At rest condition	Operational condition	Class	Operational condition
A	1	1	5	29
B	1	2 000	7	2 930
C	2 000	20 000	8	29 300
D	20 000	Not defined		

Table 4.3. Maximum permitted airborne particles (≥ 0.5 μm) per cubic metre (m^3) in pharmaceutical cleanrooms.

EU GMP Annex 1			*U.S. FDA and USP*		*ISO 14644-1*	
Grade	At rest condition	Operational condition	Area	Operational condition	Class	Operational condition
A	3 500	3 500	Critical	3 500	5	3 520
B	3 500	350 000	Background to critical area	350 000	7	352 000
C	350 000	3 500 000	Controlled	3 500 000	8	3 520 000
D	3 500 000	Not defined				

Table 4.4. Maximum permitted/recommended airborne viable particles (CFU) per cubic metre (m^3) in pharmaceutical cleanrooms.

EU GMP Annex 1		FDA 1987[1] & Draft 2003[2]		USP 27	
Grade	Operational condition	Area	Operational condition	Class U.S. Customary	Operational condition
A	< 1	Critical	< 3[1] 1[2]	100	< 3
		Background to critical area Class US Customary 1 000	7[2]		
B	10	Background to critical area Class US Customary 10 000	10^2	10 000	20
C	100	Controlled	88[1] 100[2]	100 000	100
D	200				

Table 4.5. Number of airborne particles (≥ 0.01 µm) per cubic metre (m^3) in air in various places (Gustavsson, 2002).

Places (examples)	Number of particles per m^3
Cleanroom	10^3
Artic region	10^7
Country side	10^9
City	10^{11}
Tobacco smoke	10^{14}

4.1.2 Filter types and their efficiencies

Over the years the designation of filters has been changed as have test methods (Gustavsson, 2003). In Table 4.6–4.7 some information on filter types and filter efficiencies is summarised.

Table 4.6. Information on filters of F-type as well as their efficiencies.

Filter Type	Mean efficiency	Test method
F65	≥ 65%	Mean degree of de-blackening during the lab test
F85	≥ 85%	Mean degree of de-blackening during the lab test
F7 (EU7)	$80 \leq E_m < 90$	Test acc. to EN 779:2002 particulate air filters for general ventilation – Determination of the filter performance
F95	≥ 95%	Mean degree of de-blackening during the lab test
F8 (EU8)	$90 \leq E_m < 95$	Test acc. to EN 779:2002
F9 (EU9)	$95 \leq E_m$	Test acc. to EN 779:2002

Table 4.7. Information on filters of H- and U-type as well as their efficiencies.

Filter Type	Mean efficiency	Test method
H10	85%	Test acc. to EN 1822:1999 HEPA and ULPA filters. Efficiency MPPS* (%)
H11	95%	Test acc. to EN 1822:1999 Efficiency MPPS* (%)
H12	99.5%	Test acc. to EN 1822:1999 Efficiency MPPS* (%)
H13	99.95% Leak test local 99.75%	Test acc. to EN 1822:1999 Efficiency MPPS* (%) Leak test scanning MPPS
H14	99.995% Leak test local 99.975%	Test acc. to EN 1822:1999 Efficiency MPPS* (%) Leak test scanning MPPS
U15	99.9995% Leak test local 99.9975%	Test acc. to EN 1822:1999 Efficiency MPPS* (%) Leak test scanning MPPS

* Most Penetrating Particle Size

4.2 INVESTIGATED SITES

Four cheese plants in different parts of Sweden were chosen for investigation and are referred to as A (Figures 4.1–4.4), B (Figures 4.5–4.6), C (Figures 4.7–4.9), and D. The photos from the production areas have been published with permission. At all four plants, a technical survey covering buildings and ventilation systems was carried out. The cheese-making process was considered to be equivalent at the chosen plants, although there were some differences in the processing equipment. The size and shape of curdling tanks varied. Sites A, C and D had horizontal open vats for the curd and site B applied a more closed process with a vertical tube for the curd. The curd was portioned into clean cheese moulds and transported to press tables in the processing hall. The washing machines for the cheese moulds were different, but a certain amount of hot humid air escaped from all of them into the processing area. The washing process for the press tables generated humid aerosols in the processing area. At C and D, the processing area was divided into two levels with connecting openings. The number of personnel and manual operations in the processing area seemed to be higher in plants C and D than in A and B.

The ventilation systems in the chosen cheese plants have been described together with the responsible personnel. Table 4.8 shows the main features of the different systems. In general, the buildings of the investigated plants were older than the ventilation systems in use. These ventilation systems had been upgraded over the years mainly with regard to control and monitoring.

Table 4.8. Description of ventilation system in the investigated plants.

Plant	Building of ventilation system (a)	Filter quality	Air-changes (number/h)	Filter replacement (number/a)	Ventilation principle in processing area	Additional process exhaust
A	1980	EU 7 EU 8	Approx. 7	2	High supply, high exhaust	Yes
B	2000	F95	Approx. 2	2	Displacement Low supply, high exhaust	Yes
C	1982-85	F65/ F85	Approx. 7	2	High supply, high exhaust	Yes
D	1970	F65	Approx. 7	2	High supply, high exhaust	Yes

Figure 4.1. Processing area in plant A.

Figure 4.2. Vat in plant A.

Figure 4.3. Processing area, product coming out of cheese moulds, in Plant A.

Figure 4.4. Packaging area in Plant A.

Figure 4.5. Processing area, filling cheese moulds, in Plant B.

Figure 4.6. Processing area, product being taken out of cheese moulds, in Plant B.

Figure 4.7. Sampling air in Plant C.

Figure 4.8. Processing area in Plant C.

Figure 4.9. Processing area, product coming out of cheese moulds, in Plant C.

4.3 METHODS IN AIR QUALITY STUDIES

4.3.1 Airborne particles

Measurements of airborne particles have been performed using a discrete-particle counter (DPC), which uses a light scattering device as a means of displaying and recording the count and size of discrete particles in the air. It also has a size discrimination capability which allows it to detect the total particle concentration in the appropriate particle size ranges. The same standard particle counter instrument (DPC, Hiac-Royco 245 A) has been used at all four locations. The sampling flow was one cubic foot per minute (ft^3/min). The concentrations are given as the number of airborne particles per cubic metre (m^3) equal to and larger than 0.5 μm and 5 μm respectively. The author performed the sampling and evaluation of the data.

4.3.2 Viable airborne particles

Measurements of airborne viable particles have been performed using three different active sampling devices; a slit-sampler FH3, a sieve-sampler MAS, and a centrifugal sampler RCS. These devices for active sampling of airborne aerobic colony-forming-units (CFUs) have been compared earlier with regard to their biological efficiency and evaluated as giving relatively comparable results (Ljungqvist & Reinmüller, 1998). Table 4.9 shows where the samplers were used during the survey. A summary of the media used in the collection and enumeration of CFUs is shown in Table 4.10. Measured concentrations are given as the number of airborne CFUs per cubic metre (CFU/m^3). The microbial sampling was performed by KTH. Personnel at the study plant carried out the incubation and first reading and personnel at SIK carried out the identification of isolates. Examples of the sampling devices used in this study are shown in Figures 4.10–4.11.

Table 4.9. Devices used for active air sampling.

Sampler/ Plant	FH3 Slit-sampler Theoretical impaction velocity 35 m/s	MAS Sieve-sampler Theoretical impaction velocity approx. 10 m/s	RCS Centrifugal sampler Theoretical impaction velocity 4 m/s
A	X	X	
B	X		X
C	X		X
D	X		X

Figure 4.10. Sampling equipment used in plant A.

Figure 4.11. Air sampling in the production area at Plant B.

4.3.3 Temperature and relative humidity

Temperature and relative humidity were registered with data loggers at representative locations in the processing area during the survey for periods of not less than three days.

Table 4.10. Media used for active air sampling during the survey.

Media	Sampling utensils	Characteristics	Recommended by	Used in plant
Cloramphenicol-Glycos-Yeast extract (CGY)	9 cm agar Petri dishes	Medium for the enumeration of yeasts and moulds in dairy products and food	Milk and Milk products, IDF standard 94B, (1990)	A, B & C
Rose Bengal Agar (YM)	Agar strips (Biotest)	Medium for the enumeration of yeasts and moulds. Restricts the growth of *Rhizopus* and *Mucor* spp. that otherwise could overgrow the agar. Streptomycin is present to inhibit bacterial growth.	Biotest Diagnostic Corporation.	B & C
Sabouraud-Dextros Agar (SDX)	Agar strips (Biotest)	Medium for the enumeration of yeasts and moulds. Neutral pH with antibiotics to inhibit contaminant bacterial flora. Has a higher recovery than YM	USP and Biotest Diagnostic Corporation	D
Mod. Creatine Sucrose Dichloran Agar (CREAD)	9 cm agar Petri dishes	Useful for distinguishing species of *Aspergillus* and *Penicillium*	Lund, F., Filtenborg, O., Frisvad, J. C., (1995)	B & D
Drigalski Agar 'Blue plate'	9 cm agar Petri dishes	Media for isolation and cultivation of Gram-negative bacteria	SIK	B & D
Tryptone-Soy-Agar (TSA)	9 cm agar Petri dishes	General enumeration of aerobic microbes	Pharmaco-poeias	B

4.4 RESULTS AND DISCUSSIONS

4.4.1 Airborne particles

In each of the four plants, airborne particles (total number and number of CFUs) were counted and registered with the methods described. Typical results from the different sites are presented in Figures 4.12–4.23. The *y*-axis in the figures is shown as a logarithmic scale in order to show the number of airborne particles equal to and larger than 0.5 µm and 5 µm and also the number of CFUs. The microbial results shown in Figures 4.12–4.14 are from the summary reported in Table A8.1 in Appendix 8.

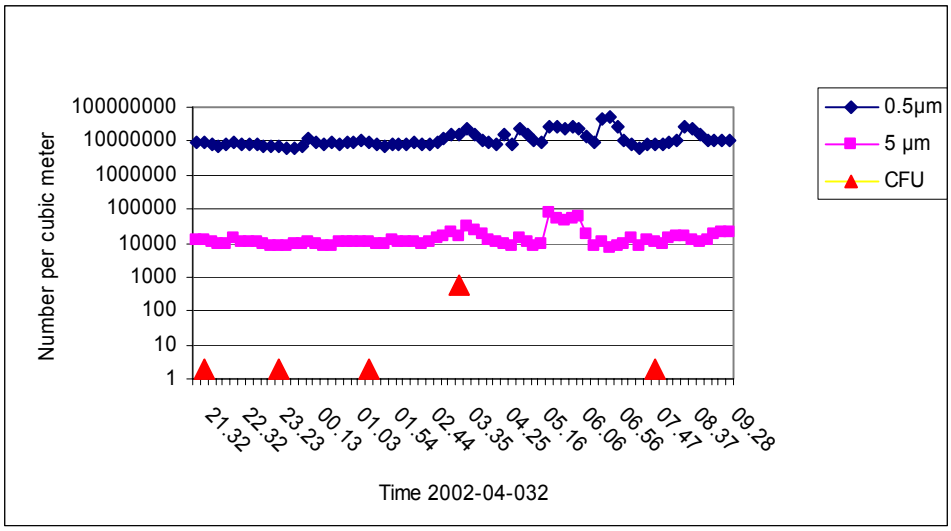

Figure 4.12. Viable and total particle counts in air from the general processing area in Plant A, where the sampling was performed on April 3–4, 2002. The filter quality used in this area was EU7.

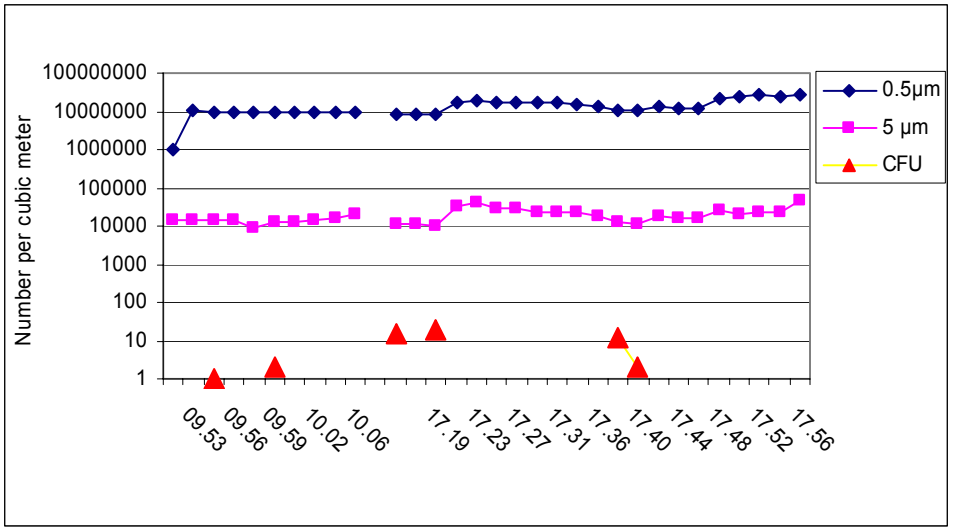

Figure 4.13. Viable and total particle counts in air from the processing area close to the open product in a vat in Plant A, where the sampling was performed on April 4, 2002. The filter quality used in this area is EU7.

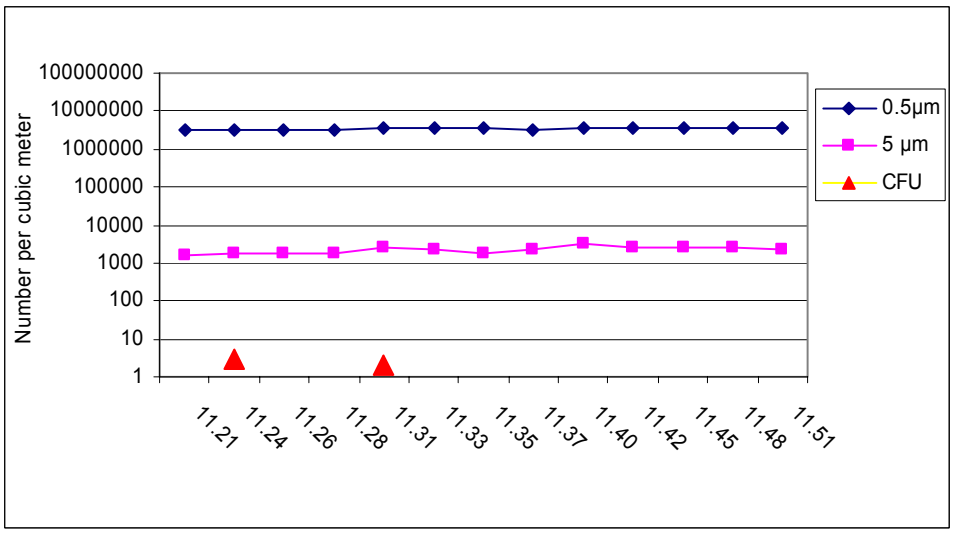

Figure 4.14. Viable and total particle counts in air from the packaging area in Plant A, where the sampling was performed on April 4, 2002. The filter quality used in this area is EU 8.

Figure 4.12 shows the results from the night shift with five microbial sampling periods, one of the periods shows significantly higher results which is also accompanied by an increased concentration of particles at both ≥ 0.5 and ≥ 5 µm sizes. Documentation showed that a washing cycle was performed at that time. Figure 4.13 shows the results sampled close to the vat during the working day. Acceptably low microbial concentrations are shown. The concentration of total particles around six o'clock in the morning is lower than shown in Figure 4.12. Figure 4.14 illustrates the concentrations of airborne particulates in the packaging area, which is supplied with air from the processing area but filtered a second time. The filter quality in the second step is EU 8. This packaging area, which is a limited access area, shows approximately a 90% reduction in the concentrations of airborne particulates compared with the concentrations measured in the processing area (Figure 4.12 and 4.13).

Figures 4.15–4.17 originates from plant B with its newer displacement ventilation system equipped with F95 filters. The microbial results shown in these figures are from the summary reported in Table A8.2 in Appendix 8.

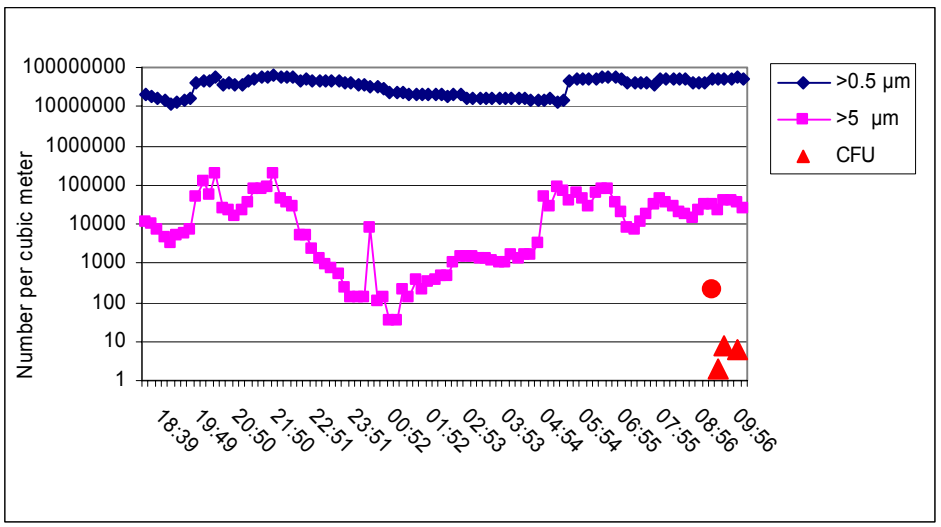

Figure 4.15. Viable and total particle counts in air from the general processing area in Plant B, where the sampling was performed on February 18–19, 2003. The filter quality used in this area is F95. The dots in the diagram indicate the total number of CFUs and the triangles represent the number of yeasts and moulds.

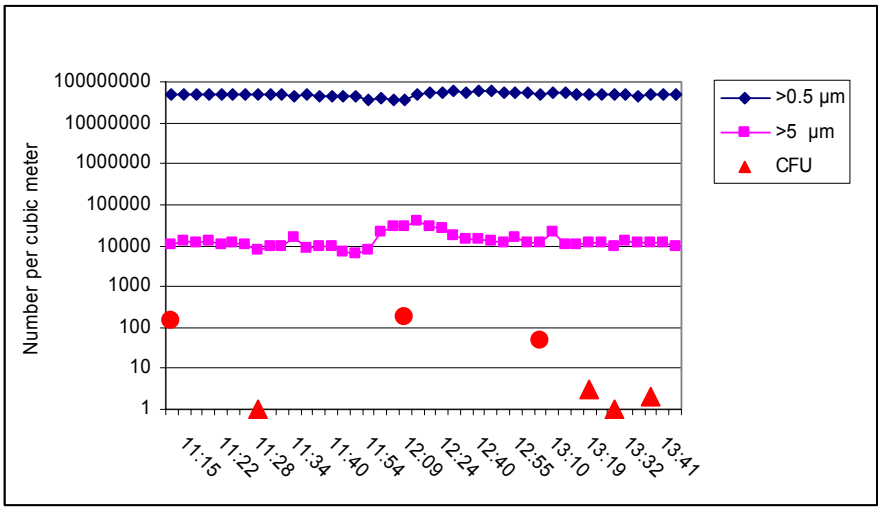

Figure 4.16. Viable and total particle counts in air from the processing area close to the open product out of the vat in Plant B, where the sampling was performed on February 19, 2003. The filter quality used in this area is F95. The dots in the diagram indicate the total number of CFUs and the triangles represent the number of yeasts and moulds.

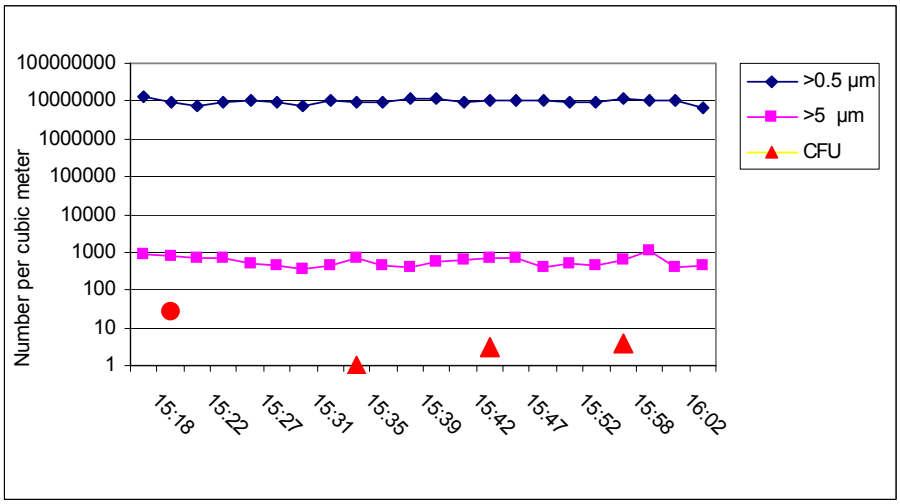

Figure 4.17. Viable and total particle counts in air from the vicinity of a supply air (recirculation) device in Plant B, where the sampling was performed February 19, 2003. The filter quality used in this area is F95. The dots in the diagram indicate the total number of CFUs and and the triangles represent the number of yeasts and moulds.

Figure 4.15 shows the general concentrations of airborne particles measured over a longer period (approx. 15 h) where the lowest concentrations are registered during the night between 11 pm and 5 am. The decrease is most observable in the number of particles of 5 µm and above. The difference between the concentration levels of particle sizes ≥ 0.5 and ≥ 5 µm is larger than the differences shown from plants with lower efficiency filters. The relationship between the number of airborne CFUs and the number of airborne yeasts and moulds seems to be 10 to 1. Figure 4.16 illustrates the concentrations measured in an area close to the open product (pressed curd in cheese mould) during ordinary day activity and shows higher concentrations than in Plant A. The number of airborne CFUs and yeasts and moulds are in the same range as given in Figure 4.15. Figure 4.17 shows concentrations measured in the supply air (close to the air inlet); here the concentrations are almost 90% lower than in the area close to the open product. This result indicates that the higher concentrations in the general area are affected by the processes and work performed in the area. In Figures 4.18–4.20 showing the results from Plant C, the air filters are of lower quality than in Plants A and B.

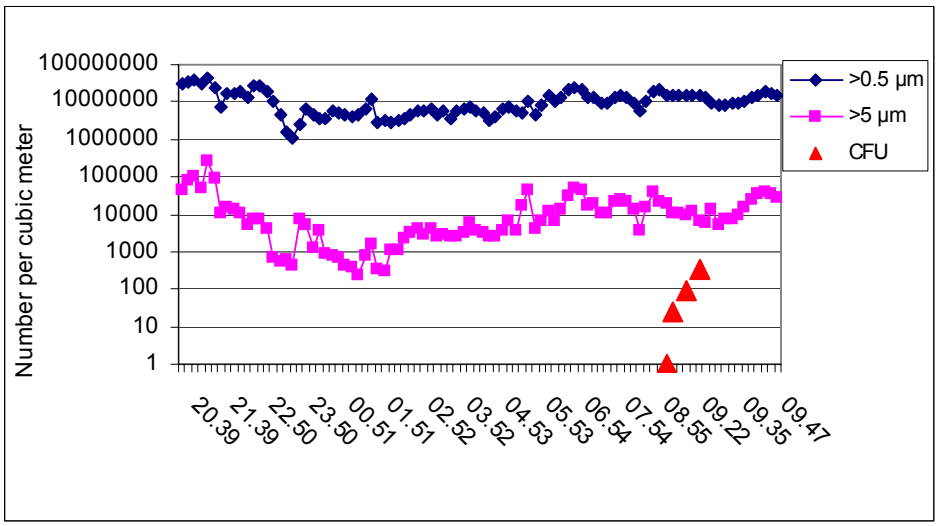

Figure 4.18. Viable and total particle counts in air from the general processing area in Plant C, where the sampling was performed on January 18–19, 2003. The filter quality used in this area is F65.

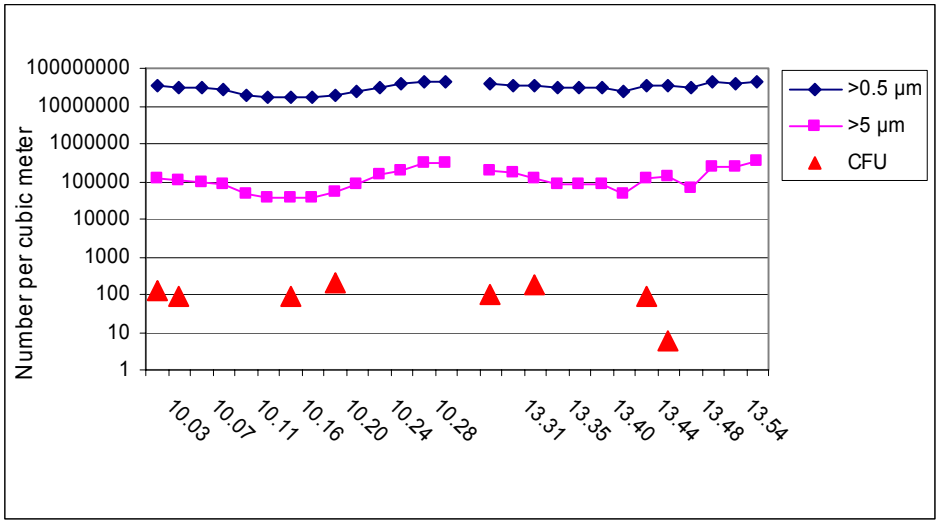

Figure 4.19. Viable and total particle counts in air from the processing area close to the open product and the washing machine in Plant C, where the sampling was performed on January 19, 2003. The filter quality used in this area is F65.

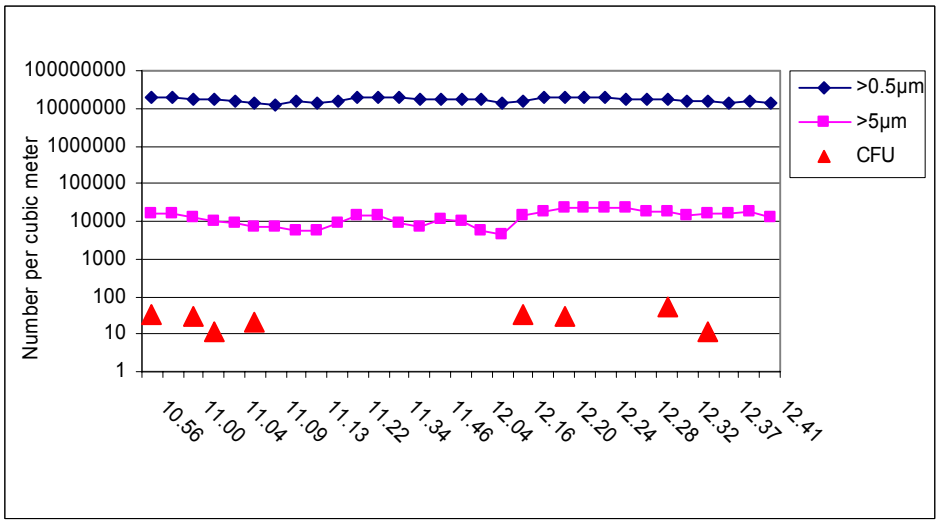

Figure 4.20. Viable and total particle counts in air from the processing area close to a supply air device in Plant C, where the sampling was performed on January 19, 2003. The filter quality used in this area is F65.

The concentrations during the night in the general processing area in Plant C are shown in Figure 4.18. It can be seen that the decrease in particle concentration during periods of low activity is more pronounced for the larger particle size. Figure 4.19 and 4.20 show that the number of yeasts and moulds are relatively high close to open products and the washing machine and also in the supply air. The relatively high number of airborne viable particles could be caused by the process and the recirculation of air. The bioburden in outdoor air is usually low during wintertime and the sampling was performed in January. Results from Plant D, which used the same filter quality as Plant C, are shown in Figures 4.21–4.23.

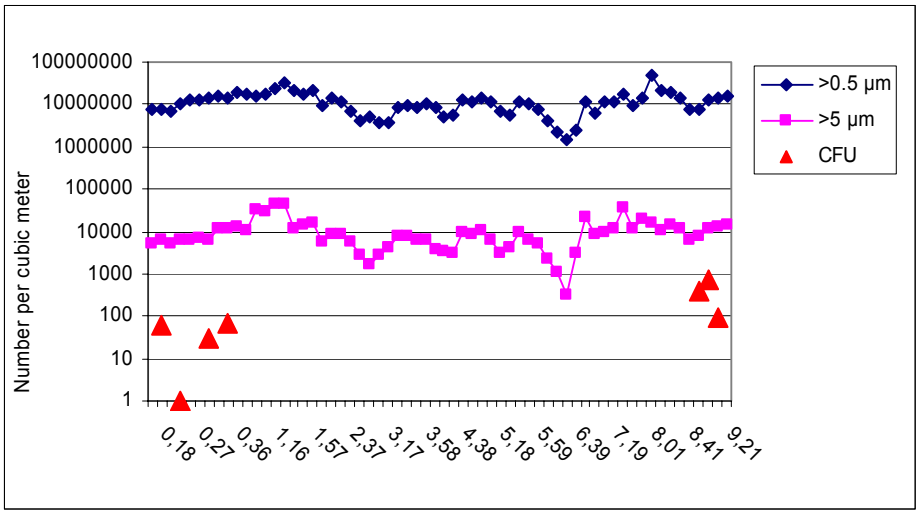

Figure 4.21. Viable and total particle counts in air from the general processing area in Plant D, where the sampling was performed on March 13, 2003. The filter quality used in this area is F65.

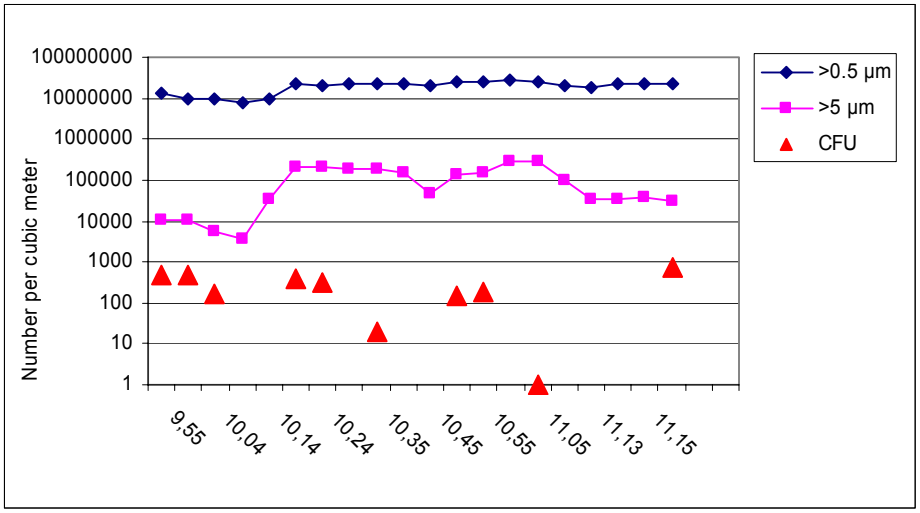

Figure 4.22. Viable and total particle counts in air from the processing area close to the open product and the washing machine in Plant D, where the sampling was performed on March 13, 2003. The filter quality used in this area is F65.

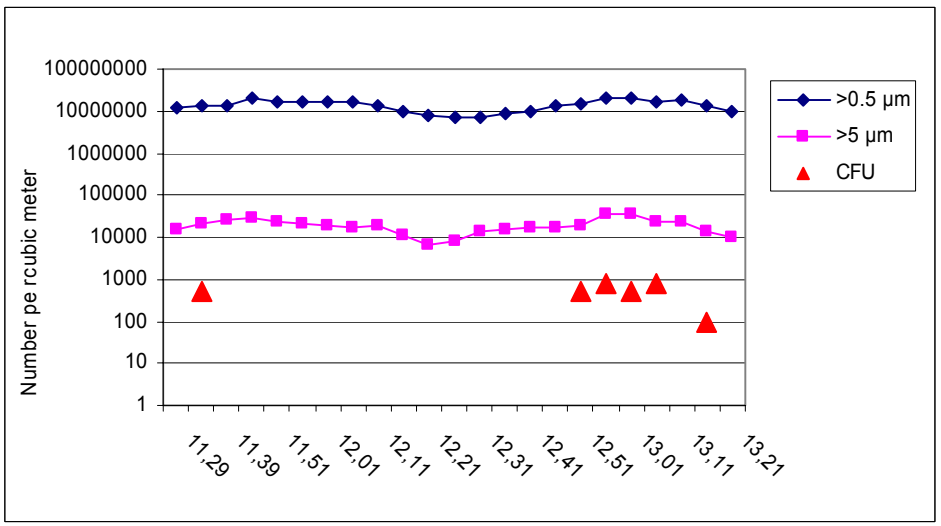

Figure 4.23. Viable and total particle counts in air from the processing area close to a shaft to the floor below the main production floor in Plant D, where the sampling was performed on March 13, 2003. The filter quality used in this area is F65.

Figure 4.21 shows similar levels during the night as shown in Figure 4.18, the concentrations of airborne yeasts and moulds seem high in relation to the processes performed. Figure 4.22 shows high levels of particles of 5 μm and above, and airborne yeasts and moulds. This is probably caused by the presence of a blowing device that disperses pressurised air and water drops from the cheese and the cheese moulds into the air. Figure 4.23 shows high levels of airborne yeasts and moulds. The open shaft between the two production floors contributes to a lower control of air movements and a higher degree of dispersion of airborne particles in the whole processing area.

4.4.2 Airborne viable particles

A summary of the results from the microbial sampling is presented in Appendix 8. In the processing areas of plant A, 30 samples of air were taken for the microbial monitoring of fungi using MAS and FH3 air-samplers, respectively. The medium for collecting and enumerating CFUs was Cloramphenicol-Glucose-Yeast extract (CGY). On 10 of the samples, single colonies of yeasts and moulds were detected. Only two of the samples showed an approximate concentration of 10 airborne yeasts and moulds per cubic metre and one sample showed an approximate concentration of 600 airborne fungi (yeasts) per cubic metre. Among the yeasts, *Candida* species were the majority. Among the single colonies of moulds *Penicillium*, *Cladosporium*, *Phoma* and *Geotrichum* species were identified. Due to an effective ventilation system supplied with filters of acceptable quality (EU7 and EU8), and a low challenge level in outdoor air; satisfactory low concentrations of airborne CFUs were found in the processing area.

In the processing areas of plant B, a total 32 samples were taken using RCS and FH3 air samplers. Different media for collecting and enumerating CFUs were used: Tryptone-Soy-Agar (TSA) as a general medium for both bacteria and fungi, Drigalski Agar (Blue plate) for Gram-negative bacteria, Rose Bengal Agar (YM), CGY and Modified Creatine Sucrose Dichloran Agar (CREAD) for yeasts and moulds. Around 20 samples showed contamination levels of less than 10 CFU/m^3. The results from the general and selective media compared indicated that the number of yeasts and moulds in relationship to the total number of microbes was in the range of 1 to 10. Only single colonies of Gram-negative bacteria were detected. However, the reason for this might be the sampling method and the growth conditions (media choice, incubation time, and temperature). Species of *Penicillium* were identified.

In plant C, 24 samples were taken in the processing area using RCS and FH3 air samplers. The media for collecting and enumerating yeasts and moulds were YM and CGY. Three samples showed less than 10 CFU/m^3 and the rest of the samples showed a concentration of between 10 and 200 CFU/m^3. The dominant yeasts were *Candida* species found in samples from both the media and air samplers. Species of *Penicillium*, *Aspergillus* and *Cladosporium* were identified from the YM agar and the RCS air sampler, whereas species *Paecilomyces*, *Phoma*, *Rhizopus* and *Trichoderma* were identified from the CGY and the FH3 air sampler.

In plant D, 28 samples were taken in the processing area using RCS and FH3 air samplers. Different media for collecting and enumerating CFUs were used: Blue plate for Gram-negative bacteria, SDX, and CREAD for yeasts and moulds. The highest number of Gram-negative bacteria were found close to the shaft down to the floor below. High numbers of fungi were found both on SDX and CREAD. Species of *Alternaria*, *Phoma*, and *Penicillium* were identified from the SDX agar and the RCS air sampler. *Fusarium*, *Phoma*, *Penicillium*, *Phialophora*-like, and single *Cladosporium* species were identified from CREAD agar and the FH3 air sampler. The identification of yeasts was not reported.

The microbial results reported were compared with regard to the influence of the air sampler and the different media used. Some differences were observed which require further investigation. Earlier investigations have shown that the selection of the air sampler influences the results (Ljungqvist & Reinmüller, 1998). The SS-EN ISO 14698-1 (2003) recognizes this and describes in annex B 'Guidance on validating air samplers', which is a technique for determining the physical collection efficiency of samplers used for counting airborne microbes (CFU). Physical collection efficiency means the ability to collect particles of various sizes. The test method described recommends the use of five particle sizes. The range of particle diameters should cover approx. 0.8–15 µm. The biological collection efficiency will be lower than the physical efficiency for a number of reasons, such as the survival of microbes during collection and the capacity to grow on the collection medium. None of the air samplers used for this investigation had a stated record of physical collection efficiency. It is advantageous to have such a record before choosing air samplers for environmental monitoring in processing areas.

At each site investigated approximately 30 samples were taken in the processing areas during normal operation. All samples were taken when the microbial load from outdoors was estimated to be lower than in the summer and autumn. Data in Figures 4.24–4.27 are based on the results compiled in Appendix 8. Figure 4.24 shows the values reported from sampling points in Plant A where the same media was used for two air samplers. Figure 4.25 illustrates the results from Plant B and shows variations depending on the different media and on the two types of air sampler used. Figure 4.26 shows the variation between the same two air samplers (FH3 and RCS) as in Figure 4.25 and a similar variation in the media for fungi (YM and CGY). Figure 4.27 shows the comparison between FH3 and RCS in combination with two media for enumerating fungi and one medium for enumerating of Gram-negative bacteria.

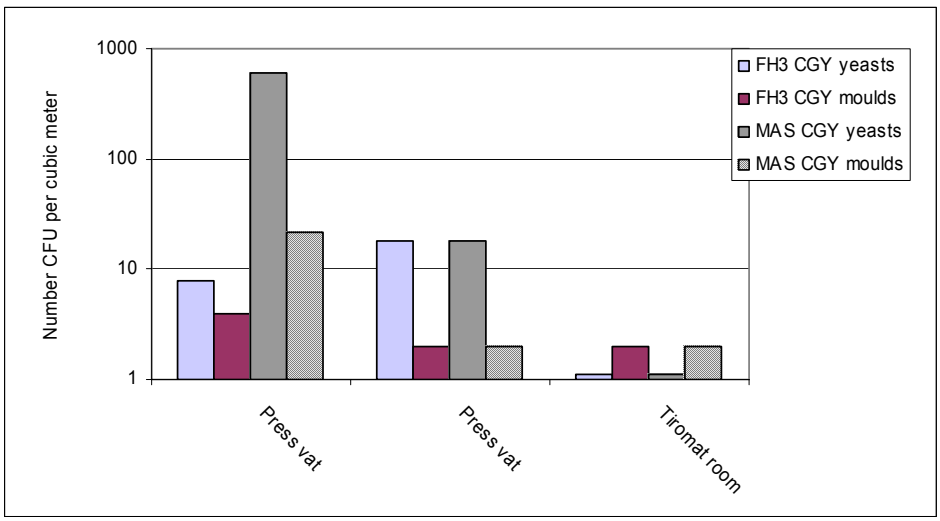

Figure 4.24. Variations in the results (number of airborne CFU/m^3 of moulds and yeasts) between two air samplers, FH3 and MAS. Data is taken from the Table 'Summary of microbial results from Plant A'. Sampling was performed on April 3–4 2002.

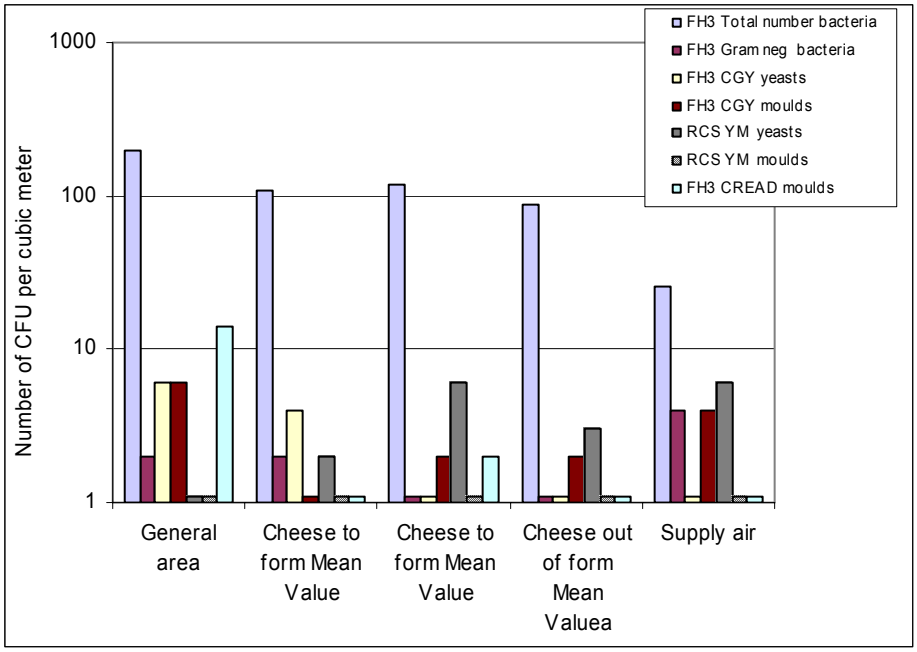

Figure 4.25. Variations in the results (number of airborne CFU/m^3) between two air samplers, FH3 and RCS and among different media. Data is taken from the Table 'Summary of microbial results from Plant B'. The sampling was performed on February 19, 2003.

Figure 4.24 shows the values reported from the sampling points. A reasonable agreement is indicated in the concentration achieved from the two samplers (FH3 and MAS). Then again, most of the samples from the plant showed no growth. Figure 4.25 shows variations in the air samplers and in the media, thus illustrating the importance of thorough risk assessment and media selection. The results shown in Figure 4.25 are highly influenced by the media. The number of total CFUs (bacteria, yeasts and moulds) seems to be around ten times higher than the number of yeasts and moulds. The number of detected Gram-negative bacteria CFUs is low. The CFU number of yeasts and moulds found using the FH3 sampler on CGY or CREAD medium seem to be in the same range as the CFU number found using the RCS with YM in corresponding locations.

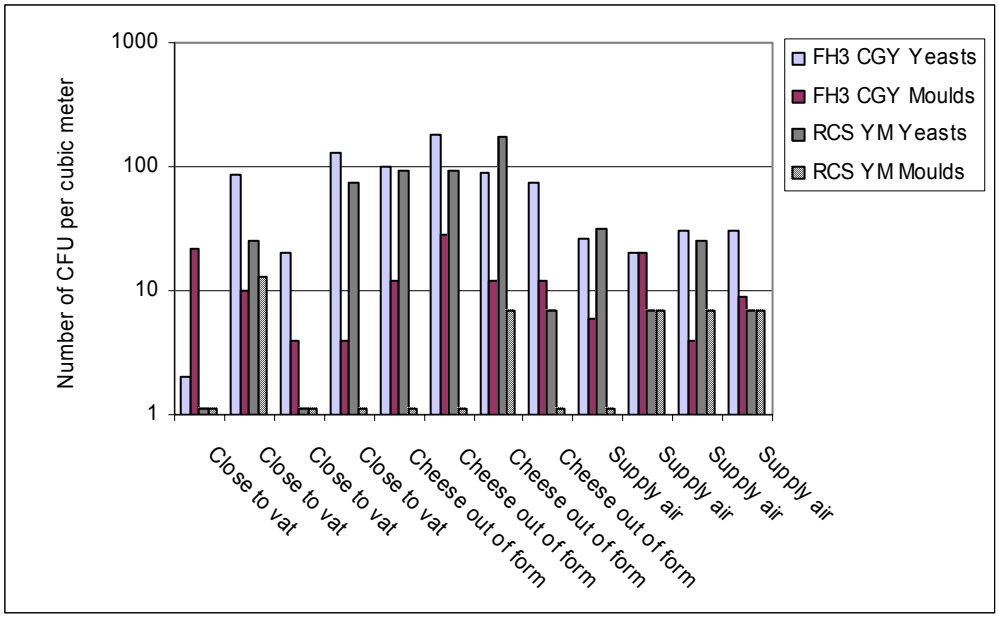

Figure 4.26. Variations in the results (number of airborne CFU/m^3, yeasts and moulds) between two air samplers, FH3 and RCS and between two media. Data is taken from the Table 'Summary of microbial results from Plant C'. The sampling was performed on January 19, 2003.

Figure 4.26 shows results from the same air samplers (FH3 and RCS) as in Figure 4.25. The variation in media (YM and CGY) was restricted to media for fungi. The results indicate that the CGY medium seems to be more effective in detecting different types of moulds. In Figure 4.27 the same comparison between FH3 and RCS is shown using two media for enumerating fungi and one medium for enumerating Gram-negative bacteria. Figure 4.27 shows high levels of airborne contamination in the plant D. In some of the samples there was too much growth to be able to give an actual number of CFUs and in these cases the counts were estimated. In spite of this, it seems to be possible to differentiate between different types of moulds more easily with the CREAD medium. The results from this investigation should only be seen as indications and should be followed by further investigations to verify the trends. The development of a special 'cheese-agar' would improve the prospects of monitoring cheese making plants if it was better adopted to identifying the risks of contamination of the manufactured product.

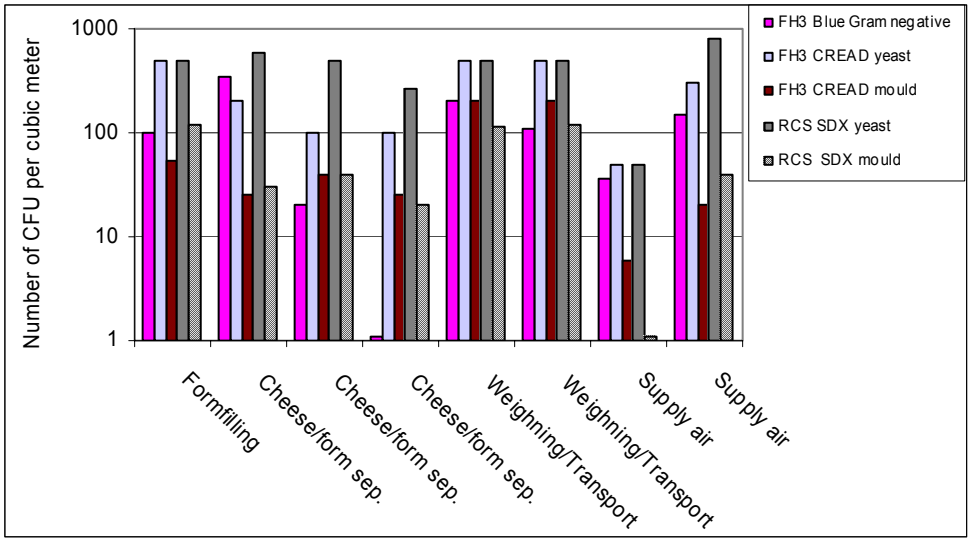

Figure 4.27. Variations in the results (number of airborne CFU/m^3, yeasts and moulds) between two air samplers, FH3 and RCS among three media. Data is taken from the Table 'Summary of microbial results from Plant D'. Sampling was performed on March 13, 2003.

4.4.3 Temperature and relative humidity

Temperature and relative humidity were recorded by data loggers at different locations in the processing area during the period of the survey. Typical graphs from the plants investigated are presented in Figures 4.28–4.32. It should be noted that the scales are different in different graphs.

Figures 4.28 and 4.29 from plant A show the temperature and relative humidity at two locations in the processing area. At plant A the ventilation is usually reduced by 50 % at nights and weekends. Fewer people (1–2) are present during production than at the other plant. The effect of the reduced ventilation at weekends (2002-04-01) can be seen from the figures and the general agreement between the two measuring locations. The washing process performed close to 'cheeses out of the mould' gives peak values up to 100% RH. When high relative humidity is measured, both supply and exhaust air volumes are increased and the re-circulated air volume is decreased. Thus, the temperature in the processing area is affected by the outdoor temperature and during winter can fall for shorter periods as recorded here.

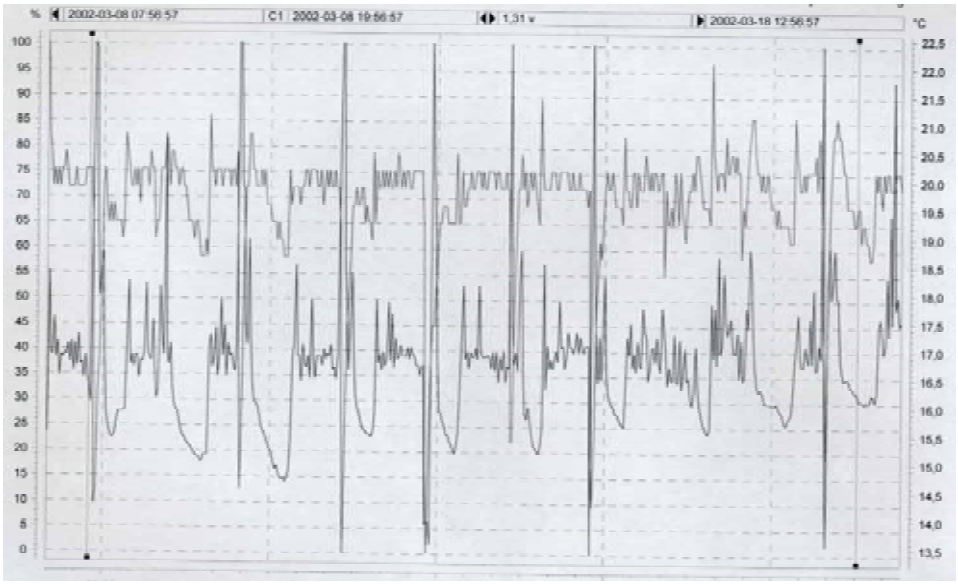

Figure 4.28. Plant A in the processing area, with the logger placed close to where cheese is taken out of the moulds. The relative humidity (lower mean line, left scale) varies from below 10% to 100% and the temperature (upper mean line, right scale) varies from 14°C to 22°C. The measurements were performed on March 9–17, 2002.

Figure 4.29. Plant A in the processing area, with the logger placed close to vat. The relative humidity (upper mean line, left scale) varies from 25% to 60% and the temperature (lower mean line, right scale) varies from 18°C to 23°C. The measurements were performed on March 31 to April 4, 2002.

Figure 4.30 shows the temperature and humidity variations in the processing area of plant B over a week. The temperature varies between 18 and 20ºC and seems not to be influenced by the processes. On the other hand, the relative humidity varies with the process from a low of 30% to peak values of over 70%. The day-to-day pattern seems to be regular. When washing and rinsing processes take place, the relative humidity rises.

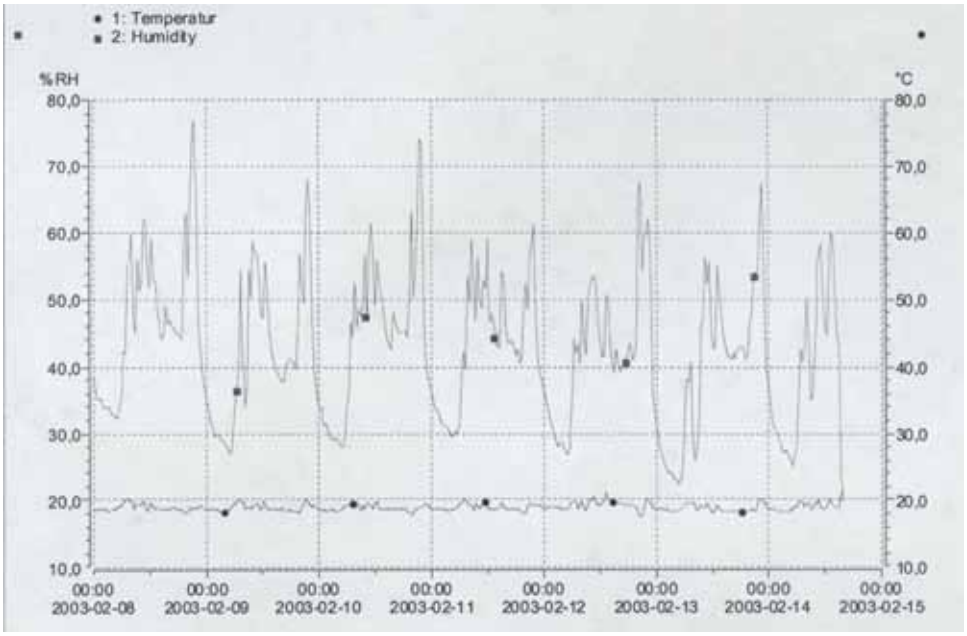

Figure 4.30. Plant B in the processing area, with the logger placed close to where the cheese is taken out of the mould. The relative humidity (upper mean line, left scale) varies from 25% to 75% and the temperature (lower mean line, right scale) varies from 18°C to 20°C. The measurements were performed on February 8–15, 2003.

Figure 4.31 illustrates the temperature and humidity levels at plant C. During the weekend, with lower activity, the temperature and the relative humidity go down to 12ºC and 50%, respectively. Peak levels of over 70% RH are measured regularly, which indicates that the washing process releases hot humid air into the production environment.

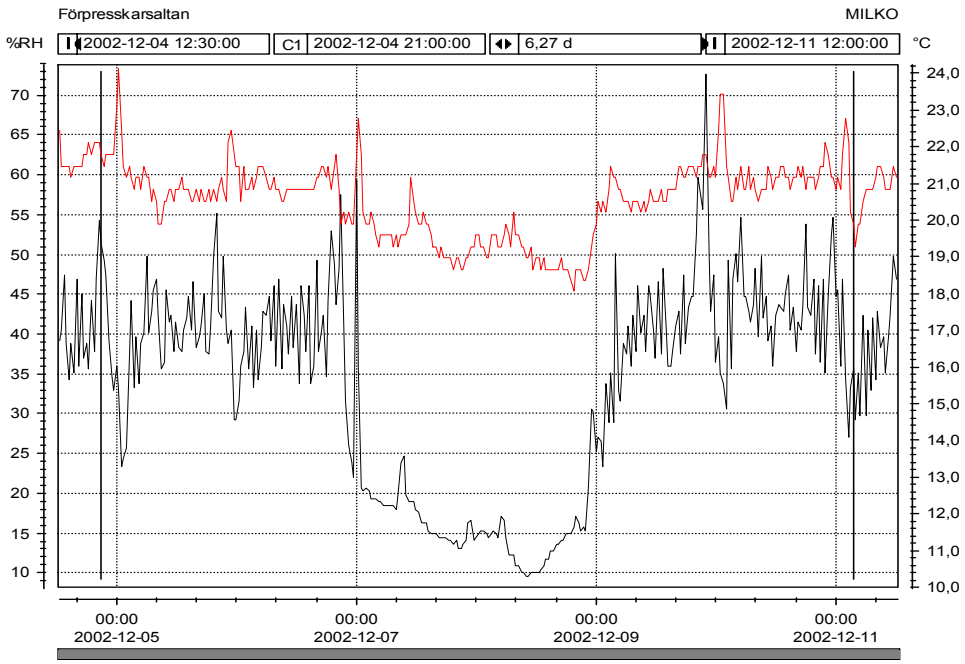

Figure 4.31. Plant C in the processing area; with the logger placed close to the pre-press area where the cheese is put into the vats. The relative humidity (lower mean line, left scale) varies from 10% to 70% and the temperature (upper mean line, right scale) varies from 12°C to 25°C. The measurements were performed on December 5–11, 2002.

Figure 4.32 shows the temperature and humidity levels at plant D during a period of two weeks. Temperature sensors in the processing areas regulate the ventilation system. Recirculation of air occurs, but is limited to a maximum of 70%. Production runs for seven days per week in three shifts, with 2–4 people working per shift. Production processes are performed during the night shift, and washing and rinsing cycles mainly during the daytime.

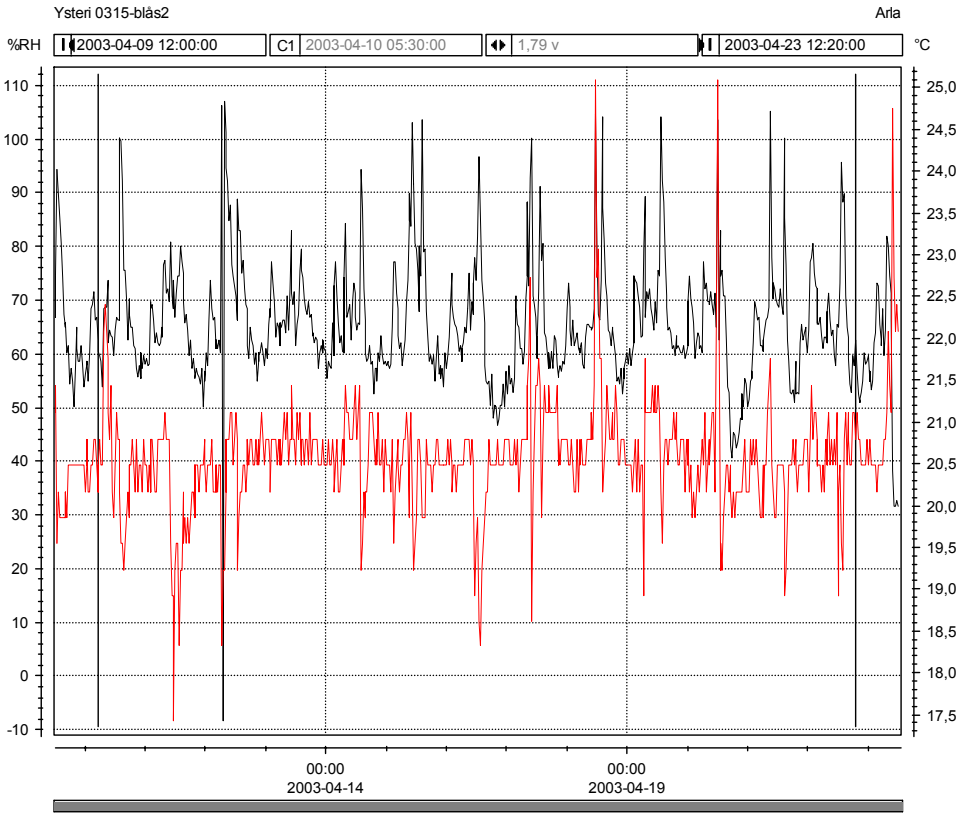

Figure 4.32. Plant D in the processing area, with the logger placed close to where the cheese is taken out of the moulds. The relative humidity (upper mean line, left scale) varies from 10% to 100% and the temperature (lower mean line, right scale) varies from 17°C to 25°C. The measurements were performed on April 14–19, 2003.

4.5 Observations Based on the Air Hygiene Survey

Specifications for the processing areas with regard to air quality must be established and maintained by the person in charge at each plant. Frequent monitoring in a representative way is advantageous. Results from this study show that older ventilation systems need to be upgraded to reach the desired air flows and pressure differences. The quality of the filters needs to be improved in order to separate the outdoor challenge from indoor processing areas as well in winter as in the autumn. Locations where the open product is processed are

vulnerable to airborne contamination; local zones supplied with HEPA-filtered re-circulated room air would consequently improve the air quality and reduce airborne product contamination.

When planning filter installations to achieve good indoor air quality, it is considered adequate items of hygiene that inlet air is filtered in two steps. The first filter in the air intake must be of at least F5 quality but preferably F7. The second stage filter should be at least F7 but preferably F9 quality. If there is only one filtration step, the minimum requirement is F7 quality. When the outdoor burden of particles and microbes is high; higher filter quality should be used.

For reasons of hygiene, the filters should be replaced after the pollen and spore season in the autumn. If requirements are stringent, filters should also be changed in the spring, after the heating season, to eliminate combustion products.

If the general ventilation system does not work at full capacity, it will be unable to take care of process generated contaminants such as humid air. To avoid the dispersion of warm humid air after, e.g., washing cycles in all of the processing areas, effective process exhaust equipment should be added. This process exhaust equipment need not be operated continuously. High humidity air should not be recirculated.

Contamination of air from less controlled areas or from outdoors should be avoided. A system of locks could guard the processing areas. The results reported show that openings between floors such as shafts or conveyers contribute to an area more difficult to control. The most critical processing zones should be situated in the middle of the building in order to achieve better control of the cleanliness of the air and pressure differences.

In cases where compressed air is used in the process for blowing off or drying the product or components, it is important that the contaminated air is collected close to the source by an adequately designed local exhaust device. The contaminated air should not be diluted in the whole processing area but should be expelled by the general exhaust systems. Obviously, the compressed air should be of appropriate cleanliness.

Environmental monitoring should be based on risk assessment. Air samplers used for monitoring the air in processing areas should be suitable for the environment. To improve the evaluation of environmental monitoring an appropriate combination of media should be used. The ability to detect and make a rapid analysis of microbes that are considered harmful to the product is of vital importance to the monitoring lab.

The microbial results could also be used as a base for subsequent investigations concerning comparisons of seasonal changes in the microbial challenge from outdoors and/or comparisons of the media used for collecting and enumerating microbes in cheese processing areas. Data collected in this investigation could be used as references to follow-up investigations, e.g., at different seasons or after changes in ventilation systems or changes to the process.

5. AIR QUALITY AT HERAJOKI DAIRY

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5.1 BACKGROUND TO THE AIR QUALITY STUDY

At Herajoki dairy there are 9 big filtration units in the production hall. The oldest one dates back to 1967 and the latest to 2003 (Figure 5.1). The filters are sock filters and EU 7. Only air from the atmosphere outside is used; recycled air is not used. Continual improvements have been made to the old units, the latest concentrating on protecting the filling spaces in the packing machines (Figure 5.2). It has been observed that it is easier to protect just the small area around the product instead of trying to keep the whole production area extremely clean microbiologically.

The aim of this study was to measure the air quality and the airflow by sampling with different particle and microbial samplers and a smoke detector. The microbiological results were compared with the number of particles found in the air. This study was a part of a Nordic co-operation project DairyNET. Background information to this study was collected by familiarisation with the air filtration systems and with the history of the building at Herajoki dairy (Kontula *et al.,* 1978; Flannigan, 1992; Nuutinen, 1997; Ruotsalainen *et al.,* 1997; Hakala & Säteri, 1999). In addition, the results achieved were compared with the results from previous air measurements. Changes in production and their effect on the air quality in the production hall have been dealt with as well. The air quality seemed to be microbiologically good according to those references, which are available from different literature sources (Kang & Frank, 1989b; Ljungqvist & Reinmüller, 1999). The number of particles exceeded corresponding references. In this study, the seasonal variation and the construction work at the dairy have to be taken into account when the results are examined. By examining the airflow in the production area and by steering clean air round the product, contamination of the product can be reduced.

Figure 5.1. Air filtration system in the stock of returning material.

Figure 5.2. Doso-packing machine with HEPA filters installed. The whole filling space has been protected with a plastic cover.

5.2 SAMPLING PROTOCOL IN STUDYING AIR QUALITY

The airflows were measured by VTT's Cumulus Air Current Tester Flow Check (Dräger Sicherheitstechnik GmbH, Germany) smoke generator. The smoke was produced with a cartridge. The movements of the smoke were observed and on the basis of that conclusions could be made. This smoke generator proved to be so small that the amount of smoke produced was equivalent to a cigarette.

Microbiological measurements were carried out with two air samplers: VTT's MAS air sampler (Merck) and Valio's RCS air sampler (Biotest Hycon), with settle plates and HygiCult® dipslides (Orion Diagnostica Ltd.) as well. The total bacterial count, yeasts and moulds were measured from the air samples. The agars used were HygiCult® TPC (total count agar), HygiCult® Y&F (yeast & moulds), PCA (plate count agar) and THG (tryptone-soy-glucose) for the air sampling with MAS and RCS samplers and settle plates. The plates for the total bacterial count were incubated at +30°C for 3 days and yeast and moulds at +25°C for 5 days. The RCS sampler is based on centrifugal forces in which the air sample is collected onto the agar strip placed in the air sampler. Both the amount of air sampled and particles sorted can be fixed according to the measurement point. The MAS equipment is an impactor i.e. an air sampler in which the air sampled is steered through a perforated plate on top of the Petri dish (Ø 90 mm) containing agar. In addition, the total number of particles in the air were measured with Kojair's METONE 237 particle sampler (Hach Ultra Analytics). The METONE sampler is a laser particle counter which counts all the particles existing in the air and divides them according to size (see Chapter 4 written by Reinmüller).

The first measurements were taken in May 2001. At first it was planned to measure the airflow into and inside the production hall and from there to the other spaces (offices, entrances etc.). However, the smoke generator proved to be so small that it was decided to measure only the airflow in the packing machines. Samples were taken from the yoghurt incubation room, nitrogen line pipe, lid stock, yoghurt room air, and the filling spaces of the following yoghurt packing machines: Gasti 82, Ampack, Erca, Shikoku and PP7 (milk packing machine), smetana (ripened sour cream) room air and the open air (outdoors). Microbiological results were collected with the air samplers mentioned above as well as with settle plates (settling time 10 min) and HygiCult® dipslides. The total number of airborne particles was measured with the METONE particle sampler.

In June 2001, the number of particles in the filtered air in the yoghurt incubation and packing tanks was measured. All samples were clean so those results have not been discussed further. The installed HEPA filters work extremely well, if they are well maintained and filters are replaced at regular intervals.

The second measurements were taken in October 2001. As the samples taken from the nitrogen line were clean (and they always are), it was decided not to take samples from there this time. This time, several samples from the air after filtration (smetana, yoghurt control room, yoghurt maintenance platform, yoghurt incubation room, returning material and the new area) were also taken. There were also special holes made in certain packing machines to make it easier to take air samples without having to open the doors and interrupt the packing.

5.3 RESULTS IN THE AIR QUALITY STUDY AT HERAJOKI DAIRY

The airflow in packing machines was appropriate. There was clearly overpressure in machines which were equipped with some kind of air filtration. The airflow between departments could not be measured because of the inadequate capacity of the smoke generator.

The total number of particles was high, especially in October (Figure 5.3). The highest number s of particles were found in the air in the yoghurt room, on the yoghurt maintenance platform and in the outdoor air. Surprisingly, also in the sample taken from the filtration unit in the new area, the number of particles was as high as in the samples mentioned above. In the new area there was a construction site that was not separate from the old part of the building. This meant that all the dust and other dirt coming from the construction site floated into the old part of the building. The number of particles in the smetana room air was also very high in spite of the fact that the smetana department was situated on the other side of the building i.e. not in the new part. Smetana production was moved to the dairy in Oulu at the end of 2003.

The number of particles was much lower in samples taken from the filling spaces of the milk and yoghurt packing machines. Most of those machines have HEPA filters. But in these samples taken, the level was, on average ten times higher in October than in May.

The recommended maximum number of particles (>0.5 µm) in cheese plants is 4,000,000 per m^3 (Radmore & Lück, 1984; Rossi, 1992). The average number of particles at Herajoki dairy was 4 600 000 particles per m^3 (>0.5 µm) in May and 10,900,000 particles per m^3 (>0.5 µm) in October. According to this recommendation, the numbers were too high.

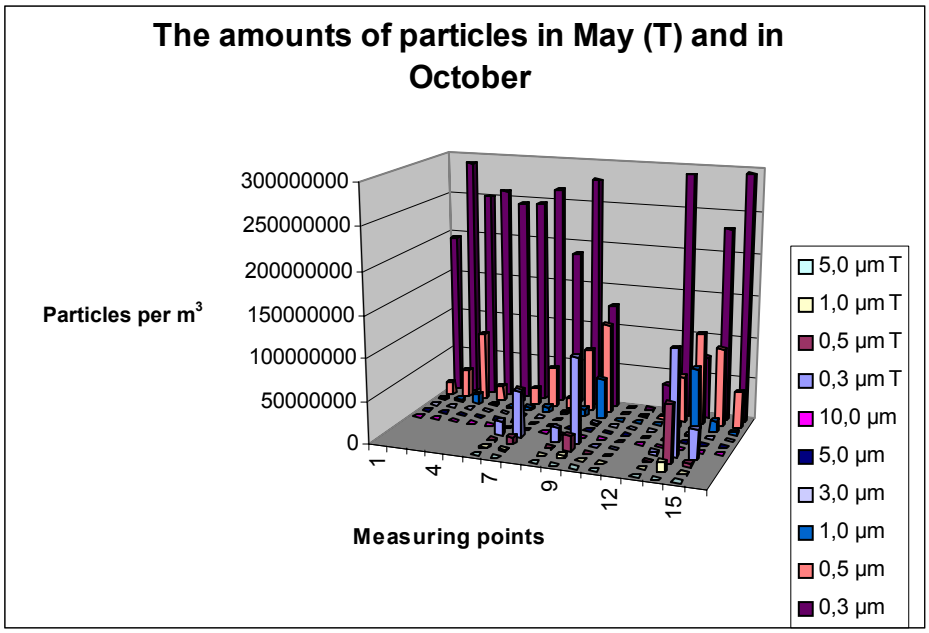

Figure 5.3. The numbers of airborne particles measured in various places in the process in May 2001 and in October 2001.

The total number of bacteria was at an elevated level as well (Table 5.1). There are no regulations on the number of microbes in the air in dairies and, therefore, the total bacterial count is normally not defined at Herajoki dairy. Because of this it was hard to say whether these results indicated good or poor air quality. The amount of yeast and moulds varied. The filling spaces of the packing machines were mostly clean. Otherwise, the level was generally higher in October than in May. In this case, the construction site in the new area raised the amount of yeast and moulds throughout the building. Those microbes came into the dairy-processing area with tools, machinery and equipment installed in the new production area. In many samples, the number of particles and total bacteria was high: the amounts of yeast and moulds were at a tolerable level. The amounts of yeast and moulds are of much more interest to us in Herajoki than

the numbers of total bacteria, since the main product is yoghurt, and fungi can be a problem in sour milk products. The construction site should have been separated properly from the production and packing area in order to minimise the air movements between these two areas. There are some recommendations on cheese plants: the upper limit values for total bacteria in the air vary in different studies between <200 CFU/m^3 and <500 CFU/m^3 as well as <100 CFU/m^3 for yeast and moulds (Radmore & Lück, 1984; Rossi, 1997; Keskikuru *et al.,* 2000). According to these limits, the air quality in Herajoki is good. In the measurements based on settle plates and HygiCult® dipslides the settling period should be long enough and the sampling places should be chosen with care, so that the measurements give a realistic view of the situation, if these methods have to be used. The HygiCult® dipslides were placed both vertically and horizontally, but for our purpose this method and the settle plates did not give reliable results.

Table 5.1. Microbiological average results.

Determination, air sampler	**Microbiological average results., CFU/m^3**			
	May 2001		October 2001	
	all samples	all samples excl. places with HEPA filters	all samples	all samples excl. places with HEPA filters
Total bacterial count, MAS	303	386	71	84
Total bacterial count, RCS	ND	not defined	103	113
Yeasts and moulds, MAS	54	72	75	91
Yeasts and moulds, RCS	19	25	31	37

5.4 BUILDING AND PROCESSING SITUATION AT HERAJOKI IN SPRING 2004

Now, three years later, the construction site is even bigger though the biggest projects are no longer being performed in the production hall. The building activities will continue until the spring of 2005, when the new cold store will be completed. In the production hall, there are several new packing machines that were introduced after 2001, when the air quality study was performed. All the new packing machines are equipped with either HEPA or ULPA filters. Also some of the old packing machines have now been equipped with those kinds of filters (Figure 5.2). Altogether, it can be said the air quality at Herajoki dairy has

improved. This can be seen especially in those packing machines which did not previously have any air filtering. These machines are now equipped with air filtration and the air quality at the yoghurt packaging phase is now better than before the installation. The number of particles most probably will decrease when all the construction work is finished. The development programme at Valio will continue until 2008, and it is, therefore, likely that several changes will still happen in production at Herajoki due to the centralisation of production.

6. MICROBIAL CONTAMINATION IN WATER, AIR AND CHEESE

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6.1 BACKGROUND FOR THE STUDY ON CHEESE PLANT HYGIENE

In 1999, the Swedish cheese group (Ostfrämjandet; www.ostframjandet.se) decided on a new strategy with the long-term objective of providing Swedish cheese that is free from additives such as natamycin E235, sodium nitrate E251, and potassium sorbate E202. The production of cheese without natamycin and sorbate (mould and yeast inhibitors), and nitrate (prevents gas formation by coliforms) demands a high level of hygiene during processing. The production of cheese is partly an open production process, which gives ample opportunity for contamination of the product while it is being processed. Surface contamination can be reduced in some of the processing steps. Aerosols may spread microbes from one processing area to another. In order to obtain cheese with a high microbial quality a high standard of hygiene must be maintained throughout the production line. The use of natamycin, sorbate and nitrate at the four cheese dairies included in the study is shown in Table 6.1.

Table 6.1. Use of additives.

Plant	Natamycin	Sorbate	Nitrate
A	Yes	Yes	One product type
B	One product type	Yes	Certain products
C	Yes	No	No
D/one cheese dairy	No	Yes	Yes

The aim of the study was to determine the microbial contamination of water, air and cheese at four cheese plants. These were analysed for Gram-negative bacteria, yeast and mould. The identities of several isolates were determined and the growth ability on cheese agar and CREAD was determined for a selection of isolates.

6.2 Sampling and Microbial Analyses of Water

The purpose was to focus on microbial biofilm that might exist in a tap or connected hose. Water samples were collected by taking the first water coming from a water tap. If a hose was connected, it was not removed before sampling. Samples were also taken from taps, hoses and connectors used for cleaning (in foaming central and high-pressure equipment).

Two 25–200 ml samples from each water sample were membrane-filtered and the two filters were transferred to Violet Red Bile Glucose agar (VRBGA) and Chloramphenicol Glucose Yeast extract-agar (CGY), respectively. The agar plates were incubated (VRBGA at 30°C for 1 day; CGY at 22°–25°C for 5 days). Colonies were counted, and the agar plates were sent to SIK for colony identification.

6.3 Sampling and Microbial Analyses of Air

Air sampling was performed using sampling devices such as: slit sampler FH3, centrifugal sampler RCS and MAS100. Microbial enumerations were made for yeast and moulds (CGY), Gram-negative bacteria (Drigalski agar) and cheese spoilage associated moulds (CREAD).

6.4 Microbial Identification

Isolates grown on VRBGA were grouped together according to colony appearance and counted. Where possible, five colonies from each group were transferred to Tryptone Glucos Extract agar (TGE), blood agar and in some cases Drigalski agar. The agar plates were incubated at 30°C for 1–2 days. The isolates were then Gram-stained and analysed using a microscope and isolates were tested for oxidase activity. Isolates from each group were also selected for API-profiling.

Isolates grown on CGY were grouped together according to colony appearance and counted. Where possible, five colonies from each group were transferred to Yeast-extract Peptone D-glucose agar (YPD) and in some cases to Blood agar, and incubated at 25°C for 1–2 days. The isolates were then Gram-stained and analysed using a microscope and isolates were selected for API-profiling. The profiles were clustered using Systat.

Isolates grown on CGY were grouped together according to colony appearance and counted. One from each group was transferred to 2% Malt Extract Agar (MEA) or Czapek Yeast Autolysate agar (CYA), and incubated at 25°C for 5–7 days. The isolates were then identified according to the morphological appearance, growth rate, colour and growth pattern.

6.5 Characterisation Methods for Microbial Contaminants

Cheese agar was produced by pouring a layer of agar supplemented with 2% glycerol (a_w=0.95), into a Petri dish. A thin slice of cheese was placed on the agar and a thin layer of agar was poured on top. Chlomaphenicol was added to the agar when yeast and mould were tested in order to inhibit bacterial growth. Crystal violet was added to the agar when bacterial growth was studied in order to inhibit the growth of Gram-positive bacteria. Single strains were inoculated on the agar surface, and the cheese agar was incubated at 8°C or 22°C for yeast and moulds and at 30°C for bacteria.

Creatine sucrose dichloran agar (CREAD) was prepared according to Frisvad *et al.* (1992). The agar was surface-inoculated with single strains of moulds, incubated at 25°C for 7–10 days, and checked for the extent of growth.

6.6 Microbial Load in Water Samples

The water sources (taps, hoses, nozzles) sampled are used during cheese processing and cleaning. As shown in Table 6.2, the water contained varying concentrations of different types of microbes. The results are summarised in Figure 6.1. Gram-negative bacteria were found in > 20% of the water samples at all plants, except for plant C. High yeast levels were found at plants B and D, while mould was found at plants B, C and D.

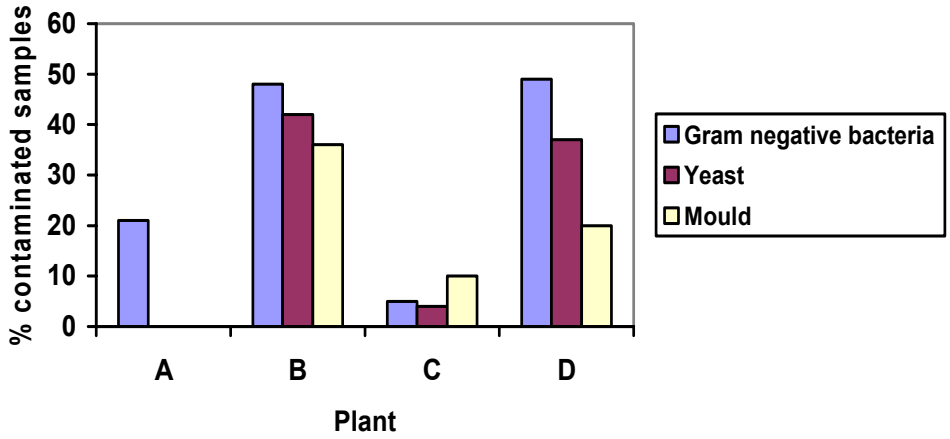

Figure 6.1. The percentage of contaminated water samples at plants A, B, C and D. A sample was regarded as contaminated when the microbial count was ≥ 10 CFU/100 ml.

Table 6.2. Microbiological investigation of water samples.

Cheese plant	Date	Samples	% Samples containing								
			Gram negative bacteria (CFU/100 ml)			Yeast (CFU/100 ml)			Moulds (CFU/100 ml)		
		n	1–9	10–99	≥ 100	1–9	10–99	≥ 100	1–9	10–99	≥ 100
A	011128	25	72	8	20	100	0	0	100%	0	0
A	020117	28	85.7	3.6	10.7	100	0	0	100%	0	0
B	011128	30	53.3	10	36.6	56.7	23.3	20	70	20	3.3
B	020121	29	48.3	13.8	37.9	55.2	31.0	10.3	58.6	31.0	10.3
B	020305	31	54.3	12.9	32.3	61.3	9.6	25.8	64.5	22.8	12.9
C	011128	18	94.4	0	5.6	100	0	0	88.9	5.6	5.6
C	020123	24	95.8	0	4.2	91.7	0	0	91.7	0	8.3
D	020204/05	38	55.3	18.4	26.3	68.4	15.8	18.4	81.6	15.8	0
D	020104/05	38	47.4	7.9	44.7	57.9	15.8	21.1	78.9	18.4	0

The cleaning equipment at plants B and D was found to be contaminated with Gram-negative bacteria, yeast and to a lesser extent with mould (Figure 6.2).

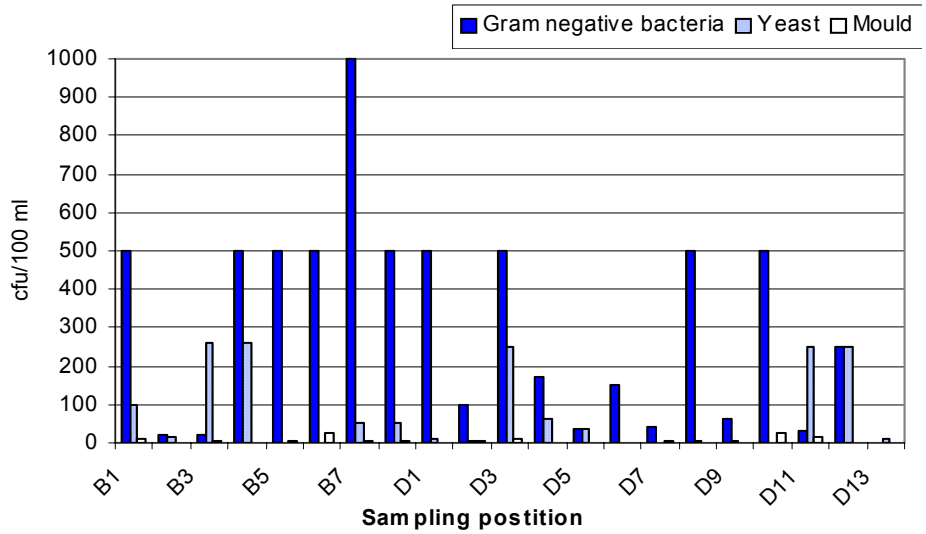

Figure 6.2. Microbial count in water sampled from equipment (taps, hoses, nozzles) used for cleaning and foaming at plants B and D.

The identity of the bacterial isolates is presented in Table 6.3. The most frequently isolated bacteria were *Acinetobacter* spp. and *Pseudomonas* spp. These were retrieved at all four plants. The higher the occurrence of Gram-negative bacteria, the greater the varieties of bacteria that were isolated.

The identity of yeast is presented in Table 6.4. *Candida* spp. and *Rhodotorula* spp. were the most frequently isolated genera. In particular *C. intermedia*, *C. famata* and *R. minuta* were isolated, each constituting about 5–10% of the isolates. These two genera were retrieved at all plants.

The identities of the moulds are presented in Table 6.5. *Phoma* spp. dominated and constituted about 30% of the mould isolates. *Acremonium* spp. and *Fusarium* spp. each constituted about 10% of the isolates. *Phoma* spp. were found in all plants. The diversity was fairly high at all plants.

The conclusion is that the results demonstrate the possibility of water-borne contamination at processing plants due to insufficient hygiene and maintenance of water sources, such as hoses, nozzles and taps. The microbes most likely to become established in these environments are bacteria such as *Acinteobacter* spp. and *Pseudomonas* spp.*;* yeast such as *Candida* spp. and *Rhodotorula* spp.;

and mould such as *Phoma* spp., *Acremonium* spp. and *Fusarium* spp. There is a high diversity of microbes with the ability to become established.

Table 6.3. Identity of bacterial isolates in water sampled at four cheese plants.

Bacterial strain	**% of isolates at all plants**	**Retrieved at plant**			
		A	B	C	D
Acinetobacter spp.	32.1	+	+	+	+
Pseudomonas spp.	18.1	+	+	+	+
Klebsiella spp.	6.4	-	+	+	+
Stenotrophomonas maltofilia	5.9	+	+	-	+
Comamonas spp.	5.4	+	-	+	+
Enterobacter spp.	4.4	+	+	-	+
Aeromonas spp.	3.9		+		+
Citrobacter spp.	2.5	-	-	-	+
Serratia spp.	2.5	-	+	-	+
Shewanella putrefaciens	2.5	-	+	-	+
Chrysomonas indolegenes	1.5	-	+	-	+
Pantoea spp.	1.5	-	+	-	+
Sphingomonas pancimobilis	1.5	+	-	-	+
Alcaligenes denitrificans	1	+	+	-	-
Agrobacterium radiobacter.	0.5	-	-	-	+
Brevundimonas vesicullaris	0.5	-	-	-	+
Ochrobacterium spp.	0.5	-	-	-	+
Yersinia kristensenii	0.5	-	-	-	+
Unidentified	9.3				
No. of isolates	193				

NT, not tested

Table 6.4 Identity of yeast isolates in water sampled at 4 cheese plants.

Yeast strain	% of isolates at all plants	Retrieved at plant			
		A	B	C	D
Candida spp. *(C intermedia, C. famata)*	45.3 (9.3, 4.7)	+	+	+	+
Rhodotorula spp. *(R. minuta)*	14 (9.3)	+	+	+	+
Geotrichum spp.	8.1	-	+	-	+
Trichosporon spp.	8.1	-	+	+	+
Saccharomyces spp.	5.2	-	+	-	+
Cryptococcus spp.	1.2	-	+	+	-
Unidentified	20.9				
No. of isolates	172				

Table 6.5 Identity of mould isolates in water sampled at four cheese plants.

Mould strain	% of isolates at all plants	Retrieved at plant			
		A	B	C	D
Phoma spp.	28.9	+	+	+	+
Fusarium spp. *(F. oxysporum)*	11.3 (4.1)	-	+	+	+
Acremonium spp.	9.8	+	+	-	+
Exophiala spp.	8.2	-	+	-	+
Phialophora spp.	6.7	-	+	+	+
Lecythophora lignicola	3.1	-	+	-	-
Penicillium spp.	2.6	+	-	+	+
Trichoderma spp.	2.6	-	+	+	-
Verticillium spp.	2.5	-	+	+	+
Cladosporium spp.	1.5	+	-	-	-
Phialemonium spp.	1.5	-	+	-	-
Zygomycet	1.5	-	-	-	+
Geotrichum spp.	1.0	-	+	-	-
Paecilomyces spp.	0.5	-	+	-	-
Plectoshaerella spp.	0.5	+	+	-	-
Unidentified	12.9				
No. of isolates	194				

6.7 MICROBIAL LOAD IN AIR SAMPLES

The content of Gram-negative bacteria in air was low at plants B and D, the only plants where it was analysed. Only a few colonies/m^3 air were found on 9 cm Drigalski plates using a FH3 slit sampler.

Yeast was detected in the air samples. At plant A, one sample position was heavily contaminated with yeast and 600 CFU/m^3 was detected (Figure 6.3). At plant C, two sampling positions contained > 50 CFU/m^3 air. At plant D, four positions were detected with high levels of yeast (Figure 6.4). In contrast, the level of airborne yeasts was low at plant B (Figure 6.3).

Mould was detected at most sampling position,s but at lower levels than yeast (Figures 6.3–6.4). The sampling positions with high levels of yeast frequently harboured increased levels of mould.

The conclusion is that airborne contamination with yeast and mould was demonstrated, but not with Gram-negative bacteria. Air sampling may be used for the identification of positions along the processing line that contribute to airborne contamination.

The identified yeast isolates found at plants A and C are listed in Table 6.6. *Candida* spp. was found at both plants and a few isolates of *Debaryomyces polymorphus*, *Rhodotorula* spp., *Trichosporon* spp. and *Zygosaccharomyces* spp. at one of the plants.

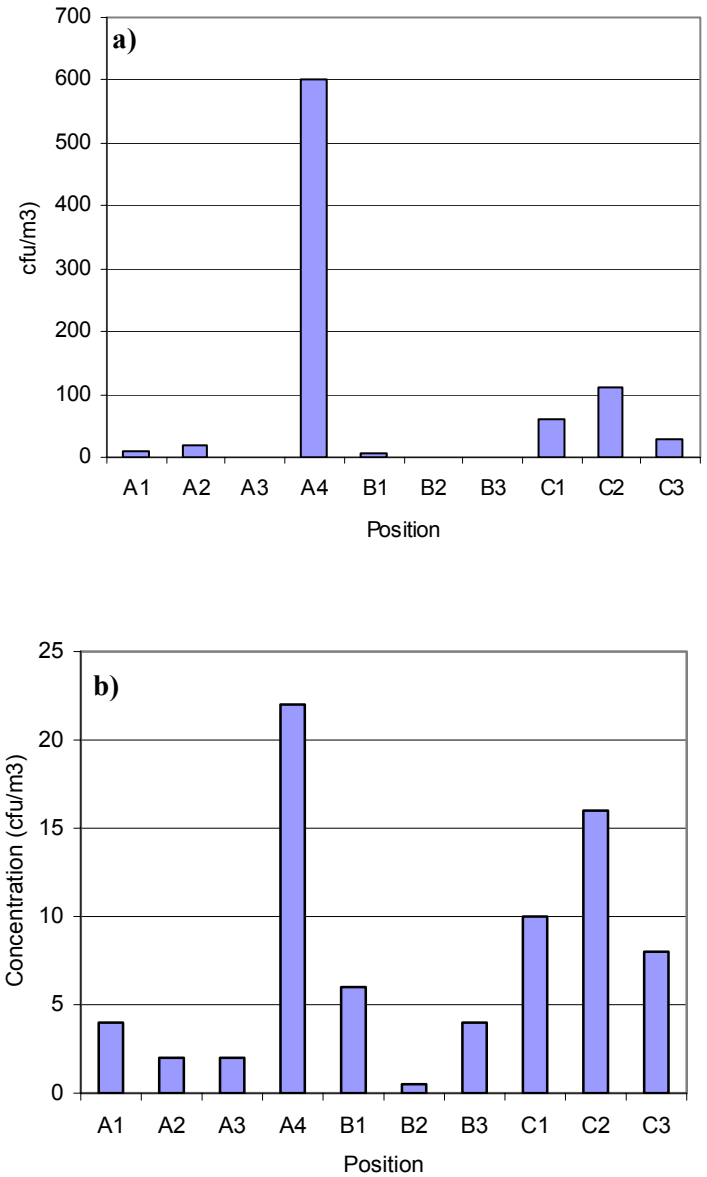

Figure 6.3. Concentrations of (a) yeast and (b) mould in air sampled at plant A, B and C, using a FH3 slit sampler fitted with a 9 cm CGY agar plate.

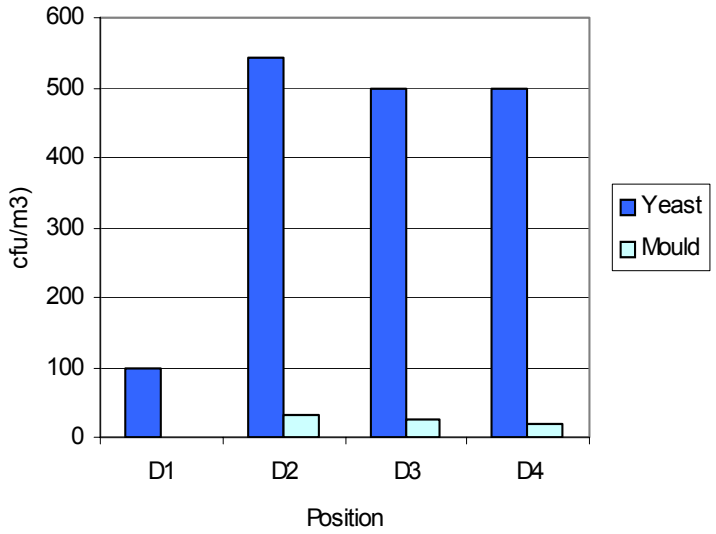

Figure 6.4. Concentrations of yeast and mould at plant D measured using a centrifugal air sampler with agar strips.

Table 6.6. Yeast strains found in air sampled at cheese plants A and C.

Yeast strain	No. of isolates at plant	
	A	C
Candida colliculosa	-	2
Candida curvata	1	-
Candida famata	-	3
Candida intermedia/famata	-	3
Candida intermedia	3	3
Candida lipolytica	-	1
Candida parapsilosis	2	2
Candida rugosa	1	1
Candida sphaerica	-	9
Candida sp.*5*	1	-
Candida spp.	-	2
Debaryomyces polymorphus	-	2
Rhodotorula sp.*3*	1	1
Trichosporon spp.	1	-
Zygosaccharomyces spp.	-	2

Mould isolates were identified (Table 6.7). At plant A, the air contained moulds identified as *Cladosporium* spp., *Geotrichum* spp., *Penicillium* spp. and *Phoma* spp. At plant B, only a few isolates were made, all belonging to *Penicillium* spp. At plant C, *Penicillium* spp. dominated, followed by *Cladosporium* spp., *Trichoderma* spp. At plant D, *Phialophora* dominated, followed by *Penicillium* spp. and *Phoma* spp. (Table 6.7).

Table 6.7. Moulds found in air sampled at cheese plants A, B, C and D.

Mould strain	No. of isolates at plant			
	A	**B**	**C**	**D**
Alternaria spp.	-	-	-	1
Aspergillus spp.	-	-	2	-
Cladosporium spp.	1	-	7	2
Fusarium spp.	-	-	-	1
Geotrichum spp.	1	-	-	-
Paecillomyces spp.	-	-	2	-
Penicicllium steckii	-	-	1	-
Penicillium brevicompactum	-	3	6	-
Penicillium chrysogenum	-	3	-	-
Penicillium decumbens	-	-	1	-
Penicillium solitum	-	-	2	-
Penicillium spp.	1	1	11	27
Phialophora spp.	-	-	-	30
Phoma spp.	5	-	2	14
Rhizopus spp.	-	-	1	-
Trichoderma spp.	-	-	4	-

6.8 MICROBIAL CONTAMINANTS IN CHEESE SAMPLES

Table 6.8 compares the identity of moulds isolated from cheese at three plants to findings in air and water. The few samples of cheese analysed indicate air as the primary source for moulds that contaminate cheese, and water as an additional source for *Geotrichum* spp. and *Trichoderma* spp. The limited number of cheeses investigated does not permit a general conclusion to be made.

Table 6.8. Microbial contaminants in cheese compared with findings in water and air.

Microbe	In cheese			At plant in	
	Plant A (n=9)	Plant B (n=3)	Plant C (n=8)	Air	Water
Geotrichum spp.	-	-	**X**	A	B
Penicillium brevicompactum	**X**	-	-	B, C	-
Penicillium crustom	-	**X***	-	-	-
Penicillium decumbens	**X***	-	-	C	-
Penicillium solitum	**X***	-	-	C	-
Trichoderma spp.	**X***	-	-	C	B, C

*, isolated from cheese in the ripening room

6.9 MICROBIAL GROWTH ON CHEESE AGAR

The ability of isolates to grow on cheese agar was used in order to obtain an indication of the role of selected isolates in cheese spoilage. It should, however, be noted that growth on cheese agar does not necessarily prove cheese spoilage activity since it is a complex process with many factors affecting the outcome.

All yeast isolates were able to grow on cheese agar incubated at 22°C and at 8°C (except for one isolate; Table 6.9). In contrast, few bacterial isolates were able to grow on cheese agar at 8°C; only *Klebsiella oxytoca, Pseudomonas fluorescens* and *Serratia marcescens* and *Stenotrophomona maltofila* showed growth (Table 6.10). Among the *Penicillium* spp. tested*, P. brevicompactum, P. chrysogenum, P. crustosum* and *P. solitum* grew on cheese agar at 8°C (Table 6.11).

The conclusion is that cheese agar incubated at 8°C can be used for further characterisation of the isolates, in order to separate those of minor importance (non-growers) from alarming ones (growers). The collection of microbial isolates from water, air and cheese samples will enable the identification of the critical species at a plant. The growth ability on cheese/cheese agar at cold storage temperature is one important criterion for critical species. The growth ability of isolates should be identified at species level.

Table 6.9. Growth of yeast isolates on cheese agar.

Microbe	Incubation at 22°C	Incubation at 8°C	Origin	No. of isolates
Candida famata	++	++	Water	1
Candida famata	++	++	Air	2
Candida intermedia	++	+	Water	1
Candida intermedia	++	++	Air	1
Candida parapsilosis	++	++	Water	1
Candida parapsilosis	++	++	Air	1
Candida rugosa	++	++	Water	2
Candida sphaerica	++	++	Air	1
Cryptococcus laureutii	++	++	Water	1
Geotrichum capitatum	++	++	Water	2
Geotrichum spp.	++	++	Water	2
Rhodotorula minuta	++	+/-	Water	4
Rhodotorula minuta	++	+	Air	1
Rhodotorula mucilaginosa	++	++	Water	1
Saccharomyces spp.	++	++	Water	4
Trichosporon asakii	++	+	Water	2
Trichosporon mycoides	++	+	Water	2

++, good growth, + growth, - no growth

Table 6.10. Bacterial growth on cheese agar.

Microbe	Incubation at 30°C	Incubation at 8°C	Origin	No. isolates
Acinetobacter calcoaceticus	++	-	Water	1
Acinetobacter junii/woffi	-	-	Water	1
Aeromonas hydrophila	-	-	Water	1
Chrysomonas indolegenes	++	-	Water	1
Comomonas acidovoranas	++	-	Water	1
Enterobacter cloace	++	-	Water	1
Klebsiella oxytoca	++	++	Water	2
Pantoea sp.	++	-	Water	1
Pseudomonas fluorescens	++	++	Water	2
Pseudomonas stuzeri	-	-	Water	1
Serratia marcescens	++	+	Water	1
Shewanella putrefaciens	-	-	Water	1
Stenotrophomona maltofilia	++	++/-	Water	2

++, good growth, + growth, - no growth

Table 6.11. Fungal growth on cheese agar.

Organism	Incubation at 22°C	Incubation at 8°C	Origin	No. isolates
Penicillium brevicompactum	++	++	Air	8
Penicillium brevicompactum	++	++	Cheese	1
Penicillium decumbens	++	-	Air	1
Penicillium chrysogenum	++	++	Air	2
Penicillium crustosum	++	+	Cheese	3
Penicillium decumbens	++	++	Cheese	1
Penicillium solitum	++	++	Air	2
Penicillium solitum	++	++	Cheese	2
Penicillium steckii	++	-	Air	1
Trichoderma spp.	++	-	Cheese	3

++, good growth, + growth, - no growth

6.10 MICROBIAL GROWTH ON CREAD AGAR

The ability of a selection of isolated and identified moulds to grow on CREAD was evaluated (Table 6.12). Genera/species showing good growth on CREAD were *Aspergillus versicolor, Cladosporium* spp., *P. chrysogenum, P. crustom* and *P. solitum*. Other penicillium species, such as *P. brevicompactum, P. decumbens* and *P. steckii,* showed weak growth. Among these, *P. decumbens* and *P. steckii* were able to grow on cheese agar at 22°C, but not at 8°C (Table 6.11). *P. brevicompactum* grew on cheese agar at 8°C.

6.11 SUGGESTIONS FOR IMPROVED HYGIENE SURVEYS

Production of cheese is partly an open process which provides ample opportunities for contamination of the product while it is being processed. There are few processing steps where surface contamination can be reduced. Aerosols may spread microbes from one processing area to another. In order to obtain cheese with a high microbial quality a high standard of hygiene must be maintained throughout the entire production line.

Table 6.12. Fungal growth on CREAD agar.

Microbe	Growth on CREAD	No. of isolates	Origin
Aspergillus versicolor	++	1	Air
Cladosporium spp.	++	1	Air
Geotrichium capitatum	Weak	1	Air
Penicillium brevicompactum	Weak/+	9	Air, cheese
Penicillium chrysogenum	++	2	Air
Penicillium crustosum	++	3	Cheese
Penicillium decumbens	Weak	1	Air
Penicillium solitum	++	4	Air, cheese
Penicillium steckii	Weak	1	Air
Phialophora spp.	Weak/+	2	Air, water
Phoma spp.	Weak/+	2	Air, water
Rhizopus spp.	-	1	Cheese
Trichoderma spp.	Weak/++	1	Cheese

++, good growth, + growth, - no growth

Yeast spoilage causes discolouration, production of gas, structural changes and off-flavours (Loureiro & Querol, 1999; Vasdinyei & Deák, 2003). In recent years, yeasts have also been connected with positive effects on the cheese maturation process (Vasdinyei & Deák, 2003). *Geotrichum candidum* and *Debaryomyces hansenii* were the two most common species isolated from Hungarian cheeses (Vasdinyei & Deák, 2003). Moulds (*Cladosporium cladosporiodes, C. herbarum, P. commune; P. glabrum* and *Phoma* sp.) cause 'thread mould' defects, which appear as black, brown or green spots or threads (Lund *et al*. 1995; Fox *et al.* 2000). Among the bacteria, coliforms are associated with early gas formation (Fox *et al.* 2000). Several genera of *Enterobacteriaceae* are capable of producing biogenic amines in cheese, and it is recommended that the presence of biogenic amines such as cadaverine in cheese is limited by controlling the *Enterobacteriaceae* count (Marino *et al.,* 2000).

The present study demonstrated that several isolates grow on cheese agar incubated at 8°C. Even though these strains have not been shown to produce spoilage or biogenic amines, it is sensible to restrict the presence of all yeast,

mould and *Enterobacteriaceae* as much as possible.Based on the results of the present study, recommended analyses include Gram-negative bacteria, yeasts and moulds. CREAD may be useful for the enumeration of mould associated with cheese spoilage (Lund, 1996); a high level on CREAD indicates a problem with mould.

It is important to know about the critical species at a plant. This can be achieved by testing the growth ability of isolated strains on (i) cheese agar incubated at cold storage temperature (e.g. 8°C), and on (ii) CREAD. According to the present study, isolates from cheese production plants, including *A. versicolor, Cladosporium* spp*., P. chrysogenum, P. crustom* and *P. solitum* were able to grow on CREAD. Among these, *P. chrysogenum, P. crustom* and *P. solitum* also grew on cheese agar incubated at 8°C (only *Penicillium* strains were tested on both agars). In contrast, *P. brevicompactum* showed good growth on cheese agar 8°C, but not on CREAD.

In order to perform a hygienic survey, the sampling of water, air, surfaces and cheese is recommended. It is crucial that the sampling points are randomly selected, and not biased by what is believed to be a likely problem. As the present study shows, the four cheese plants studied had different problems. The groups of microbes that are important to analyse are Gram-negative bacteria, yeast and moulds. Water should be sampled on a routine basis, including water sources, such as taps and nozzles. Preferably, sampling should be performed in order to evaluate the presence and composition of a biofilm. In particular, water sources used for cleaning should be included. Air sampling should be performed with the purpose of identifying critical points in the processing environment where air is contaminated. The collection of microbial isolates from water, air and cheese samples will enable the identification of critical species at a plant. The growth ability on cheese/cheese agar at cold storage temperature is one important criterion for critical species. Isolates that are able to grow should be identified at species level. Further typing of isolates at subspecies level may be used in order to determine specific contamination sources or routes (Kure et al. 2003). If CREAD is used for hygiene surveys, it is advisable to test identified critical species for growth on this agar in order to obtain information for interpretating a situation of no counts on CREAD.

7. MONITORING HYGIENE IN CHEESE PROCESSING – SPECIAL ATTENTION ON EQUIPMENT

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Cheese production is partly an open process providing ample opportunities for contamination. Processing steps in which air, water, and utensils can contaminate the product must be reduced so that microbes from one processing area cannot be spread to another. In order to obtain cheese of high microbial quality a high standard of hygiene must be maintained throughout the entire production line.

7.1 METHODS FOR ASSESSING CLEANING OF CHEESE MOULD

A survey of the outcome of cleaning of cheese moulds was conducted. At four cheese plants and on three occasions, the cleaning results were checked in three different ways: (i) the last rinse water was sampled (100 ml) directly from the mould cleaning machine, (ii) the remaining water in the cleaned moulds was sampled and collected into a single one sample (100 ml), and (iii) the cleaned moulds were rinsed with sterile water supplemented with 0.9 % NaCl, and subsequently collected into a single sample (100 ml). Samples of 50 ml were filtrated through a filter membrane (0.45 μ) and subsequently analysed. Analyses were performed for Gram-negative bacteria (VRBGA; incubated for 2 days at 30°C), yeast and mould (CGY; incubated for 5 days at 22–25°C).

7.2 RESULTS AND SUGGESTIONS FOR CHEESE MOULD CLEANING

The occurrence of Gram-negative bacteria, yeast and mould was determined in the final rinsing water originating from the cheese mould cleaning machine. The rinse water was often contaminated; Gram-negative bacteria, yeast and mould were found in six, nine, and eight samples out of 11, respectively (Table 7.1). The results indicate that somewhere in the cleaning machine contamination with microbes takes place, possibly from a biofilm not being removed during regular cleaning and

disinfection of the cheese mould cleaning machine. The possible formation of a biofilm is likely to be the result of bad hygienic design of the cleaning machine, and is most likely to be found in the final stages of the cleaning machine.

Two more test procedures were used in order to evaluate the cleaning of cheese moulds; the remaining water in cleaned cheese moulds and sterilised water used for rinsing cleaned cheese moulds (Table 7.2). The microbial status of the remaining water in the cheese moulds reflects the quality of the rinsing water and the cleanliness of the moulds. The quality was similar to that of the final rinsing water in the cleaning machine. Four, seven, and six samples out of nine were found to be contaminated with Gram-negative bacteria, yeast and mould, respectively. The microbial status of the sterilized water used to rinse the already cleaned cheese moulds, is an indication of the overall cleanliness of the moulds, and consequently the efficiency of the cheese mould cleaning operation. In six, seven and eight samples Gram-negative bacteria, yeast and mould, respectively, were found in the samples.

Table 7.1. Microbial contaminants in the final rinse water from the cheese mould cleaning machine.

Plant	Levels of microbes (CFU/50 ml)			Sampling date
	Gram-negative bacteria	Yeast	Mould	
A	20	2	12	2003-10-24
A	14	12	14	2003-11-04
A	500	<1	500	2003-11-25
B	<1	14	<1	2003-10-15
B	15	4	<1	2003-10-27
B	50	<1	<1	2003-11-03
C	<1	18	2	2003-10-09
C	44	>250	1	2003-10-24
D	<1	#	#	2003-10-29
D	<1	1	1	2003-11-05
D	<1	#	#	2003-11-12

= over growth

The microbial flora resposible for causing problems varied in the dairies (Tables 7.1–7.2):

- in plant A, Gram-negative bacteria and mould were found at increased levels
- in plant B, mainly Gram-negative bacteria and to some extent yeast were found
- in plant C, contamination with Gram-negative bacteria and yeast was found and
- in plant D, yeast and mould contamination was a particular problem.

Table 7.2. Occurrence of Gram-negative bacteria (GNB), yeast and mould in the remaining water in cleaned cheese moulds and sterilized water rinsed through cleaned cheese moulds.

Plant	Microbes in remaining water (CFU/50 ml)			Microbes in presterilised water used for rinsing moulds (CFU/50 ml)			Sampling date
	GNB	**Yeast**	**Mould**	**GNB**	**Yeast**	**Mould**	
A	<1	8	2	<1	<1	<1	2003-10-24
A	<1	<1	4	2	8	4	2003-11-04
A	78	<1	100	14	<1	50	2003-11-25
B	1	5	<1	<1	3	4	2003-10-15
B	19	5	<1	6	5	<1	2003-10-27
B	50	2	3	11	<1	1	2003-11-03
C	-	-	-	>250	>250	9	2003-10-29
D	<1	700	<1	<1	450	15	2003-10-29
D	<1	200	2	<1	110	10	2003-11-05
D	1	#	#	3	350	15	2003-11-12

= over growth

In summary, the cheese moulds were not satisfactorily cleaned during the cleaning procedure. It is of particular importance that the water used for the final rinsing of the moulds is clean. In this study all three test procedures gave similar results. In cases where the rinsing water actually is clean, the procedure using sterilized water for rinsing of cleaned cheese moulds could give additional information.

In order to prevent contamination, procedures for inspecting and cleaning the cheese mould cleaning machine must be established. Sampling and analysing the last rinse water is a good indicator to check whether the procedures are in place and working.

7.3 METHODS FOR ASSESSING CLEANING OF CONVEYOR BELTS

The cleanliness of conveyor belts for cheese transportation was assessed using swab and microbiological tests. Samples were taken from conveyor belts that had recently been cleaned. A defined area of the conveyor belt, roller, one link of conveyor chains and lamellae belts were swabbed using moisturized cotton swabs. The swab was transferred to sterile peptone water, whirl mixed and 0.1 ml was subsequently analysed for Gram-negative bacteria (VRBGA), yeast and mould (CGY).

7.4 RESULTS AND SUGGESTIONS FOR CONVEYOR BELT CLEANING

Cleaning conveyor belts and their beams is a well known problem. Another problem is the method used for assessing the cleanliness. The tests were carried out using swabs but there are some sritisisms of this method. A more effective way to assess and improve cleaning has been developed at Arla Foods, and is presented below.

7.4.1 Swabbing test results

Conveyor belts were sampled in plant A and B. In plant A, two (7%) samples (n=27) with mould, but no samples with Gram-negative bacteria or yeast were found. Figure 7.1 shows samples being taken from conveyor belts in plant B. Almost 20% of the samples contained yeast and mould, and the occurrence of Gram-negative bacteria was less frequent.

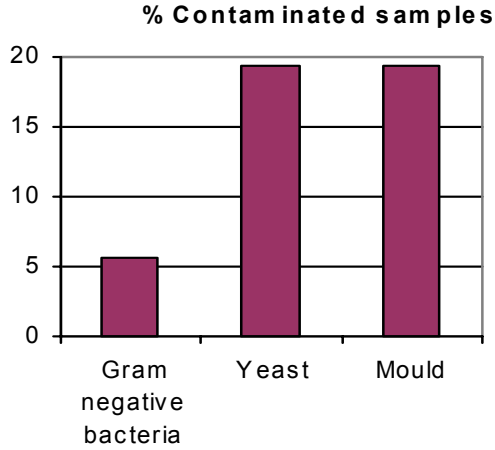

Figure 7.1. Microbial contamination rate in swab samples from conveyor belts in plant B. (n= 3x12 sampling positions, three sampling occasions).

A similar contamination pattern that was found between the different areas where samples were taken in plant B. The samples revealed that three positions were contaminated, when cleaned using ordinary cleaning procedures. The critical spots were identified as being under the conveyor belt (Figure 7.2) and on two rollers.

Figure 7.2. A critical spot contaminated with fungi was found under the conveyor belt.

7.4.2 Cleaning of conveyor belts

The question remains as to how effective the sampling procedure using swabs is, in order to assess the cleanliness of conveyor belts. At Arla Foods, an alternative method for assessing the cleanliness of, for example, conveyor belts and beams has been introduced. The procedure involves: definition of the critical areas/spots, daily checking by an independent person not belonging to the cleaning group, documentation, auditing of the cleaning effect on a regular basis, actions taken when deviations are found and feedback to the cleaning group.

To achieve the best cleaning results, it is advisable to dismantle the conveyor belt, clean it in a cleaning machine and let it dry before being put back together. When the belt has been taken away it is also easier to reach the conveyor beam for cleaning. This is not always possible due to technical reasons, and lack of

time and resources. The second best method is to clean the belts using fixed cleaning, that is CIP, with nozzles for both the belt and the beam. Foam cleaning is normally used. It is very important to encapsulate or adjust the jet to avoid aerosols from forming. The third alternative is foam cleaning from a foam station or a mobile unit. This will generally create more aerosols and result in less effective cleaning.

In some cases the conveyor belt travels through a bath with detergent or disinfectant. In this way the mechanical effect of the cleaning procedure will be absent. The bath can easily become a source of contamination because microbes can easily grow in solutions. To disinfect the conveyor belt it is better to spray disinfectant directly onto the belt, but be aware that if an unwrapped product is transported, rinsing with potable water must be performed after disinfection. the The hygienic design of the belt, the design and choice of cleaning programmes, and the type of preventative maintenance are important parameters for the effectiveness of the cleaning.

7.4.3 Verification of cleaning effectiveness

Verification must be performed on a regular basis, and also when a hygienic problem occurs. There are several tests/methods available to verify the effectiveness of the cleaning. There are also some simple methods available to follow-up daily cleaning. The best method is to make a visual inspection of the surface using clean hands and fingers to feel underneath the conveyor belt. The inspection should also try to identify any damage or bends. It is also advisable to use a torch or a UV lamp during the visual inspection. A further analysis includes ATP (Table 7.3) or microbial determinations (Table 7.4) at certain, selected spots.

There are several different methods to choose from, none of which are optimal. The best method has to be chosen by specifying the desired objective and by assessing the advantages and disadvantages of each method. A combination of methods is often the best choice and should be based on the following important questions:

- What is to be tested?
- What kind of results are to be presented?
- How sensitive (accurate) must the method be, and what specificity is needed?
- How quickly must the result be ready?

- How many samples should be analysed?
- How much may it cost? Investments, consumable supplies, start up costs, how simple is the method to perform, who will perform the analysis?

Table 7.3. Commercial ATP tests available.

Test	Company
Clean-Trace	Biotrace
Aqua-Trace	Biotrace
PocketSwab	Charm
Pocket H2O	Charm
Lightning	BioControl
LuciPac	Kikkoman
HY-LiTE	Merck
HY-LiTE	Merck

It is important to remember that only a small amount of the microbes that are attached to the surface are likely to be retrieved. Only about 10% of the attached microbes will loosen using the swab method (Wirtanen, 1995). However, the methods will give an indication if the surface is clean or not.

Table 7.4. Commercial tests for microbial testing of surfaces.

Test	Company
Hygicult	Orion Diagnostica
HYcheck	Difco
SPC sampler	Millipore
Envirocheck Contact slides	Merck
Envirocheck Rodac plates	Merck

7.5 UV LIGHT FOR DETECTING RESIDUES AFTER CLEANING

The use of a UV lamp (MegaLight^{TM} UVA 14.5, Drivdon AB, Delsbo, Sweden) was evaluated for use in hygiene checks in a cheese producing plant. The lamp is a hand-held, high-performance UVlamp with extremely high UV intensity. It has an instantaneous start-up, and is robust and shock resistant with good durability. This lamp produces a beam of nearly 10 times the intensity and more than 4

times the reach of a conventional 100 W UV lamp. The lamp has a portable 12 V battery, making it very easy to carry, even where no main supply is available. However, the equipment is quite heavy.

It was very easy to detect fluorescent materials and pigments in the cheese plant using the lamp,. Most of the fluorescent material was cheese material remaining after cleaning. It was easy to detect residues on stainless steal. Plastic materials were auto fluorescent, but it was possible to detect contamination since this gave off a different kind of light. The UV lamp was very easy to use and gave immediate results on the efficiency of the cleaning. Being able to give direct feedback to the cleaning group on the cleaning is a great advantage in particular when inspecting critical spots on site. Immediate evaluation of the cleaning is superior to a discussion based on laboratory results coming some days later. The usage of the UV lamp and the results are is illustrated in Figure 7.3.

Figure 7.3. Usage of the UV lamp and the results.

7.6 HYGIENIC ROUTINES FOR WATER SOURCES

It is vital that dairies have control over the water quality at production sites, as this water is a potential source of infection. A new Swedish Water Act came into force on 25th December 2003. Among other things, the Act states that sampling points must be varied when sampling water and that the water must be checked in the same way as it is used. Some advice is given below as to how the water should be sampled and how water hoses should be maintained in order to protect the water quality.

7.6.1 Procedures for water tapping points

All tapping points in the white zone must be marked on site and documented in the register. A diagram of the sections showing where the water tapping points are marked is also needed:

- Only drinking water may be present at these tapping points.
- No dead ends may exist.
- The tapping point in question must be used regularly, so that no stagnant water will be contained in these taps.
- Thermostatic taps may not be used – there must be separate taps for hot and cold water.

7.6.2 Procedures for water hoses

Water hoses must be made from Food Grade materials i.e. materials marked with the glass and fork symbol. This means that the materials meet the requirements for migration, i.e. they do not release chemical substances into the water in quantities, which could be harmful. However, Food Grade approval of the hose does not give any indication of hygienic properties from a microbiological perspective:

- Water hoses must be stored in a suspended position.
- No jets may be fitted onto the hoses unless it is absolutely necessary.
- Hoses may not be bent in order to create a more powerful jet. Bending damages the hose and shortens its in-use life.
- Hoses must be as short as possible.

- Transparent hoses should not be used, because such hoses enable algae to grow.
- Hoses should be replaced once a year, even if they are not damaged. Damaged hoses must be replaced immediately. The replacement of hoses must be documented.

7.6.3 Effect on water quality of hoses and jets

There are many different types of hose, varying from those in which high pressure and temperature can be used to simpler alternatives. The rate at which a hose ages, depends on the temperature and purpose for which it is used. For example, if a water hose is used for water at temperatures of 80ºC, it will last for 12 months. If the same type of hose is used for steam, it will last only for 1 month. The ageing of hoses can be monitored on the basis of the change in the number of microbes and particles in the water over time. If a hose has aged, the plasticizer leaks out and the hose becomes stiffer. This leakage accelerates with ageing. CIP cleaning speeds up the ageing process of the hose. Unfortunately, there is no general information on when hoses should be replaced and each situation must be monitored separately. PVC is a common material in hoses. The disadvantage of this material is that free vinyl groups are constantly released and the plasticizer is drawn out at an accelerating rate, which means that they age quickly. When hoses are folded and bent, migration of the plasticizer increases and the aging process accelerates at the point of bending. Hose jets may contain brass components.

7.6.4 Procedures for sampling cold water at tapping points

Water samples must be taken at least twice a year at all tapping points in the white zone. The sample must be taken after it has passed through the hose. The sample must be taken in the same way as the hose/water is used. The sampling process must also include a check to ensure that the water really does feel cold. The water must be analysed for coliforms (100 ml) and heterotrophs (1 ml). The results must be documented.

7.6.5 Follow-up procedures

The hose must be inspected if results in excess of the maximum limits are obtained. If the hose appears to be intact, only about 10 cm of the extreme end should be cut off. Otherwise, the entire hose must be replaced. Further sampling must be carried out after the hose has been shortened. Internal cleaning of the hose can be carried out using an injector, which is connected between the tap and the hose. External cleaning of the hose is easy to overlook but is important given the large surface areas that are covered. A dirty hose can contaminate both products and the surroundings. The easiest, if not the most efficient way, is to cover the entire suspended hose in foam.

7.6.6 Procedures for hot water

Hot water must be heated to at least 65°C in connection with production (heat exchanger). The temperature of the hot water at the tapping point should be 50–55°C in order to obtain a good cleaning effect. This does not pose scalding risks. Stagnant water leads to rapid microbial growth in the hot water pipes/hoses.

7.6.7 Procedures for sampling in connection with special requirements

On occasion, additional checks of the water may be necessary depending on the department and the problem being experienced with the product. The following can be used as guidance in these circumstances:

- Take a sample of the water that comes out of the hose first.
- Take a sample of the hot water.
- Analyse for yeast and mould.
- Take a sample from the cleaning point and foam sprayers.
- Product-related sampling is carried out in the event of a product fault where water is suspected to cause the problem. The analyses to be carried out should be selected on the basis of the type of problem being experienced with the product.

8. OPTIMIZATION OF CIP-CLEANING AND PURITY CONTROL OF CIP-WATERS – A CASE STUDY

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The main target in the optimisation of CIP-cleaning was the milk reception system and the yoghurt production line. The target included the tank cleaning system, which included big raw milk tanks (120 m^3), truck tanks, some pipelines and one production line for yoghurt. The cleaning procedure consists of pre-rinsing, alkaline cleaning, rinsing, acid cleaning and final rinsing. In tank truck cleaning only alkaline or acid cleaning is used. In the process at Herajoki dairy we use about 1.5% alkaline (NaOH) and approx. 1% acid (HNO_3), furthermore there is some chlorine added in the final rinsing water. In the purity control of CIP-water the adenosine triphosphate (ATP) method was tested to find out whether this rapid method is reliable in this application. The ATP results were compared to traditional culturing. The water samples in this part of the study were taken after cleaning the tank trucks and from the pre-rinse and final rinse tanks as well as from the secondary water tank and some taps.

8.1 CIP-CLEANING

CIP cleaning was monitored using a measuring device, which was equipped with a conductivity meter, a flow meter and a thermometer. The device was installed in the pipeline just before the point where the liquids are returned to the flow or the drain. This device was also used in tracking the pumps and the valves in the system. All the information was saved automatically to the computer system and all the changes in the cleaning protocol were saved and checked a few times with this device.

8.2 OPTIMISATION OF CIP-CLEANING

Seven (7) CIP-cleaning lines, 6 for milk reception and 1 in the yoghurt production line, were studied with the device mentioned above. There were 35 different concepts for cleaning these lines. The optimisation started in early June

2002. The first targets were the tank trucks for which the cleaning procedure is quite simple. The cleaning steps performed are pre-rinsing with water, alkaline cleaning and final rinsing. Twice a week the alkaline step is replaced by acidic cleaning. The device collected information from one CIP-cleaning line for approx. 3 days. After the information had been collected, the cleaning procedures were changed to make the cleaning procedure more efficient. The most common types of changes made in the procedures involved the pump, for example, when pumping to empty the tank, even if the tank is already empty. Other mistakes in the procedures were also found e.g. in places where liquids flow back to the wrong tank. A very important device in CIP-cleaning is the conductivity meter, which controls where liquids are going. If the conductivity meter is in bad condition, chemicals are wasted, which loads the wastewater at the same time. The condition of the conductivity meters should therefore be checked at least once a year.

In the milk reception system problems have sometimes occurred with the alkaline cleaning agent, because a gel has sometimes formed. This was a result of the alkaline agent reacting with the milk to form a gel. This most probably happens when small amounts of milk react with the alkaline agent returning to the tank. All the cleaning procedures were changed so that the alkaline solutions are directed to the drain for the first 30 s. After these 30 s the solution is flows back to the alkaline tank.

8.3 ATP METHOD TESTING IN PURITY CONTROL OF CIP-WATERS

The water used for the final rinsing in tank truck cleaning, pre-rinsing of tanks, tap water and secondary water was also examined. The ATP method was used to assess the purity of the water samples collected (Figure 8.1). The method is based on measuring all ATP in living material e.g. in microbial, plant and animal cells. The luciferin reagent reacts with the ATP, releasing light and adenosine diphosphate (ADP). The purity of one hundred (100) samples assessed using traditional culturing and ATP tests, which is based on testing using cuvettes and two kinds of snapshots in which the principle of the testing methods is the same. The testing was performed in late June 2002. The analyses were comparable. These snapshot tests are designed for hygiene control of surfaces. One test device was light and small and only allowed for snapshots, while the other was larger and allowed for both snapshot and cuvette measurements. The snapshot

tests were very simple and rapid. These sticks include all reagents. The cuvette test, which is more sensitive, is not as rapid and easy, because pipetting of water samples and reagents must be carried out.

Figure 8.1. Sampling process water from the funnel at Herajoki milk dairy.

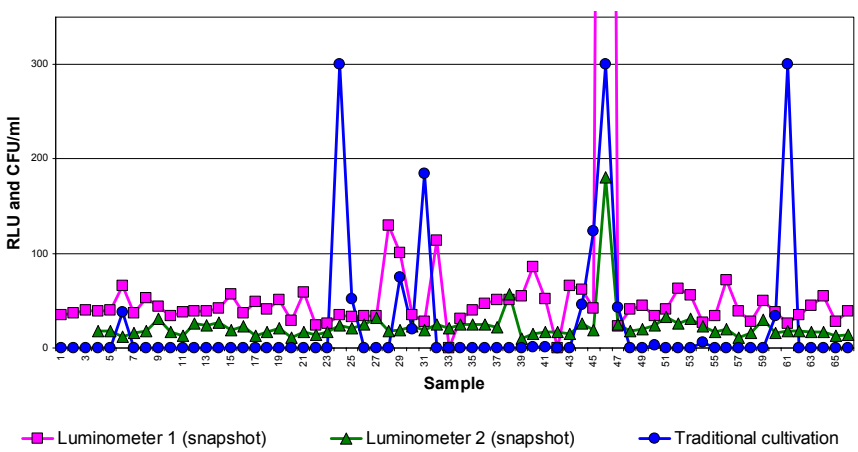

Figure 8.2. ATP and total count results from CIP water samples at Herajoki dairy.

9. APPLICABILITY OF AN ULTRASONIC WASHING SYSTEM IN THE CLEANING OF RETURNABLE PLASTIC CRATES

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Cleaning by ultrasonics has been known and used for a long time commercially in certain specific areas where the level of cleanliness is high, e.g. electronics and optics manufacturing, aviation and maintenance. The first ultrasonic cheese mould washing system was introduced in 1986. Goods to be cleaned are immersed in the liquid and subjected to a high ultrasonic intensity developed by ultrasonic transducers. Small, underpressurized bubbles are generated by cavitation. Those bubbles collide with material surfaces and finally collapse with pressure shocks of up to 1,000 bar. This is the mechanical energy, which is removes the dirt. As long as the goods to be cleaned are submerged, ultrasonics is effective at every point of liquid penetration (Kivelä, 1996). The cleaning result depends on the amplitude and frequency of the ultrasound as well as the depth and temperature of the washing solution, cleaning time and detergent and the target material (Heino, 2000).

9.1 HYGIENE OF RETURNABLE TRANSPORTATION PACKAGES

The hygiene of equipment surfaces plays an important role in dairy manufacture. In particular, the environment in which products are packed can easily become contaminated. Returnable transportation packages or crates can be one source of contamination. The cleaning of these packages is often incomplete, and pathogens and spoilage organisms attached to surfaces can easily survive the washing process. Returnable transportation packages like plastic trays, wooden/plastic pallets, plastic containers, crates, cages etc. can, therefore, be a hygiene risk in the food industry. They are often difficult to clean because of their construction. Lange *et al.* (1999) found that plastic trays with a simple construction and smooth surface were the easiest to clean. Trays with rugged and cracked surfaces were hard to control for cleanliness and returnability after cleaning (Parkes, 1988). There are some limitations in the cleaning procedure

(e.g. temperature, detergents and pressure) because of materials (Kane *et al.*, 2001a, 2001b). Returnable transportation packages can also be hard-soiled or contaminated by pathogens, particularly if they are misused.

The aim of this study was first to make a study of the literature about traditional and new cleaning methods for returnable transportation packages e.g. plastic crates and trays used in the supply chain of fresh milk products. The next step was to make pilot studies of the ultrasonic cleaning of artificial soiled and contaminated plastic crates. And the third aim was to find out the hygiene status of different cleaning processes – the washing tunnel used at Herajoki dairy and ultrasonic cleaning (Arpiainen, 2002).

9.2 CLEANING EXPERIMENTS WITH PLASTIC CRATES

9.2.1 Industrial-scale Tests

The hygiene quality of plastic crates (materials: HDPE high-density polyethene; blue; external dimensions 430 mm x 340 mm x 280 mm) was monitored at Herajoki dairy. The washing tunnel with two lines (Oy Hackman Ab, Koltek, Finland) was installed in the early 1970s. Altogether, 140 crates were investigated visually and by the Hygicult® method (Orion Corporation Orion Diagnostica, Finland) and swabbing before and after the washing tunnel. New, unused crates were also washed to find out if they had been contaminated by the washing liquid in the washing tunnel. 70 returned and unwashed crates were analysed for *Listeria* using the traditional method (7473AYF) and Vidas analyser at Valio's microbiological laboratory.

The washing liquid (1% concentration, pH 13, alkali-based combined detergent obtained from Orion Corporation Noiro Farmos Tecnochemicals) used in the washing tunnel was monitored for soiling by means of the COD method (Chemical Oxygen Demand; ready-to-use cuvette by LCK 514, DrLange, Düsseldorf, Germany) and for microbiological quality with Drycult® (Orion Corporation Orion Diagnostica, Finland) and Petrifilm™ as well as by spread plating. The temperature of the washing liquid and total cleaning time of a crate were registered in three different cleaning periods. The cleaning procedure of the washing tunnel consists of pre-rinsing, cleaning and post-rinsing sections.

9.2.2 Pilot Scale Tests

In the pilot cleaning tests, the wall section of some crates was cut into pieces (10 cm x 12 cm). The pieces were contaminated with NordFood soil (Wirtanen *et al.*, 1997) in combination with either *A. niger* (VTT D-81078) + *E. coli* (VTT E-97836) bacteria or *B. cereus* spores (VTT E-96727) + *L. innocua* (VTT E-98101) bacteria. The contaminated pieces were washed in the ultrasonic cleaner (m40, FinnSonic Ltd., Finland) with an ultrasonic frequency of 30 kHz and ultrasonic power of 600 W. Cleaning agents and concentrations and cleaning programmes were variable parameters in the tests (Figure 9.1).

The surviving bacteria combination was enumerated by selective agars. The ATP method (HY-LITE®) was also used. Some whole, NordFood soiled crates were also washed in a bigger ultrasonic cleaner (volume 300 litres with two ultrasonic elements, FinnSonic Ltd., Finland).

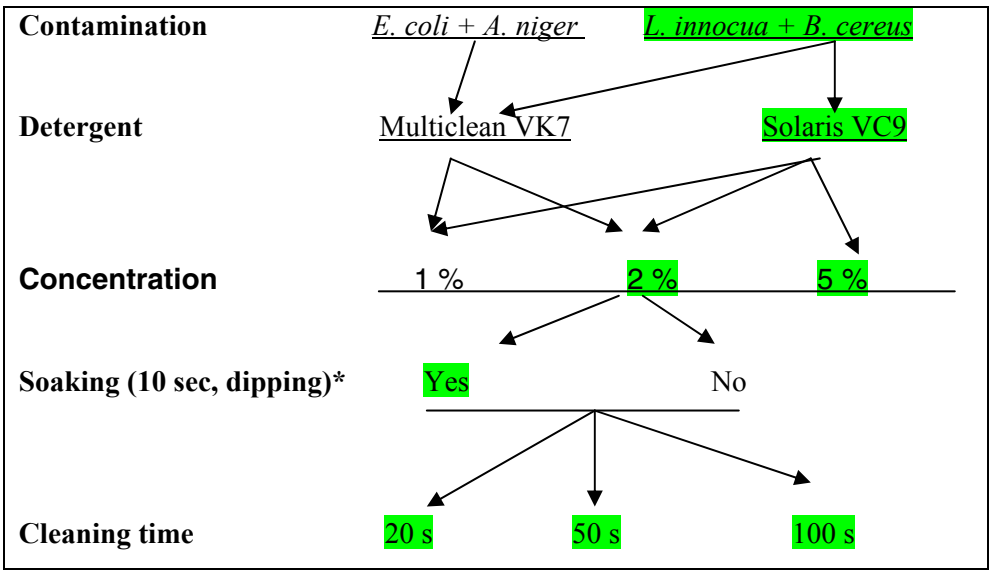

*Figure 9.1. Cleaning procedures in pilot ultrasonic cleaning tests of crate pieces. * Soaking with either 2% Multiclean VK7 (< 5% anionic tensides, pH 12.5) or 5% Solaris VC9 (15–30% NaOH, < 5% EDTA, pH 12.5) depending on the concentration of the detergent (Suomen Unilever Oy DiveserseyLever, Finland). Grey-coloured parameters were chosen in the cleaning of whole crates.*

Design of Experiment (DOE, Taguchi method) was used to find the best cleaning procedure. Whole crates were contaminated, washed and controlled as previously mentioned. The experiment consisted of 11 factors at 2 levels; the total number of experiments was 12 with 3 trials per experiment (Table 9.1). After the calculation of the experiment results (averages, analysis of variation and S/N ratio) the main effects analysis (S/N response tables) can be done and the best cleaning procedure can be confirmed using confirmation tests.

Table 9.1. Description of experiment. Standard Orthogonal Array Model used: L12 (2^{11}).

Label	Description of factor	Level 1	Level 2
A	Soaking, detergent	Detergent (5%)	SU989 Backdisk (1%)
B	Soaking, time	10 s	30 s
C	Soaking, temperature	10°C	20°C
D	Standing time before cleaning	10 s	30 s
E	Cleaning, temperature	45°C	60°C
F	Cleaning, detergent	Multiclean	Solaris
G	Cleaning, concentration	2%	5%
H	Cleaning, time	50 s	100 s
I	After rinsing, agent	Water	A8 Sumabrite Zerospots
J	After rinsing, temperature	10°C	20°C
K	After rinsing, time	10 s	30 s

9.3 Observations on the Cleaning of Returnable Transportation Packages

The study of the lliterature gave only some hits for new cleaning. It does not appear to be of interest to evaluate cleaning degree of returnable transportation packages. The hygiene status of the washing tunnel at Herajoki dairy was as follows:

- The temperature of the washing liquid ranged from 32°C to 60°C, being lowest when milk packaging was most hectic.
- The total cleaning time of a crate ranged from 40 s to over 7 min, being 80 s on average.

- Total bacteria counts of the washing liquid ranged from 100 CFU/ml to 300 CFU/ml, there was no difference between the detection methods.
- New, unused crates were contaminated during washing in the tunnel.
- Returned unwashed crates were visually evaluated into three categories of dirtiness: soiled by milk, soiled by dust and ground and visually clean; the crates soiled by dust and ground had the lowest cleaning degree 30.5%, being 60% on average.
- The external walls of crates were cleaned better than the internal walls; the bottoms were the dirtiest parts with the worst cleaning result; yeast and moulds were easily washed out.
- *Listeria monocytogenes* was found in one heavy soiled crate and *Listeria innocua* in three crates.

These results focus attention on the need for continuous control of the automatic refreshing and concentration of the washing liquid. Cleaning parameters should be constant. Investment in a new cleaning system for returnable crates should take these results into serious consideration. There is a real risk of getting *Listeria* inside a dairy plant with the returnable transportation packages. The level of cleanliness depends on the use of the returnable transportation packages – if they are in direct contact with the product or if they carry packed products (Colditz & Sowa, 1997). According to the zoning of dairy production, the packaging process of milk products demands the strictest hygiene requirements. Returnable transportation packages like plastic trays, crates and cages should be stored and completed in the green zone area with less strict hygiene requirements (Heggum *et al.*, 1997). This means minor requirements for their cleanliness but they should at least be visually clean.

In the pilot tests the vegetative bacteria cells were easily detached by the ultrasonic cleaning method, but *Bacillus* spores were still left on the washed crate surfaces. ATP measures correlated well with the count of bacterial cells. The cleaning effect of Solaris VC9, a NaOH and EDTA-based detergent, used as a 2% or 5% concentration with a 100 s cleaning time and without any pre-dissolving was better than the cleaning effect of Multiclean VK7 used as a 1% or 2% concentration. According to DOE, a washing temperature of 45°C was better than 60°C in the ultrasonic washing system.

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10. COMPARISON OF CULTURING METHODS FOR MONITORING MICROBIAL LOADS ON PLASTIC CRATES

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The hygiene of equipment surfaces and the packaging environment is an important part of overall dairy packaging hygiene. Returnable transportation packages or crates may be one source of contamination. All dairy processes must be planned and implemented in a way that does not jeopardise hygiene of the products. The purpose of the study was to verify the suitability of three cultivation methods, DryCult®, Petrifilm™ and conventional culturing for monitoring the microbial load of a cleaning liquid used for cleaning of returnable plastic crates.

10.1 HYGIENE MONITORING OF THE CLEANING SOLUTIONS IN A PLASTIC CRATE LINE

The test series was carried out at Herajoki Dairy on three consecutive nights. The test methods used were DryCult® TPC (Orion Diagnostica Oy, Espoo, Finland), Petrifilm™ for total plate count (3M, St. Paul, MN, USA) and conventional culturing using Trypticase Soy agar (Becton Dickinson, Cockeysville, USA). All the tests were performed in accordance with valid written instruction manuals. The test volume for each test was 1 ml. Samples were taken at 30 min intervals for a total of 13 h each day. Altogether 81 samples were collected for each of the three methods. All samples were analysed separately. The three culturing methods were compared using paired t-tests with the entire material from the 81 samples tested separately by each method. In this paired t-test, the statistical significance of the average colony count for each of the different culturing methods was compared with the others.

10.2 COMPARISON OF THE CULTURING METHODS IN-LINE

Figure 10.1 shows a summary of the microbial counts of the cleaning solutions monitored using the three methods for three consecutive days.

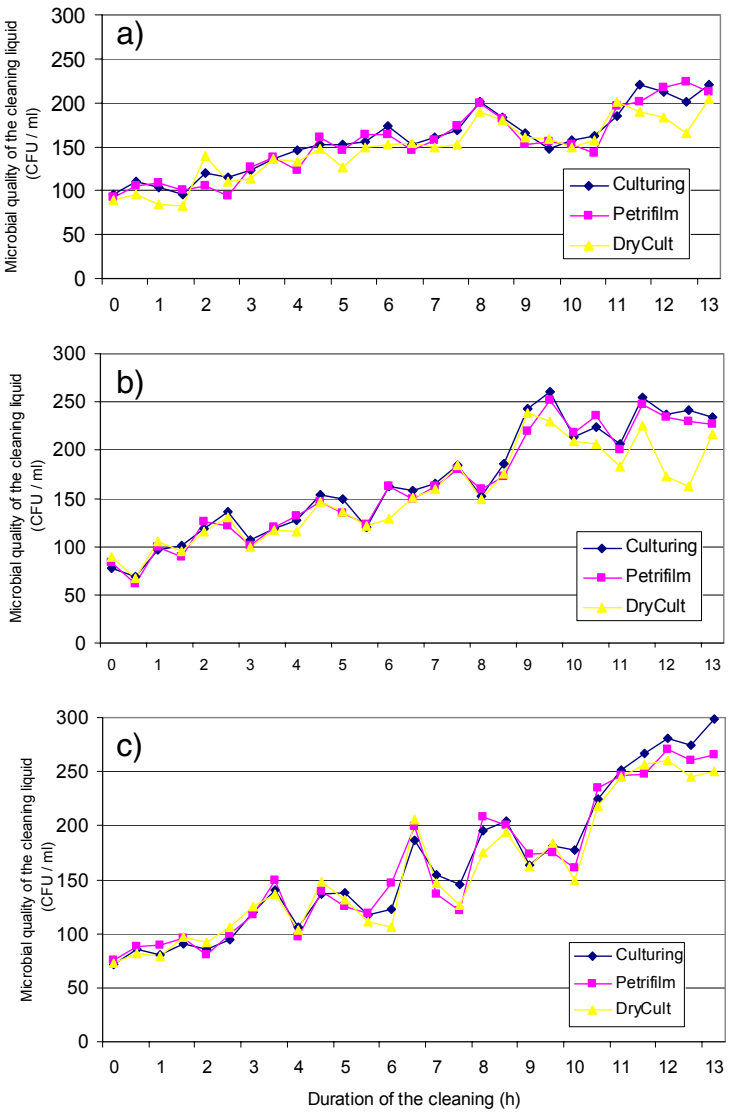

Figure 10.1. Microbial counts for the cleaning solutions monitored using DryCult® TPC, Petrifilm™ aerobic count and conventional culturing on Trypticase Soy agar for three consecutive days: a) 1st day, b) 2nd day and c) 3rd day.

The comparison of conventional culturing with Petrifilm™ was carried out using paired t-test samples. As a conclusion from this comparison, the 0-hypothesis was not valid because the t-statistic value clearly fell between the confidentiality limit (CL) 95%. The CL and the two-tailed p-value were clearly above 0.001. Statistically the average colony count values did not differ from each other significantly. Table 10.1 shows the results from the paired sample t-test for the comparison of conventional culturing and Petrifilm™.

Table 10.1. The results from the paired sample t-test for the comparison of conventional culturing and Petrifilm™.

CFU/ml	n	Mean	SD	SE
Conventional culturing	81	163.07	57.66	6.41
Petrifilm™	81	158.60	52.95	5.88
Difference	81	4.47	16.13	1.79
Difference between means	4.47			
95% CL	0.90 to 8.04			
t-statistic	2.49			
2-tailed p	0,0147			

The comparison of Petrifilm™ with DryCult® TPC was also carried out using the paired sample t-test. As a conclusion from this comparison, the 0-hypothesis was not valid and the average colony counts did not significantly differ from each other statistically. Table 10.2 shows the results from the paired sample t-test for the comparison of Petrifilm™ and DryCult® TPC.

Table 10.2. The results from the paired sample t-test for the comparison of Petrifilm™ and DryCult® TPC.

CFU/ml	n	Mean	SD	SE
Petrifilm™	81	158.60	52.94	5.88
DryCult® TPC	81	151.86	47.86	5.32
Difference	81	6.74	17.15	1.91
Difference between means	6.74			
95% CL	2.95 to 10.53			
t-statistic	3.54			
2-tailed p	0.0007			

The comparison of conventional culturing with DryCult® TPC was carried out using the same test. As a conclusion from this comparison, the 0-hypothesis was valid. Statistically, the average colony counts differed significantly from each other. The two-tailed p-value was <0.0001 and the t-statistic value of 4.26 fell outside 95% CL. Table 10.3 shows the results from the paired sample t-test for the comparison of conventional culturing and DryCult® TPC.

Table 10.3. The results from the paired sample t-test for the comparison of conventional culturing and DryCult® TPC.

CFU/ml	n	Mean	SD	SE
Conventional culturing	81	163.07	57.66	6.41
DryCult® TPC	81	151.86	47.86	5.32
Difference	81	11.21	23.68	2.63
Difference between means	11.21			
95% CL	5.97 to 16.45			
t-statistic	4.26			
2-tailed p	<0.0001			

10.3 Sensitivity and Performance of the Culturing Methods

The sensitivity between the three methods compared varied a little, but considering the level of bioburden in the cleaning liquid samples one can conclude that all three methods are equally well suited for this type of monitoring. The high sensitivity of DryCult TPC made its growth curve bend a little earlier than that of the other two methods. Monitoring processes with colony counts of 80–300 CFU/ml demonstrates that minor differences in sensitivity, such as those obtained in this study, do not play a major role in the outcome. The main properties to focus on, when choosing a monitoring method are ease of use and costs. If the tests have to be carried out on site, DryCult® TPC is the most practical choice.

11. SOURCE AND CONTAMINATION ROUTES OF *LISTERIA MONOCYTOGENES* IN THE DAIRY INDUSTRY

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Listeria monocytogenes is an invasive foodborne pathogen that can cause serious disease in immuno-compromised individuals and pregnant women (Farber & Peterkin, 1991). It is common in many natural and manmade environments and can grow at refrigerated temperatures (Fenlon, 1999; Lou & Yousef, 1999). Although *L. monocytogenes* rarely causes diseases in the majority of the population, there is a zero-tolerance ruling issued by the U.S. Food and Drug Administration (1999) in ready-to-eat foods (including dairy products) because of high mortality rates. High prevalence of *L. monocytogenes* in food processing environments and in ready-to-eat food (Arimi *et al.,* 1997; Autio *et al.,* 1999) that has undergone pasteurisation, indicates that the process environment represents a significant source of *L. monocytogenes* in the finished products. The ability of *L. monocytogenes* to adhere to various food contact surfaces (Blackman & Frank, 1996; Hood & Zottola, 1997; Smoot & Pierson, 1998) and unusual growth and survival properties of the bacterium have supported these findings (Lou & Yousef, 1999). Other investigations, however, suggest that raw food material is the most likely source for *L. monocytogenes* (Eklund *et al.*, 1995; Giovannacci *et al.*, 1999).

11.1 *LISTERIA MONOCYTOGENES* IN DAIRY PROCESSING

More recently, there has been an increase in listeriosis outbreak linked to the consumption of dairy products. Dairy products contaminated with *Listeria* include pasteurised milk (Fleming *et al.*, 1985), soft cheese (Fleming *et al.*, 1985; Loncarevic *et al.*, 1995), soft and hard cheese from raw milk (Goulet *et*

al., 1995; Dalton *et al.*, 1997), ice cream (Miettinen *et al.*, 1999), pasteurised chocolate milk (Ryser, 1999) and butter (Lyytikäinen *et al.*, 1999). The occurrence of *L. monocytogenes* in raw milk can vary to a great degree. Studies carried out in Western Europe and the US have mostly shown that the occurrence of *Listeria* is between zero and five percent (Hird, 1987; Farber & Peterkin, 1991; Guerra *et al.*, 2001), although the figure is considerably higher in other areas, as recently reported in Mexico (Carlos *et al.,* 2001). Although there have been reports concerning the survival of *L. monocytogenes* during HTST pasteurisation (Doyle *et al.*, 1987; Fernández-Garayzábal *et al.,* 1987), it is generally accepted that pasteurisation temperatures kill the bacteria (Farber *et al.,* 1988). *Listeria monocytogenes* has, however, been shown to exist and be persistent in dairy factory environments (Charlton *et al.*, 1990; Frank *et al.*, 1990). This is a particularly important issue in dairies where many products are produced in open areas in a relatively small space, due to possible cross contamination risks.

The advent of molecular methodology has revolutionised the possibilities of investigating the ecology and pathogenicity of foodborne bacteria such as *L. monocytogenes*. These new methods include multilocus enzyme electrophoresis, Pulsed-Field Gel Electrophoresis (PFGE), random amplification of polymorphic DNA and ribotyping (Jacquet *et al.,* 1995; Destro *et al.,* 1996; Salamina *et al.,* 1996; Arimi *et al.,* 1997; Ralyea 1998; Autio *et al.,* 1999). Using this methodology it has been possible to investigate the contamination patterns in foods and in the food processing environment. Ribotyping is a highly automated and standardised method that has been used for molecular sub-typing and data analysis and was first introduced by DuPont Qualicon TM (Wilmington, DE, USA) in 1995. DuPont ribotyping system characterises and identifies bacterial strains by generation of ribosomal RNA fingerprints from bacteria (Bruce, 1996). The manufacturer has analysed more than 1300 *L. monocytogenes* strains to develop a database (Bruce *et al*., 1995).

Characterisation of *L. monocytogenes* isolates by PFGE has been widely used in epidemiological studies of foodborne outbreaks. (Jaquet *et al*., 1995; Ericsson *et al*., 1997; Autio *et al*., 1999; Miettinen *et al*., 1999). Typing by PFGE has demonstrated high discrimination, accuracy and reproducibility for *L. monocytogenes* (Brosch *et al*., 1994; Destro *et al*., 1996; Kerouanton *et al*., 1998; Autio *et al*., 1999). In no instances has listeria been found to be non-typeable by PFGE (Brosch *et al*., 1994). The disadvantages of this method are its laborious

and time-consuming nature, although a protocol has recently been described that enables typing in 30 h, which will make the PFGE method more competitive with other methods (Graves & Swaminathan, 2001). Using PFGE it has been shown that the same *L. monocytogenes* clone survived in a dairy for up to 7 years (Unnerstad *et al*., 1996).

The aim of this study was to examine the occurrence and contamination routes of *Listeria* throughout the production line (farm environment → dairy products) in a small dairy in Iceland. Isolates were subsequently genotyped using ribotyping and PFGE to allow for epidemiological analysis of possible contamination. These two typing methods were also compared with each other.

11.2 ISOLATION OF *LISTERIA* IN A DAIRY ENVIRONMENT

11.2.1 Dairy products

The products investigated for the presence of *Listeria* were: pasteurised milk with three different fat content (0.1%; 1.5%; 3.8%), hard cheeses (Gouda; two fat contents, 17% and 26%), cottage cheese, soft blue cheese, pasteurised juice (three types), cottage cheese, butter, skyr (made from whey), brown soft cheese, cream and sour milk. The products were tested for five successive weeks in summer and winter. The total number of product samples was 200. Each sample represented ca. 5 ml or 5 grams of five units of each product, which were added together to total exactly 25 ml or 25 grams. 225 ml of UVM broth was added to each sample and incubated for 24 h at 30ºC. From these enrichments, 0.1 ml was transferred to 10 ml of Fraser broth and incubated for 48 h at 35ºC. Inoculation loops were used to spread samples from each Fraser broth tube onto MOX agar plates, which were then incubated for 48 h at 35ºC. Suspected colonies (black/silver) were transferred onto Tryptic Soy Yeast Extract agar plates and incubated for 24 h at 35ºC before Gram-stained and catalase activity testing. Finally, after growing for 24 h at room temperature in Tryptic Soy Yeast Extract broth, the isolates were checked for mobility with the hanging drop method. To distinguish *L. monocytogenes* from other *Listeria* species, a MiniVidas (Model 12 BioMérieux Vitek, Inc. France) was used in accordance with the manufacturer's instructions.

Modified Listeria Enrichment Broth (UVM) and Fraser broth were used for the enrichment of *Listeria* bacteria. For further characterization, Modified Oxford agar (MOX) was used. For gram staining and catalase tests, the bacteria were grown overnight on Tryptic Soy Yeast Extract agar. For mobility tests, Tryptic Soy Yeast Extract broth was used. All media were obtained from Difco.

11.2.2 Dairy plant environment

The occurrence of *Listeria* in various dairy plant environments was investigated for five successive weeks in both winter and summer. Samples were collected from various locations in the dairy, including various drainage systems, floors, cartons, packaging material and process surfaces. The number of samples was 250. The samples were taken with sterile cotton swabs. Two hydrophobic and two hydrophilic cotton swabs were used for each sampling site. At dry places the cotton swabs were dipped into a phosphate buffer before swabbing. After swabbing, the swabs were placed in sterile 30 ml plastic bottles and transported to the laboratory. 25 ml of sterile UVM broth was added to every sample. Thereafter, the isolation procedure for *Listeria* was as described above.

11.2.3 Raw milk

The number of farms delivering milk to the dairy was 143, producing from 10.000 to 600.000 L of milk per year. Samples of raw milk were taken from all farms during a period of three weeks in summer and winter. Because of the large number of farms included in this study, 4–5 samples (farms) were mixed together. Portions of the original samples were kept intact at refrigerated temperatures to trace eventual positive samples. A sample of 25 ml was added to 225 ml of UVM broth and the subsequent isolation procedure was performed as described above.

11.2.4 On the farm

At one farm where raw milk was positive for *L. monocytogenes* on three successive sampling occasions, it was decided to collect milk from all the farm's milking cows and from the environment of the shed. Samples were aseptically taken from each udder of every milking cow and from the stable environment, including the silage, drinking water and floor. Samples were treated as described above.

11.3 IDENTIFICATION OF PROCESS ISOLATES

11.3.1 Ribotyping

Ribotyping was performed using the standard method of the automated ribotyping device RiboPrinter System (DuPont QualiconTM) according to the manufacturer's instruction (Bruce, 1996). Cells were grown on Tryptic Soy agar plates (Difco, Detroit, MI, USA) overnight at 30ºC. Colonies were picked, suspended in lysing buffer, and heat-inactivated at 90ºC (10 min) and placed in the RiboPrinter®. The sequence of steps in the analysis comprised the lysis of cells, digestion of DNA with *Eco*RI, separation of DNA fragments on agarose gel, transfer onto a nylon membrane and hybridization with a chemio-luminescent-labelled rRNA probe. The RiboPrinter System generates its own ribogroups in the instrument's database.

11.3.2 Pulsed-Field Gel Electrophoresis (PFGE)

The PFGE was performed as described by Maslow *et al.* (1993) with the following modification. The use of insert molds was substituted by syringes. In addition the plugs were lysed for 4 h, and washed once in ES buffer (0.5 M EDTA, 1% sodium lauroyl sarcosyl) containing 50 mg/ml Proteinase K, which was rotated overnight at 50°C. 5U *Asc*I and 10U *Apa*I (New England Biolabs, Beverly, MA) were used for restriction endonuclease digestion. The samples were electrophoresed through 0.9% (*Asc*I) and 1.0% (*Apa*I) (w/v) agarose (SeaKem GTG, FMC Bioproducts, Rockland, Maine) in 0.5 x Tris-borate EDTA (45 mM Tris, 4.5 mM boric acid and 1 mM sodium EDTA) at 210V and 200V respectively, at 14°C in a CHEF DRIII system (BioRad Laboratories, Richmond, CA). The pulse times for *Asc*I ramped from 1s to 28s for 10h and 28s to 30s for 10h and from 1s to 18s for 20h with *ApaI*. The gels were stained with ethidium bromide and photographed under UV transillumination using GelDoc 2000 documentation system (BioRad Laboratories). The photos were stored as TIFF files. Lamda ladder PFG marker (New England Biolabs) was used for fragment size determination.

PFGE pattern analysis: The analysis of gels was performed visually and using GelComparII (Applied Maths, Kortrijk, Belgium) to compare strains. A dendogram was created using the similarity between restriction patterns based on

the bands position expressed as Dice coefficient correlation, and clustered by the unweighted pair-group method with arithmetic averages (UPGMA). Any differences between two PFGE profiles were considered sufficient for distinguishing the profiles. The *Asc*I profiles were identified using arabic numericals starting with 1 and *Apa*I profiles were identified with capital letters starting with A.

11.3.3 Discrimination index for typing methods

The discrimination power of the typing methods was determined by calculating the discrimination index (DI) using the formula developed by Hunter and Gaston (1988).

$$DI = 1 - \frac{1}{N(N-1)} \sum_{J=1}^{s} n_j(n_j - 1)$$

where N is the total number of isolates in the same sample population,
s is the total number of types described, and
n_j is the number of isolates belonging to the type.

11.4 ISOLATION OF *LISTERIA*

No positive *Listeria* samples were observed in any dairy product or in any of the samples collected from the factory environment (Table 11.1). However, thirteen (13) samples were found positive for *Listeria* (all *L. monocytogenes*) in the raw milk samples that were collected during winter. Three farms were found to be positive on all three winter-sampling occasions and four farms were positive on one occasion. To be able to trace the isolates, it was decided to choose one positive farm and collect samples from all milking cows and the shed (environment and silage). One animal was found to be positive for *L. monocytogenes* and suffering from sub-clinical mastitis. Nine (9) samples of *Listeria* were detected in the stable environment, eight (8) of which were identified as *L. monocytogenes* and one as *L. seeligeri* (Table 11.1).

All *Listeria* isolates were identified immunologically by MiniVidas and genotyped using RiboPrinter (chapter 11.5) and PFGE (chapter 11.6). The DI was 0.656 for ribotyping and 0.695 for PFGE.

Table 11.1. The total number of samples taken for the isolation of Listeria monocytogenes at one farm (shed environment, silage and raw milk), in raw milk from all farms, in the dairy process environment and in various dairy products. Number of positive Listeria spp. and L. monocytogenes are also shown.

Sampling site	**Number of samples**	**Number of positive *Listeria* samples**	**Number of positive *L. monocytogenes***
Dairy products	200	0	0
Dairy environment	250	0	0
Raw milk (cows)	153*	13	13
Farm I: Raw milk from cows	30	2	2**
Farm I: Drinking place	13	5	5
Farm I: Silage samples	7	3	2
Farm I: Floor samples	5	1	1
Total	**658**	**24**	**23**

* samples were taken six times (three times in summer and three times in winter)

** sample taken twice from the same animal

11.5 IDENTIFICATION OF *LISTERIA* STRAINS USING RIBOTYPING

Based on the RiboPrinter System software and visual assessment of the data, three distinct ribogroups were observed from *L. monocytogenes* strains (Figure 11.1). Each ribotype pattern generated between five to seven fragments. The sizes of the most typical fragments were: two between 2.0 and 2.5 kbp (kilobase pairs used in the system), one at 4.0 kbp, two bands between 5.0 and 6.0 kbp and one between 9.0 to 9.5 kbp. The distinction between ribogroup I and the other ribogroups is an extra band located at 12.0–13.0 kbp in ribogroup I, whereas in ribogroup II, extra bands are located at 3.6 and ca. 35 kbp and in ribogroup III extra bands are located at 3.2 kbp and ca. 35 kbp. Finally, no bands are located between 6.0 and 7.0 kbp in ribogroup III as is the case with the other ribogroups.

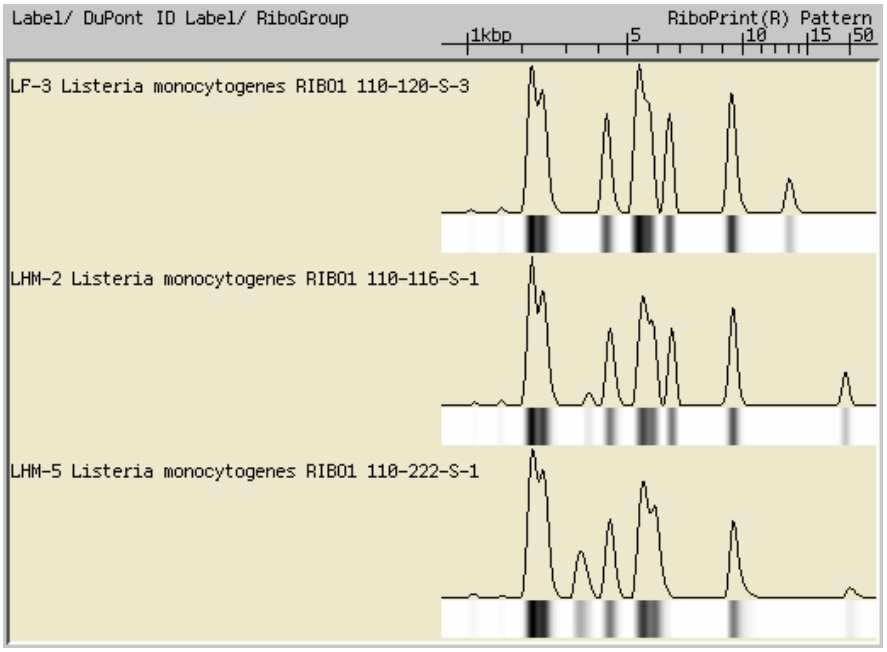

Figure 11.1. RiboPrint patterns of three ribotypes of Listeria monocytogenes. The patterns are composites of individual patterns, the number varying from 1 to 15. From top: Ribogroup I, Ribogroup II and Ribogroup III.

The isolates from raw milk from seven different farms (13 isolates) were grouped into three ribogroups (Figure 11.1; Table 11.2). In ribogroup I, isolates represented farms 1, 3 and 7 (five isolates). In ribogroup II, isolates represented farms 2, 4 and 6 (seven isolates) and in ribogroup III, one isolate represented farm 5. All isolates from the environment and from one milking cow (10 isolates) from farm 3 belonged to the same ribogroup as the isolates from raw milk from the same farm (ribogroup I). The dendogram pattern generated from the ribotype data also shows the three ribogroups generated (Figure 11.2).

Most of the isolates in ribogroup I (12 out of 15) were identical with DUP-1030 database isolates. The other three were identical with DUP-1023 isolates. All seven isolates in ribogroup II were identical with DUP-1039 database isolates and the isolate from ribogroup III was identical with DUP-1045 database isolates (Table 11.2).

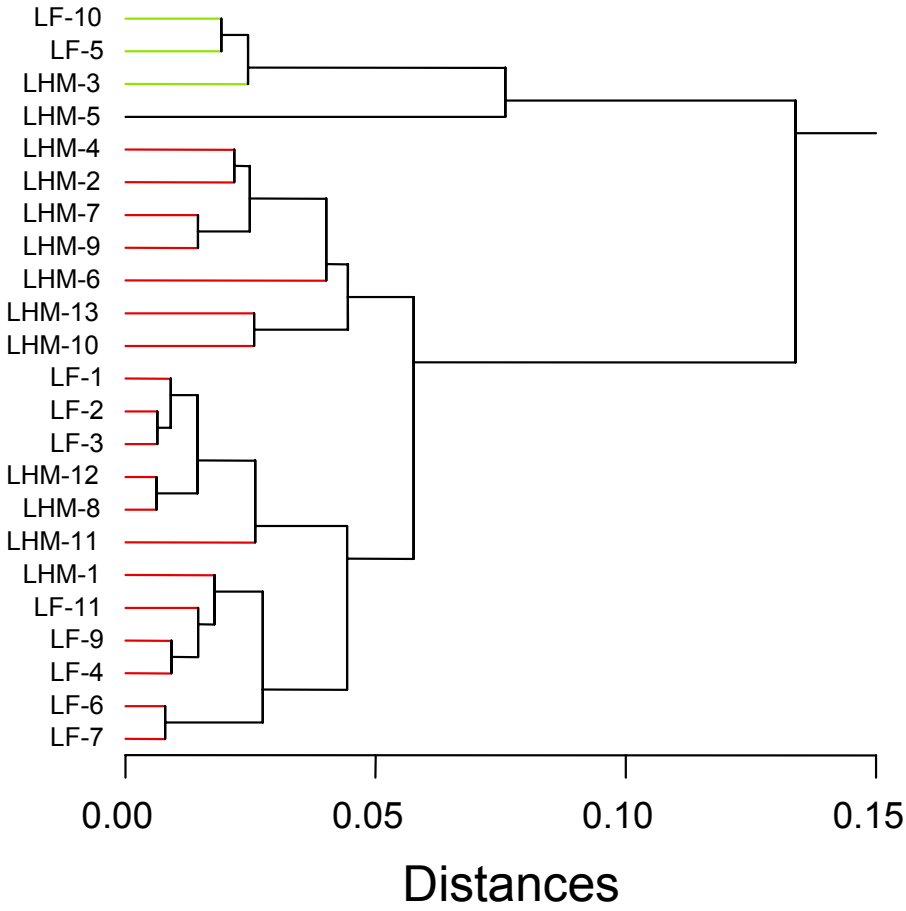

Figure 11.2. Dendogram of the generated individual ribotypes: all LF strains and LHM-1, 3, 8, 11 and 12 strains were grouped into ribogroup I. Strains in LHM-2, 4, 6, 7, 9 and 13 are grouped into ribogroup II and strains in LHM-5 were grouped into ribogroup III.

Table 11.2. Listeria monocytogenes ribotypes compared with DuPont database patterns and pulsotypes.

Strains	Isolated from	Similarity between patterns	Identical with DUP strains	Lineage*	Pulso-types
LHM-1	Raw milk (Farm 1)	0.92	DUP 1030	II	1A
LHM-2	Raw milk (Farm 2)	0.94	DUP 1039	II	2B
LHM-7	Raw milk (Farm 2)	0.90	DUP 1039	II	2B
LHM-10	Raw milk (Farm 2)	0.92	DUP 1039	II	2B
LHM-3	Raw milk (Farm 3)	0.91	DUP 1023	II	1A
LHM-8	Raw milk (Farm 3)	0.93	DUP 1030	II	4D
LHM-12	Raw milk (Farm 3)	0.93	DUP 1030	II	4D
LHM-4	Raw milk (Farm 4)	0.94	DUP 1039	II	3C
LHM-9	Raw milk (Farm 4)	0.94	DUP 1039	II	3C
LHM-13	Raw milk (Farm 4)	0.92	DUP 1039	II	3C
LHM-5	Raw milk (Farm 5)	0.96	DUP 1045	II	2E
LHM-6	Raw milk (Farm 6)	0.94	DUP 1039	II	3C
LHM-11	Raw milk (Farm 7)	0.95	DUP 1030	II	5D
LF-1	Farm 3: Environment swab	0.95	DUP 1030	II	4D
LF-2	Farm 3: Environment swab	0.94	DUP 1030	II	4D
LF-3	Farm 3: Environment swab	0.95	DUP 1030	II	4D
LF-4	Farm 3: Environment swab	0.94	DUP 1030	II	4D
LF-5	Farm 3: Environment swab	0.90	DUP 1023	II	4D
LF-6	Farm 3: Silage	0.92	DUP 1030	II	4D
LF-7	Farm 3: Silage	0.89	DUP 1030	II	4D
LF-8	Farm 3: Silage	nd	DUP 1065**	nd	
LF-9	Farm 3: Raw milk (container)	0.93	DUP 1030	II	4D
LF-10	Farm 3: Environment swab	0.90	DUP 1023	II	4D
LF-11	Farm 3: Raw milk, cow	0.95	DUP 1030	II	4D

* From Wiedmann *et al*. (1997).

** *Listeria seligeeri*

nd: not detected

11.6 IDENTIFICATION OF *LISTERIA* STRAINS USING PFGE

The PFGE of *L. monocytogenes* DNA digested with *AscI* showed 3-7 fragments ranging from approximately 50 kbp to 533 kbp in size, while 11–18 fragments of approximately 44 to 340 kbp were obtained following digestion using *ApaI*. PFGE typing divided the isolates into 6 pulsotypes using the two restriction enzymes (Table 11.2). Pulsotype 4D dominated containing 12 isolates from farm 3 (environmental swabs, silage, raw milk, raw milk container and cow). Only one isolate from farm 3 belonged to another pulsotype (1A), one other isolate from farm 1 belonged to that group. *AscI* type 2 was further divided into two *ApaI* types, pulsotypes, pulsotype 2B, which contained isolates from farm 2 and 2E, which contained isolates from farm 5, Pulsotype 3C contained 3 isolates from farms 4 and 6. Pulsotype 5D differed from 4D only by one band in the *AscI* profile. The dendogram was generated with *AscI* restriction enzyme (Figure 11.3).

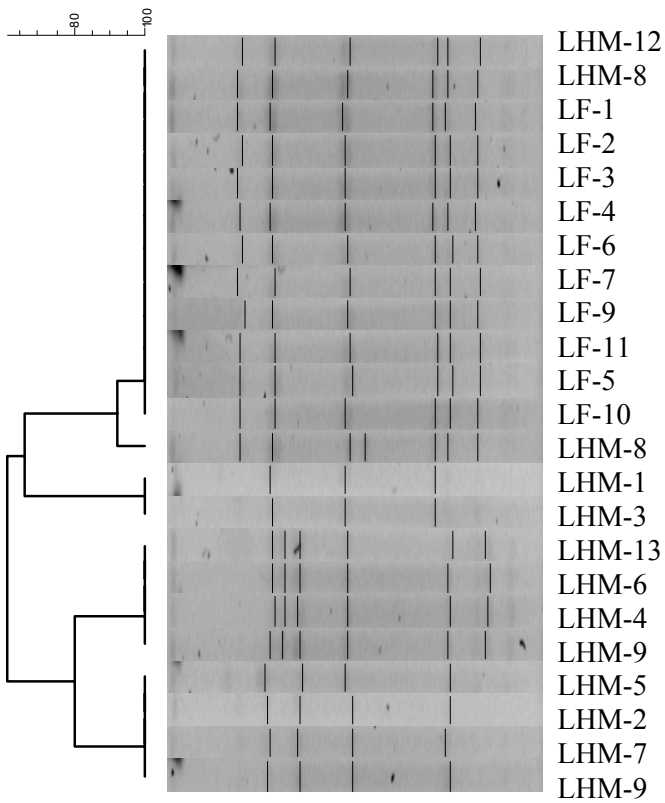

Figure 11.3. Dendogram of the AscI pulsotypes.

11.7 TRACEABILITY OF LISTERIA IN THE DAIRY PRODUCT CHAIN

This study is part of a project carried out in the Nordic countries that focuses on hygiene in the dairy industry. In the investigation on Iceland the research concentrated on presence and contamination routes of *Listeria* in the dairy industry.

L. monocytogenes can be transmitted through the consumption of raw milk and raw milk products or through the consumption of contaminated dairy products (Goulet *et al.,* 1995; Loncarevic *et al.,* 1995; Lyytikäinen *et al.,* 1999; Miettinen *et al.,* 1999). In the food industry, it is not clear whether ready-to-eat products are contaminated by *Listeria* through the process environment (Arimi *et al.,* 1997; Autio *et al.,* 1999) or through the raw food material (Eklund *et al.,* 1995; Giovannacci *et al.,* 1999). The situation in the dairy industry probably differs slightly from that in other food sectors as in most cases, the milk is pasteurised. Thus it is reasonable to conclude that dairy contamination products aquire the pathogen somewhere along the process line.

The main aim of this study was to establish the occurrence and distribution of *L. monocytogenes* throughout the distribution line, from the farm environment to the final products. The survey observed no cases of positive *Listeria* samples in the 450 samples taken from the factory environment, process equipment or from any of the selected dairy products (Table 11.1). This was rather surprising since other reports have shown that *L. monocytogenes* is relatively common in the food industry, both in the process environment and in products (Arimi *et al.,* 1997; Norton *et al.,* 2001; Suihko *et al.,* 2002). However, recent investigation has shown positive occurrence of *L. monocytogenes* in drains at this same dairy (results not shown). Over 70% of the raw milk from the dairy investigated in this research is used for cheese production, and it is indeed mostly in cheeses that *L. monocytogenes* has been reported (Fleming *et al.,* 1985; Goulet *et al.,* 1995; Loncarevic *et al.,* 1995; Miettinen *et al.,* 1999).

However, *L. monocytogenes* was detected in 13 samples of raw milk (1.5%; Table 11.1), which is in keeoing with previously reported from Western Europe and the US (Hird, 1987; Farber & Peterkin, 1991; Guerra *et al.,* 2001). In order to trace *L. monocytogenes* isolates from the raw milk, samples were collected from the raw milk of all the milking cows and from the shed environment at a farm that was found positive on all three winter-sampling

occasions. Molecular typing is a useful tool for identifying and tracking different strains, and it defines possible relationships among isolates (Swaminathan *et al.,* 1996; Wiedmann *et al.,* 1996). In this work, all isolates were typed using two molecular methods, namely Ribotyping and PFGE. In this study the ribotyping resulted in four different ribogroups and 6 pulsotypes from the 23 isolates. All of the generated ribotypes were identical with *L. monocytogenes* patterns available with the exception of one ribotype, *L. seligeeri* (Table 11.2). Wiedmann *et al.,* (1997) classified *L. monocytogenes* into three genetic lineages based on ribotyping and allelic polymorphism in the virulence genes *hly*A, *inl*A and *act*A. Lineage I consisted of isolates from humans, animals and foods, lineage II primarily from foods and animals and lineage III from animals (Wiedmann *et al.,* 1997). All *L. monocytogenes* isolated in this investigation belonged to lineage II (Table 11.2) and were probably contaminated through silage, as has been reported by others (Low *et al.,* 1992; Wiedmann *et al.,* 1994, 1996). The four different DUP isolates (1023, 1030, 1039 and 1045) also seem to be very common in the food industry, as reported in various investigations (Norton *et al.,* 2001; Suihko *et al.,* 2002). The relationship between different isolates is shown in Figure 11.2 with a dendogram. The three ribogroups are clearly distinct from each other with the exception of three isolates belonging to Ribogroup I (LF-5, LF-10 and LHM-3, all DUP 1023 isolates) (Table 11.2, Figure 11.2). These isolates seem to be genetically closer to LHH-5, which belongs to another ribogroup. This was probably caused by insufficient cell material used for the isolates. This can be seen by running these three isolates separately. The peak, at 12–13 kb, was significantly lower as compared with the 1030 isolates (results not shown), thus allowing the programme software to group them together further from the DUP 1030 isolates despite being grouped into the same ribogroup.

It has been shown that *L. monocytogenes* is able to establish itself and persist for a long period of time (Unnerstad *et al.,* 1996; Miettinen *et al.,* 1999; Vogel *et al.,* 2001), which suggests that an in-house flora becomes established. This can be seen on farm 3, where one predominant genotype exists. In some cases, isolates from different farms belonged to the same genotype. All of the farms are geographically far apart. This study revealed that as in previous reports PFGE had better DI than ribotyping (Louie *et al.,* 1996; Kerouanton *et al.,* 1998; Aarnisalo *et al.,* 2003).

Surprisingly, no *L. monocytogenes* strains were isolated from the dairy factory environment, but it would be interesting to compare presumable *L. monocytogenes* isolates that are present in dairy environment with the isolates this project. Thus, further efforts will be made to isolate *Listeria* from the dairy factory environment. It is also worth noting that there appear to be a seasonal variation in the occurrence of *L. monocytogenes* in raw milk. The most reasonable explanation is the higher risk of contamination from the shed environment and contaminated silage during wintertime caused by long periods when the animals are kept in close proximity with each other. Other reports have indicated a higher prevalence of *L. monocytogenes* in the spring (Hassan *et al.,* 2001; Yoshida *et al.,* 1998).

12. EFFICACY OF DISINFECTION IN FOOD PROCESSING

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The general aims for microbial control including biofilm removal are to prevent the spoilage of products and to ensure that the quality specifications of the product are met. The most important means for maintaining efficient microbial control include: 1) minimizing the microbial load from outside sources to the process, 2) efficient control of growth at microbiologically vulnerable sites and 3) adequate cleaning and disinfection of the process lines (Wirtanen *et al.,* 2000). Physical, chemical and microbiological cleanliness is essential in food plants (Gould, 1994):

- ⇒ physical cleanliness means that there is no visible waste, foreign matter or slime on the equipment surfaces
- ⇒ chemically clean surfaces are surfaces from which undesirable chemical residues have been removed and
- ⇒ microbiologically clean surfaces imply freedom from all types of microbes.

Attached microbes or microbes in biofilms can be a problem in food processing, because they adhere to the surfaces, and if the cleaning is insufficient the remaining cells start to grow and contaminate the product (Hood & Zottola, 1995). The selection of detergents and disinfectants in the food industry depends on the efficacy, safety and rinsability of the agent and whether if it is corrosive or affects the sensory values of the products manufactured. An independent quality control system to monitor the cleaning results for a food plant can be integrated into the Hazard Analysis Critical Control Points (HACCP) program. The key to effective cleaning and disinfection of food plants is an understanding of the type and nature of the soil (sugar, fat, protein, mineral salts etc.) and the microbial growth to be removed from the surfaces (Czechowski & Banner, 1990; Troller, 1993).

12.1 CLEANING AND DISINFECTION PROCEDURES

Cleaning and disinfection is carried out in order to produce safe products with acceptable shelf life and quality. In the food industry there is a trend towards longer production runs with short intervals for sanitation. The cleaning programmes should be performed as cost-effectively and safely as possible, which means as infrequently as possible, in the shortest possible time, with low chemical, energy and labour costs, producing as little waste as possible and with no damage to the equipment (Lelieveld, 1985; Holah, 1992). The mechanical and chemical power, temperature and contact time in the cleaning regime should be carefully chosen to achieve an adequate cleaning effect (Czechowski & Banner, 1990; Mosteller & Bishop, 1993).

The use of effective cleaning agents and disinfectants on surface-attached microbes minimises contamination of the product, enhances shelf life and reduces the risks of foodborne illness. Prolonged exposure of the surfaces to cleaning agents and disinfectants improves removal (Troller, 1993). Attention should also be paid to the quality of the processing water, steam and other additives. Using additives of poor quality easily spoils the process. Furthermore, the tools and methods used must also suit the process and the personnel must be properly trained and responsible to for maintaining a high level of plant hygiene (Lelieveld, 1985; Mattila-Sandholm & Wirtanen, 1992).

An efficient cleaning and disinfection procedure consists of a sequence of rinses and detergent and disinfectant applications in various combinations of temperature and concentration (Frank & Koffi, 1990; Holah, 1992; Troller, 1993; Gould, 1994; Wirtanen, 1995). In a wet open process the gross soil should be removed by dry methods, e.g. brushing, scraping or vacuuming and visible soil rinsed off with low-pressure water. Using water that is sufficient in terms of volume and temperature increases the cleaning effect. However, a pure water washing system is not practical due to ineffectiveness and cost limitation. Surfactants, which loosen the particles and microbes adhering to the surfaces and suspend them in the water, are added to increase the washing effect. After a production run, the equipment should be dismantled and the cleaned utensils should be stored on racks and tables, not on the floor (Mattila-Sandholm & Wirtanen, 1992; Holah & Timperley, 1999). The cleaning of open process surfaces and surfaces in the processing environment is carried out using either foam or gel cleaning. The foam-units are constructed to form foam of varying wetness and

durability depending on the cleaning to be performed. The application of gels extends the contact time with a soiled surface and can be used with low-pressure systems. The cleaning is mostly carried out in combination with a final disinfection, because there are likely to be viable microbes on the surfaces that could harm continued production. Furthermore, good ventilation in the process facilities is needed to enable drying of the process equipment and process lines (Holah, 1992; Wirtanen *et al.,* 2000).

In the cleaning of closed processes, pre-rinsing with cold water is carried out to remove loose soil. The CIP treatment is normally performed using hot cleaning solutions, but cold solutions can also be used in the processing of fat-free products. The warm alkaline cleaning solution, normally of 1–2% sodium hydroxide, is heated to 75–80°C and the cleaning time is 15–20 min. The equipment is rinsed with cold water before the acid treatment is performed at approximately 60°C for 5 min. The cleaning solutions should not be re-used in processes aiming at total sterility because the re-used cleaning solution can contaminate the equipment. The design of the tank should ensure that parts directly above the spray ball are also cleaned. The drainage, minimisation of internal probes, crevices and stagnant areas, arrangement of valves, couplings and instrument ports and instrumentation should be planned carefully so that the equipment can be cleaned easily. Problems caused by equipment constructions and materials cannot be eliminated with CIP, because the CIP treatment was not designed to eliminate biofilms (Czechowski & Banner, 1990; Brackett, 1992; Chisti & Moo-Young, 1994; Zottola & Sasahara, 1994; Wirtanen *et al.,* 1997).

12.2 COMMONLY USED DISINFECTANTS IN FOOD INDUSTRY

Disinfectants have been developed to destroy microbes. Microbes have nevertheless been found in disinfectant solutions, which is the result of their ability to form resistant strains and the build-up of protective biofilms (Gilbert & Allison, 1999; McBain *et al.,* 2000; Wirtanen *et al.,* 2002a). This means that microbial contaminants can be spread on the surface to be cleaned instead of being cleaned. Findings as early as 1967 reported that chlorhexidine mixtures were contaminated with *Pseudomonas* sp.. *Pseudomonas* sp. have also been found in concentrated iodine solutions (Marrie & Costerton, 1981). *Serratia marcescens* was found to be viable even after 27 months in a disinfectant containing 2% chlorhexidine. A concentration of 0.1% chlorhexidine is

sufficient to kill the cells of *S. marcescens* if they are freely suspended in liquid (Costerton & Lashen, 1983; Marrie & Costerton, 1981). Microbial contamination of e.g. *Alcaligenes faecalis*, *Enterobacter cloacae*, *Eschericia coli*, *Flavobacterium meningosepticum* and *Pantoea agglomerans* has also been found in solutions of quaternary ammonium compounds, aldehydes and amfotensides (Heinzel, 1988).

Disinfection is required in food plant operations, where wet surfaces provide favourable conditions for the growth of microbes. The aim of disinfection is to reduce the surface population of viable microbes after cleaning and to prevent microbial growth on surfaces before restarting of production. Disinfective agents do not penetrate the biofilm matrix left on the surfaces after an ineffective cleaning procedure very well, and thus do not destroy all the living cells in biofilms (Bloomfield, 1988; Pontefract, 1991; Brackett, 1992; Holah, 1992; Carpentier & Cerf, 1993). Disinfectants are most effective in the absence of organic material, e.g. fat-, sugar- and protein-based materials. Interfering organic substances, pH, temperature, concentration and contact time generally control the efficiency of disinfectants (Czechowski & Banner, 1990; Mosteller & Bishop, 1993; Gould, 1994). The disinfectants must be effective, safe and easy to use, and easily rinsed off surfaces, leaving no toxic residues or residues that affect the sensory values of the product. The use of disinfectants in food plants depends on the material used and the adhering microbes. Disinfectants (Table 12.1) approved for use in the food industry are alcohols, chlorine-based compounds, quaternary ammonium compounds, oxidants (peracetic acid, hydrogen peroxide, ozone), persulphates, surfactants and iodophors. They should be chosen on the basis of the process (Sequeira *et al*., 1989; Larson & Morton, 1991; Troller, 1993; Wirtanen, 1995):

⇒ Is the agent effective in the pH range used?
⇒ Is the agent stable when diluted? Does it vaporize?
⇒ Is the agent toxic, safe or irritating?
⇒ What is the spectrum of the agent?
⇒ How does temperature affect the activity of the agent?
⇒ Is the agent corrosive on the surface?
⇒ Is the agent surface active?
⇒ Is the agent stable when reacting with organic material?
⇒ Is the agent effective, and what are the costs?

Table 12.1. Advantages and disadvantages of some disinfectants used in the food processes (according to Flemming, 1991; Troller, 1993 & Wirtanen, 1995).

Disinfectant type	Advantages	Disadvantages
Alcohols	effective against vegetative cells, non-toxic, easy-to-use, colourless, harmless on skin, soluble in water, volatile	microbistatic, ineffective against spores
Peracetic acid	effective in low concentration, broad microbial spectrum, kills spores, penetrates biofilms, non-toxic (→ acetic acid and water)	corrosive, unstable
Hydrogen peroxide	decomposes to water and oxygen, relatively non-toxic, easy to use; weakens biofilms and supports detachment	high concentrations needed, corrosive
Chlorine	effective in low concentration, broad microbial spectrum, easy to use, supports microbial detachment, cheap	toxic by-products, resistance development, residues, corrosive, reacts with EPS, discoloration, explosive gas
Hypochlorite	cheap, effective in a broad microbial spectrum, easy to use, supports detachment	unstable, toxic, oxidative, corrosive, rapid regrowth, no prevention of adhesion, discoloration of products
Chlorine dioxide	effective in low concentration, can be produced on-site, low pH dependency	toxic by-products, explosive gas
Quaternary ammonium agents	effective, non-toxic, prevents regrowth, supports microbial detachment, non-irritating, non-corrosive, odourless, tasteless	inactivated in low pH and by salts (Ca^{2+} & Mg^{2+}), resistance development, ineffective against Gram-negative bacteria
Iodophor	non-corrosive, easy to use, non-irritating, broad activity spectrum	expensive, flavour, odour, forms purple compounds with starch
Ozone	Similar effect as chlorine, decomposes to oxygen, no residues, decomposes biofilm	corrosive, inactivated easily, reacts with organics (→ epoxides)
Glutaraldehyde	Effective in low concentrations, cheap, non-corrosive	low penetration in biofilms, degrades to formic acid, increased DOC

There are several chlorine or chlorine-based compounds that are approved for use in food plants, e.g. gaseous chlorine, chlorine dioxide, sodium and calcium hypochlorites. The antibacterial active moiety is formed when the chlorine compound is added to water. Hypochlorous acid is formed and it dissociates further into protons and hypochlorite anions. Stabilised hypochlorites are used when a long duration is required. The range of microbes killed or inhibited by chlorine-based compounds is probably broader than that of any other approved sanitizer (Troller, 1993; Stewart *et al*., 1994; Sanderson & Stewart, 1997).

Hydrogen peroxide has been found to be effective in removing biofilms from equipment used in hospitals. The effect of hydrogen peroxide is based on the production of free radicals, which affect the biofilm matrix. The microbicidal effect of peracetic acid on microbes in biofilms was shown to vary (Exner *et al*., 1987; Christensen, 1989; Kramer, 1997). Aldehydes did not break the biofilm, but rather seemed to improve its stability. The biofilm must be disrupted in some way before chemical agents such as peracetic acid and aldehydes can be used effectively (Exner *et al*., 1987). The effect of ozone treatment has been found to vary depending on the processing circumstances and the microbes tested, e.g. ozonation proved very effective in the treatment of cooling water systems (Lin & Yeh, 1993).

Iodophors are also used in the food industry. In the disinfection, the iodine compound takes part in the oxidation of essential parts of the microbial cells. Like chlorine-containing products, iodophors are active against Gram-positive bacteria and Gram-negative bacteria, yeasts and moulds (Holah *et al*., 1990; Holah, 1992; Troller, 1993). Bacterial spores, however, are highly resistant to iodophors. Iodophors cannot be used in food plants where starch-containing products are produced because iodine forms a purple complex with starch. Quaternary ammonium compounds are used as sanitizers in dairies and in the food industry, because they have good wetting properties and are non-specifically described as cationic surface active agents in which the cationic part is hydrophobic. The greatest effect of quaternary ammonium compounds is observed against Gram-positive bacteria, whereas Gram-negative bacteria organisms, many of them significant in the contamination of food, may not be affected (Troller, 1993).

Fogging can be defined as chemical disinfection using automatic spraying of disinfectant in a closed room. The aim of disinfection testing on an industrial scale using fogging was to study the efficiency of the disinfection on surfaces at different places in the room. Controlled experiments were carried out at two

cheese-producing dairies. Neither of the fogging trials showed a clear reduction in the microbial load. Critical control points in fogging were the amount of fog used, the disinfectant concentration used for the fog, thorough rinsing of equipment and the drying of facilities (Wirtanen *et al.,* 1997, 2002a).

12.3 METHODS IN EFFICACY TESTING OF DISINFECTANTS

The efficacy of disinfectants and antimicrobial agents is usually determined in free cell suspensions which do not mimic the growth conditions on surfaces where the agents are required to inactivate the microbes (Frank & Koffi, 1990; Wirtanen, 1995). The agent must reduce the microbial populations by 5 log units in suspensions in order to be considered effective. The goal for the reduction of surface-attached bacteria with disinfectants is 3 log units (Mosteller & Bishop, 1993). The standard suspension tests have proved sufficiently reliable because the variations in results are within acceptable limits when replication is adequate. There can, however, be problems with the repeatability and reproducibility of suspension tests performed with organic loading. It is obvious that the surface tests are even more difficult to perform than suspension tests because of the carrier material used and the viability of dried cells on the surfaces (Bloomfield *et al*., 1994). In developing a proposal for the testing of disinfectants on surfaces to an analytical standard, it is important to identify the major sources of variation in the procedure. Microbes growing or dried on surfaces are not susceptible to disinfectants from all sides as they are in suspensions, and due to the requirement for penetration, disinfectants are used in higher concentrations on surfaces than in suspensions (Mattila-Sandholm & Wirtanen, 1992).

12.3.1 Suspension tests

The determination of disinfectant efficiency is often performed in suspension tests with ready-to-use dilutions. The European Committee for Standardization (CEN) has launched many standards. The microbes used are standard test organisms as well as spoilage bacteria, pathogens and spores of concern in hygiene. All disinfectants passing the efficacy test should reduce the number of vegetative cells by ≥ 5 log units and the number of bacterial spores by ≥ 1 log unit (Wirtanen, 1995). The suspension tests in European Standards are:

⇒ in EN 1040:1997 the basic bactericidal activity of a disinfectant is tested against both Gram-negative bacteria (*Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus*),

⇒ in EN 1275:1997 the basic fungicidal activity of a disinfectant is tested against both yeast (*Candida albicans*) and mould (*Aspergillus niger*),

⇒ in the quantitative suspension test EN 1276:1997 the bactericidal activity of a disinfectant for use in food, industrial, domestic and institutional areas is tested against both Gram-negative bacteria (*P. aeruginosa,* and *Escherichia coli)* and Gram-positive bacteria (*S. aureus* and *Enterococcus hirae*) (additionally the following bacteria *Salmonella* Typhimurium, *Lactobacillus brevis* and *Enterobacter cloacae* can be used if needed) in hard water,

⇒ in the quantitative suspension test EN 1650:1997 the fungicidal activity of a disinfectant for use in food, industrial, domestic and institutional areas is tested against both yeast (*C. albicans* and if necessary *Saccharomyces cerevisiae* can also be used) and mould (*A. niger*) in hard water,

⇒ in the quantitative suspension test EN 1656:2000 the bactericidal activity of a disinfectant for use in the veterinary field is tested against both Gram-negative (*P. aeruginosa,* and *Proteus vulgaris)* and Gram-positive (*S. aureus* and *Enterococcus hirae*) bacteria in hard water with an organic load of bovine albumin or a mixture of bovine albumin and yeast extract or skimmed milk,

⇒ in the quantitative suspension test EN 1657:2000 the fungicidal activity of a disinfectant for use in the veterinary field is tested against both yeast (*C. albicans)* and mould (*A. niger*) in hard water with an organic load of bovine albumin or a mixture of bovine albumin and yeast extract or skimmed milk,

⇒ in the quantitative suspension test prEN 13704:1999 the sporicidal activity of a disinfectant for use in food, industrial, domestic and institutional areas is tested against bacterial spores of *Bacillus subtilis* (if necessary spores of *B. cereus* and *Clostridium sporogenes* can also be used) in hard water,

⇒ in EN 1499:1997 the basic activity of hygienic handwash products is tested against *E. coli* on test persons' hands,

⇒ in EN 1500:1997 the basic activity of hygienic handrub products is tested against *E. coli* on test persons' hands.

VTT Biotechnology has tested the activity of disinfectants using a Dutch 555-suspension test protocol. The 555-suspension test is performed to find out the bactericidal, fungicidal and sporicidal activity of the disinfectant. The activity is measured after a challenging time of 5 min. The product has both bactericidal and fungicidal activity if the microbial reduction is at least 5 log-units for vegetative cells and sporicidal activity if the reduction of spores is at least 1 log-unit. The microbes used are *Salmonella* Choleraesuis, *P. aeruginosa, S. aureus, B. cereus* (spores) and *S. cerevisiae* and the test is carried out using bovine albumin as the organic load. In a modified 555-suspension test the disinfectant is tested against a chosen panel of process contaminants (consisting of bacteria, yeasts and/or moulds) in bovine albumin as organic soil (Wirtanen, 1995; Wirtanen & Juvonen, 2002).

12.3.2 Tests based on microbes in biofilms

Various surface tests have shown that surface-attached cells are more resistant to disinfectant treatment than are cells in suspension (Wirtanen, 1995; Wirtanen *et al.,* 1997). Results obtained using only one assessment method in testing can be inaccurate. For example, cultivation and CTC-DAPI staining in a comparison based on biofilms showed an underestimation of viable bacteria in the cultivation (Wirtanen *et al.,* 1997). Microscopy techniques have often been used as a reference method for cultivation based on swabbing. It has been reported that counting cells by direct microscopy consistently gives a result at least one log unit higher than the cultivation method (Holah *et al.,* 1988). This may be because the bacteria do not grow under the conditions provided in the plate count method or because large numbers of bacteria remain on the surfaces after swabbing (Holah, 1992). In our experiments epifluorescence microscopy clearly revealed that even vigorous swabbing detached only a small portion of the actual biofilm and the cells within it (Wirtanen, 1995). Therefore, methods used in assessment and in detachment should both be chosen carefully. One testing procedure, based on cultivation, image analysis, impedance and metabolic indicators e.g. CTC-DAPI staining, seems to give a good estimation of both removal of biofilm from surfaces and killing of bacteria on surfaces (Wirtanen *et al.,* 1997). Microbial cells dried on test surfaces have also been used in disinfectant carrier tests e.g. Draft prEN 13697. The model biofilms have many of the characteristics of ‘wild’ biofilms. The microbial cells are adhered to test surfaces, they produce slime and show increased resistance to disinfectants. In

response to the need for a relatively realistic, simple and reliable test for disinfectant efficacy Charaf *et al.* (1999) have developed a method for culturing laboratory model biofilms. The method involves growing biofilm on test coupons using inoculated filter papers placed on top of a suitable nutrient agar (Figure 12.1). These coupons are after the removal of the filter paper subjected to disinfectant testing as given in suspension tests. The above-mentioned methods for disinfectant testing, however, require further validation.

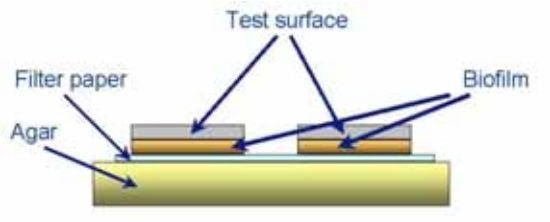

Figure 12.1. Diagram of the biofilm-based disinfectant test: growing biofilm on test coupons using an inoculated filter paper placed on top of a suitable nutrient agar (Charaf et al. 1999).

12.3.3 Tests based on biofilm constructs

The poloxamer hydrogels demonstrate thermo-reversible gelation properties, being liquid and fully miscible with water at temperatures <15°C but firm gels at temperatures >15°C. This means high cell densities can be cultured within the gels at 30°C and subsequently exposed to a disinfectant. After treatment, a full recovery of the individual cells can be achieved simply by moving the hydrogels into neutraliser solutions/diluents at <15°C (Wirtanen *et al.* 1998, 2001, 2003). Poloxamer F127 is a di-block co-polymer of polyoxyethylene and polyoxy-propylene. It has been investigated earlier for its potential as an agar substitute in microbiology. Solutions are unaffected by autoclaving and appear to be non-toxic to all the bacterial species so far tested (Gilbert *et al*. 1998; Wirtanen *et al*. 1998, 2001). The poloxamer matrices in the present study not only reproduce the reaction-diffusion resistance properties of the biofilms but also simulate other aspects of the biofilm mode of growth (Figure 12.2). The use of artificial biofilms, i.e. biofilm-constructs inoculated with process contaminants in disinfectant testing, is suitable for screening the activity of various disinfectants.

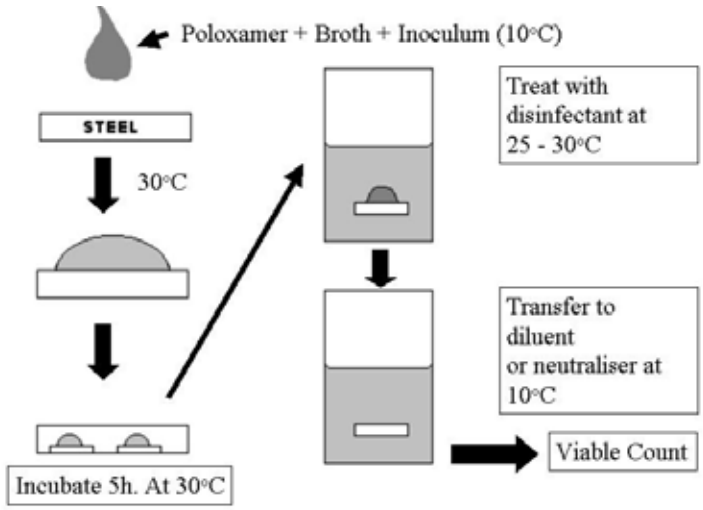

Figure 12.2. Test protocol in efficacy testing of disinfectants using biofilm-constructs (Wirtanen et al. 1998, 2001, 2003).

12.4 Efficacy of Disinfectants Used in Food Industry

12.4.1 Suspensions

Tests carried out at VTT Biotechnology have shown that microbes in suspensions are easily destroyed, which is in agreement with several studies from other areas (Table 12.2). LeChevallier *et al.* (1988) showed that unattached Gram-negative bacteria in drinking water were susceptible to chlorine disinfectants whereas attached cells were not. Eagar *et al.* (1988) reported that planktonic cells of *Pseudomonas fluorescens* were more sensitive to glutaraldehyde than sessile cells. Best *et al.* (1990) stressed that the selection of appropriate disinfectants for use on contaminated surfaces should be carried out carefully. They tested the effects of disinfectants on *Listeria monocytogenes* in carrier and suspension tests and found that sodium dichloroisocyanurate was the only effective solution in the presence of milk containing 2% fat.

Table 12.2. The microbicidal effect of four commercial disinfectants (alcohol-based, hydrogen peroxide-based, hypochlorite-based and persulphate-based products) against Listeria innocua, L. monocytogenes, E. coli, Salmonella Infantis, S. Choleraesuis and Bacillus cereus grown in suspension (reduction unit is log CFU/ml).

Disinfectant /	Alcohol-based		Hydrogen peroxide-based			Hypochlorite-based			Persulphate based	Control
Concentration	100%	75%	1%	0.50%	0.25%	1.40%	0.70%	0.35%	1/1.25 l	
Escherichia coli	>6.7	>6.7	>6.7	>6.7	>6.7	>6.7	>6.7	>6.7	>6.7	7.46
Listeria innocua	>6.4	>6.4	>6.4	>6.4	>6.4	>6.4	>6.4	>6.4	>6.4	7.11
Listeria monocytogenes	>6.3	>6.3	>6.3	>6.3	>6.3	>6.3	4.9	>6.3	>6.3	7.04
Salmonella Choleraesuis	>6.5	>6.5	>6.5	>6.5	>6.5	>6.5	>6.5	>6.5	>6.5	7.29
Salmonella Infantis	>6.8	>6.8	>6.8	>6.8	>6.8	>6.8	>6.8	>6.8	>6.8	7.67
Bacillus cereus (spores)	ND	0.14	0.20	0.26	ND	ND	ND	ND	ND	6.04

ND = no microbicidal effect observed

Available reports about the susceptibility of foodborne yeasts to various chemicals used in the food industry are sporadic and cover only a few disinfectants against some spoilage yeasts (Juvonen *et al*., 2001). McGrath *et al.* (1991) showed that ready-to-use concentrations of a hypochlorite disinfectant killed most of the *S. cerevisiae* tested in suspension in 15–20 min. The ascospore-containing cultures of *Pichia* and *Saccharomyces* species are more resistant to disinfectants than the vegetative populations (McGrath *et al.,* 1991). Hypochlorite, peracetic and phosphoric acid as well as an anionic compound in ready-to-use concentrations were effective against suspended yeasts isolated from orange juice (Winniczuk & Parish, 1997). Chlorine dioxide appeared to be effective against yeast and mould contaminants (Han et al., 1999). The efficacy of various types of disinfectants against food-spoilage was thoroughly studied using modified 5-5-5 suspension tests against yeast contaminants isolated from various food processing environments. The results of the suspension tests (Table 12.3) showed that the alcohol, peroxide and tenside-based disinfectants were efficient. The disinfectants containing chlorine and persulphate did not destroy suspended yeast cells (Wirtanen & Juvonen, 2002).

12.4.2 Biofilms

The microbicidal effect of the four commercial disinfectants (hydrogen peroxide-based, alcohol-based, persulphate-based and hypochlorite-based products) was also tested with biofilms of 6 different bacteria (*L. innocua, L. monocytogenes, E. coli, S.* Infantis, *S.* Choleraesuis and *B. cereus)*. The bacteria were allowed to

form a biofilm on the surface of steel coupons (Figure 12.1) according to the method described by Charaf *et al.* (1999). When the results of the suspension test and the biofilm test were compared, the protection of the biofilm against the disinfectants was shown clearly. In the suspension tests all disinfectants had sufficiently microbicidal effect on all vegetative bacterial types, but in the biofilm tests the microbicidal effect of the same disinfectants was lower. Only

Table 12.3. The in-use concentrations of alcohol-based (A1-A3), hydrogen peroxide-based (H1-H3), chlorine-based (C1-C2), tenside-based (T1-T2) and persulphate-based (S1) disinfectants needed to kill various yeast isolates obtained from different food processes. The disinfectant efficacy was tested using a modified 5-5-5 suspension test that used Saccharomyces servazzii, S. cerevisiae, Zygosaccharomes rouxii, Candida krusei, C. lambica, C. lipolytica, C. boidinii, C. intermedia, C. parapsilosis, Cryptococcus albidus, Debaryomyces hansenii, Dekkera anomala, Rhodotorula glutinis, R. rubescens and R. mucilaginosa (Wirtanen & Juvonen, 2002).

Yeast	Control	Disinfectant treatments*										
		A1**	A2	A3	H1	H2	H3	C1	C2	T1	T2	S1
S. servazzii C-00362	4.7 - 5.1	100%	75%	75%	0.5%	1.0%	0.3%	0.3%	1.0%	0.2%	2%	—
S. cerevisiae C-00370	5.8 - 6.4	100%	75%	75%	0.5%	0.25%	1.3%	1.0%	ME 4.3	2%	2%	—
S. cerevisiae C-96203	5.0 - 5.6	100%	75%	75%	0.25%	0.25%	0.3%	0.7%	1.0%	0.2%	2%	—
Z. rouxii C-00363	5.9 - 6.1	100%	75%	75%	0.25%	0.25%	0.3%	ME 3.7	0.1%	0.2%	2%	—
Z. rouxii C-00367	4.6 - 4.7; 6.1 - 6.3	100%	75%	75%	0.25%	0.25%	0.3%	0.3%	1.0%	0.2%	2%	—
Z. rouxii C-95218	4.6 - 5.1	100%	75%	75%	0.25%	0.25%	0.3%	0.3%	1.0%	0.2%	4%	—
C. lambica C-00365	5.6 - 6.0	ME 2.0	75%	75%	ME 1.4	0.25%	0.3%	0.7%	ME 4.0	2%	2%	—
C. lipolytica C-00365	5.0 - 5.7; 6.2 - 6.5	100%	75%	75%	ME 1.5	ME 3.6	ME 2.2	ME 2.1	ME 2.8	0.2%	2%	—
C. lipolytica C-00380	4.9 - 5.1; 6.7	100%	75%	75%	1.0%	0.25%	1.3%	1.0%	ME 3.1	0.2%	2%	ME 1.1
C. boidinii C-00366	5.7 - 6.1; 6.5 - 6.6	100%	75%	75%	0.25%	0.25%	0.3%	0.7%	ME 4.3	0.2%	2%	—
C. intermedia C-00372	6.3 - 6.5	100%	75%	75%	1.0%	0.25%	ME 2.8	1.0%	1.0%	2%	2%	—
C. parapsilosis C-00373	6.0 - 6.7	100%	75%	75%	1.0%	0.25%	1.3%	ME 1.3	ME 3.5	2%	2%	—
C. parapsilosis C-00381	5.9 - 6.4	100%	75%	75%	1.0%	0.25%	ME 3.3	1.0%	ME 1.7	2%	2%	ME 0.9
C. krusei C-00371	5.9 - 6.1	100%	75%	75%	0.5%	0.25%	0.3%	ME 3.7	ME 3.0	ME 4.4	ME 3.7	—
C. albidus C-00392	4.9 - 5.4	100%	75%	75%	0.25%	0.25%	0.3%	0.7%	ME 2.3	0.2%	2%	—
C. albidus C-00397	4.6 - 5.2	100%	75%	75%	0.25%	0.25%	0.3%	0.3%	ME 2.7	0.2%	2%	—
D. hansenii C-00382	5.3 - 6.1	100%	75%	75%	0.25%	0.25%	0.3%	0.3%	ME 3.9	0.2%	2%	1tab/ 1.25l
R. glutinis C-00391	5.3 - 6.0	100%	75%	75%	0.25%	0.25%	0.3%	0.3%	1.0%	0.2%	2%	—
R. rubescens C-00393	5.7 - 6.0	100%	75%	75%	0.25%	0.25%	0.3%	ME 3.0	ME 3.0	2%	2%	—
R. mucilaginosa C-00396	5.8 - 6.1	ME 2.7	75%	75%	0.25%	0.25%	1.3%	—	ME 2.5	0.2%	2%	—
D. anomala C-91183	6.3 - 7.0	100%	75%	75%	0.25%	0.25%	0.3%	0.3%	1.0%	0.2%	2%	ME 3.5
R. mucilaginosa	5.6 - 6.1	100%	75%	75%	0.5%	0.25%	1.3%	—	ME 4.4	0.2%	2%	—

* the percentage given is the lowest in-use concentration of the agent tested, which kills the yeast tested; if a microbial effect (ME) value is given in a shadowed cell, the highest concentration of the agent tested is given (ME unit is log CFU/ml).

** the agent A1 was tested only in undiluted form (recommended concentration).

the alcohol-based and the hydrogen peroxide-based agents were efficient enough giving a log-reduction greater than 3 for most vegetative bacteria tested. Spore forming bacteria were also used in biofilm test. The test showed (Table 12.4) that the biofilm also protects spores against the disinfectants.

Table 12.4. The microbicidal effect of four commercial disinfectants (alcohol-based, hydrogen peroxide-based, hypochlorite-based and persulphate-based products) against biofilms of Listeria innocua, L. monocytogenes, E. coli, Salmonella Infantis, S. Choleraesuis and Bacillus cereus (reduction unit is log CFU/cm^2).

Disinfectant /	Alcohol-based		Hydrogen peroxide-based			Hypochlorite-based			Persulphate based	Control
Concentration	100%	75%	1%	0.50%	0.25%	1.40%	0.70%	0.35%	1/1.25 l	
Escherichia coli	>5.07 [0.00]	>5.07 [0.00]	4.29 [0.80]	2.81 [2.10]	1.44 [3.18]	2.11 [0.84]	0.44 [0.77]	0.29 [0.65]	1.54 [0.29]	5.39 [0.51]
Listeria innocua	>2.99 [0.00]	>2.99 [0.00]	>2.99 [0.00]	>2.99 [0.00]	>2.99 [0.00]	>2.99 [0.00]	1.76 [0.63]	2.68 [0.55]	2.35 [1.11]	3.32 [0.37]
Listeria monocytogenes	>5.30 [0.00]	>5.30 [0.00]	>5.30 [0.00]	4.72 [1.01]	4.20 [1.91]	-0.47 [0.99]	-0.91 [0.24]	-1.19 [0.08]	0.00 [0.00]	5.62 [0.00]
Salmonella Choleraesuis	>3.14 [0.00]	>3.14 [0.00]	1.81 [1.20]	2.47 [1.17]	1.80 [1.31]	1.98 [2.02]	1.81 [1.26]	2.22 [0.67]	0.58 [1.15]	3.47 [0.32]
Salmonella Infantis	>6.06 [0.00]	>6.06 [0.00]	5.09 [1.68]	4.30 [1.95]	5.48 [1.01]	1.55 [0.52]	0.34 [0.15]	0.43 [0.06]	1.61 [0.13]	6.38 [0.25]
Bacillus cereus (spores)	ND	ND	ND	ND	ND	ND	ND	ND	0.36 [0.23]	5.21 [0.59]

ND = no microbicidal effect observed

Abundant growth of unwanted yeast during production can lead to defects in the final products and, furthermore, problems in process hygiene (McGrath *et al.,* 1991; Winniczuk & Parish, 1997; Han et al., 1999; Juvonen *et al.,* 2001). A low pH, or a high sugar or salt content favours the growth of yeast. Spoilage yeast is a contributor to the deterioration of syrups, pralines, jams, berries and fruits, fruit juices, pickled vegetables and dairy products. In many cases the risk caused by a growth in spoilage yeast in products has been underestimated because many of these yeast are not known to be opportunistic pathogens (Wirtanen & Juvonen, 2002). The yeast also readily forms biofilms on process surfaces at both low and elevated temperatures. The spoilage yeast can, therefore, be a hygiene risk, when failures occur in the cleaning and disinfection procedure. In this study the efficacy of various types of disinfectants against yeast isolated from various food processes growing on surfaces was assessed (Charaf *et al.,* 1999). The results of the surface test showed that the alcohol-based agent was the most efficient disinfectant against the yeasts tested (Table 12.5). After 5 min treatment the hydrogen peroxide-based disinfectant was also effective in some cases, and particularly when the duration of the disinfectant treatment was extended to 15

min, the peroxide-based and quaternary ammonium containing disinfectants were also efficient (Table 12.5). The disinfectants containing chlorine and persulphate were ineffective in destroying yeast biofilms.

Table 12.5. Efficacy of disinfectant treatments on yeast biofilms grown on stainless steel after 5 min (upper table) and 15 min (lower table): the yeast isolates used were Saccharomyces cerevisiae, Candida lipolytica, C. intermedia, C. parapsilosis, C. krusei, Cryptococcus albidus, Debaryomyces hansenii, Rhodotorula glutinis, R. rubescens and R. Mucilaginosa, Dekkera anomala and Trichosporon asahii.

Yeast	Disinfectant treatments*											
	Alcohol-based			Hydrogen peroxide-based			Hypochlorite-based		Quaternary ammonium			Persulphate-based
Concentration	100%	75%	50%	1.0%	0.5%	0.25%	1.4%	0.7%	4%	2%	0.2%	1 tab / 1.25 l
S. cerevisiae C-00370	■	■	■	ND	ND	ND	ND	ND	ND	ND	ND	ND
S. cerevisiae C-96203	■	■	■	■	■	ND	ND	ND	■	ND	ND	ND
C. lipolytica C-00380	■	■	■	■	▒	▒	▒	ND	■	ND	ND	ND
C. intermedia C-00372	■	■	■	ND	ND	ND	ND	ND	ND	ND	ND	ND
C. parapsilosis C-00373	■	■	■	▒	ND	ND	ND	ND	ND	ND	ND	ND
C. parapsilosis C-00381	■	■	■	▒	ND	ND	ND	ND	ND	ND	ND	ND
C. krusei C-00371	■	■	■	▒	ND	ND	▒	▒	▒	ND	ND	ND
C. albidus C-00392	■	▒	▒	ND	ND	ND	ND	ND	ND	ND	ND	ND
D. hansenii C-00382	■	■	■	■	■	ND	ND	ND	▒	ND	ND	ND
R. glutinis C-00391	■	■	■	■	■	▒	ND	ND	▒	ND	ND	ND
R. rubescens C-00393	■	■	■	■	▒	▒	■	▒	▒	▒	▒	▒
R. mucilaginosa C-00396	■	■	■	■	■	▒	ND	ND	■	▒	ND	ND
D. anomala C-91183	■	■	■	■	■	■	■	ND	■	■	ND	ND
T. asahii G-10	■	■	■	■	▒	ND	ND	ND	ND	ND	ND	ND

Yeast	Disinfectant treatments*											
	Alcohol-based			Hydrogen peroxide-based			Hypochlorite-based		Quaternary ammonium			Persulphate-based
Concentration	100%	75%	50%	1.0%	0.5%	0.25%	1.4%	0.7%	4%	2%	0.2%	1 tab / 1.25 l
S. cerevisiae C-00370	—	—	—	■	■	■	▒	ND	■	■	ND	ND
C. lipolytica C-00380	—	—	—	■	■	▒	▒	▒	■	▒	ND	ND
C. intermedia C-00372	—	—	—	▒	ND	ND	—	—	ND	ND	ND	ND
C. parapsilosis C-00373	—	—	—	▒	▒	ND	ND	ND	▒	▒	ND	ND
C. parapsilosis C-00381	—	—	—	■	■	▒	ND	ND	■	▒	ND	ND
C. albidus C-00392	—	—	—	■	■	ND	ND	ND	ND	ND	ND	ND
R. mucilaginosa C-00396	—	—	—	—	—	—	ND	ND	ND	ND	ND	ND

■ = reduction greater than 3 log-units
▒ = reduction below 3 log-units but exceeding 1.5 log-units
ND = reduction lower than 1.5 log-units
— = not tested

12.4.3 Biofilm constructs

The biofilm construct test is a severe test of disinfection efficiency, and it gave reproducible results. Whilst the results do not necessarily reflect the likely effects of a formulation against microbial contamination *in situ,* they make it possible to discriminate between the disinfectant formulations at normal use level and choose the most effective. The survival levels obtained in the test made it possible to distinguish the performances of many different disinfectant formulations against a variety of test strains, whereas this had been impossible with conventional testing methods (Wirtanen *et al*. 1998, 2001, 2003). Conventional suspension tests fail to discriminate between the agents in terms of their efficacy and do, therefore, not assist in the final selection of agents (Gilbert *et al*. 1998; Wirtanen *et al*. 1998). The results showed that Gram-negative bacteria are more resistant to disinfectant treatments than Gram-positive bacteria. This is in conformity with the general observation (Nikaido and Vaara 1985; McKane and Kandel 1996) as well as studies using surfaces with dried bacterial cells (Grönholm *et al*. 1999) and biofilms (Wirtanen, 1995; Wirtanen *et al*. 1997, 2002a).

13. MIC Determination of Dairy Isolates using Different Types of Disinfectants

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In order to provide consumers with safe products, it is essential to control microbes in food products and on the surfaces of processing equipment (Lelieveld, 1985; Pontefract, 1991). The main aim of disinfection within the food industry is to minimise the number of microbes on food contact surfaces, thus avoiding contamination of raw materials and products. Disinfection failures are usually caused by incorrect temperature, low disinfection concentration, incorrect exposure time or failure in the cleaning process. Another common reason is that bacteria may be resistant towards the disinfectants used. By definition, resistant strains in the food industry are usually those that survive the disinfection procedure used at a given place. Recent articles by Russell (2004), Chapman (2003) and Cloete (2003) are excellent reviews on bacterial adaptation to disinfectants, resistance mechanisms and cross-resistance.

Disinfectants are classified according to the specific action they perform on different microbes: bactericides kill vegetative bacteria, sporocides destroy spores, virocides kill viruses and fungicides kill fungi. The chemical agents used can affect cells in various ways, such as causing proteins to coagulate. However, most proteins in cells are enzymes, which can precipitate in the presence of specific chemicals, inactivating cell functions. Other agents may disrupt the cell membrane, altering its physical and chemical properties. Some agents remove free sulphydryl groups, which are important components in the amino acid cysteine, thus damaging many of the cell's proteins. Finally, chemical agents can cause chemical antagonism, i.e. the disinfectant compound resembles a substrate in structure, and the enzyme that acts on the natural substrate has an affinity with the disinfectant (Chapman, 2003; Cloete, 2003; Russell, 2004).

13.1 SANITATION PROCEDURES IN DAIRIES

Sanitation procedures naturally differs from one dairy to another. However, closed systems are usually cleaned using cleaning-in-place (CIP) systems, which consist of prerinsing, circulation of an alkaline detergent, water rinsing, circulation of an acid solution and a final cold water rinse. Open surfaces are often treated with foam cleaning and QACs are sometimes left on conveyor belts and other surfaces over the weekend. Surfaces are then rinsed with chlorinated water before the process start-up. As a result indoor bacterial flora in a dairy is in constant contact with various disinfectants, and can therefore develop resistance towards them.

The efficiency of disinfectants is often measured by suspension tests. These tests do not, however, take into account the increased resistance which may result from the attachment of bacteria to surfaces or biofilm formation. Therefore, minimum inhibitory concentration (MIC) is used in a similar way as in determining resistance to antibiotics. The application of this method is limited as many disinfectants cannot be tested because their pH is too high or too low for growth, or because precipitation occurs in the medium (Nicoletti *et al.,* 1993; Sundheim & Langsrud, 1995).

In this investigation, 140 strains of bacteria were isolated from various locations in a dairy, including drainage systems, floors, cheese moulds, wagons, cheese conveyor belts, ceilings, tanks and other determined critical control points in the process, The bacteria were tested for minimum inhibitory concentration in four disinfectants (hypochlorite, ethanol, per acetic acid and a quaternary ammonium compound containing benzalkonium chloride (QA). Several strains were investigated more closely by measuring the kinetics during growth in different concentrations of the disinfectants.

13.2 ISOLATION OF STRAINS FOR MIC TESTING

Methods

For sampling the process and its environment, cotton-wool swabs with hydrophilic and hydrophobic properties were used. Four sticks were used for each sampling location. Streaking was always performed in the same way in

experimental areas and collected into sterile bottles. 30 ml of phosphate buffer was added to the bottles at the laboratory. For the characterisation of various strains in a factory environment, dilutions were carried out in a buffer and samples inoculated on PCA agar plates, grown for 48 h at 30°C and colonies were obtained from the highest dilutions. Preliminary characterisations were carried out and the samples were frozen using glycerol. A total of 140 strains were isolated, 80 during the summer and 60 during the winter. Samples were collected at various locations inside (drainage, pasteurisation equipment, floor area, various wheels in the dairy, cheese moulds) and outside (cold storage rooms, milk trucks, floors) the dairy process environment.

Results

All strains were Gram-stained, 32 of which were Gram-positive and 108-Gram negative. Some of the summer strains (39) were also characterised by 16 S rRNA analysis. Nine were characterised as *Pseudomonas putida*, seven as *Acinetobacter*, three as *Burkholderia*, three as *Moraxella osloensis*, three as *Klebsiella oxytoca*, three as *Ralstonia*, two as *Bacillus pumilus*, two as *Brucella sius*, two as *Kocuria* spp., two as *Stenotrophoomonas spp*., and one each of *Aeromonas veroni, Delftia acidovorans, Klyvera, Lactobacillus curvatus, Ochrobactrum anthropi, Sphingomonas spp., Spirochaeta spp.* Table 13.1 shows the locations within the dairy where they were isolated.

Table 13.1. Number of strains isolated from various locations in the dairy, inside and outside the production working area.

Sampling location in the dairy	**Strains isolated during**	
	summer	**winter**
Drainage systems inside production line area	13	29
Drainage system outside production line area	32	19
Critical points*	23	2
Floor	10	4
Other	2	6
Total	80	60

* Locations that are regularly checked as part of the internal hygiene system at the dairy including packaging machines, cheese moulds and knives.

13.3 ASSESSMENT OF MIC

Methods

The MIC determinations were performed using the two-fold broth dilution method. Starting from a chemical agent solution, serial dilutions were prepared in Tryptic Soy Broth inoculated with various bacterial strains. This was carried out at a volume of 2 ml with an inoculation size of 10 µl, yielding ca. 10^6 CFU/ml. The MIC was identified as the lowest concentration of the chemical agent, which resulted in confirmed inhibition of growth of the bacteria after 24 h of optimal incubation conditions. In some cases, additional phosphate buffer was included in the medium to keep the pH within the bacterial growth range. The disinfectants used were hypochlorite, ethanol, per acetic acid and benzalkonium chloride (QA).

Results

More than 90% of the strains were inhibited at 2500 mg/l of hypochlorite concentrations or less, whereas around 8% were inhibited at 5000 mg/l (Figure 13.1A). Most of the tolerant strains were isolated during the summer (8 out of 12 strains). Five of the tolerant strains were isolated within the production line area and seven from the drainage systems at the dairy. Over 90% of the isolates from the dairy environment were inhibited at 8.75% (87,500 mg/l) ethanol concentration or less, whereas 9% were inhibited at 17.5% ethanol concentration (Figure 13.1B). Most of the more tolerant strains were isolated during the summer (11 out of 12 strains). Six of the tolerant strains were isolated within the production line area and six from drainage systems at the dairy. Around 75% of the strains were inhibited at 625 mg/l or less of peracetic acid concentrations and 25% of the strains were inhibited at 1250 mg/l (Figure 13.1C). Most of the tolerant strains were isolated during summer time (31 out of 46 strains). Then majority (58%) of the tolerant strains was isolated within the production line area and 43% from drainage systems at the dairy. Four strains showed considerable tolerance (> 32 mg/l) towards the QA used, all of which were isolated during summer time (Figure 13.1D). However, most of these strains tolerated less than 2 mg/l of QA.

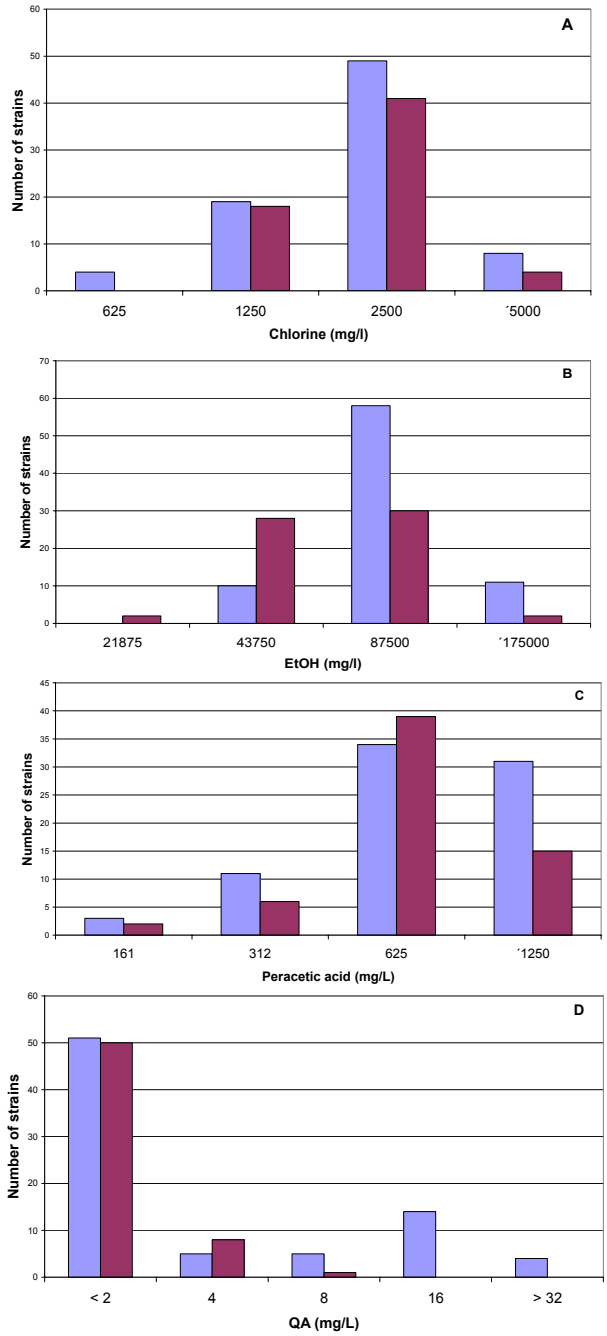

Figure 13.1. Minimum inhibitory concentration (mg/l) for hypochlorite (A), ethanol (B), per acetic acid (C) and quaternary ammonium compound (D). The blue column shows the number of summer isolates and the brown column the number of winter isolates.

13.4 KINETIC STUDIES ON SELECTED STRAINS

Methods

Seven isolates were selected for further kinetic studies with chemical agents: 3 sensitive isolates and 4 isolates with high tolerance. Those with high tolerance were selected according to high MIC-values on at least two of the disinfectant used. These isolates were grown in a Bioscreen system for 96 h at 30°C with different concentration of disinfectants (hypochlorite, ethanol, per acetic acid and QA). The generation time (and μmax) was calculated based on the 'growth curves' formed.

Results

Figure 13.2 shows the growth curve of one tolerant strain (A) and one sensitive strain (B) in the presence of different concentrations of hypochlorite. The generation time for the tolerant strain is about 3 h without any addition of disinfectants (Figure 13.2A). By increasing disinfectants in the medium, the generation time decreases threefold until the minimum inhibitory concentrations are obtained (2500 mg/l). A similar spectrum is observed for the sensitive strain apart from a smaller difference in generation time; the generation time decreases only twofold, but these strains have lower MIC-values than the tolerant strains (Figure 13.2B).

Figure 13.3 simplifies the results for all seven strains for all disinfectants used in different concentrations. The generation times for the tolerant isolates without the addition of any chloride were between 1.9 h and 2.8 h but decreased to 2.7 to 12.7 h at the highest concentrations where growth occurred (4–8%; Figure 13.3A). A decrease in generation time for the sensitive isolates was not observed except for isolate no. 8 (from 19.8 h to 53.3 h; Figure 13.3B). The MIC-values for hypochlorite were between 0.031% and 0.125%. The generation times for the tolerant isolates without the addition of any ethanol were between 1.1 h and 7.5 h, but decreased to 3.6 to 27.7 h at the highest concentrations where growth occurred (4–8 %; Figure 13.3C). The decrease in the generation times for the sensitive isolates was more drastic with higher concentrations of ethanol (3.2–7.3 → 26.7–34.6; Figure 13.3D). The generation times for the tolerant isolates without the addition of any peracetic acid were between 1.7 h and 19.3 h, and did not change very much at higher concentrations until the MIC values were reached (Figure 13.3E). A similar observation was made for the more sensitive

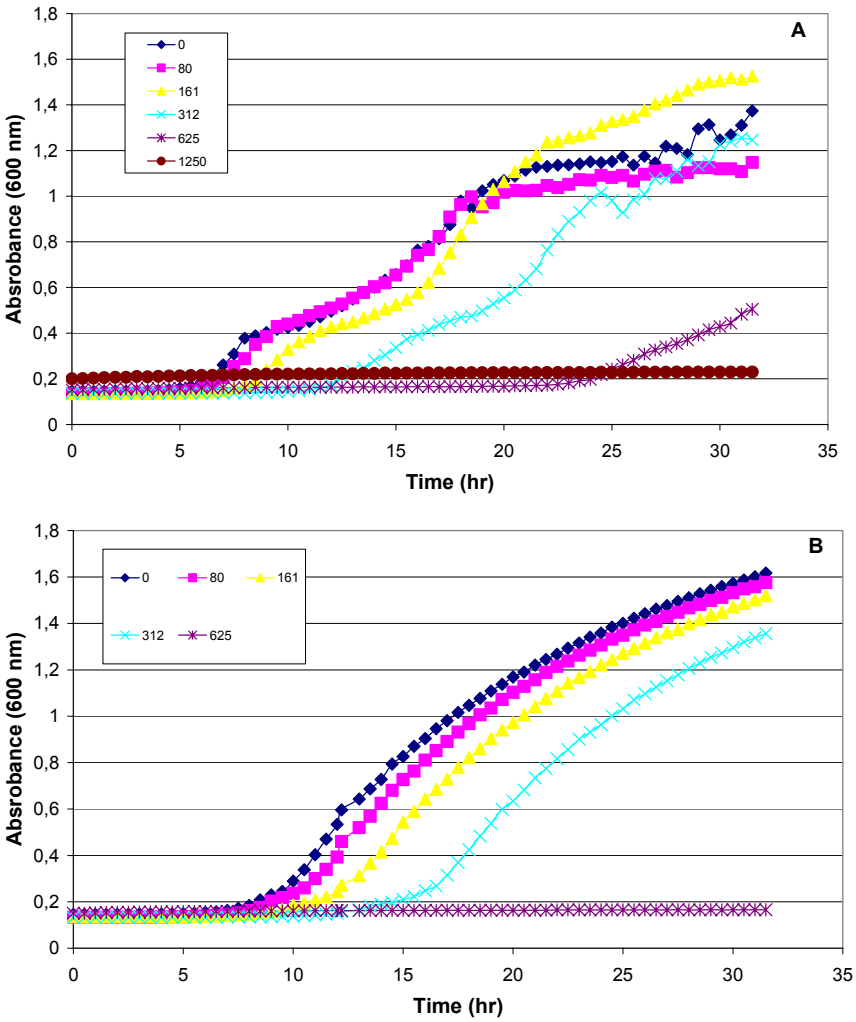

Figure 13.2. Growth curve of a tolerant strain (A) and a sensitive strain (B) in the presence of different concentrations of hypochlorite (mg/l).

strains, although generation times were generally higher (Figure 13.3F). Strain 8 did not grow under any conditions in this experiment. The generation times for the two tolerant isolates (no. 3 and no. 4) that grew without the addition of any QA were 1.1 h and 11.9 h. The generation times did not decrease significantly with higher concentrations. In fact, in the case of strain no. 3, the generation time seemed to decrease with higher concentrations until the MIC value was reached (Figure 13.3G). Similar results were obtained with one sensitive isolate (no. 6).

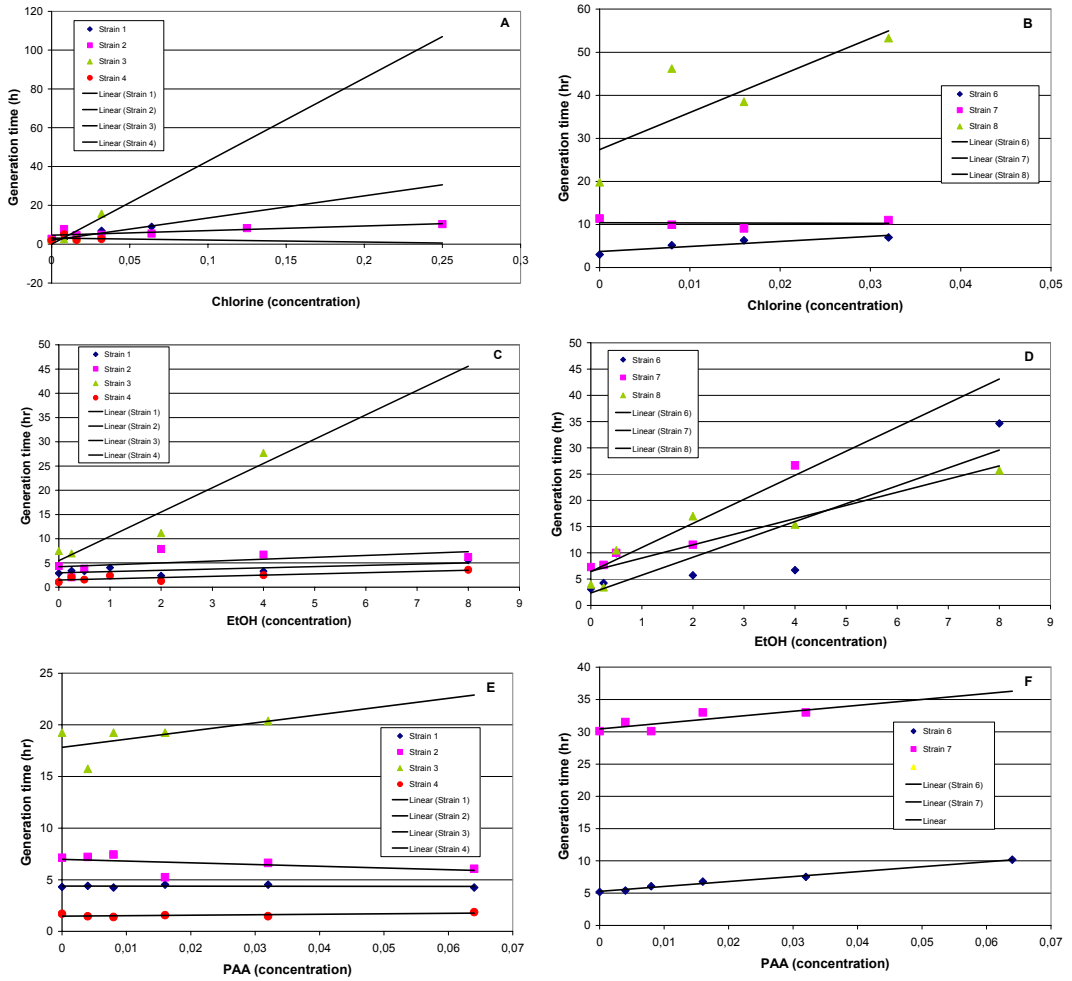

Figure 13.3. Generation time of selected isolates at different concentrations of hypochlorite (A = tolerant strain; B = sensitive strain), ethanol (C = tolerant strain; D = sensitive strain), peracetic acid (E = tolerant strain; F = sensitive strain) and quaternary ammonium compound (G = strain no. 3 and no. 4 are tolerant and strain no. 6 is sensitive).

Isolates no. 1, no. 2, no. 7 and no. 8 were tested for lower concentrations of the QAC used compared with the other isolates. There was no difference in the generation times for the various concentrations of these four isolates (results not shown). Thus, the inhibition is more of an on/off mechanism for this compound. The MIC value was usually around 0.3 mg/l for all isolates.

13.5 DISINFECTANT RESISTENCE PHENOMENA

Relatively little is known about disinfectant resistance among the bacterial flora present in the food industry. Some reports have indicated that the use of tenside-based disinfectants has resulted in a selection of resistant bacteria in clinical areas (Leelaporn *et al*., 1994; Nakashima *et al*., 1987), but less attention has been focused on resistance in food production environments. Hypochlorite is used at the dairy on a daily basis at a concentration of 3000 mg/l. It is therefore interesting to note that 12 strains had an MIC value of 5000 mg/l. Reports have shown that the frequency of resistance of *Listeria* spp. isolated from food towards QAC is 19% (Lemaitre *et al.,* 1998). Aase *et al.* (2000) showed that 4% of *Listeria monocytogenes* isolated within the food industry were tolerant and 13% were resistant.

Sundheim *et al.* (1998) reported that QACs were frequently used in the food industry. Thus bacterial resistance to QACs is the disinfectant resistance mechanism that has been studied most (Chapman, 2003). A huge difference in resistance against QACs for bacteria has been reported (Langsrud *et al.,* 2003b). It has also been reported that bacteria isolated from food process equipment (after cleaning and disinfection) are more likely to be resistant in comparison with bacteria isolated from raw food material or food products. Quaternary ammonium compounds are often left on surfaces, so bacteria that survive these conditions are more likely to develop resistance to these compounds. The present study observed great variation in MIC values for QACs. Strains that tolerated more than 36 mg/l were all isolated from places that are cleaned and disinfected regularly (results not shown). It is not possible to determine whether or not a strain is resistant on the basis of MIC values alone. Most staphylococci isolated within the Norwegian food industry had MIC values below 2 mg/l, but strains with MIC values above 4 mg/l were regarded as resistant (Sundheim *et al.,* 1998). The situation is different for the more tolerant Gram-negative bacteria. In the same investigation the authors classify pseudomonads as resistant if they tolerated 200 mg/l of QAC. Most of the strains classified as tolerant towards QAC were indeed isolated from places where QACs are used (results not shown). Some authors have shown that better results are obtained by using higher temperatures with QACs (Merianos 1991; Russell *et al.,* 1986).

14. RESISTANCE PHENOMENA IN DAIRIES DUE TO DISINFECTION

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The aim of disinfection is to reduce the number of microbes present on food-contact surfaces, thereby avoiding the contamination of raw materials and products with pathogens and spoilage organisms. When the disinfection process fails, this can in most cases be explained by the use of a too low disinfectant concentration, temperature, exposure time or a failure in the cleaning process that leaves soil on the surfaces to be disinfected. However, in some cases bacteria survive after an apparently effective cleaning and disinfection programme. One explanation for this could be that the susceptibility of the bacteria present in the process facilities is lower than expected. The recommended in-use concentration of disinfectants is often based on laboratory suspension tests and laboratory strains. Therefore, the range of bacteria tested in the documentation of the disinfectant applied may not reflect the flora present on the food production equipment.

For the users of disinfectants in the food industry and in other applications it is most relevant to define resistance as survival in practical use. However, since the efficacy of disinfectants is strongly related to the testing method used, resistance should be used as a relative term in a scientific context. If a microbe (or species) survives or grows in a higher concentration of disinfectant than another microbe (or species), it is said to have higher resistance. Within a species, strains that survive (or are not inhibited by) a concentration of disinfectant that kills (or inhibits) the majority of the strains of that species will be termed resistant. The level of resistance varies considerably between different species, and the natural level of resistance of a species is termed the intrinsic resistance. An example of a microbe with relatively high intrinsic resistance is the sporeform of *Bacillus* spp. Spores will survive most chemical disinfection processes used in the food industry. Another type of intrinsic resistance is the phenotypic, physiological adaptation of a microbe resulting from the growth conditions. Bacteria growing as biofilms on surfaces may survive 10-1000-fold higher concentrations of disinfectants than bacteria in suspension. Resistance may also be acquired by

mutation or acquisition of genetic material (plasmids, transposons). An example of microbes with acquired resistance is *Staphylococcus* sp. harbouring plasmids with genes encoding the efflux of quaternary ammonium compounds.

14.1 Resistance to Disinfectants among Food-Related Bacteria

Quaternary ammonium compounds (QACs) are commonly used disinfectants in the Norwegian food industry. The screening of food-associated *Pseudomonas* spp., coliforms, *Listeria monocytogenes*, *Staphylococcus* spp. and lactic acid bacteria showed huge differences in the level of resistance and the frequency of resistant strains within each species. It was also demonstrated that bacteria isolated from food processing equipment after cleaning and disinfection are more likely to be resistant than bacteria isolated from raw materials and food products. Gram-positive bacteria were in general more susceptible to QACs than Gram-negative bacteria. The isolates with the highest resistance to QAC among all the strains tested were isolated after disinfection with a QAC and identified as *P. fluorescens* and *S. marcescens*. (Langsrud *et al*., 2003b).

Resistance to QACs and other tenside-based disinfectants is well documented in the scientific literature, but less attention has been paid to oxidative antibacterial agents, such as hypochlorite- and peroxygen-based disinfectants. Enhanced resistance has been described for bacteria surviving disinfection with hypochlorite (Higginbottom *et al.,* 1964).

14.2 Resistance to Disinfectants in Dairy Isolates

14.2.1 Bacteria isolated from disinfecting footbaths

Disinfecting footbaths are used for the elimination of microbes on footwear to prevent cross-contamination between areas with a different hygienic level. A questionnaire about the use of footbaths was distributed to 30 Norwegian dairies. The most commonly used disinfectant was hypochlorite, followed by amphoteric tensides. A microbial analysis of samples from the footbaths showed that bacteria could be isolated from all types of disinfectants used, and bacteria were isolated from about 75% of the footbaths tested (Wirtanen *et al.,* 2002a). *S.*

marcescens isolated from footbaths containing the amphoteric tenside TEGO was resistant to the recommended in-use concentration. A laboratory strain of *Serratia marcescens* was susceptible. One of the strains tested multiplied in user concentrations of TEGO. The isolates were cross-resistant to a QAC, but could be eliminated by hypochlorite or peracetic acid. The conclusion from the investigation was that disinfecting footbaths should not be used without regular cleaning and hygienic monitoring (Langsrud *et al.,* 2003a).

14.2.2 Bacteria isolated after fogging disinfection

Fogging disinfection is the use of finely dispersed droplets of a disinfectant within the production facilities. It has been demonstrated that fogging disinfection can reduce the microbial counts in the air and on surfaces (Burfoot *et al.,* 1999). In this study, the effect of fogging disinfection was tested by means of contact agar plates before and after fogging disinfection in 5 dairies. A total of 10–19 control points were sampled in each dairy. The efficacy of the cleaning and the extent to which the fog filled the room varied greatly among the dairies and this was reflected in the microbial counts (Wirtanen *et al.,* 2002a). Six microbes isolated after apparently effective cleaning and disinfection in two dairies using rotational fogging with a peracetic acid-based disinfectant and an alkyl amino acetate-based disinfectant were identified and further characterised. The microbes were identified as *Methylobacterium* sp. (3 strains), *R. erythropolis*, *Sphingomonas* sp. and *R. mucilaginosa*. Four isolates as well as the two laboratory strains of *P. aeruginosa* and *Staphylococcus aureus* were subjected to bactericidal suspension tests that employed the recommended user-concentrations of six different commercially available disinfectants. The *Sphingomonas* sp. and the *S. aureus* showed little resistance, while the other isolates showed resistance to several disinfectants. Biofilm growth experiments on stainless steel indicate that the *Sphingomonas* sp. has a much higher ratio of early attachment than the other isolates, indicating the possibility that biofilm growth serves as a survival mechanism for this less resistant isolate.

15. Industrial Cases of Poor Hygienic Design!

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The requirements for the hygienic design of equipment are becoming increasingly strict, and there are many guidelines for hygienic design, including those by EHEDG and FDA. The guidelines are excellent, but hygienic design cannot be learned in theory alone: an important precondition for being able to design equipment for specific tasks is that the designer has detailed practical knowledge of the conditions where the equipment is used. This is where things can go wrong! Most food processing companies are becoming increasingly closed to visitors. This means that designers of food processing machinery receive less and less feedback over time. As a result, it becomes more difficult for designers to obtain the right practical understanding of problem areas, which in turn is essential if they are to keep up with developments required to optimise the hygienic design of equipment.

15.1 Examples of Poor Hygienic Design

Below, five examples of equipment with typical problems relating to hygienic design are reviewed. The examples focus on equipment that is not good enough and equipment that could be improved. In my evaluation I will also point out changes in equipment with regard to hygienic design and the effect of the design is assessed in relation to the following areas: product contact surfaces, product close contact surfaces and production environment/surroundings. The examples are:

1. Static fluid bed
2. DuoSafe plate heat exchangers (PHEs)
3. Cheese slicer
4. Bag filler
5. Plastic bottle filler.

15.2 Design Problems in a Static Fluid Bed

A static fluid bed is a unit located immediately below the spray tower of a powder production plant, which keeps the powder suspended for further transport. Two static fluid beds were evaluated for a project in the workshop immediately after manufacture. They were well made, but there was a design fault, which meant that four identical areas in each static fluid bed could not be cleaned properly. The static fluid bed illustrated shows that hygienic design is not always more expensive, and keeping things simple often provides good hygienic design at lower cost (Figures 15.1–15.4).

Figure 15.1. The Static fluid bed seen from the outside.

Figure 15.2. The perforated sheet above which the powder is kept suspended.

Figure 15.3. The underside of the perforated sheet in the static fluid bed including bearers. Note the cross bearer above the supporting bottom bearer.

Figure 15.4. A close-up of one of the four identical problem areas: a patch has been welded on to connect the cross bearer with the cylinder side of the static fluid bed. The patch serves no purpose, as the perforated sheet is itself welded to the cylinder side of the static fluid bed. The problem is that the jets from the CIP nozzles do not reach behind the welded patch, as the supporting bearer and the cross bearer on both sides shelters it. This produces a 30° pocket, which cannot be cleaned. If the four small patches had not been welded on, the result would have been a saving of approx. 6 h of work and a more hygienic design.

15.3 HYGIENE IN A DUOSAFE HEAT EXCHANGER PLATE

A DuoSafe PHE is a plate apparatus in which each individual DuoSafe plate consists of two thinner plates punched and pressed at the same time (Figure 15.5). A normal plate is usually 0.6 mm thick, while a DuoSafe plate is 2 x 0.35 mm thick. DuoSafe plates for PHEs were introduced in the early 1990s to act as a safeguard in a plate pasteur for instance, to ensure that non-pasteurised milk

does not mix with pasteurised milk. The safeguard entails that if a hole appears in the plate, the product will drip onto the floor and reveal the leak.

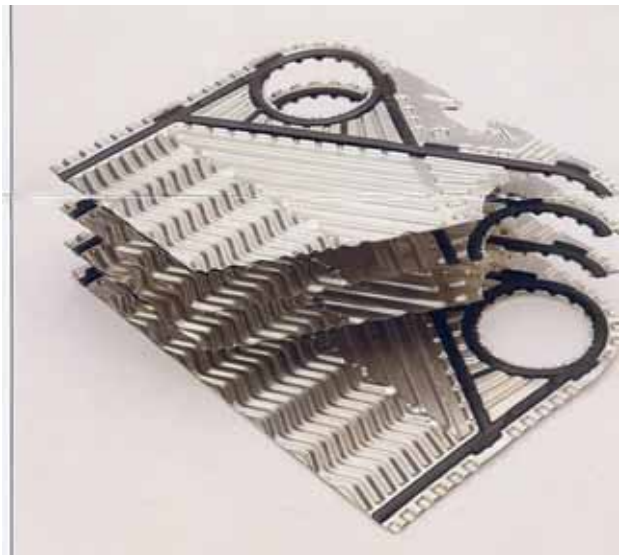

Figure 15.5. A DuoSafe plate.

The result of testing DuoSafe plates showed that the plates cannot be recommended for milk-type products which may coagulate. The test itself was carried out by drilling one 0.8 mm hole in 3 different sections of a cream pasteur, the pasteur section, the regenerative-2 section and the regenerative-1 section. The test period was three weeks and the result showed that drips appeared in the pasteur section and in the regenerative-2 section for 1½ h after start-up, after which drips did not occur during the remaining test period, not even after CIP cleaning. In the regenerative-1 section drips appeared for the first 3 h after start-up, after which they did not occur during the remaining test period, even after CIP cleaning. After 3 weeks, the apparatus was dismantled and the space behind the single DuoSafe plate with drill holes appeared as shown below. To understand the actual problem what happens in a plate apparatus when leaks occur must be examined. Typically, a hole begins as a very small crack – i.e. less than 1 x 10 μm. According to my argument, a hole in a plate does not start as a hole of 0.8 mm in diameter, but as a small crack of less than 1 μm x 100 μm. In other words, the 0.8-mm holes used for the test covered an area almost 5000 times larger than an initial hole during practical use. As a result, a DuoSafe plate rarely reveals whether there is a hole in it.

Other arguments exist against the use of DuoSafe plates, including the findings of a visual inspection which showed that corrosion easily occurs in the space of the an individual DuoSafe plate, as water penetrates the space when the plate apparatus is rinsed externally with water. Here, it will evaporate during the operation of the Pasteur. During the next rinse, more water will penetrate and each time, any chloride in the water will remain after evaporation. After repeated evaporations the chloride content in the space will be so extensive that corrosion is inevitable. The following illustrations are of the spaces in a used DuoSafe Pasteur. My visual inspection involved 650 plates from a Pasteur which had been used for 5 years, without any drips observed from the apparatus. I picked out a plate at random and took it apart. It was tested for cracks and one half was found to have 3 holes, the other 2. One wonders what the result would have been if the other 649 plates had also been tested for cracks (Figures 15.5–15.8).

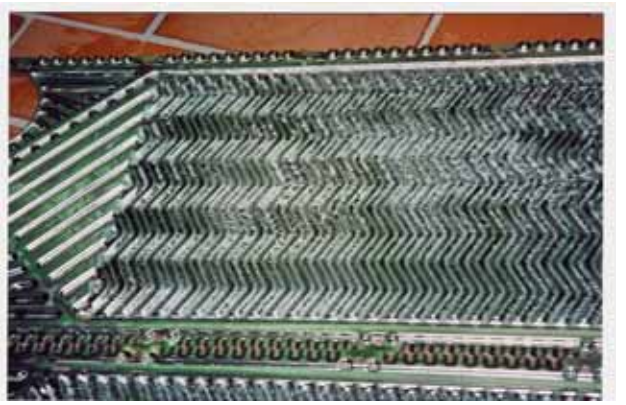

Figure 15.6. A greasy surface with an unpleasant odour from an opened DuoSafe heat exchanger.

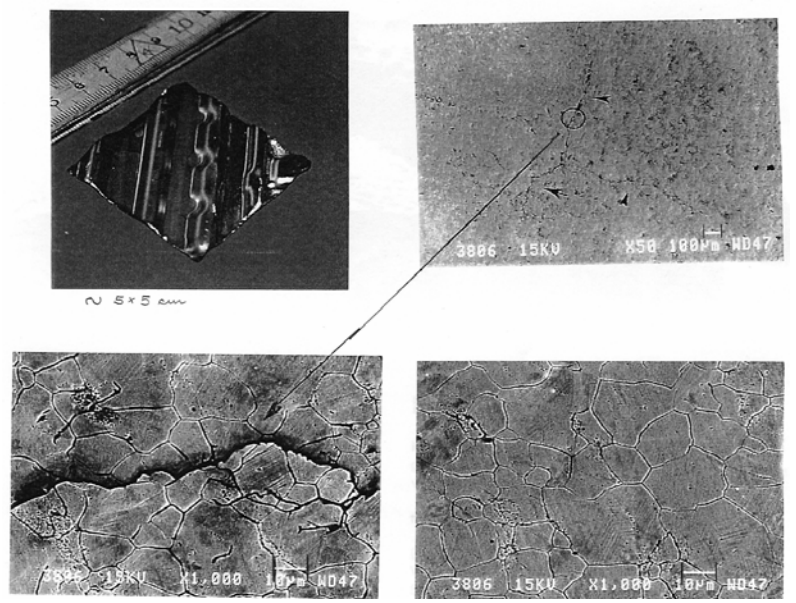

Figure 15.7. A 50x50 mm section of a plate from a leaky plate apparatus (top left); magnification of 50x shows the crack as a faint line in the picture (top right). Magnification 1000x shows how small the crack is (bottom).

Figure 15.8. Visual inspection of a plate from a Pasteur used for 5 years.

The problems of using DuoSafe plates in PHEs:

- A leak in DuoSafe plates cannot be observed.
- Cracks often appear in plates in PHEs, which results in accumulation of bacteria inside the DuoSafe plate as CIP cleaning does not clean the space within the plate.
- Corrosion easily occurs as a result of water penetrating the space in the individual DuoSafe plates.

DuoSafe PHE cannot be recommended for processing dairy products. Requirements for equipment producers:

- Equipment producers should pay more attention to the user comments regarding new equipment.
- Equipment producers should pay more attention to user comments regarding existing equipment.
- Equipment producers must investigate the risks associated with the use of the equipment they produce.

15.4 Cleaning a Cheese Slicer

The machinery in the slicer is part of the equipment evaluated in connection with monitoring an external-cleaning contractor. The machine was not hygienically designed, which makes cleaning difficult (Figures 15.9–15.11).

Figure 15.9. A cheese slicer with the access door open. First impressions were good, but closer inspection revealed some problems.

Figure 15.10. The underside of the conveyor belt. The conveyor belt is not easy to dismantle, as it should be and it is virtually impossible to clean underneath.

Figure 15.11. The access to the far corners inside the slicer is awkward and the corners are therefore not cleaned properly.

The illustrations of the problem areas in the slicer show that at least the following requirements must be established and fulfilled for the design of slicer:

- Belts should be easy to dismantle for cleaning the belt itself and the surfaces covered by the belt.
- Visible surfaces must be are cleaned – the machine should therefore have access doors on both sides.
- Corners and edges should be rounded more – rounding makes cleaning easier.

15.5 Quality Problems Caused by Bag Filler in Powder

The next example is a bag filler for powder (Figure 15.12–15.14), which is badly designed and very likely to contaminate the product at some point. The bag filler needs to be redesigned.

Figure 15.12. The filling funnel, which doses the powder into bags. A rotating 'snail' is fitted to keep the powder 'flowing' and it is driven by a geared motor containing oil. The gear is difficult to inspect.

Figure 15.13. The geared motor, which is located in the middle of the powder funnel. If a leak develops in the gear, oil will seep into the powder and therefore pass on to the consumer. Due to this, it is necessary to redesign the bag filler so that the gear is located outside the powder funnel. The gear must have a collecting tray on which any oil leaking out of the gear is collected so that it cannot run down inside the foil into the bag.

Figure 15.14. The level sensor that ensures the correct powder level in the funnel. If the sensor is removed, it can be refitted in several ways. If it is fitted incorrectly, the rotating snail may hit the level sensor and break off metal, which is mixed into the powder. This should not happen. A safety device, which is extremely easy to construct, must be installed to ensure that the level sensor is fitted in the correct position.

If the gear motor develops an oil leak in the bag filler, oil will drip down inside the foil and may reach the powder, if the gear motor pulling the foil roll is positioned as shown in Figure 15.14. If the gear motor was to be turned 180 degrees, the oil would only drip along the outside of the foil and not reach the powder. A better design would be to fit the gear motor outside the foil roll to eliminate the risk of oil getting into the product. The example shows that carelessness in the designing can lead to major risks in product quality. It is important to design equipment in such a way that the risk of foreign bodies/foreign matter getting into the product is minimised. At the same time, any risk areas and frequency of inspection should be listed in the user manual. Inspection must also be easy to carry out.

15.6 Defects in a Plastic Bottle Filler

The final example is a machine for filling plastic bottles with milk. This story dates from 1995, and is a good example of how our perception of hygienic design may vary. I often visited the UK in connection with various projects in 1995. During one of my visits, I had a look at some new filling machines that were being installed. According to our own staff, the filling machines met the strictest requirements in relation to hygienic design. I took a closer look at the filling machines and noticed that the machine had various problem areas. My observations led to a request for a report on the hygienic design of such a machine. When writing the report, I received a newsletter from the producer of the filling machines whose hygienic design I was in the process of criticising. The newsletter included an article on the above-mentioned filling machine stating that: 'Dairyfill complies with the very highest contemporary industrial norms relevant to hygiene'. Surely they must have got something wrong, because I was claiming that their 'newly-developed' machine was defective in several areas (Figure 15.15). An excerpt from my report follows below.

The points are followed by a letter to indicate the reason for comment, e.g. F. for 19 – h = hygienic risk, G. for 7 – hh = high hygienic risk, H. for 1 – pq = product quality, I. for 4 – s = safety and J. for 11 – o = others. A total of 42 points was obtained. About four weeks after the start-up of the filling machines, I had a meeting with the development staff of the equipment producer regarding my findings and claims about the machine. This was a case of a poorly designed filler. We first crawled under the machine and took the following photo, which

illustrates that the machine was not hygienically designed. After a review of the report, the producer initiated redevelopment of the new machine.

Figure 15.15. A machine producer's idea of hygienic design may be very different from that of a food producer. In this case, it meant that a newly designed machine had to be completely redesigned, costing time and money.

It is rather worrying that no specialised knowledge is required for designing and producing food-processing equipment. As a result comparison, sausages cannot be served in a cafeteria or kiosk in Denmark unless the person serving them has an appropriate certificate. Good advice for purchasing equipment:

- Set up a hygienic design group.
- Participate in the entire process from the basic idea through the design and development phases to installation and start-up.
- Demand that developers and designers representing the equipment supplier take part in the dialogue. These are the people who determine whether the equipment is hygienically designed or not. It is important that these people especially get a first-hand impression of the user requirements (ERFA).
- Evaluate the equipment thoroughly after 2–3 months of use. All partners should take part in this evaluation dealing with all problems noted.

16. HYGIENIC CONDITION OF DOUBLE-WALLED PLATE HEAT EXCHANGERS IN DAIRY USE

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Plate heat exchangers (PHEs) are widely used in the dairy industry for heating, cooling and regenerative duties both for utilities and products. The tightness of the unit is essential for the hygienic conditions of production. Modern PHEs are designed in a way that gasket leakage cannot lead to contamination between the different sections of the heat exchanger. The risk of recontamination is associated with leakage through the plate. Sometimes fatigue cracks, wear or corrosion occur and a hole appears in the plate.

16.1 FEATURES OF PHES IN DAIRIES

Suppliers of processing equipment have developed different methods to detect the condition of PHEs and security during production. One frequently used method is to use differential pressure in the sections so that product pressure is higher than media pressure or pasteurised product pressure is higher than unpasteurised product pressure in the regenerative section of the PHE. This is normally solved with a booster pump and constant pressure modulating valve (CPM). Pressure difference is also monitored at critical control points and records are kept for detection purposes.

There is also another way to reduce the risk of recontamination in case of leakage through the plate. This concept is based on the idea that if you press two plates at one time, the pattern of the plates is not absolutely similar and thus a leakage channel is formed between the plates.

This concept was originally used to create more security in cases where there, was a danger of explosion i.e. electrical transformer plants where the leakage of water into the oil causes a short circuit in the unit. In those PHEs the layers of double-walled plates were welded together around the corner channel. Because it must be possible to open food heat exchangers for inspections, the usage of those

PHEs in food processing was not possible. Moreover, also the double edge of the plate was facing the media in the corner channel hole. This is not in accordance with the food design guidelines.

Some years ago double-walled PHEs appeared on the market for use at dairies. Their plates were no longer welded together but just pressed together by means of screws in the frame of the PHE. The corner hole diameters were different in the primary and secondary plates of the double-wall plate and the product could not enter between the plates, as was the ease with the welded one.

A test was made by a German institute to check whether leakage takes place if one layer of the double-wall plate is pierced. The test showed that a hole in one of the layers of the double-wall caused a leakage detectable by visual inspection. However, the experience of using double-wall heat exchangers in dairy applications is quite limited. Because there are no scientific studies available and information given in magazines is limited, it was decided to make a series of tests on the hygienic conditions of double-wall heat exchangers.

16.2 BACTERIA GROWTH BETWEEN THE LAYERS OF DOUBLE-WALL PLATES IN PHES

Dummy plates were installed in the double-wall heat exchanger at two points: one in the hot end and the other in the cold end of the PHE. The surface of the double-wall layer facing the space between the layers was contaminated with milk and cream. The heat exchanger was opened five weeks later and swab samples were analysed in the laboratory. Some swab tests were also taken from the original plates in the PHE (Table 16.1). The incubation was performed at 30ºC for 3 days.

Bacteria growth is limited in the PHE. The unit is heated up during CIP (approx. 80ºC). Heating prevents the growth of the bacteria. The test did not cover thermo-resistant spores because of the single incubation temperature. During the opening of the PHE, corrosion was found between the double-wall plate layers. Valio Ltd decided to send a plate to be analysed at VTT. In the report VAL74-012841/EN, dated January 16, 2002, the conclusion was that the stress corrosion cracking of the milk lamellas studied was caused by the combined action of the enrichment of chlorine and high operating temperature. The pitting in sour milk

lamellas, aggravated by high temperature, is caused by the enrichment of chlorine. Chlorine is enriched on the surface of lamellas by moist air.

Table 16.1. Microbial test on original and dummy plates in the PHE after 5 weeks in operation.

Sample	Location	Total plate count (CFU)	Remarks
1.	Cold, original plate	< 1	
2.	Cold, original plate	< 1	
3. contaminated with product	Cold, dummy plate	2	
4. contaminated with product	Cold, dummy plate	>300	Very small seats
5. contaminated with detergent	Hot, dummy plate	< 1	
6. contaminated with detergent	Hot, dummy plate	< 1	

16.3 RECOMMENDATIONS FOR OPERATION AND PREVENTIVE MAINTENANCE OF PHES

As a result of the test that we carried out and the study made by VTT it can be said that the use of double-wall PHEs requires some additional consideration in plant design, operation and preventive maintenance:

- Because the thermal effect of the double-wall plate is poor, more plates are needed to perform the same duty as single-wall plates. This causes either a greater pressure drop in the PHE or lower flow per channel. Lower flow per channel may cause cleaning problems in hot sections. Sometimes narrower channels (more shallow pressing depth) are used to obtain better heat transfer. This may cause early clogging of the PHE due to the fouling in the hot section. A shallow pressing depth is normally used in industrial PHEs and water heaters.

- Product and cleaning detergent flushes on the PHE should be avoided because of the risk that capillary forces will suck it into the leaking chamber and may cause corrosion or bacteriological growth between the plates. It is highly recommended that covers are added to the PHE for this reason.
- The leakage chamber is in contact with the atmosphere. Temperature changes in the PHE cause a ‘pumping’ phenomenon. This may make the surfaces in the leaking chamber soiled. For that reason the double-wall PHE must sometimes be opened for cleaning. The plates can be visually checked for leakage at the same time.
- If leakage takes place in the hot section, there is a risk that the leaked product will dry in the chamber and cause clogging. This prevents leakage into the atmosphere and makes it impossible to detect visually. In particular, a small crack in the plate may cause limited leakage.
- Regular pressure testing is recommended to find small cracks. If the product leakage dries in between the plates, there is a high risk that corrosion will also make a hole in the second plate.
- For these reasons, the purchase and operating costs of a double-wall PHE are higher compared with the traditional PHE. It is recommended that a double-wall PHE is used only if there is no other way to solve the problem.

17. SURFACE ROUGHNESS OF STAINLESS STEEL – DOES A VERY LOW R_A-VALUE ENSURE BETTER HYGIENE?

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Modern food processing requires top hygiene; pathogenic and destructive microbes must be strictly controlled. And that is difficult. Bacteria and other microbes are very adept at adhering to surfaces and hiding in minute cracks in processing equipment. There are numerous examples of bacteria adhering so well that they are not removed by ordinary cleaning and disinfection and therefore can become a permanent source of pollution of the products, for instance, within the food processing industry.

17.1 MODERN FOOD PROCESSIGN REQUIRES TOP HYGIENE

For the past decade, there has been a strong focus on processing equipment and lay-out from a hygiene point of view and the concept of 'hygienic design' has become a mantra. There are many excellent guides and guidelines (such as the machine directive) on the design and layout of processing equipment under the auspices of, interalia, EHEDG (the European Hygienic Engineering Design Group, www.ehedg.org). Brief descriptions of these are available on www.staalcentrum.dk. Typically such guides are phrased very generally – for instance 'surfaces must be smooth and easy to clean...'. For a producer specifying requirements to an equipment supplier, such terms are far too imprecise and there is a great need for more measurable definitions, for instance, of what is hygienically satisfactory for a surface. The more difficult it is for dirt and microbes to adhere to a surface – and the easier it is to remove both again – the more hygienic a surface will be.

Today a surface is generally characterised by a series of parameters describing the two-dimensional topography. Examples are R_a, R_t and R_z values. The R_a

value, which is the average value of variances from the average height of the surface, is particularly often used and it is generally recommended that the R_a value be 0.8 μm or less. This recommendation, like others, implies that it is generally assumed that the more uneven and rough a surface is, the more unhygienic it will be. We have examined the importance of the R_a value in relation to the adhesion of bacteria to stainless steel surfaces, their cleanability and their corrosion resistance. We have mainly focused on R_a values under 0.8 μm in order to assess whether such very smooth surfaces are more hygienic – and for instance suitable for use in inaccessible corners. This research was carried out under the auspices of a 'Centre for Hygienic Design' supported by the Danish Agency for Trade and Industry.

17.1.1 Stainless steel surfaces

Stainless steel was polished with granule 4000 or electro-polished, which resulted in surfaces with R_a values between <0.08 μm. Polishing with granule 120 and granule 80 produced R_a values of approx. 0.5 μm and approx. 0.9 μm respectively. In order to assess corrosion resistance, glass-blown surfaces with an R_a value of approx. 2.0 μm were also produced. The surfaces were assessed by means of electron microscopy and polishing scratches were evident on granule 120 and granule 80 polished surfaces, while the electronically polished surfaces appeared completely smooth, almost mirror-like.

17.1.2 Model systems and bacteria quantification

Stainless steel surfaces were examined in two systems. One is a very simple laboratory system, where small surfaces (typically 1 x 2 cm) are placed vertically in a holder and dipped in various bacteria suspensions (Figure 17.1). We cultivated both bacteria and yeast, harvested the cells and re-suspended them in buffer, which is poured over the vertical steel-sheets. At regular intervals over a 24 h period, sheets were removed and rinsed, and the bacteria on the surface quantified. The other is a flow-system (the Kolding test rig), where holes have been drilled in a square pipe so that various surfaces can be placed in them in a kind of plug-arrangement (Figure 17.1). Dirt and bacteria suspension can be pumped through the system and in these tests a growing culture of *Pseudomonas aeruginosa* was flushed through the system for 24–48 h. After soiling and possibly cleaning, the plugs can be removed and the number of bacteria

assessed. A complicated step in such work is the quantification of the adhering bacteria. They stick and one cannot be sure of picking up all the bacteria with, for instance, agar-contact-surfaces or swab methods. If there are sufficient bacteria, they can be coloured with fluorescent materials and subsequently quantified in fluorescence microscopes. As these tests have also included surfaces with relatively few bacteria (after cleaning), the fluorescence microscopic method is inadequate. We have used the conductometric method, where the entire surface (after non-adhering bacteria have been rinsed off) is placed in a nutrient medium. A couple of electrodes are inserted and changes in the conductivity of the medium are measured. The speed with which the conductivity changes is proportionate to the original number of bacteria. A standard graph can be constructed and the number of bacteria on the surface calculated on that basis.

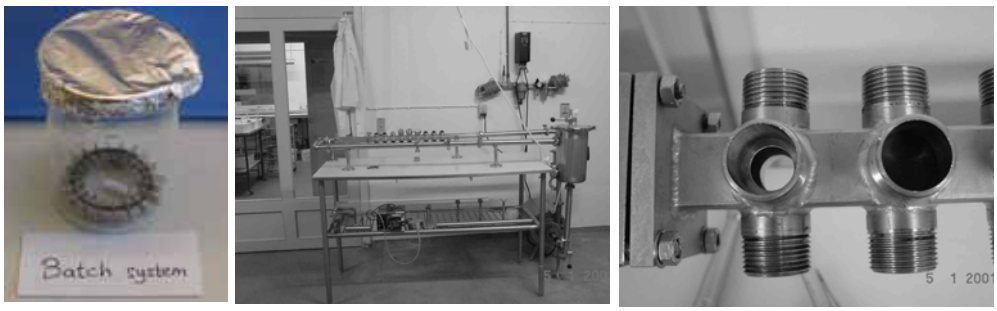

Figure 17.1. Simple laboratory system for testing bacteria adhesion (left), Kolding test rig for testing hygiene (middle), close-up of Kolding test rig (right).

Both bacteria and yeast adhered equally well to the different surfaces in the simple laboratory system, and there was no difference in the flow system either (Figure 17.2). In other words, the R_a values were irrelevant to the number of adhering microbes. The flow system was also used to test if the cleanability of the surfaces differed and they were cleaned with both low and high flow. There was no difference in the removal of bacteria dependent on the smoothness of the material. After cleaning, the largest number of bacteria was found on surfaces cleaned with low flow. It should be added that the R_a value may influence bacteria-adhesion; especially when the values are very high i.e the surfaces are very rough (Hilbert *et al.,* 2003).

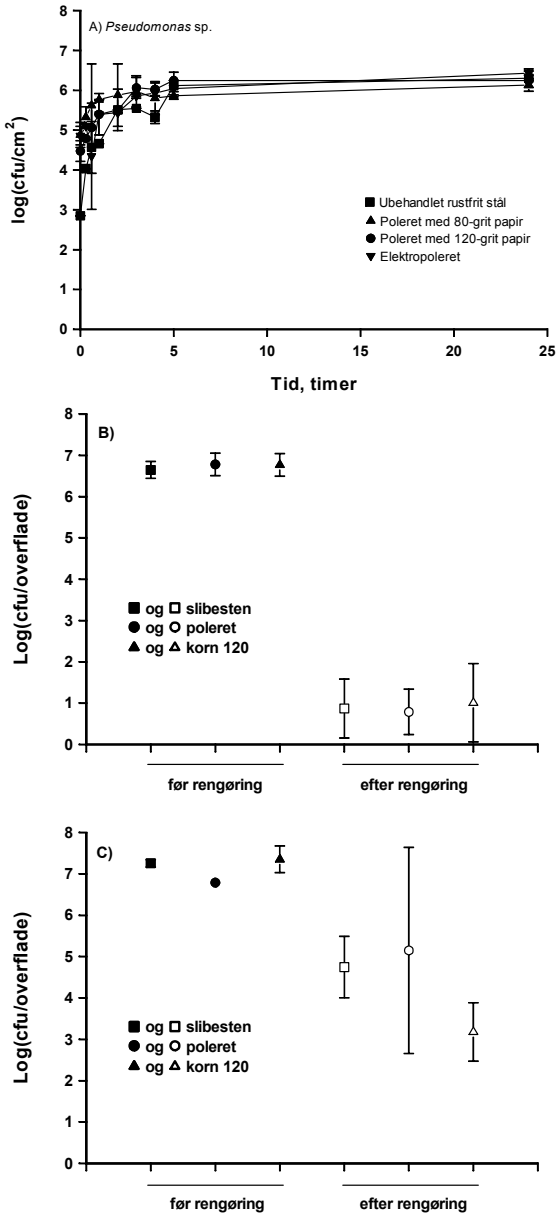

Figure 17.2. Adhesion of bacteria to stainless steel surfaces in a simple laboratory system (top) and to surfaces in a flow system (middle and bottom). Open symbols (middle and bottom) indicate the number of bacteria after cleaning with high flow (middle) and low flow (bottom) respectively.

17.2 CORROSION RESISTANCE OF SURFACES

The corrosion resistance of the surfaces was assessed by measuring the pitting potential through electro-chemical polarisation. The surfaces were treated with either an oxidising disinfectant or with 1 M sodium chloride. In contrast to the bacteria adhesion and removal, the smoothness/roughness of the surface was here found to have a definite effect, as the roughest surfaces had the lowest pitting potential and, therefore, the lowest corrosion resistance (Figure 17.3). Pitting potentials cannot on their own describe the corrosion resistance of a surface; the stability of the current should also be measured. Thus a surface polished with granule 80 had a high pitting potential, but the current varied considerably, indicating formation and termination of micro-pits which in the long term will lead to corrosion (Hilbert *et al.,* 2003).

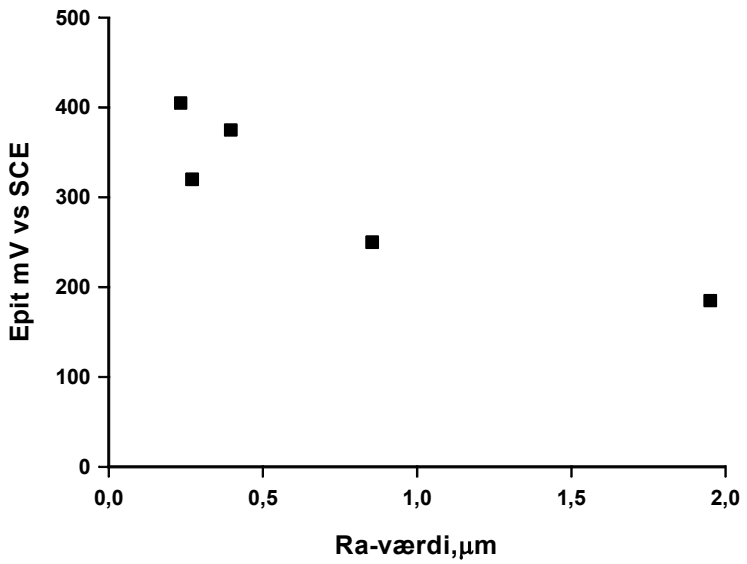

Figure 17.3. Pitting potentials for AISI 316 steel in 1 M sodium chloride as a function of R_a value.

18. DANISH CENTRE FOR STAINLESS STEEL

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A new competence centre for the stainless steel industry has been set up in Kolding, Denmark in 2003. The consortium consists of the Centre for Small Company Research at the University of Southern Denmark, CEU Kolding, the Trade and Industry Council, the Steel Group Triangle Area and the Danish Technological Institute, with financial support from the Ministry for Science, Technology and Development for 2003–2005. The purpose of the centre is to enhance the future competitiveness of the stainless steel industry regionally and nationally by:

- Promoting innovative, knowledge-based product development
- Developing and expanding existing positions of strength within the food processing industry
- Creating access to new market segments, such as the pharmaceutical and biotechnological industries
- Establishing a forum where users and producers can meet around technological and business challenges
- Increasing companies' access to relevant, knowledge-bearing institutions
- Offering relevant training at all levels.

A network of some 130 people has been established with representatives from the entire value chain. From the participants, six working groups have been set up to prepare guidelines on 1) material specification, 2) trade specifications, 3) mechanical joints, 4) conveyors, 5) cabling and 6) flow components. Through these activities, any barriers to collaboration will be broken down.

An important part of the centre's work is to take the producers' need for knowledge as a starting point and then distribute it to the participants. The centre analyses and assesses the critical properties of stainless steel for industrial use (called 'Priority Analysis'). The purpose of the analysis is to obtain information about the specific challenges and competence requirements of the industry

providing an indication of the specific contents and initiatives that the centre should initiate and implement. In the analysis, the companies are categorised as sub-suppliers (equipment and components), installation suppliers and buyers (producers of food, medicine and biotech). The report is available on the centre's information portal www.staalcentrum.dk under the heading *Viden TANK* (Knowledge TANK). Process optimisation and cleanability of production equipment are significant competitive parameters within the food processing industry. As result competencies such as hygienic design, material technology and production techniques are important. The centre will develop hygiene testing methods which companies can subsequently apply themselves. The tools and methods that are developed must be capable of testing e.g. the cleanability of material surfaces, welds and the adhesion power of bacteria. This will allow companies to test design, properties and quality during the construction phase and the subsequent quality control phase.

The centre creates an overview of standards, guidelines and legislation within the fields of hygienic design and safety. It continuously gathers knowledge about requirements and guidelines for the hygienic design of equipment, installations and production areas and the information gathered is structured and made available on the information portal www.staalcentrum.dk. The information will be used in connection with training and project activities at the centre and to form the basis for new guidelines that are being drawn up by the working groups. The centre is in an ongoing dialogue with market-led standard-setters. The centre offers short and long courses in hygiene, material selection and hygienic design, partly in order to incorporate new knowledge into existing training, and partly to develop new in-service training for managers, owners, designers, quality-testers, smiths, etc. within stainless steel companies. The courses will be offered in collaboration with the Danish Technological Institute and CEU Kolding.

19. THE INTEGRATED APPROACH TO HYGIENIC ENGINEERING

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The demands placed on products and processes within the food production industry are interconnected. Therefore, it is not appropriate to use a mainly sequential approach when designing a production facility, where the first thing is to address the primary function of the product, i.e. product quality, while considering other issues as add-ons to the process. These include issues such as safety, hygienic design, cleaning, agility, careful processing and control with respect to traceability. It is not likely that an optimal process is obtained from optimising one aspect at a time. These issues need to be integrated in order to arrive at the best decisions and balance different needs.

19.1 HYGIENIC ENGINEERING

Poor decisions are often made during the sequence of designing, fabricating, installing, contracting and making design changes, or when maintaining a production assembly, a line, or a facility, because the sequential approach to problem-solving is adopted. This way, hazards may be unintentionally created in the process line, such as leaving a valve on a branch closed, thus creating a dead end, or simply placing equipment inexpediently, making cleaning very difficult.

Another important issue for obtaining a line that runs optimally is to make sure it is operated systematically. One way to ensure high performance is to implement HACCP and GMP, which primarily deal with hygiene, cleaning and critical control point monitoring. Furthermore, high performance is ensured by employing changes in management, by establishing and maintaining documentation with regard to installation, automation, operation, maintenance, and cleaning as well as by testing the operation and performance of the equipment before routine use.

A subgroup of the European Hygienic Engineering and Design Group (EHEDG) is currently producing a guideline on Hygienic Systems Integration (HSI). The

ideas presented here are part of the imminent EHEDG guideline on HIS. The EHEDG guideline has the task of linking and supporting current guidelines on hygienic design regarding specific equipment and hygienic tests, and which can be viewed as vertical guidelines. The HSI guideline, on the other hand, is classed as a horizontal guideline, which is a completely new approach. Neither the EN1672-2 nor the HACCP standards are replaced by the HSI guideline.

19.2 HYGIENIC INTEGRATION

The integrated approach to hygienic design is a systematic way of combining hygienic entities into a hygienic facility. This may be a new design or reassignment of existing entities. An entity is a component, which is part of a hygienic system, and can be a part, an assembly, a module, a line, or a factory. Part of the scope of the HSI guideline is:

- ⇨ to describe the integration of entities, including the manufacture and supply of goods, in order to produce safe food or related products cost effectively, and
- ⇨ to describe integration topics that can affect hygienic design, including installation, operation, automation, cleaning and maintenance, especially those that are common or a frequent cause of failure.

The guideline defines ‘hygienic integration’ as a process of combining or arranging two or more entities to work together while eliminating or minimizing hygiene risks. While the focus is on the hygienic standard of the equipment, there are many surrounding issues that must be controlled in order to complete ‘hygienic integration’. For example, a facility must conform with all specified requirements, which may originate from legislation, users, product quality or safety. The integrated approach also involves determining specifications for product flow, control strategy, automation, maintenance, change management and training of personnel. Furthermore, implementation of HACCP and GMP is a necessity. A failure mode and effect analysis (FMEA), which is a structured, equipment-based safety tool based on risk assessment of the consequences of failure of any parts of a process may also be carried out.

The integration process comprises a set of actions, which are given in Figure 19.1. Each step is carried out by following a flow diagram, which takes the user

through the necessary steps in order to complete each particular action properly. Examples of such flow diagrams are given in Figures 19.2 and 19.3.

Each integration-action must have at least a prospective validation identifying probable failure modes. Hygienic integration should be carried out on a modular basis with entities that have already passed the functional requirement for integration. Instructions must cover: installation, operation, cleaning, sterilisation (if applicable) and maintenance. Concurrency with design and validation activities other than those concerned with hygiene is naturally a prerequisite. For an unassigned module or assembly, the provisionally intended process or processes and product(s) must be defined in a prospective list.

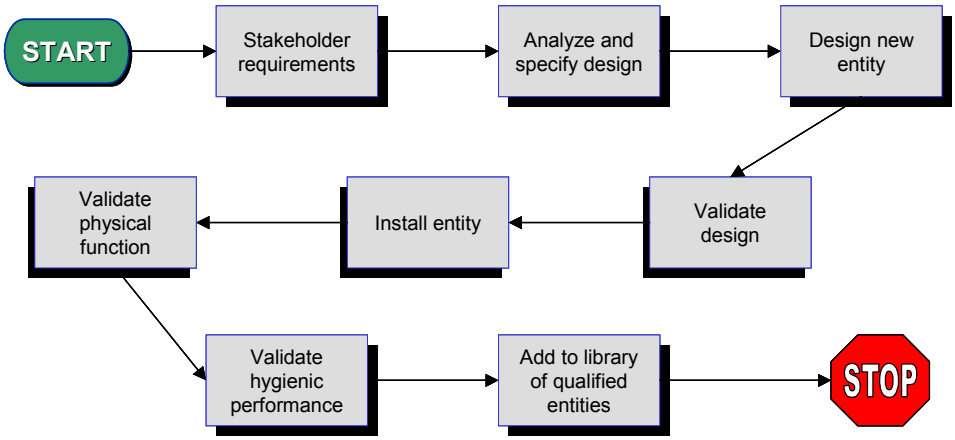

Figure 19.1. The figure shows a single integration displaying the required integration actions.

The first action is to determine the stakeholders' requirements, which can originate from customer, food safety, environmental legislation, or some other type of constraint. After listing the stakeholders' requirements the user goes through the first flowchart: 'Analyse and specify the design', given in Figure 19.2.

Going through the stakeholders' list of requirements should produce a conceptual design for the entity or entities under examination. Every time such a stage is completed, the flow diagram takes the user through a confirmation step, making sure there is compliance between the information obtained and the outcome of the analysis. For example, if the user forgot to take some legislation issues into consideration in the conceptual design, the user should be able to

notice this before going on to specify the design in more detail. The flow chart also asks to record data produced during the decision process and to record the decision itself (Figure 19.2). The user then continues through the integration 'snake' (Figure 19.1), and goes on to design the new entity, validate physical function, install the entity, validate the design, and the hygienic performance. There is a separate flowchart for each of these actions taking the user through the necessary steps to complete a particular action.

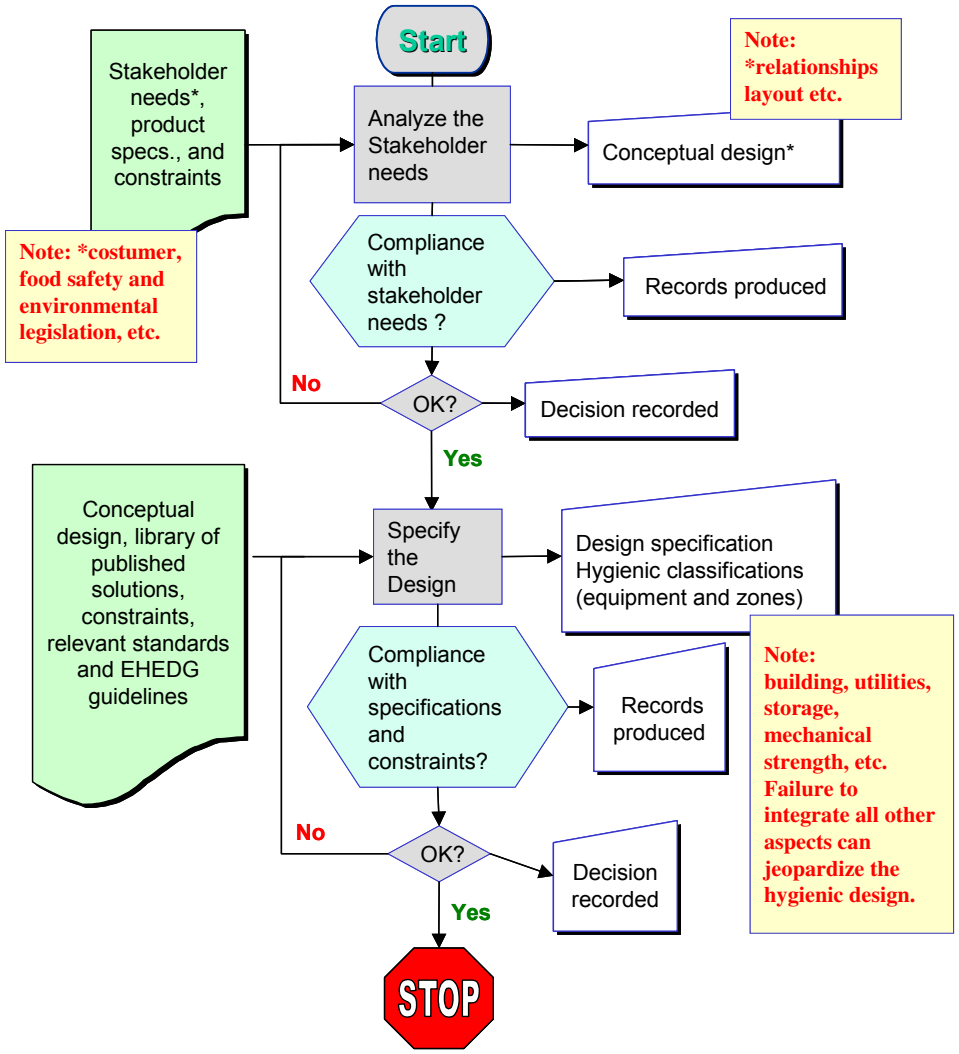

Figure 19.2. Flow Diagram for the integration action: 'Analyse and specify the design'.

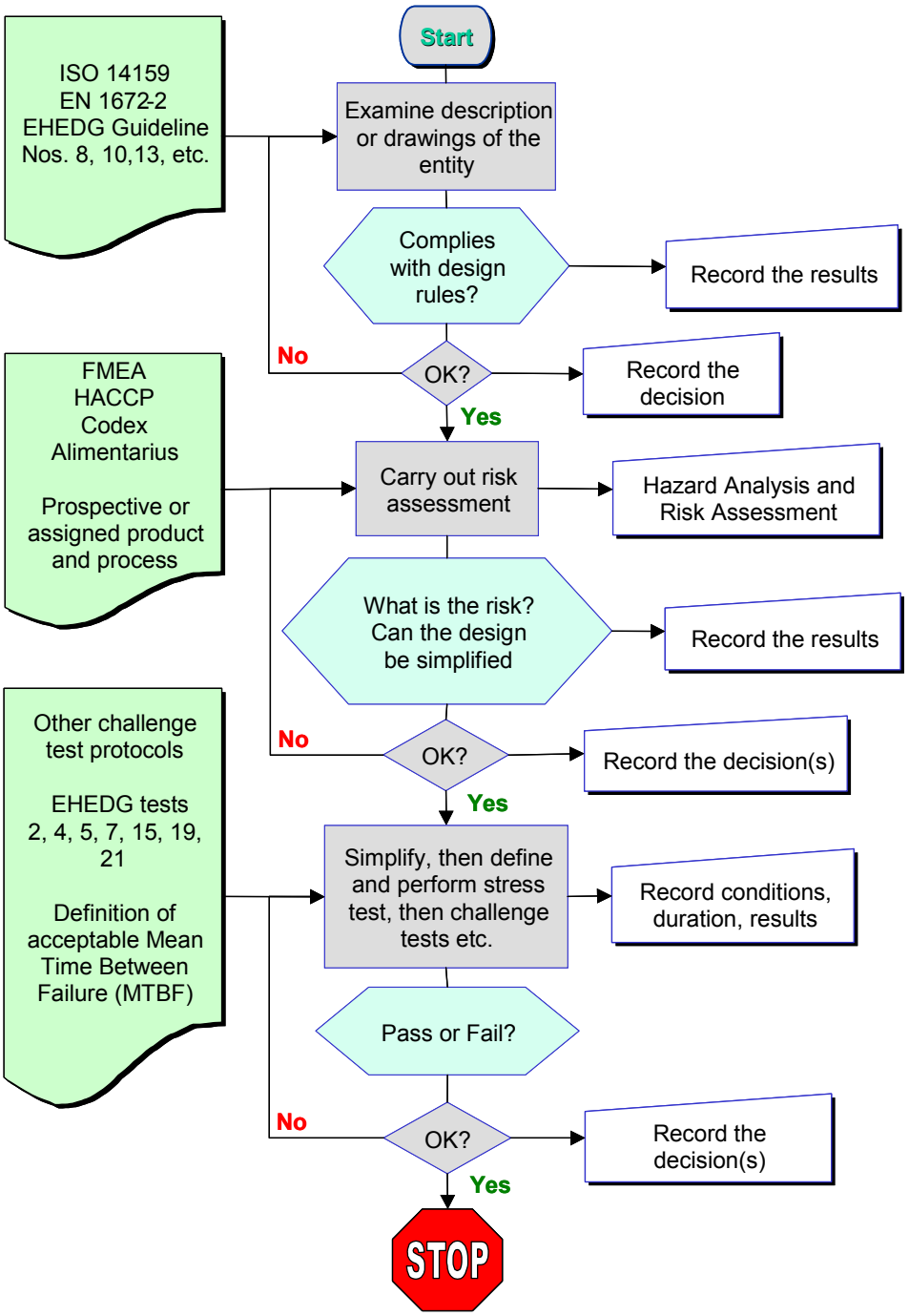

Figure 19.3. Flow diagram for the integration action 'Validate hygienic performance'.

An example is given here for the 'Validate hygienic performance' flowchart (Figure 19.3). The incoming information for the first step is provided by examining the description or drawing of the entity with respect to the guidelines on the safety of machinery, i.e. NSF 14159 or EN 1672-2, as well as the EHEDG guideline on 'Hygienic equipment design criteria'. Depending on the entities to be integrated, EHEDG has published guidelines on:

⇒ 'Hygienic design of closed equipment for the processing of liquid food',
⇒ 'Hygienic design of equipment for open processing',
⇒ 'General hygienic design criteria for the safe processing of dry particulate materials'
⇒ 'Hygienic engineering of plants for the processing of dry particulate materials' or similar.

The second step is to perform a risk assessment, which in practice means performing a FMEA and HACCP analysis, while the third step is to test the entity. Depending on intended use, one or more of the EHEDG tests for sterilisability, in-place cleanability, or bacteria tightness may be applicable. The acceptable mean time between failure may also be determined at this time.

After completing the validation of the hygienic performance, the entity has been integrated successfully, and can be implemented for the specific process to which it was assigned. If the entity has not been assigned to a particular product or process, it can simply be added to the library of unassigned entities.

20. Assessing Residues of Detergents and Disinfectants with a Photobacterial Test

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The focus on hygiene in the food industry has resulted in an increased use of chemical disinfection. It has been questioned if misuse of disinfectants has imposed a selective pressure contributing to resistant microbes. The total cleanliness of the process facilities prior to production start-up is mainly based on measuring the bacterial load with traditional microbial tests. It is possible that the test result is not an indicator of the cleanliness, it only shows that there are no viable microbes becuase of chemical residues of sanitisers left on the surfaces. This is not allowed but usually no tests are run to avoid it. The method based on light inhibition of luminescent bacteria can be used to measure residues both in liquids and on surfaces.

20.1 Detection of Detergents and Disinfectants

The luminescence inhibition method with *Vibrio fischeri* photobacteria is a useful tool to estimate the toxicity of different chemicals and effluents by measuring the reduction of light production due to interactions between bacteria and toxic compounds (Dutka *et al.,* 1991). The method is rapid, because the incubation time is from 5 to 30 min. The test is standardized for water and effluent samples (ISO 11348-3, 1998). The test method can be used to measure residual cleaning agents and disinfectants on production surfaces (Lappalainen *et al.,* 2000). According to the findings there could be a need for this kind of test. However, there is a very limited amount of published data available about the situation in the food industry regarding the residues, because the chemical methods available are difficult and expensive to perform. Therefore, a study was performed during 2001–2002 in Sweden, Finland, Norway, Denmark and Iceland as a part of the 'DairyNET – Hygiene control in dairies' project to obtain more data about the performance and usefulness of the test.

Because most of the people involved in this study had no previous experience about toxicity tests and photobacteria tests, the study was performed in three different phases. Phase one was the introduction to the method, reagent and instrumentation. The users were given detailed instructions and reagents to perform the test and, after

receiving acceptable results, they moved onto the second phase. In this phase 10 unknown samples were sent to the laboratories for an intercalibration study. In the third phase samples from the production facilities were tested.

20.1.1 Principle of the BioTox test

The test was performed by combining specified volumes of the test sample or diluted sample with the luminescent bacteria suspension in a cuvette. The parameter measured is the decrease in luminescence after a contact time of 5 min. The values measured are compared with a control sample and the intensity chances in the control sample are taken into account by using a correction factor when the results of the samples are calculated.

The test was carried out using *V. fischeri* bacteria from the BioTox kit (Aboatox, Turku, Finland). A standard ISO protocol was followed with the exception that the pH of the samples was not adjusted and the salinity was not adjusted for swab samples. The pH should be between 6–8 and salinity should be 2%. The light-producing *V. fischeri* bacteria were reconstituted from the freeze-dried vials with the rehydration solution and stabilized for 1 h at 4ºC. Instead of adjusting the salinity of the liquid samples, the salinity of the test bacteria solution was adjusted with 1 ml of 20% NaCl solution from the kit. The NaCl addition gives a final NaCl concentration of 1.7% during the measurement. For the swab samples no salinity adjustment was done, because the sample volume is about 100 µl and the resulting NaCl concentration during the measurement, as with liquid samples. The dilution series of disinfectants and cleaning agents was made with tap water.

The test was performed using 500 µl *V. fischeri* suspension, which was transferred into the measuring cuvette and the light output was measured with a luminometer. This reading represents the light production of unstressed bacteria and it was performed for all sample tubes including the controls. After this first measurement the samples (500 µl) were mixed with the bacteria, and the tubes were incubated for 5 min. The second reading was taken after the incubation, and the light production was measured from the control and samples. The effect of agents causing inhibition in light production was calculated and compared with the light output of unstressed bacteria. The results are expressed as an inhibition percentage (INH%), which was calculated with BioTox software according to formulas [1] and [2]:

$CF = IC_5/IC_0$ [1]

$INH\% = 100-[IT_5/(CF*IT_0)]*100$ [2]

where
CF = correction factor
IC_5 = light production of the control after incubation (5 min)
IC_0 = light production of the bacterial suspension (control tube)
IT_5 = light production of the sample after incubation (5 min)
IT_0 = light production of the bacterial suspension (sample tube)

The effective concentration of the detergent tested that resulted in an inhibition of 50% of light production (EC-50 value) was measured and calculated according to a standard using results from the dilution series of the detergents. For the factory samples only the inhibition percentage was calculated.

20.1.2 Measurement of detergents/disinfectants in dilution series

Laboratories were given instructions to test a dilution series from three different types of detergent or disinfectant samples normally used in the dairy. Results were calculated and assistance given if needed. This was done in order to see that the instrumentation was suitable for the testing in each laboratory and that the reagent handling was acceptable. A dose response curve was the target result for each chemical.

20.1.3 Measurement of supplied samples of detergents/disinfectants

Ten coded samples were supplied in 4.5 ml tubes (chemicals and concentrations in Table 20.1) to six different users in five different laboratories. The concentrations were chosen so that the results should show all types of results from clear inhibition to no inhibition. Swabs were also supplied except, to one laboratory. One of the samples was water (= zero sample and diluent for the samples). If a swab sample was measured, it was first inserted into the sample liquid and the stick was then cut with scissors and left to the bacteria cuvette during the incubation and measurement. The samples were run in duplicate. Chemicals that were thought to disintegrate rapidly in a diluted concentration were shipped in stronger concentrations, and laboratories were instructed to do more dilutions of these. FIN 2 was the reference laboratory. They prepared the samples and tested the samples first.

20.1.4 Measurement of the samples from the dairy

The participating laboratories took samples from a dairy and they tested the samples with the Biotox kit. Both swab samples and liquid samples were tested.

20.1.5 Measurement of microbial load

Total plate count results were obtained from the same locations where the residue samples were taken. This was done in order to find out if there is a correlation between the residues and viable organisms.

20.1.6 Instrumentation

The test is based on the inhibition of light and, therefore, most luminometers can be used for the test. The instruments used in this study were Unilite (Henkel Ecolab), Luminator (Henkel Ecolab), Lumax (Lumac), Charm LUMinator T (Charm Sciences), Luminometer 1253 (Bio-Orbit) and PD10 (Kikkoman).

20.2 RESIDUE ASSESSMENT RESULTS

20.2.1 Prepared test materials

All participating laboratories were able to obtain a typical dose response curve from different types of chemicals with the photobacteria test. They were able to perform the test reliably and repeatably. Prepared samples were then sent to the laboratories. Most of the problems encountered in the first stage were caused by the unsuitability of the laboratory's luminometer in this test. Three out of five laboratories had luminometers not suited for the measurement. The photobacteria produce a lot of light with the volume used and, therefore, some of the instruments were overloaded or not operating properly. Detailed information about the problems and possible corrective actions are not available. Those laboratories that were unable to use their own instrument borrowed an instrument suitable for these measurements.

The results of the prepared samples are presented in Table 20.1. The chemicals used were isopropanol, hypochlorite and tenside-based agents. All laboratories produced similar results (in the same order of magnitude) for the isopropanol

and tenside based. With the hypochlorite-based sample there was much more variation in the results. One reason for this may be that the laboratory with the smallest inhibition tested its sample several weeks later than the other laboratories. It is possible that the chlorine in the sample is no longer reactive. The correction factor is reasonably low (from 0.562 to 0.981) in every test series and, therefore, the results are reliable.

With swab samples the variation between different users is much higher than with liquid samples. The correction factor with the water samples is much higher (0.124–0.660) and therefore the results are comparable only intraseries, not interseries. A small correction factor means in practice that a clean water sample together with the swab inhibit the production of light. The sample volume is about 100 µl, when swab samples are used and therefore the sensitivity of the test is different compared with the 500 µl liquid samples. A five-fold difference can cause a very high increase in the inhibition especially because the light inhibition of the bacteria is linear only in the double logarithmic presentation. It was noted during the testing that different users dipped the swab into the tube in different ways and the sample volume is not the same with everybody.

The negative values in the table represent induction of the light production compared with the control instead of inhibition. This is a very common phenomenon with different chemicals within a certain concentration range.

20.2.2 Dairy environment samples

Most of the samples taken in dairies were swab samples because it is easier to obtain a swab sample than a liquid sample. There were totally 501 samples from different locations in different countries. The inhibition values are presented in Figure 20.1a and 20.1b. In Figure 20.1a the samples are divided into different groups according to the result, and in Figure 20.1b the results are sorted according to the inhibition. The results are spread over the whole possible area under 100% inhibion. If we compare the results obtained for the prepared samples with theoretically diluted chemicals, it is interesting to see that the situation is the same in all factories. There are samples with a very clear inhibition (inhibition >50%), samples with moderate or no inhibition (inhibition 20–50% or 2–20%) and also samples that induce the light production (inhibition value negative). About 40% of the samples showed a clear inhibition. In other

words this means that there are residues before starting the production if no preventive rinsing is performed.

Table 20.1. Results from the common prepared samples. H = hypochlorite-based disinfectant, I = isopropanol-based disinfectant, P = surface active detergent. The samples marked with a D were diluted by the operator. FIN 2 was the reference laboratory which prepared the samples. Therefore, their results are considered as 'correct'.

Sample				Inhibition (%)			
Liquid	Tube	Sweden	Finland 1	Norway	Denmark	Iceland	Finland 2
CF	10	**0.562**	**0.769**	**0.610**	**0.685**	**0.981**	**0.612**
0.1% H	1	99.4	100	100	-	100	100
0.02% H	8	100	99.5	2.5	1.9	12.2	100
0.005% H	9	19.0	6.4	7.6	10.4	14.9	97.3
0.0017% H (D)	9	29.8	13.3	-3.4	16.6	23.8	-25.0
10% I	4	100	100	100	100	100	100
5% I	6	100	99.7	100	100	100	100
1% I	2	96.5	92.5	99.8	99.6	94.2	99.6
0.3% I (D)	2	77.3	73.6	92.5	97.1	63.5	64.9
0.17% I	2	33.0	42.8	47.8	65.0	34.4	12.9
0.15% P	5	99.9	99.2	100	100	99.6	99.9
0.07% P	7	99.8	99.2	99.8	100	99.1	99.8
0.015% P	3	61.4	57.4	82.2	71.5	33.4	50.2
	Sample					Inhibition (%)	
Swab	Tube	Sweden	Finland 1	Norway	Denmark	Iceland	Finland 2
CF	10	**0.340**	**0.124**	**0.531**		**0.660**	**0.353**
0.1% H	1	67.6	87.0	75.5	-	85.3	99.9
0.02% H	8	3.9	49.8	-8.5	-	-8.9	43.0
0.005% H	9	-30.9	93.7	0.3	-	-25.4	-7.8
10% I	4	56.6	99.9	94.5	-	20.9	99.9
5% I	6	45.5	99.1	64.0	-	71.0	99.4
1% I	2	-15.1	58.2	18.2	-	25.2	47.3
0.15% P	5	31.1	99.9	72.2	-	77.5	89.9
0.07% P	7	32.8	63.0	47.4	-	55.0	80.6
0.015% P	3	4.1	87.9	20.6	-	22.8	24.4

The best results regarding the residues were obtained with CIP systems. If the cleaning process is optimised, there are no residues (and also no viable organisms) after the cleaning. The highest inhibition values were usually obtained from places that are difficult to wash and automated washing cannot be performed.

One of the dairies was monitored several times. When the residues were found during the first tests, more attention was paid to the rinsing. The inhibition values dropped significantly in the last series.

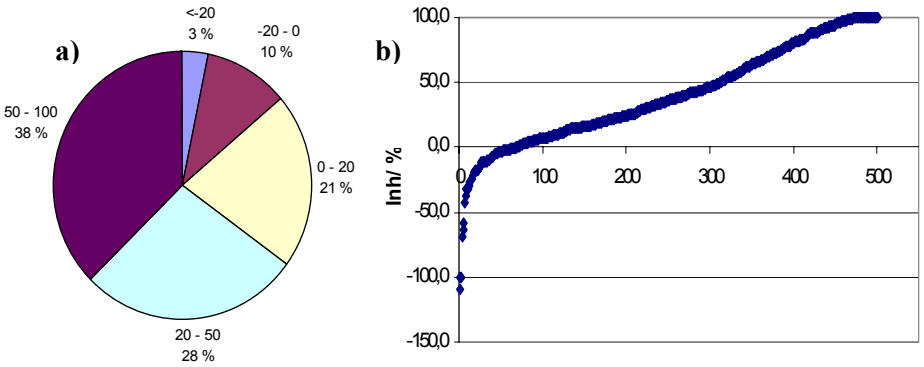

Figure 20.1. Residue test results from dairies. 501 samples were tested: a) the results are divided in 5 groups according to the inhibition result & b) the distribution of all results shows clearly that there are residues left on the surfaces in about 40% of the samples (inhibition >50%).

20.2.3 Residue results compared with TPC results

The preliminary results of the residue tests and total plate count results show that there is no correlation between the TPC results and residue test results (Figure 20.2). The microbial cleanliness is describes the whole cleaning process whereas the residue test measures only the residues after the last cleaning step. If only one production line is followed with the method during the washing cycles, it shows that the microbial load drops at the same time as the chemicals reach the location and the photobacteria are killed totally in five minutes.

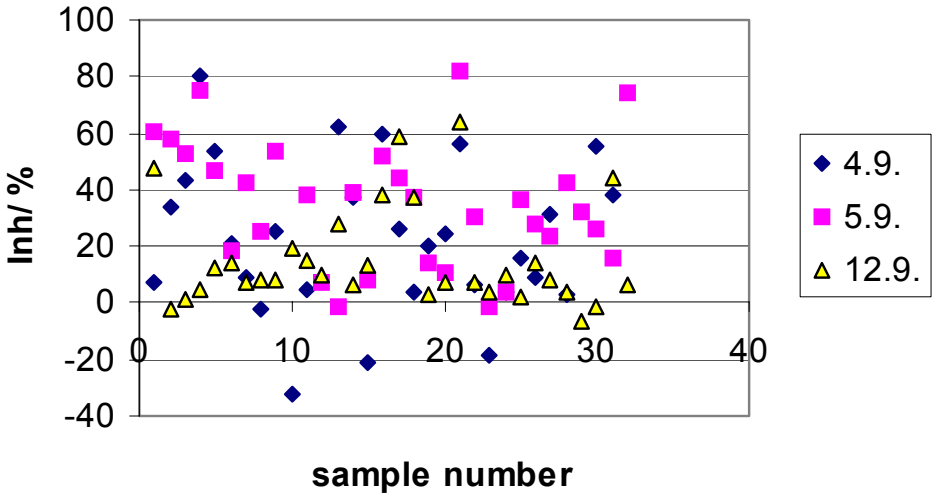

Figure 20.2. One dairy was tested several times. The inhibition values are significantly smaller during the last series of samples (September 12) compared with the previous ones (September 4 and September 5) due to enhanced rinsing.

The photobacteria method used in this study was performed rapidly because the incubation time is only 5 min. The distribution of the result shows that residues do exist on the production surfaces prior to starting the production. The method offers a useful alternative for residue testing.

21. A METHOD FOR ASSESSING THE CLEANABILITY OF OPEN PROCESSING EQUIPMENT

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The demand for hygienic design has been brought into focus by the HACCP wave which swept through Europe, leading to a preoccupation with hygienic design instead of finished product control, combined with the environmental movement demanding fast, easy cleaning with minimal use of water and cleaning agent. The machinery must not only be functional, but also easily accessible, and all parts must be drainable. In line with this came the demand for standardised verification test procedures to identify the critical areas with poor design, so that they can be eliminated in a redesigned process, and also to ensure that the equipment/machinery can be cleaned efficiently with minimal use of water.

21.1 CLEANABILITY TESTING

The EHEDG together with the A3 organisation in the USA has established a set of guidelines that describe these hygienic design criteria. EHEDG guidelines have been used for several years to verify bacteria tightness to ensure that no microbes can enter the equipment (EHEDG Document no. 7) and that the equipment can be pasteurised (EHEDG Document no. 4) or sterilised (EHEDG Document no. 5) in-line. In-place cleanability of closed food-processing equipment (EHEDG Document no. 2) and of open moderate-sized equipment (EHEDG Document no. 15) has also been published. As microbes are used in all these tests, it is not advisable for untrained persons to be involved in their implementation. The Danish Technological Institute is the only institute in the Nordic countries which can issue a certificate for equipment based on the cleanability test.

The aim is to establish a reference method for cleaning open production equipment that can be used to test the hygienic standard of equipment before purchasing or evaluation during the construction phase. NSF has developed a specified method for validation of cleaning of open equipment e.g. mechanical belt conveyors. The method uses sucrose water and riboflavin for soiling and

water for rinsing. Hot spots or critical points are detectable by using UV light. The staff at the Danish Technological Institute has developed this method further for use in evaluating the hygienic design of open processing equipment. The research institutes participating in DairyNET have tested the method in the second synergy task and in this report a comparison has been carried out. The continuing development within the EHEDG also in results new test guidelines e.g. for testing the cleanability of equipment, and the aim is to publish this method in the EHEDG guideline series.

21.2 Reference Plate in the Test Procedure

DTU has produced a reference plate for each test institute. The plate contains a bent steel plate 50 cm * 50 cm horizontal and 10 cm vertical. A plate with two 5-cm welds is mounted with two umbraho bolts.

The intention in using a reference device is that we shall obtain a standardised result when trained personnel in different locations carry out the test. EHEDG test centres use a similar procedure, in which a reference pipe is used. The EHEDG method is developed to avoid as much individual judgement as possible. CIP cleaning is not as sensitive to individual procedures as manual cleaning procedures. The aim of synergy task II is to find a method of training in a standard procedure, which will make it possible to evaluate the cleanability of new equipment highlighting the 'hot spots' needing special attention during clean-up procedures.

21.3 Test Procedure

In principle, the methods are based on the EHEDG testing methods for closed equipment. The equipment parts are soiled with cultured sour milk and Uranine; all the parts are dried and then cleaned by a specified procedure. An evaluation using UV light will highlight the non-hygienic areas requiring either a redesign of the equipment or a new cleaning procedure. The evaluation is based on even small traces of Uranine lighting up when exposed to UV light. The sour milk contains protein, fat and sucrose; it adheres well to the surfaces and is liquid-based so that it flows into cracks.

21.4 RESULTS OF THE SOILING PROCEDURE

Some variation in the soiling was seen. On the basis of the reporting, it is not possible to explain this variation in thickness of the layer. Sour milk is a natural product and the age of the cultured milk might be of importance. It has been seen that there is a large variation in the soiling ability of the cultured sour milk used by the EHEDG laboratories for the cleanability of closed equipment. A variation is to be expected but a procedure creating a more uniform soiling is preferred. None of the laboratories have had problems with burned proteins, i.e. it seems that drying with the "hairdryer" as well as simply leaving it to slowly dry in the room worked well. The following is an evaluation of three levels of soiling:

- heavy soiling is seen at Matforsk and at VTT Biotechnology,
- medium soiling is seen at the Icelandic Fisheries Laboratories and
- light soiling is seen at SIK and Biotechnological Institute.

21.5 RESULTS OF THE CLEANING PROCEDURE

The cleaning procedure is meant to be 'mild' i.e. some of the soil has to be left on the reference plate. The objective is also to be able to make a procedure that can be used as a standard by all participating laboratories. In comparison with the EHEDG cleaning procedure, as described in Guideline 2, the intention is that small amounts of soil are supposed to stay after the mild cleaning procedure. The areas with a lack of hygienic design are shown with heavier residual soiling after cleaning. The inspection of the cleaned surfaces was done under UV light as well as in standard light. In general, soiling can be seen with the use of standard light, but, with UV light in particular the Uranine gives stronger visual proof of residual soil. All the test institutes have shown results where there is evidence of a lack of hygienic design around the test objects: cheese mould itself, around the edges of the steel plate and mould. Other general areas are around the edges of the entire plate and the area where the plate is bent. The following results were obtained:

- at **Matforsk**, the plate was heavily soiled and the cleaning was so mild that widespread soil was left on the plate. Even though this shows that there are problems in the above-mentioned areas, it will be difficult to argue for the differences obtained (no electronic picture available),

- at **VTT Biotechnology** the plate was heavily soiled and the cleaning device not considered to be effective. The result was that there was a great deal of residual soil on the plate. Especially, the cheese mould and the area around the steel plate showed a great deal of residual soil and there were problems in cleaning these areas,
- at **SIK** the plate was lightly soiled and after the cleaning there were still spots with soil, indicating a mild cleaning. The problem areas to clean were the cheese mould and the edge around the steel plate,
- at the **Biotechnological Institute** the plate was lightly soiled. After cleaning, the problem areas to clean were on the cheese mould, around the steel plate as well as the holes for the umbraho bolt. Soil around the bolt head was also seen after cleaning.
- at **IFL** the result here highlights all the spots indicated at the other test institutes.

21.6 Discussion about the Test Procedure

The reports from the institutes suggest that it is possible to soil a reference plate, including building in elements of hygienic design problems, with a procedure that produces a soil so 'tough' that it can be used to simulate production conditions. The following programmed 'mild' cleaning procedure shows that the places with known hygienic design problems can be visualised. It has been demonstrated that, within the variation of the participating laboratories, it is possible to use the method to show where the hygienic design problems are located. Visualising these known 'hot spots' is important in order to be able to point out 'hot spots' on a more complex device/machine where special attention is needed during the specified cleaning procedure. The project results showed that it is possible to develop a test procedure for reporting the cleanability of open surface equipment as a guideline. As soon as the soiling and cleaning method is established, it can be used as a method for testing equipment before it is installed in the production environment. The result, however, showed that more standardisation of the procedure to obtain a uniform result from different persons in different locations is needed. The tests performed in the second synergy task show that the same areas were pinpointed as problems within the hygienic design. Using a generally accepted test method will improve the design of new equipment because of competition between equipment manufactures.

22. MOULD CONTAMINATION IN CHEESE PRODUCTION

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Mould contamination of semi-hard cheeses is periodically a significant quality problem, and thereby poses economic consequences for cheese producers. In order to reduce the problem, a study was performed to obtain information about the fungi associated with consumer-packed cheese and blocks of Norwegian semi-hard cheese and to identify their main mould contaminants. A survey was also performed in four cheese factories in order to obtain information about fungi in the indoor environment and to identify critical points for mould contamination in factories. In order to identify the critical points, the two major mould contaminants were classified below species level using M13 finger-printing and AFLP (Kure et al. 2002; Kure et al. 2003).

Air was found to be the major source of mould contaminants during the production process (Kure *et al.,* 2004). It is therefore important to monitor fungi in air at dairies. A number of mould species may be present in the production facilities in a dairy, but only a few of these are able to grow on the cheese and spoil it. The most important moulds found on cheese are the *Penicillium* species *P. palitans, P. commune* and *P. roqueforti* (Lund *et al.,* 1995; Kure & Skaar, 200; Kure *et al.,* 2001).

22.1 EFFECT OF CULTURING MEDIA ON MOULD DETECTION

Non-selective agar media are often used to measure air quality, but the results do not necessarily reflect the quality of the final product. Consequently, the selective Creatine Sucrose Dichloran Agar (CREAD) medium has been developed (Frisvad, 1992), which supports the growth of moulds that spoil high fat and protein content food. The more widely used mould agar Dichloran 18% glycerol (DG18) agar (Hocking & Pitt, 1980) supports the growth of all types of moulds. *Penicillium* species, which are commonly found on cheese, grow well on the selective agar medium CREAD (Frisvad, 1992), while *P. brevicompactum* will not grow on CREAD. *P. brevicompactum* is commonly present in indoor air

in dairies, and was the most frequent species found in the air in Norwegian dairies (Kure *et al.,* 2004).

Since house flora varies between dairies, it is always possible that a selective medium will not support the growth of a problem organism. It is therefore recommended that CREAD is used together with a general agar medium for fungi (Lund, 1996). In the present study, the CREAD agar medium, DG 18 and a general agar medium selected by each dairy were tested at a number of dairies.

22.2 MOULD SAMPLING OF CHEESE

Samples of air, brine and cheese were collected at 8 dairies producing semi-hard cheese. Air-sampling was carried out using an air-sampler with Petri dishes (9 cm in diameter) at a total volume of 500 l air. Three different agar media were used at each sampling point; DG18 (Hocking and Pitt, 1980), CREAD Frisvad, 1992) and one agar medium normally used at the dairy (Potatoe dextrose agar (PDA), Dichloran Rose Bengal Chloramphenicol agar (DRBC), Oxytetracycline-glucose-yeast extract agar (OGY) and yeast extract-glucose-chloramphenicol agar (YGC) (Mossel *et al.,* 1970; King *et al.,* 1979, Engel & Rosch, 1999)

Samples of cheese were collected using a sterilised slicer. The cheese slices were approximately 4.5 cm * 11 cm and taken from cheese surfaces exposed to air. 10 ml of brine was collected with a sterile tube.

The agar plates and the cheese slices were incubated in darkness for 7 days at 25°C and 7–10 days for CREAD at 25°C. The colonies were inspected and counted. The number of *Penicillium* sp., *Aspergillus* sp. and *Cladosporium* sp. was determined by visual inspection according to 'Introduction to food and air-borne fungi' (Samson *et al.,* 2002).

22.3 CREAD FOR IDENTIFICATION OF CHEESE CONTAMINANTS

The mould levels varied between the dairies with the highest level of mould at dairy 5. The total number of moulds was higher on general agar medium DG18 and on local general medium than the total number on CREAD. This finding is

as expected since only a few species grow well on CREAD; DG18 and the local medium (PDA, DRBC, OGY) are general agar media for moulds. However, the difference between the general agar medium and CREAD was not statistically significant. At dairy 4 the total number of moulds was higher on CREAD than on the local general agar medium (PDA). This is due to a high number of moulds on CREAD at one of the sample points. The local medium in dairy 4 was PDA. The results are given in Figures 22.1 and 22.2.

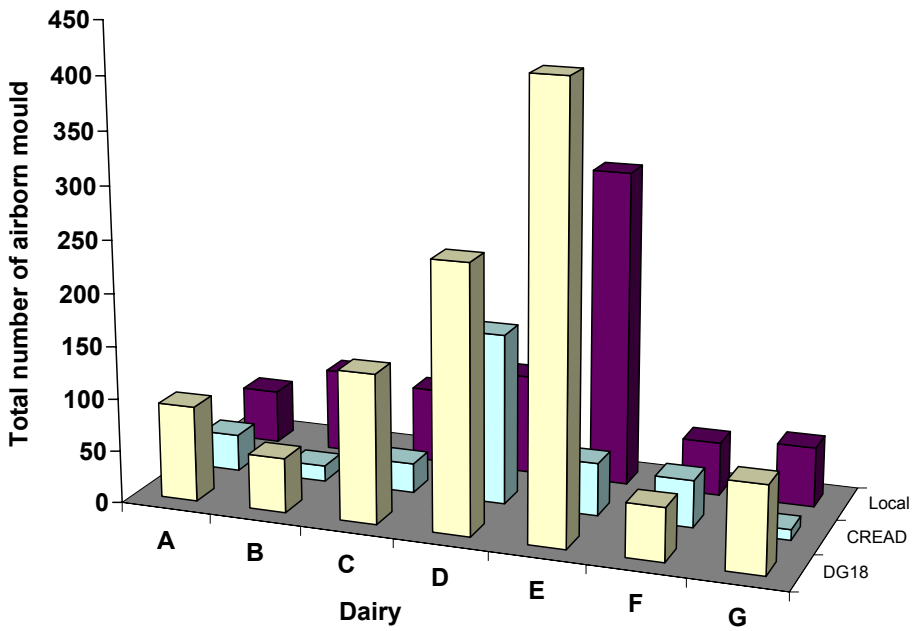

Figure 22.1. Total number of mould found in the samples of 500 l air from 7 different dairies, on DG 18, CREAD and the local general agar medium.

Penicillium sp. dominated at the dairies. Figure 22.2 shows that at 5 of the 7 dairies *Penicillium* species made up over 80% of the mould species found in air. At dairies 5 and 6, *Cladosporium* species and *Aspergillu*s species dominated respectively, and *Penicillium* sp. made up approximately 30% of the moulds. Since *Cladosporium* dominated the mould flora at dairy 5, the total number of moulds on CREAD was compared with growth on DG 18 and the local medium and it was found that they differed significantly. This feature was also expected from dairy 6, where *Penicillium* species only made up approximately 25%.

However, the difference between the media was not significant. The *Aspergillus* species, which was predominant in dairy 6, grows well on CREAD.

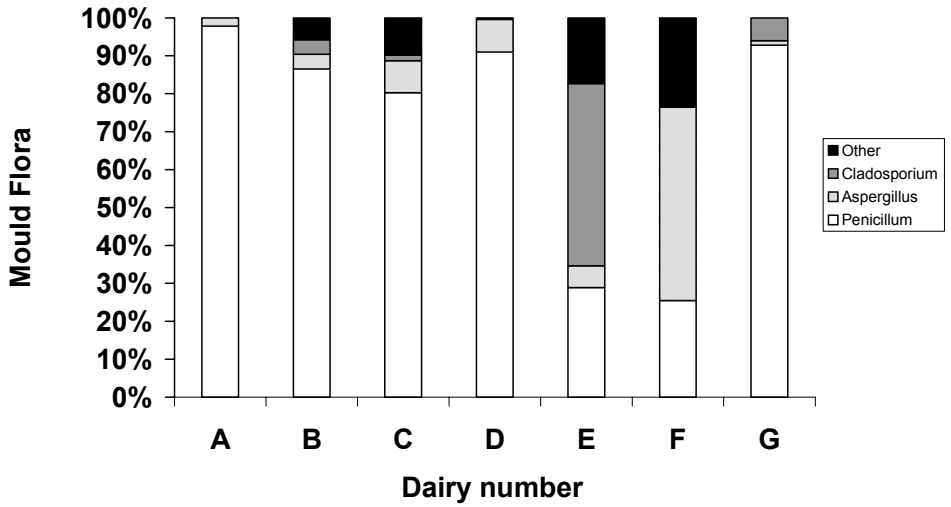

Figure 22.2. Frequency of Penicillium *sp.,* Aspergilus *sp.,* Cladosporium *sp. and other moulds found at the seven dairies.*

The results show that mould flora in general is dominated by *Penicillium* species. Since the number of moulds is higher on DG 18 and on the local medium than the total number on CREAD, the air contains *Penicillium* species or other genera that most probably do not grow on cheese, and therefore do not represent a problem for it. The use of CREAD together with DG18 or another general agar medium for mould detection at the dairies in this study gave important information on monitoring cheese contaminants in air.

23. CONCLUSIONS

23.1 RAW MILK QUALITY

Five Norwegian dairies were visited. Three dairies used cleaning with alkali and acid followed by disinfection with hot water. The two remaining dairies cleaned with alkali and disinfected with peracetic-acid-based acid disinfectants. Processing equipment and cleaning stations were inspected visually under UV light and microbial samples were taken. In addition, the cleaning and processing routines and results from quality control were inspected. Methods for sampling, the choice of sampling points and investigation of microflora were adapted throughout the project period, on the basis of the experience acquired. The results showed that the dairies using traditional cleaning routines in this survey obtained the best hygienic level. Visual control showed that there were points with unsatisfactory hygiene in all dairies, but neither the microbiological samples nor the visual control uncovered common problem areas for all dairies. Important checkpoints are cleaning solutions, gaskets and areas with problematic design e.g. cleaning nozzles, manhole lid areas, sensors, lids on air separators and tank trucks. The microbial flora on equipment after cleaning and disinfection varied and belonged to common spoilage bacteria in raw and pasteurised milk. None of the dairies could prove a connection between the microbial level in raw milk from farm and dairy silotanks and the level in the pasteurised product.

23.2 *LISTERIA* CONTAMINATION ROUTES

This project focused on dairy hygiene in the Nordic countries and the study concerning Iceland was directed towards contamination routes of *Listeria. L. monocytogenes* can be transmitted by the consumption of raw milk products or contaminated dairy products. The situation in the dairy industry is slightly different compared with other food sectors, because the milk is pasteurised. Thus it is reasonable to conclude that dairy products become contaminated with *Listeria* somewhere along the process line. In this survey, no positive *Listeria* samples were observed from the 450 samples taken in the factory environment, from process equipment and selected dairy products. However, *L. mono-*

cytogenes was detected in 13 samples of raw milk (1.5%), which is in the same range as previously reported from western Europe and the USA. To trace *L. monocytogenes* isolates from the raw milk, samples were collected from the raw milk of all milking cows and from the shed environment at the farm that was positive for all three winter-sampling occasions. In this investigation, all the generated ribotypes were identical to the *L. monocytogenes* patterns available except for one ribotype, which was *L. seligeeri*. It has been confirmed that *L. monocytogenes* can establish itself and persist for a long period of time, which suggests that an in-house flora become established. Additionally, it is interesting to note that there seems to be a seasonal variation concerning the occurrence of *L. monocytogenes* from raw milk. A reasonable explanation for this is contamination from the shed environment and contaminated silage during wintertime caused when animals are kept relatively close together for long periods.

23.3 AIR QUALITY ASSESSMENT

In the food industry, settle plates and active air sampling based on impaction or centrifugation are the most commonly used routine monitoring methods. Monitoring effectiveness is dependent to a great extent on the monitoring methods and active sampling has been shown to be more accurate. There are two types of solid-surface impactors: slit samplers and sieve samplers. The microbial air sampler MAS-100 is an impactor that aspirates air either horizontally or vertically through a perforated nozzel onto the agar surface of a standard Petri dish. The Reuter Centrifugal Sampler (RCS sampler) is convenient to use but does not quantitatively recover very small viable particles. The filtration methods do not collect all vegetative cells in a viable form because of the stress the method has on cells through dehydration during sampling. Sampling time affects dehydration and is therefore crucial in bacterial recoveries of sensitive microbial strains. Gelatine filter membranes composed of gelatine foam are designed to prevent vegetative microbes from being deactivated by desiccation during filtration, thus making the method more reliable. Each of the proposed monitoring methods has its limitations. It should also be noted that airborne counts recorded using different sampling techniques are not directly comparable but they most probably show similar trends. To improve the efficacy of the

microbiological monitoring an appropriate combination of media and monitoring devices should be used.

The test results from the four Swedish cheeseries indicate that a more controlled environment is eligible. To achieve a more controlled environment in cheese plants, specifications for the processing, storage, and packaging areas have to be established by the manufacturer. Old ventilation systems need to be upgraded to achieve the desired air quality. Correctly installed systems with over-pressure and air-flows are needed in the process areas where open products vulnerable to contamination are kept. In such process steps local zones supplied with HEPA filters would improve the process facilities. The environmental monitoring should be based on risk assessment. In the Finnish sub-project the air quality in the Herajoki dairy plant was examined in 2001, when construction and rebuilding work were performed in the processing facilities. In this study, the level of yeast and moulds was approximately the same when measured using both RCS and MAS air samplers. The RCS air sampler is very useful for measuring microbial air quality in a dairy although it is not recommended as the primary measuring device for measuring yeast and moulds. It was shown that by dividing the production area and by maintaining overpressure in critical departments, contamination from contiguous spaces could be avoided.

23.4 HYGIENE IN DAIRY PROCESSING

In order to perform a hygienic survey, a sampling plan for water, air, surfaces and cheese is recommended. It is crucial that the sampling points are randomly selected and not prejudiced by what is believed to be a likely problem. The collection and further characterisation of microbial isolates will reveal the identity and source of the critical species in a plant. These results demonstrated the possibility of waterborne contamination in processing plants due to insufficient hygiene and maintenance of water sources e.g. hoses, nozzles and taps. Some advice is given in the Swedish Water Act dated December 25, 2003 on how to take samples and how water hoses should be maintained in order to protect the water quality coming through the hose. Product-related sampling should be carried out in the event of a product fault where water is suspected of causing the problem. The analyses to be carried out should be selected on the basis of the type of problem being experienced with the product. The microbes

most likely to become established in these environments are bacteria such as *Acinteobacter* spp. and *Pseudomonas* spp.*;* yeasts such as *Candida* spp. and *Rhodotorula* spp.; moulds such as *Phoma* spp. and *Fusarium* spp. *Penicillium* spp. was rarely isolated from water.

Positions with increased contamination were detected by sampling water sources. At two plants, several contaminated water sources used for cleaning were detected. It was also shown that air sampling can be used for the identification of process positions contributing to improper hygiene. The CIP water purity can be tested using the ATP method, in which the ATP reacts on all organic material and shows very quickly if the water is dirty or clean. Traditional culturing can show that the water is dirty i.e. containing a high microbial load even though the ATP method shows that the water is clean, which is due to chemical residues. In a few cases the ATP method can also show that water is not clean while traditional culturing shows that water is very clean. This is also due to the fact that the chemicals interfere in the ATP reaction.

Mould contamination of semi-hard cheeses is periodically a significant quality problem and thereby has financial consequences for cheese producers. Most of the fungi found in the hygiene surveys performed in the indoor environment in the cheeseries did not represent a contamination problem in cheese products. The selective CREAD agar together with a general agar medium for the isolation of moulds was tested in order to obtain data on moulds in the environment and see if these moulds are able to grow on the cheese surface. It is advisable to test identified critical species for growth on the CREAD agar in order to know how to interpret the mould growth. The growth ability on cheese/cheese agar e.g. CREAD at cold storage temperature is one important criterion for the critical species spoiling cheese products.

The synergy result showed that it is possible to develop a test procedure based on UV light for reporting the cleanability of open surface equipment as a guideline. The visualisation was performed using reference plates with elements of bad hygienic design. These reference plate, which were soiled with sour milk and cleaned using a “mild” cleaning procedure, showed that the places with known hygienic design problems could be visualised. Visualising these known “hot spots” is important in order to be able to pinpoint problematic spots in complex equipment. Using a generally accepted test method will improve the

design of new equipment as a result of competition between equipment manufactures. With a UV lamp it was easy to detect residues on stainless steel. Plastic materials were auto-fluorescent, but it was possible to detect contamination since this gave a different kind of light. The UV lamp gave immediate results on cleaning efficiency. An immediate evaluation of the cleaning is superior to a discussion based on laboratory results coming some days later.

Cheese moulds were found to be not cleaned satisfactorily during the cleaning procedure used. In order to prevent contamination, procedures for inspection and cleaning of the cheese-mould cleaning machine must be established. Good indicators for checking that the procedures are in place and working properly, are sampling and analysing the last rinse water. The cleaning of conveyor belts is also a known problem. Almost 20% of the samples contained yeast and mould; the occurrence of Gram-negative bacteria was less frequent. Critical spots where contamination was found were under the conveyor belt and on the rollers. An assessment procedure introduced at Arla Foods involves the definition of critical areas/spots, daily checking by an independent person not belonging to the cleaning group, documentation, auditing of the cleaning effect on a regular basis, action taken when deviations are found and feedback to the cleaning group.

Results obtained on washed returnable crates showed that a hygienic classification, both visual and microbial, of the crates can be determined. To improve washing results the cleaning parameters should be maintained at a constant level. When microbial monitoring was being performed in the process, the colony-forming units in the cleaning liquid varied between 80 and 300 CFU/ml. The ultrasonic cleaning method is applicable to cleaning returnable plastic crates and is an important method compared with the washing tunnel method. The DryCult® TPC is a practical choice, if the tests have to be carried out onsite.

23.5 HYGIENIC DESIGN IN INDUSTRIAL CASES

The primary goal of implementing the integrated approach is to avoid the hazards that might otherwise produced. These hazards can be of a microbial,

chemical or particulate nature in the consumable product. The second goal is to minimise the risk of recalls, lawsuits, bad reputation and ruining a good image. Thirdly, by implementing the integrated approach we hope to reduce other non-safety problems such as negative impacts on the environment, excessive costs, and excessive use of resources such as water, chemicals or energy. Lastly, the integrated approach should make it more likely that we can also maximise the cost efficiency of the finished design. The work on the coming EHEDG guideline "Hygienic Systems Integration" is being carried out by Roland Cocker (Cocker Consulting), Stefan Åkesson (TetraPak), Alan Friis (BioCentrum-DTU), Paul Bartels (A & F Netherlands), Hans Hoogland (Unilever), Gerd Klimmeck (Johnson Diversey), Hans Oosterom (DSM Bakery Ingredients) and Jeff Wilkinson (PGA).

Results have shown that the adhesion and removal of bacteria do not appear to be affected by the surface topography at very low R_a-values (< 0.8 μm). This might lead to the assumption that ultra-smooth surfaces do not provide hygiene benefits. However, hygiene is not only a matter of bacteria or dirt but also the resistance of the surface to the chemical agents used in production. The purpose of the new Danish competence centre for the stainless steel industry, is to enhance the future competitive power of the stainless steel industry e.g. in food processing. Smooth surfaces are clearly more expensive and our research does not suggest that the general recommendation of R_a < 0.8 μm needs to be changed. Very fine polished surfaces may be advantageous, e.g. for surfaces in pipeline products, which are not heated anymore.

The requirements for the hygienic design of equipment are based on stringent EHEDG and FDA guidelines. These guidelines are in themselves excellent, but hygienic design cannot be learned only in theory. An important precondition of being able to design equipment for specific tasks is that the designer has detailed practical knowledge of the conditions where the equipment will be used. Most food-processing companies are becoming increasingly closed to visitors, which means that the designers of food-processing machinery receive less and less feedback. As a result, it becomes more difficult for the designers to obtain correct practical understanding of the area, something that is essential for keeping up with the developments required for new optimised equipment designs. In this publication, five examples of equipment (a static fluid bed, a DuoSafe PHE, a cheese slicer, a bag filler and a plastic bottle filler) showing

typical hygienic design problems have been reviewed. In the evaluation, the effect of the design is assessed in relation to the following areas: product contact surfaces, product close contact surfaces and production environment.

The use of a double-wall PHE has shown that some additional consideration in the plant design, operation and preventive maintenance is needed. More plates are needed, because the thermal effect of a double-wall plate is poorer than that of a single-wall plate. The problems caused are either a pressure drop in the PHE or a low flow per channel, and a lowered flow may cause cleaning problems. Narrow channels can also be used to improve heat transfer, but in the hot section fouling may cause early clogging. Flushing the PHE should also be avoided because of the risk that capillary forces might suck liquid out of the product and/or detergent residues into a leaking chamber, thus causing corrosion or bacteriological growth. Therefore, it is highly recommended that covers be added to the PHE. There is a high risk that corrosion will also make a hole in the second plate if soil dries between the plates. Regular pressure testing is, therefore, recommended to find small cracks causing leakage. The use of a double wall PHE is recommended only if no other way can be found to solve the process requirements.

23.6 DISINFECTANTS – RESIDUES AND RESISTANCE

The aim of disinfection is to reduce the number of microbes present on food-contact surfaces, thereby avoiding contamination of raw materials and products with pathogens and spoilage organisms. When the disinfection process fails, this can in most cases be explained by the use of a too low disinfectant concentration, temperature, exposure time or failure in the cleaning process, which leaves soil on surfaces to be disinfected. Spores and some resistant vegetative bacteria can survive disinfection because of a build-up of resistance against the disinfectants used. The residue assessment method used in the 1st synergy task is based on an assessment of the luminescence output of photobacteria with an incubation time of only 5 min. The studies showed that chemical residues are left on the surfaces prior to production. The results from the photobacterial method can be categorised into the following residue levels based on the inhibition: very clear, moderate and no residues. It, therefore, offers a useful alternative for testing the chemical residue on surfaces.

Various laboratory studies have shown that surface-attached cells are more resistant to disinfectant treatment than suspended cells. This has led to the development of various types of carrier tests e.g. using microbial cells dried on surfaces, biofilm-constructs and biofilm grown on test coupons. Some bacteria isolated from the process persist whereas others adapt to the disinfectant used. Biofilm growth experiments on stainless steel indicated that the *Sphingomonas* sp. has a much higher ratio of early attachment than the other isolates. In the Icelandic hygiene survey, 140 strains were isolated during summer and wintertime from a dairy factory environment. It was interesting to note that some of the strains isolated have a broad range of tolerance towards disinfectants. Several of these strains were identified as *Psedomonas putida, Moraxella*, *Klebsiella oxytoca* and *Bacillus pumilus*. It is also interesting to note that 24 out of these 25 further characterised strains were isolated during summertime. Most of these strains were Gram-negative bacteria (23), which correlate well with Gram-negative bacteria being more resistant to disinfectants than Gram positive bacteria.

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Wirtanen, G., Salo, S., Maukonen, J., Bredholt, S. & Mattila-Sandholm, T. 1997. Sanitation in Dairies. Espoo: VTT Publications 309. 47 p. + app. 22 p. ISBN 951-38-5055-2. http://www.vtt.fi/inf/pdf/publications/1997/P309.pdf

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APPENDIX 1: VOCABULARY AND ABBREVIATIONS

A.	*Aspergillus;* mould belonging to the *Aspergillus* family (e.g. *A. niger* (often used in the air disinfection experiments) and *A. versicolor)*
Acinetobacter spp.	Gram-negative rod-shaped bacteria, strict aerobes, belonging to the *Neisseriaceae* family; *Acinetobacter calcoaceticus, Acinetobacter junii/woffi*
Acremonium spp.	filamentous fungi
ADP	adenosine diphosphate
Aeromonas spp.	Gram-negative rod-shaped bacteria, facultative anaerobes belonging to the *Vibrionaceae* family; *Aeromonas hydrophila, Aeromonas veroni*
AFLP	Amplified fragment length polymorphisms
AGI	all glass impinger
Agrobacterium spp.	Gram-negative rod-shaped bacteria, aerobic, belonging to the *Rhizobiaceae* family; *Agrobacterium radiobacter*
AISI	American Iron and Steel Institute
Alcaligenes spp.	Gram-negative bacteria, rods, coccal rods or cocci, strict aerobes; *Alcaligenes denitrificans, Alcaligenes faecalis*
Alternaria spp.	filamentous fungi
*Apa*I	restriction enzyme
API	identification products which can identify a wide variety of micro-organisms. The products comprise strips that generally contain 20 miniature biochemical tests.
*Asc*I	restriction enzyme
ATP	Adenosine triphosphate
a_w	water activity
B.	*Bacillus*; Gram-positive bacteria, spore-forming rods belonging to the *Bacillus* family (e.g. *B. cereus, B. flavothermus, B. licheniformis, B. pumilus* and *B. subtilis*)
BHI	Brain heart infusion
Blue plate	Drigalski agar
Brevundimonas vesicularis	basonym *Pseudomonas vesicularis*

Brucella spp.	Gram-negative bacteria, strict aerobes, coccobacilli or short rods; *Brucella suis*
Burkholderia spp.	*Burkholderia cepacia* basynom *Pseudomonas cepacia*
Campylobacter spp.	Gram-negative bacteria, spirally curved rods, microaerophilic to anaerobic, belonging to the *Spirillaceae* family
Candida spp.	yeast *(C. albicans, C. boidinii, C. colliculosa, C. curvata, C. famata, C. intermedia, C. krusei, C. lambica, C. lipolytica, C. parapsilosis, C. rugosa* and *C. sphaerica)*
CEN	The European Committee for Standardization
CF	correction factor
CFU	colony-forming units
CGY	Cloramphenicol-Glycos-Yeast extract
Chrysomonas	taxonomic synonym *Chromulina (Chrysomonas indolegenes)* algae
CIP	cleaning-in-place
Citrobacter spp.	Gram-negative bacteria, rods, aerobic and facultatively anaerobic, belonging to the *Enterobacteriaceae* family
CL	confidentiality limit
Cladosporium spp.	filamentous fungi *Cladosporium cladosporiodes, C. herbarum*
Clostridium spp.	Gram-positive bacteria, rods, strictly anaerobic, belonging to the *Bacillaceae* family
COD	chemical oxygen demand
Comamonas spp.	*Comamonas acidovorans* basonym *Pseudomonas acidoovorans*
CPM	constant pressure modulating valve
CREAD	creatine sucrose dichloran agar
Cryptococcus spp.	yeast: *Cryptococcus albidus*, *Cryptococcus laureutii*
CTC	5-cyano-2,3-di-p-tolyltetrazolium chloride (5-cyano-2,3-bis(p-methylphenyl)-2H-tetrazolium chloride)
CYA	Czapek yeast autolysate agar
D. anomala	*Dekkera anomala,* yeast used in disinfectant efficacy studies

DAPI	4',6-diaminidino-2-phenylindole (CA index name: 2-[4-(aminoiminomethyl)-phenyl]-1H-indole-6-carboximidamide)
Debaryomyces spp.	yeast; *Debaryomyces hansenii, Debaryomyces polymorphus*
Delftia spp.	*Delftia acidovorans* basonym *Pseudomonas acidoovoranas, Comamonas acidovorans*
DG18	Dichloran 18% glycerol
DI	discrimination index
DNA	deoxyribonucleic acid
DOE	Design of Experiment
DPC	discrete-particle counter
DRBC	Dichloran Rose Bengal chloramphenicol agar
E. coli	*Escherichia coli*, bacterium used in disinfectant efficacy studies. Gram-negative bacteria, rods, facultatively anaerobic, many members may show opportunistic pathogenicity, belonging to the *Enterobacteriaceae* family
EC-50 value	the effective concentration of the detergent tested that resulted in an inhibition of 50% of light production
*Eco*RI	restriction enzyme
EDTA	organic chelating agent; ethylenediaminetetraacetic acid (CA index name: N,N'-1,2-ethanediylbis[N-(carboxy-methyl)-glycine)
EHEDG	European Hygienic Engineering and Design Group
Enterobacter spp.	Gram-negative rods, belonging to the *Enterobacteriaceae* family; *Enterobacter cloace*
Enterobacteriaceae	family of Gram-negative bacteria rods; aerobic and facultatively anaerobic; Genera belonging to *Enterobacteriaceae: Escherichia, Edwardsiella, Citrobacter, Salmonella, Shigella, Klebsiella, Enterobacter, Hafnia, Serratia, Proteus, Yersinia* and *Erwinia*
Enterococcus	lactic acid bacteria, Gram-positive bacteria, previously designated “group D streptococci” or “fecal streptococci”, *Enterococcus hirae*
ES buffer	0.5 M EDTA, 1% sodium lauroyl sarcosyl
Exophiala spp.	filamentous fungi

FDA	Food and Drug Administration
Flavobacterium	Gram-negative bacteria, cells vary from coccobacilli to slender rods, facultatively anaerobic; *Flavobacterium meningosepticum*
FMEA	failure mode and effect analysis
ft^3/min	cubic foot per minute
Fusarium spp.	filamentous fungi *F. oxysporum*
Geotrichum	yeast; *Geotrichium capitatum, Geotrichum candidum* and *Geotrichum capitatum*
GMP	Good Manufacturing Practices
GNB	Gram-negative bacteria
HACCP	Hazard Analysis Critical Control Point
HDPE	high-density polyethylene
HEPA	high-efficiency particulate air
HSI	Hygienic Systems Integration
HTST	high temperature short time
HygiCult® TPC	dip slide for detection of total bacteria count
HygiCult®Y&F	dip slide for detection of yeast & moulds
IC_0	light production of the bacterial suspension (control tube)
IC_5	light production of the control after incubation (5 min)
IDF	International Dairy Federation
INH%	inhibition percentage
ISO	International Standardization Organization
IT_0	light production of the bacterial suspension (sample tube)
IT_5	light production of the sample after incubation (5 min)
kbp	kilobase pairs used in the system
kHz	kilohertz
Klebsiella spp.	Gram-negative bacteria, capsulated rods, facultatively anaerobic, belonging to the *Enterobacteriaceae* family; *Klebsiella oxytoca*
Kluyvera spp.	bacteria
Kocuria spp.	bacteria *Kocuria varians*
L.	*Listeria; s*mall, coccoid, Gram-positive bacteria rods (may be Gram-negative bacteria in older cultures) facultatively anaerobic; *L. innocua, L. monocytogenes, L. seeligeri*

LAB	lactic acid bacteria
Lactobacillus spp.	Gram-positive bacteria, rods, anaerobic or facultative, belonging to the *Lactobacillaceae* family; *Lactobacillus brevis, Lactobacillus curvatus*
Lactococcus spp.	lactic acid bacteria*,* Gram-positive bacteria, earlier included in genus *Streptococcus*
Lecythophora spp.	filamentous fungi*; Lecythophora lignicola*
Legionella spp.	Gram-negative bacteria, rods, aerobes; *Legionella pneumophila*
Leuconostoc spp.	Gram-positive bacteria, cocci, facultative anaerobes, belonging to the *Streptococcaceae* family
LIDAR	light detection and ranging
M/T	mesofile/termofile
MAS	microbial air sampler
ME	microbial effect
MEA	malt extract agar
Methylobacterium sp.	*Methylobacterium mesophilicum* basonym *Pseudomonas mesophilica*
MIC	minimum inhibitory concentration
Micrococcus spp.	Gram-positive bacteria, cocci, aerobes, belonging to the *Micrococcaceae* family
Moraxella spp.	Gram-negative rod-shaped bacteria, strict aerobes, belonging to the *Neisseriaceae* family; *Moraxella osloensis*
MOX	modified Oxford agar
MPPS	most penetrating particle size
Mucor spp.	filamentous fungi
Mycobacterium spp.	Gram-positive rod-shaped bacteria, aerobic, belonging to the *Mycobacteriaceae* family; *Mycobacterium tuberculosis*
n	number of samples
N	the total number of isolates in the sample population
NaCl	sodium chloride
ND	not defined
NT	not tested
Ochrobactrum spp.	Gram-negative strictly aerobic bacteria, *Ochrobactrum anthropi*

OGY	oxytetracycline-glucose-yeast extract agar
Paecillomyces spp.	filamentous fungi
Pantoea sp.	Gram-negative bacteria, rods, facultatively anaerobic, belonging to the *Enterobacteriaceae* family; *Pantoea agglomerans*
PCA	plate count agar
PCR	polymerase chain reaction
PDA	potato dextrose agar
Pediococcus spp.	Gram-positive bacteria, cocci, microaerophilic, belonging to the *Streptococcaceae* family
Penicillium spp.	filamentous fungi; *P. brevicompactum, P. chrysogenum, P. commune, P. crustom, P. crustosum, P. decumbens, P. glabrum, P. palitans, P. roqueforti, P.solitum, P. steckii*
PFGE	Pulsed-Field Gel Electrophoresis
PHE	plate heat exchanger
Phialemonium spp.	filamentous fungi
Phialophora spp.	filamentous fungi
Phoma spp.	filamentous fungi
Pichia spp.	yeast
Proteus spp.	Gram-negative bacteria, straight rods, facultatively anaerobic, belonging to the *Enterobacteriaceae* family; *Proteus vulgaris*
Pseudomonas	Gram-negative bacteria rods, strict aerobes belonging to the family *Pseudomonadaceae (P. aeruginosa, P. fluorescens, P. fragi*, *P. lundensis, Pseudomonas putida*, *Pseudomonas stuzeri)*
PVC	polyvinyl chloride
QACs (QA)	quaternary ammonium compound
R. erythropolis	*Rhodococcus erythropolis,* bacteria
R_a	average value of variances from the average height of the surface (R_t and R_z-values)
Ralstonia spp.	Gram-negative bacteria rods (earlier *Pseudomonas)*
RCS	Reuter centrifugal sampler
rDNA	ribosomal deoxyribonucleic acid
RH	relative humidity

Rhizopus spp.	filamentous fungi
Rhodotorula spp.	yeast; *Rhodotorula glutinis, R. minuta, R. mucilaginosa*
rRNA	ribosomal ribonucleic acid
s	the total number of types described
S/N ratio	signal to noise ratio
Saccharomyces spp.	yeast; *S. cerevisiae, Saccharomyces servazzii*
Salmonella	Gram-negative bacteria, rods, aerogenic, belonging to the *Enterobacteriaceae* family; *S.* Choleraesuis, *Salmonella* Enteritidis, *S.* Infantis, *Salmonella* New Brunswick, *Salmonella* Typhimurium
Sanitation	cleaning & disinfection
SAS	Surface Air System
SD	standard deviation
SDX	Sabouraud-dextros agar
SE	standard error
Serratia spp.	Gram-negative bacteria, rods, facultatively anaerobic, belonging to the *Enterobacteriaceae* family; *S. marcescens*
Shewanella spp.	bacteria; *Shewanella putrefaciens*
Sphingomonas spp.	bacteria; *Sphingomonas pancimobilis*
Spirochaeta spp.	Gram-negative bacteria, helical cells, obligate or facultative anaerobes, belonging to the *Spirochetes* family
Staphylococcus spp.	Gram-positive bacteria, cells spherical, facultatively anaerobic, belonging to the *Micrococcaceae* family; *S. aureus, St. cohnii, St. pasteuri, St. warnerii*
Stenotrophomonas spp.	bacteria, *Stenotrophomonas maltofilia*
Streptococcus spp.	Gram-positive bacteria, cells spherical to ovoid, facultatively anaerobic, belonging to the *Streptococcaceae* family
TGE	tryptone glucos extract agar
THG	tryptone-soy-glucose
Trichoderma spp.	filamentous fungi
Trichosporon spp.	yeast; *Trichosporon asahii, Trichosporon mycoides*
TSA	tryptone-soy-agar
UHT	ultra high temperature

ULPA	ultra low penetration air
UVC	shortwave UV radiation
UV-light	ultra violet light
UVM broth	modified Listeria enrichment broth
Verticillium spp.	filamentous fungi
Vibrio spp.	Gram-negative bacteria, rods, facultatively anaerobic, belonging to the *Vibrionaceae* family; *Vibrio fischeri*
VRBGA	violet red bile glucose agar
Xanthomonas spp.	Gram-negative bacteria, rods, strict aerobes, belonging to the *Pseudomonadaceae* family
Yersinia spp.	Gram-negative bacteria, cells ovoid or rods, facultatively anaerobic, belonging to the *Enterobacteriaceae* family; *Yersinia kristensenii*
YGC	yeast extract-glucose-chloramphenicol agar
YM	Rose Bengal agar
YPD	yeast-extract peptone D-glucose agar
Zygomycetes	class of fungi
Zygosaccharomyces spp.	yeast; *Zygosaccharomes rouxii*

APPENDIX 2: LIST OF PROJECT EVENTS

Occasion	Date	Material
Books	DairyNI: 21 November, 2002	VTT Publications 481: 96 p. + 43 p. in 15 appendices
	DairyNET: 7 October, 2004	VTT Publications 545: 253 p. + 63 p. in 8 appendices
Annual reports	2001: 30 November 2001	Confidential reports 1-page public summary reports
	2002: 31December 2002	
	2003: 19 February 2004	
	2004: 30 November 2004	
Master thesis	2002	Arpiainen, M. An ultrasonic washing system and its applicability to cleaning of returnable plastic crates. University of Helsinki: EKT-series 1282. 95 p.
	2003	Skånseng, B. Identification and characterisation of *Bacillus* spp. isolated from post-pasteurisation sites in dairies. Ås: NLH, 54 p.
Bachelor thesis	2002	Nuutinen, U. Controlling the air quality and microbes in Herajoki dairy plant. Hämeenlinna: Häme Polytechnic
	2004	Jokinen, M. Optimizing of CIP-cleaning and testing of ATP-method to replace traditional cultivation Espoo–Vantaa Institute of Technology, 60 p.
	2004	Ehavald, H. Hygiene survey in Estonian dairies and pathogen analysis. Tallinn: TTU, 50 p.
Nordic dairy platform meetings	Meeting in Göteborg	October 2001
	Meeting in Stockholm	January 2002
	Meeting in Akureyri	June 2002
	Meeting in Kolding	November 2002
	Meeting in Turku	April 2003
	Meeting in Malmö	September 2003
	Meeting in Oslo	March 2004
	Meeting in Riihimäki	October 2004
Project web-site	2001–2004	www.nordicinnovation.net
NIF conference "The future for Nordic food innovation in a European context"	28–29 January 2002 in Stockholm, Sweden	Poster presentation of the Nordic Dairy Network Project P00027 "DairyNET -Hygiene Control in Dairies" (2001–2004). In proceedings", Oslo. 1 p.
NICe Forum 2004	10 September 2004 in Reykjavik, Iceland	Oral presentation "DairyNET hygiene control in dairies" and "Expericences on coordination of the network and synergy project DairyNET"

APPENDIX 3: LIST OF PUBLICATIONS

2001

- Kure, C. F., Wasteson, Y., Brendehaug, J. & Skaar, I. 2001. Mould contaminants on Jarlsberg and Norvegia cheese blocks from four factories. Int. J. Food Microbiol., Vol. 70, pp. 21–27.
- Salo, S. & Wirtanen, G. 2001. Enhanced low pressure cleaning procedures for open process surfaces. Renhetsteknik, 30, 1:15–16.
- Salo, S., Björnsdóttir, R. & Wirtanen, G. 2001. Evaluation of *Listeria* sampling methods used in food processing environments. In: Proceedings of International Conference "Food in Europe: Building in Safety" (cd-rom & www.ainia.es/safetycongress). 1 p.
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- Wirtanen, G., Heino, A. & Salo, S. 2001. Ultrasound cleaning in cheese mold hygiene. Renhetsteknik, 30, 1:26–27.
- Wirtanen, G., Aalto, M., Härkönen, P., Gilbert, P. & Mattila-Sandholm, T. 2001. Efficacy testing of commercial disinfectants against foodborne pathogenic and spoilage microbes in biofilm-constructs. European Food Research and Technology, 213, 409–414.
- Wirtanen, G., Kontulainen, S. & Salo, S. 2001. Effects of cleaners of biofouled stainless-steel surfaces in yoghurt manufacturing equipment. Renhetsteknik, 30, 1:28.
- Wirtanen, G. & Salo, S. 2001. Samarbete inom mejerihygien i Norden – Finländsk delrapport. Renhetsteknik, 30, 1:26.
- Wirtanen, G. Salo, S., Aalto, M. & Gilbert, P. 2001. Disinfectant testing using microbes grown in biofilm-constructs. Renhetsteknik, 30, 1:29.
- Wirtanen, G., Salo, S., Helander, I.M. & Mattila-Sandholm, T. 2001. Microbiological methods for testing disinfectant efficiency on *Pseudomonas* biofilm. Colloids and Surfaces B: Biointerfaces, 20, pp. 37–50.

2002

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- Langsrud, S. 2002. Taper vi bakteriekrigen? Resistens mot desinfeksjonsmider. Proceedings of the 33rd Nordic R3-symposium in Contamination Control and Cleanroom Technology. Pp. 195–197.
- Salo, S. & Wirtanen, G. 2002. DairyNET – Hygiene control in dairy environment. Internet proceedings of Bio-IMEB Biofilms in Industry, Medicine & Environmental Biotechnogy Euro Summer School. 1 p.
- Wirtanen, G., Langsrud, S., Salo, S., Olofson, U., Alnås, H., Neuman, M., Homleid, J.P. & Mattila-Sandholm, T. 2002. Evaluation of sanitation procedures for use in dairies. Espoo: VTT Publication 481. 96 p. + app. 43 p. ISBN 951-38-6017-5; 951-38-6018-3.
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- Wirtanen, G., Salo, S. & Storgårds, E. 2002. Microbial assessment methods.used.in cleaning efficacy evaluation. In: Matuszek, T. (ed.) Food, packaging,equipment and building surfaces in their contribution to food products contamination and process safety. Gdansk: Gdansk University of Technology Publishing Office. Pp. 49–57. ISBN 83-88579-40-1.
- Wirtanen, G., Salo, S., Heino, A., Hattula, T. & Mattila-Sandholm, T. 2002. Comparison of ultrasound based cleaning programs for cheesery utensils. In: Fouling, Cleaning and Disinfection in Food Processing, Wilson, D.I, Fryer, P.J. & Hastings, A.P.M. (eds.). Cambridge: City Services Design and Print. Pp. 165–171. ISBN 0 9542483 0 9.

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- Friis, A & Jensen, B. 2003. Evaluating hygiene in closed systems – state of the art. In: 34th R3-Nordic Contamination Control Symposium. Wirtanen, G. & Salo S. (eds). Espoo: VTT Symposium 229. Pp. 45–54. ISBN: 951-38-6284-4; 951-38-6285-2.
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- Klemetti, I., Arpiainen, M., Salo, S. & Wirtanen, G. 2003. An ultrasonic washing system and its applicability in cleaning of returnable plastic crates. In: 34th R3-Nordic Contamination Control Symposium. Wirtanen, G. & Salo S. (eds). Espoo: VTT Symposium 229. Pp. 37–43. ISBN: 951-38-6284-4; 951-38-6285-2.
- Kure, C.F, Skaar, I., Holst-Jensen, A. & Abeln, E.C.A. 2003. The use of AFLP to relate cheese contaminating *Penicillium* strains to specific points in the production plants. Int. J. Food Microbiol., Vol. 83, pp. 195–204.

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- Langsrud, S., Møretrø, T. & Sundheim, G. 2003. Characterisation of *Serratia marcescens* surviving in disinfecting footbaths. J. Appl. Microbiol., Vol. 95, pp. 196–195.
- Langsrud, S., Sidhu, M.S., Heir, E. & Holck, A.L. 2003. Bacterial disinfectant resistance – a challenge for the food industry. Int. Biodeterior. Biodegrad. Vol. 51, pp. 283–290.
- Lappalainen, J., Salo, S. & Wirtanen, G. 2003. Detergent and disinfectant residue testing with photobacteria. In: 34th R3-Nordic Contamination Control Symposium. Wirtanen, G. & Salo S. (eds). Espoo: VTT Symposium 229. Pp. 151–159. ISBN: 951-38-6284-4; 951-38-6285-2.
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- Salo, S. 2003. Handwashing as a tool against *Salmonella* in FOSARE Seminar 1 (QLK1 2002 30480) proceedings "Newly Emerging Pathogens, Including Risk Assessment and Risk Management". P. 25.
- Salo, S. 2003. In-line hygiene testing of process pipelines in FOSARE Seminar 3 (QLK1 2002 30480) proceedings "Food Safety in Relation to Novel Packaging Technologies". P. 37.
- Wirtanen, G., Mattila-Sandholm, T. & Salo, S. 2003. Biofilm formation and removal in the food industry (Formación y eliminación de biofilms en la industria alimentaria). In: III Seminario Internacional Fundisa Technologías Disponibles Hoy para Garantizar la Seguridad Alimentaria, 2003, Lizaso Azcárate, J. & Antón Boix, A. (eds). Madrid: FUNDISA. Pp. 299–336. ISBN: 84-607-8834-2.
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APPENDIX 4: DAIRYNET – HYGIENKONTROLL I NORDISKA MEJERIER

ABSTRAKT

Forskningsarbetet i det tredje nordiska projektet inom mejerihygien P00027 DairyNET – Hygienkontroll i mejerier, som har financierats av Nordiskt Innovationscenter (tidigare Nordisk Industrifond), har fokuserat på kvalitet av mjölk, process ytors and vattens hygien, luftens hygien i processutrymmen och produktkvalitet. Arbetet har utförts i de nordiska mejerierna Arla Foods, Milko, Norðurmjolk, Norrmejerier, Skåne mejerier, TINE och Valio Oy tillsammans med forskningsenheter vid BioCentrum-DTU, Teknologisk Institut (DTI), Rannsóknastofnun fiskiðnaðarins (IFL), Kungliga tekniska högskolan (KTH), MATFORSK, SIK, Háskólanum á Akureyri och VTT Bioteknik samt framställare av rengöringskemikalier, reagens och utrustning: Finnsonic Oy, JohnsonDiversey, Lagafors Fabriks AB, Mjöll, TetraPak Nordisk Processering och Orion Diagnostica Oy från maj 2001 till oktober 2004. Docent Gun Wirtanen, VTT Bioteknik, har koordinerat projektet. Oddur Gunnarsson har fungerat som seniorrådgivare för projektet vid Nordiskt Innovationscenter. De olika studierna har diskuterats på projektmötena i det nordiska samarbetsprojektet DairyNET för att skapa synergi mellan de 5 nationella projekten. Nya hygienprocedurer baserade på projektresultat har införts vid de olika mejerierna. Resultaten i de 5 nationella projekten och i det nordiska mejerinätverket kan summeras enligt följande:

- Ett exempel på nya processhygienindikatorer som enligt de nya direktiven bör mätas är rester av rengörings- och desinficeringskemikalier som blir kvar på ytan efter den slutliga sköljningen. Ingen mikrobiologisk tillväxt kan leda till felaktig tolkning av processhygienen, då endast den totala mikrobmängden uppmäts. I det **1. synergistudien** valde vi att testa kemikalierester i livsmedelsprocessen, eftersom inga tester normalt görs för att testa kemiakieresterna på ytorna efter sköljning. Resultatet av metoden som baserar sig på inhibering av bakterieluminescencen visar att metoden kan användas för mätning av kemikalierester både i lösning och på ytor. Målet med den **2. synergiuppgiften** var att ta fram en metod för testning av rengöringen i öppen produktionsutrustning. Problematiska eller kritiska punkter kan mätas med UV-ljus. Detta arbete visade att platser som är kända

för svår rengörbarhet kunde visualiseras med UV-ljus. Visualisering av ”kända” problempunkter är viktigt, eftersom det också möjliggör utpekning av problematiska punkter i komplex utrustning, där speciell uppmärksamhet vid rengöring behövs. Man har också diskuterat om alla mögel som påvisats i ysteriernas luft kontaminerar osten eller inte? I den **3. synergistudien** användes ett selektivt medium Creatine sucrose dichloran agar (CREAD) tillsammans med vanligen använda agar för isolering av luftburna mögel för att se om CREAD agar kan användas för en snabb detektering av skadliga mögel i ysterier. De mögel som växer på CREAD agar växer också på osten.

- Enligt norsk lag skall den bakteriella mängden i råmjölk före pastörisering inte överstiga 300.000 per ml. Mjölken från lantgården skall innehålla mindre än 100.000 per ml. Huvudorsaken till bakterietalet i mjölken är hygienen, åldern och uppbevaringshistorian. Ändamålet med denna studie var att undersöka **hygienen vid olika processpunkter i mejeriet** före pastöriseringen och att om möjligt få fram ett samband mellan rengöring, de hygieniska förhållanden och råmjölkens kvalitet före processering. I fem norska mejerier undersöktes rengörings- och processeringsrutinerna samt resultaten från kvalitetskontrollen. Resultaten av översikten visade att de mejerier som använde traditionell rengöring hade den bästa hygieniska nivån. En möjlig orsak till detta är den organiska kontamineringen genom disinficeringslösningen. Ingen av mejerierna kunde bevisa ett samband mellan den mikrobiologiska nivån i råmjölken från lantgården och mejeriets silon samt den mikrobiologiska nivån i den pastöriserade produkten.

- ***Listeria monocytogenes*** är en viktig livsmedelsburen patogen och dess ursprung, **kontamineringsrutt** samt påvisning är kritisk för livsmedelsindustrin. Nuvarande undersökning omfattade förekomst och utbredning av *L. monocytogenes* i hela processen från ladugården till mejeriet i norra Island. vid ett isländskt mejeri. Mer än 1400 prov samlades in under ett år för att också få fram säsongsvariationer. Inga positiva *L. monocytogenes*-prov fanns bland produktproven (n = 200) och i mejeri processlinjen (n = 250). I mjölken som samlades in under vintern var 13 prov positiva och inga av proven tagna under sommaren var positiva (n i båda serierna 459). De *L. monocytogenes*-positiva resultaten i obehandlad mjölk indikerar att det finns en variation beroende på årstid. För att få fram

spårbarheten av *L. monocytogenes* togs ytterligare prov från alla mjölkkor (n = 20) från en av de infekterade lantgårdarna tillsammans med ytterligare 30 prov från skjulområdet och ensilaget. Resultaten visade att en ko och 8 prov i skjulområdet var *L. monocytogenes*-positiva, isolaten från en och samma lantgård tillhörde alla samma ribogrupp och pulstyp.

- I det svenska projektet analyserades **luft, vatten och ost** från 4 ysterier för Gram-negativa bakterier, jäst och mögel. Resultaten påvisade möjligheten till vattenburen kontaminering i processutrymmena p.g.a. otillräcklig hygien och underhåll av vattenkällor ss. slangar, dysor och kranar. Mikrober som oftast funnits i vattenkällorna är bakterier ss. *Acinteobacter* spp. och *Pseudomonas* spp., jäst ss. *Candida* spp. och *Rhodotorula* spp. Samt mögel ss. *Phoma* spp., *Fusarium* spp. och *Acremonium* spp. *Penicillium* spp. Hittades ytterst sällan i vattenkällorna. Provtagning av luft kan användas för att identifiera platser längs processlinjerna, vilka är kritiska för luftburen kontamination. De få ostproven som analyserades, indikerade luften som en primär källa för mögel kontaminering av ost och vatten som en tilläggskälla för *Geotrichum* spp. och *Trichoderma* spp.

- En bioaerosol kan definieras som en suspension innehållande mikroskopiska fasta och vätskepartiklar samt mikrober. Det finns ett antal sätt på vilka biologiskt och mikrobiologiskt material kan göras luftburet, t.ex. mänskans rörelser, nysningar och hostningar. Bioaerosoler av svamp (dvs. jäst och mögel) har observerats vara mycket beroende av väderleken. Som en del av det svenska projektet inom DairyNET utfördes en **hygienstudie om luftburna mikrobiologiska partiklar** samt totala partikel mängden **i 4 svenska ysterianläggningar**. Den mikrobiologiska mätningen utfördes med 3 olika mätmetoder användande olika medier för mätning av tillväxt av bakterier, jäst och mögel. Generellt indikerar testresultaten att mera kontrollerade omgivningar är eftersträvansvärda. För att förbättra effektiviteten i den mikrobiologiska mätningen bör en lämplig kombination av medier och mätutrustning användas. I det finska projektet har **luftkvaliteten vid Herajoki mejeri** undersökts. Bakgrundsinformation för studien samlades in om luftfiltreringssystemet och byggnadens historia. I studien har årstidsvariationer och pågående byggnadsarbete beaktats. Luftkvaliteten var mikrobiologiskt bra enligt befintliga referenser.

- I en fallstudie vid Herajoki mejeri optimerades **CIP-rengöringen** samt kontrollerades renheten av CIP-vattnen. Resultaten påvisade att ATP-metoden kan användas som ett snabbt test för analys av vattnets renhet. Genom optimering av CIP-rengöriungen kan man i mejerierna spara vatten, kemikalier och tid. **Hygienen av returnerbara transportplastlådor** som rengörs i disktunneln bestämdes i Herajoki mejeri. Resultaten visade att bottendelen av transportlådorna var smutsigast och också svårast att rengöra. Design of Experiment (DOE, Taguchi metoden) användes vid optimering av den ultraljudsbaserade rengöringsproceduren. Den ultraljudsbaserade rengöringen är tillämpbar för rengöring av returnerbara transportlådor och den tål jämförelse väl med disk i tunnel. Resultat erhölls från försök i pilotskala med artificiellt nedsmutsade lådbitar. I detta arbete med returnerbara transportlådebitar användes 3 odlingsbaserade metoder dvs. **konventionell odling, Petrifilm och DryCult för mätning av det totala bakteriologiska belastningen**. DryCult® TPC är ett praktiskt val i test utförda på plats.

- **Plattvärmeväxlare** används vitt i mejerierna för uppvärmning, avkylning och regenerativa uppgifter för både material och produkter. Risker för återkontaminering anknyter till läckage genom plattorna. Ibland kan metallens utmattning, användning och korrosion leda till hålbildning i plattorna. För några år sedan togs dubbla plattvärmeväxlare, där plattorna pressats tillsammans med skruvar i ramen, i bruk också i mejerier. Test i ett tyskt institut utfördes för att kontrollera eventuella läckagen då det ena lagret i den dubbla plattan perforerats. Resultaten visade att ett hål i det ena lagret av plattan ledde till visuellt märkbara läckagen. Erfarenhet med dubbla värmeväxelplattor i mejerier är mycket begränsade.

- Framställning av ost är en delvis öppen produktion med många skeden där produkten kan kontamineras. Användning av oren **utrustningen** kan leda till **mikrobiologisk kontaminering**. Kritiska platser var under bälten och på rullar. Resulten visade att sköljvattnet i diskmaskinen för ostformar var nedsmutsad med mikrober. För att kunna motverka kontaminering, måste procedurer för inspektion och rengöring av diskmaskiner för ostformar slås fast. Mätningar av rengörbarheten bör omfatta: definition av kritiska platser, daglig inspektion av en person som inte hör till rengöringsgruppen, dokumentering, regulär auditering av rengöringseffekten, åtgärder baserade på upptäckta avvikelser samt gensvar åt rengöringsgruppen.

- Desinficering krävs i livsmedelsprocesser där fuktiga processytor fungerar som god grogrund för mikrober. Användning av effektiva desinficeringsmedel minimerar produktkontamination, förbättrar produktens uppbevaringstid och minskar risken för livsmedelsburna sjukdomar. En förlängd exponering av ytor för desinficeringsmedel förbättrar den mikrobicidala effekten men en överdriven exponering leder ofta till ökat mikrobiellt motstånd mot använda desinficeringsmedel. I det isländska projektet bestämdes **minimala inhiberingskoncentrationen** för 4 allmänt använda desinficeringsmedel med 140 isolerade mikrober från mejeriutrymmen. De isolerade och karakteriserade stammarna visade generellt att de mera toleranta stammarna hade en 2- till 3-faldig minskning i generationstid med ökad koncentration av desinficeringsmedel, medan en mindre miskning märktes för känsliga stammar. I den norska undersökningen fokuserade man på förekomsten av **resistenta mikrober** i livsmedelsindustrin. Man har ifrågasatt om missbruk av desinficeringsmedel har selektivt påverkat och medverkat till en ökning i processutrymmena av mikrober som innehar förhöjt motstånd till desinficeringsmedel. Mikrober har överlevt kemikaliebehandling ss. dimdesinfektion och disinficering i fotbad. Bakterier har isolerats från ca. 75 % av de testade fotbaden. Inga av de använda desinficeringsmedelen motverkade totalt bakteriell överlevnad. *Serratia marcescens* har isolerats från fotbad innehållande Tego i rekommenderbar koncentration. *Methylobacterium* sp., *Rhodococcus erythropolis* och *Rhodotorula mucilaginosa,* som isolerats i processen, visade sig också vara resistenta mot flera desinficeringsmedel.

- Detaljerade råd om hygienisk design av utrustning finns i många generella anvisningar och harmoniserade europeiska standarder. I 5 fallstudier om utrustning med dålig hygienisk design (en statisk fluidiserad bädd, en DuoSafe plattvärmeväxlare, en ostskivare, en säckfyllare och en plastflaskefyllare) visar att praktisk kunskap om processering och om processförhållanden behövs för att kunna planera funktionsduglig utrustning hygienisk. Till dags dato är studier i hygienisk konstruktion samt underhåll av hela processen få. En **integrerade granskning av hygienisk ingenjörskonst** är ett resultat av systematisk kontroll av hygienisk konstruktioner från enkla delar och kopplingar till hela processlinjer för att möta de hygieniska krav som ställs på hela processen. Systemet bygger på vederbörliga definitioner och identifieringar av möjliga problem relaterade till varje nivå i hela hierarkin. Denna granskningsmodell kommer att publiseras som en generell guideline av European Hygienic Engineering and Design Group (EHEDG).

23 SAMMANFATTNING

23.1 MJÖLK KVALITET

Av de 5 norska mejerierna som deltog i undersökningen hade 3 en rengöring med bas och syra samt desinficering med hett vatten. De 2 övriga mejerierna rengjorde med basiskt rengöringsmedel samt desinficerade efteråt med perättikssyrabaserat desinficeringsmedel. Processutrustning och rengöringsstationer undersöktes visuellt med UV-ljus samtidigt som man tog mikrobiologiska prov. Därtill inspekterades rengörings- och processrutinerna samt kvalitetskontrollen. I projektet fokuserade man på provtagningsmetoder, val av provtagningspunkter samt undersökning av mikrofloran och befintlig expertis användes för lösning av problem. Resultaten i undersökningen visade att mejerier med traditionella rengöringsrutiner hade den bästa hygiennivån. Den visuella kontrollen i mejerierna visade att det fanns punkter med otillfredsställande hygien, men varken de mikrobiologiska proven eller den visuella kontrollen påvisade gemensamma allmänna problemområden i mejerierna. Rengöringslösningen, tätningar och områden omfattande problematisk design ss. rengöringsdysor, lock till manluckor, givare, lock på luftseparatorer och tankbilar visade sig vara viktiga kontrollpunkter. Den mikrobiologiska floran på utrustningen efter rengöring och desinficering varierade och mikroberna tillhörde vanliga fördärvande mikrober i både opastöriserad och pastöriserad mjölk. Ingen av mejerierna kunde påvisa samband i den mikrobiologiska kvaliteten mellan obehandlad mjölk från lantgården och mejeriets silotankar samt den pastöriserade produkten.

23.2 *LISTERIA* KONTAMINERINGSRUTTER

Den isländska projektdel fokuserade på kontaminationsrutter för *Listeria. L. monocytogenes* kan överföras med konsumtion av obehandlad mjölkprodukter eller kontaminerade mejeriprodukter. Situationen i mejerierna skiljer sig en aning jämfört med andra livsmedelssektorer, eftersom mjölken pastöriseras. Det är därför rimligt att dra den slutsatsen att mejeriprodukterna kontamineras med *Listeria* längs processlinjen. I undersökningen fanns inga *Listeria*-positiva prov bland de 450 proven som togs i processomgivningen av processutrustning och av utvalda mejeriprodukter. Man fann *L. monocytogenes* i 13 obehandlad mjölk (1.5%) prov, vilket är i samma storleksordning som i andra rapporter i Väst-

Europa och USA. För att spåra *L. monocytogenes* i obehandlad mjölk togs prov av mjölk från alla mjölkkor och från skjulområdet i den lantgård där alla 3 provtagningarna på vintern var positiva. I denna undersökning var alla erhållna ribotyper identiska med de mönster som kommer från *L. monocytogenes*-stammar förutom en ribotyp, som visade sig vara *L. seligeeri*. Det har påvisats att *L. monocytogenes* kan etablera sig och bilda tålig tillväxt, vilket kan leda till biofilmbildning med egen husflora. Det är intressant att se att det finns säsongartade variationer i förekomsten av *L. monocytogenes* i obehandlad mjölk. Rimliga förklaringar är kontaminering i skjulområdet och kontaminerat ensilage under vintern då djuren hålls rätt tätt tillsammans under långa perioder.

23.3 MÄTNING AV LUFTKVALITETEN

I livsmedelsindustrin används sedimenteringsskålar samt aktiv luftprovtagning ss. impaktion eller centrifugering är allmänt använda i rutinanalys av luftens mikrobiologiska kvalitet. Effektiviteten i provtagningen är ytterst beroende av provtagningsmetoden och en aktiv provtagning är mera exakt än den passiva. Det finns två sorters impaktorer med fast yta dvs. provtagningsinstrument med fåror eller sikt. Det mikrobiologiska provtagningsinstrumentet MAS-100 är en impaktor som suger upp luften antingen horisontellt eller vertikalt genom ett perforerat munstycke på agarytan av en standard petriskål. Reuter Centrifugal Sampler (RCS) är lätt att använda men den samlar inte upp alla små levande partiklarna. Filtreringsmetoden samlar inte heller upp alla vegetativa celler i levande form eftersom metoden utsätter cellerna för stress genom dehydrering vid provtagningen. Tiden för provtagningen påverkar dehydreringen och är därför viktig vid uppsamling av känsliga bakterier. Gelatinfiltermembran består av gelatinskum som skyddar de vegetativa cellerna från torkning vid filtreringen, vilket gör metoden mera tillförlitlig. Var och en av de föreslagna mätmetoder har sina begränsingar. Man skall också komma ihåg att de uppsamlade luftburna cellerna tagna med olika metoder inte kan jämföras direkt eftersom insamlingsmetoden påverkar resultatet. De olika metoderna uppvisar högst troligt samma trender. Att förbättra effektiviteten vid mikrobiologisk provtagning bör man kombinera lämpliga medier med lämpliga mätapparater.

Testresultaten från de 4 svenska ysterierna visar att en mera kontrollerbar omgivning är efterstränsvärd. För att uppnå detta i ett ysteri bör process-, uppbevarings- och förpackningsspesifikationer för de olika områdena fastslås av

producenten. Gamla ventilationssystem måste uppgraderas för att den behövliga luftkvaliteten uppnås. Korrekt installerade system med övertryck och luftflöden behövs i processutrymmen där öppna produkter, vilka är känsliga för kontaminering, uppbevaras. I sådana processteg skulle lokala zoner med HEPA-filters förbättra processutrymmena. Provtagningen i processomgivningen skall baseras på riskuppskattning. I det finska delprojektet uppmättes luftkvaliteten under 2001 i Herajoki mejeriet, då ny- och ombygnadsarbeten utfördes i processutrymmena. I denna studie erhölls jäst och mögel i samma storleksordning med både RCS- and MAS-mätare. Provtagningen med RCS är mycket användbar vid mätning av luftkvaliteten i mejerier trots att denna metod inte rekommenderas som primär mätmetod för jäst och mögel. Man kunde också påvisa att man med en uppdelning av produktionsområdet och en upprätthållning av övertryck i kritiska processutrymmen har kunnat undvika kontamination från områden invid.

23.4 HYGIENEN I MEJERIPROCESSEN

För att kunna utföra en hygienisk kartläggning rekommenderas att man har en provtagningsplan för vatten, luft, ytor och produkter. Det är viktigt att provtagningspunkterna är slumpvist valda och att man inte på förhand slår fast vad som man antar vara problem. Uppsamling och fortsatt karakterisering av de mikrobiologiska isolaten avslöjar identiteten och källan för kritiska mikrober i anläggningen. Resultaten påvisar möjligheten av vattenburen kontaminering i processen p.g.a otillräcklig hygien och underhåll av vattenkällor ss. slangar, dysor och kranar. Några råd om hur provtagning i vattensystemet och underhåll av vattenslangar skall utföras ges i den svenska vattenakten som utkom den 25 december 2003, detta för att upprätthålla vattenkvaliteten av det vatten som tas från vattenslangar. Produktrelaterad provtagning skall speciellt utföras då felaktiga produkter framställts och då vattnet anses förorsaka problemen. Analyser som skall utföras skall väljas baserat på de problem som uppkommit i produkten. Vattenburna mikrober som högst troligt kan befästa sig i utrymmena är bakterier ss. *Acinteobacter* spp. och *Pseudomonas* spp.*;* jäst ss. *Candida* spp. och *Rhodotorula* spp.; mögel ss. *Phoma* spp. och *Fusarium* spp. *Penicillium* spp. har ytterst sällan isolerats från vatten.

Positioner med förhöjd kontamination hittades vid provtagning i vattenkällor. I 2 inrättningar upptäcktes flera kontaminerade vattenkällor vars vatten användes

vid rengöring. Man kunde också genom provtagning av luft påvisa processpositioner som bidrog till olämplig processhygien. Renheten av CIP-vatten kan snabbt testas med ATP-metoden, där ATP reagerar med allt organiskt material. I somliga fall kan traditionell odling påvisa att vattnet är mikrobiologiskt smutsigt trots att ATP-metoden ger resultatet att vattnet är rent. I några fall kan ATP-metoden också visa att vattnet inte är rent trots att den traditionella odlingen visar att det är rent. I båda fallen beror det på interferens av kemikalierester i ATP-reaktionen.

Mögelkontamination av halvhård ost är periodvis ett betydande kvalitetsproblem och leder därvid till ekonomiska förluster för ostproducenten. De flesta svampar som hittas i inomhus hygienstudier i ysterierna representerade inte sådana svampar som kontaminerar ostprodukter. Den selektiva CREAD-agarn tillsammans med en generell agar för isolering av mögel testades för att erhålla data om mögel i ysteriomgivning och om dessa mögel kan växa på ostytan. Det är värt att testa identifierade kritiska stammars tillväxt på CREAD-agarn för att kunna tyda mögeltillväxten. Tillväxtförmågan på ost/ostagar t.ex. CREAD-agar i kalla uppbevaringstemperaturer är ett viktigt kriterium för identifiering av kritiska fördärvarstammars på ostprodukter.

Synergiresultat visade att det är möjligt at utveckla testningsprocedurer baserade på UV-ljus för rapportering av rengörbarhet av öppen utrustning. Visualiseringen utfördes med en referensplatta med element av dålig hygienisk design. Referensplattan smutsades med surmjölk och diskades i en “mild” rengöringsprocedure. De punkter som var av dålig design kunde visualiseras. Det är viktigt att kunna utpeka kända ”heta punkters” med metoden för att sedan kunna känna igen problempunkter i komplex utrustning. Användning av en allmänt godkänd testmetod förbättrar designen i ny utrustning eftersom tävling mellan tillverkare av utrustning tilltar. Med en UV-lampa är det enkelt att upptäcka rester på rostfria stålytor. Plastmaterial är autofluorescerande, men det är trots allt möjligt att uppteckna kontaminering eftersom denna ger ett annat ljus. UV-lampan gav ett omedelbart resultat på rengöringseffekten. En omedelbar utvärdering av rengöringen är överlägsen en diskussion baserad på laboratorieresultat som erhålls några dagar senare.

Ostformarna blev inte tillräckligt väl rengjorda i den rengöringsprocedur som normalt används. För att motverka kontaminering bör man fastslå procedurer för

inspektion och rengöring av diskmaskinen för ostform. En god indikator för kontroll av att denna procedur fungerar är analys av sista sköljvattnet. Rengöring av transportband är också känt att vara problematiskt. Nästan 20% av proven innehöll jäst och mögel, förekomsten av Gram-negativa bakterier var mindre allmän. Kritiska kontamineringspunkter fanns under transportband och på rullor. En utvärderingsprocedure som införts på Arla Foods omfattar definiering av kritiska områden/punkter, daglig kontroll av en oberoende person till rengöringsgruppen, dokumentation, regelbunden auditering av rengöringseffekten, åtgärder p.g.a. avvikelser samt respons till rengöringsgruppen.

Resultaten som erhölls av tvättade returnerbara transportlådor visade att man kan utföra en hygienisk klassificering av dem både visuellt och mikrobiologiskt. För att förbättra rengöringsresultatet bör diskparametrarna hållas på en konstant nivå. Vid de mikrobiologiska utvärderingarna som utfördes i processen varierade det mikrobiologiska kolonitalet 80–300 st/ml. En rengöringsprocedur baserad på ultraljud är användbar för rengöring av returnerbara transportlådor av plast och metoden är beaktansvärd i förhållande till tunneldisk då man väljer rengöringsmetod. DryCult® TPC-metoden utgör ett praktiskt val i det fall att testen bör utföras i processutrymmena.

23.5 HYGIENISK DESIGN I INDUSTRIELLA FALLSTUDIER

Det eftersträvade målet vid införande av en holistiskt harmonisering av processdelar är att undvika risker i processen som vi kan införa då vi åtgärdar endast enskilda punkter. Dessa faror kan vara av mikrobiologisk, kemikalisk eller partikulär natur i den konsumerbara produkten. Det andra målet är att minska risken för återkallning av produkter, käromål, dåligt ryckte samt att erhålla en god image. För det tredje, genom att införa en holistisk harmonisering av processen hoppas vi att vi kan minska andra problem av icke-säkerhetsnatur t.ex. negativ inverkan på miljön, överdrivna kostnader, och överdriven användning av resurser ss. vatten, kemikalier eller energi. Till sist bör ett holistiskt tänkande göra det mera troligt att vi också kan maximera kostnadseffektiviteten av den slutliga designen. Arbetet i den kommande EHEDG guidelinen “Hygienic systems integration” utförs av Roland Cocker (Cocker Consulting), Stefan Åkesson (TetraPak), Alan Friis (BioCentrum-DTU), Paul Bartels (A & F Netherlands), Hans Hoogland (Unilever), Gerd Klimmeck (Johnson Diversey), Hans Oosterom (DSM Bakery Ingredients) och Jeff Wilkinson (PGA).

Krav på hygienisk design av utrustning baserar sig på anvisningar från EHEDG och FDA. Dessa anvisningar är i sig själva utmärkta, men hygienisk design kan inte läras enbart i teorin. En viktig förutsättning att kunna formge utrustning för speciella ändamål är att ha en detaljerad praktisk kunskap om de förhållanden utrustningen utsätts för under processering. De flesta livsmedelsföretag blir mer och mer slutna för besökare, vilket betyder att formgivare av livsmedelsutrustning får mindre möjligheter att bekanta sig med processen och också mindre gensvar. Som ett resultat av detta blir det allt svårare för formgivare att erhålla korrekt praktisk förståelse inom området, vilket är livsviktigt då man skall upprätthålla kunskapen om de krav som ställs vid utveckling av ny optimerad design av utrustning. I denna publikation har en översikt av fem exempel på utrustning (en statisk fluidiserad bädd, en DuoSafe plattvärmeväxlare, en ostskivare, en säckfyllare och en plastflaskefyllare) med typiska problem gjorts. I utvärderingen har hygienen i de olika formgivna utrustningarna mätts enligt följande: produktkontaktytor, ytor som är närbelägna till produkten men ej i kontakt med produkten och produktionsmiljön.

Användning av plattvärmeväxlare med dubbla plattor har visat sig kräva tilläggsriskuppskattning av inrättningens design, drift och preventiva underhåll. Flera plattor behövs eftersom värmeeffekten i dubbelplattor väggar är sämre än i enkla plattor. Förorsakade problem är antingen trycksänkning i värmeväxlaren eller lågt flöde per kanal och sänkt flöde kan förorsaka rengöringsproblem. Smala kanaler kan också användas för att förbättra värmeöverföringen, men i heta sektioner kan fouling leda till förtidiga tilltäppningar. Spolning av värmeväxlar skall också undvikas eftersom risken finns att de kapillära krafterna kan suga produktvätske- och/eller tvättlösningsrester in i den läckande kammaren med därpå följande korrosion eller bakteriologisk tillväxt. Det är därför ytterst rekommenderbart att man tillför värmeväxlaren höljen. Det föreligger hög risk att korrosion leder till hål också i andra plattan om smutsen tillåts torka fast mellan plattorna. För att hitta små sprickor som förorskar läckage är regulär trycktestning rekommenderad. Användning av värmeväxlare med dubbla plattor bör användas endast i sådana fall att man inte kan använda sig av andra tekniska lösningar enligt processens spesifikationer.

Forskningsresultaten har påvisat att adhesion och avlägsning av bakterier inte påverkas av ytstrukturen vid ytterst låga R_a-värden (< 0.8 μm). Detta kan leda till antagandet att ultraslätа ytor inte förbättrar hygienen. Hygien är emellertid inte

enbart en fråga om bakterier och smuts utan också om ytors motståndskraft till kemikalier använda i produktionen. Ändamålet med det danska konpetenscentret för den rostfria stålindustrin är att förbättra dess kommande konkurrenskraft t.ex. inom livsmedelsindustrin. Släta ytor är helt klart dyrare och vår forskning föreslår att generella rekommendationer med $R_a < 0.8$ μm inte bör ändras. Det kan vara fördelaktigt att använda fint polerade ytor i rör för transport av produkt som inte kommer att värmebehandlas mera.

23.6 DISINFICERINGSMEDEL – RESTER OCH RESISTANS

Ändamålet med desinficering är att minska antalet mikrober på kontaktytor i livsmedelsprocessen och därigenom undvika kontaminering av råmaterial och produkter med patogener och fördärvare. I det fall att desinficeringen misslyckas kan det i de flesta fall förklaras med användning av för låg koncentration eller temperatur eller för kort verkningstid av desinficeringsmedlet eller att man inte lyckats få bort den organiska smutsen från de ytor, som skall desinfekteras. Sporer och en del resistentat vegetativa celler kan överleva desinficering p.g.a. ökad motståndskraft mot det använda desinficeringsmedlet. Restmätnings-metoden som användes i den första synergiuppgiften baserar sig på mätning av luminescensen av fotobakterier efter det att de utsätts för behandling med ett prov t.ex. av sköljvätska från processen i 5 min. Detta prov jämförs med kranvatten som används som referens. Studierna påvisade att kemikalierester lämnats på processytorna före produktionen startades upp. Resultaten från fotobakterietesten kan kategoriseras i följande restklasser baserat på inhibitionen: mycket klara, medelmåttliga och inga rester. Denna metod erbjuder en användbart alternativ för testning a kemikalierester på ytor.

Olika laboratoriestudier har visat att celler som är fästa vid ytor är mera resistanta för desinficeringsmedel än suspenderade celler är. Detta har lett till att olika typers bärartest t.ex. mikrober intorkade på ytor, biofilmkonstrukt och biofilmformation på testytor. Somliga bakterier som isolerats från processen är tåliga medan andra anpassar sig till desinficeringsmedlen som används. Biofilm-tillväxten på rostfria stålytor påvisar att *Sphingomonas* sp. har en mycket högre faktor för testade desinficeringsmedel än de andra isolaten. I den isländska hygienstudien isolerades 140 stammar under sommar och vinter från mejeri-relaterade omgivningar. Det var intressant att lägga märke till att somliga stammar har ett brett toleransspektrum mot desinficeringsmedel. Flera stammar

identifierades som *Psedomonas putida, Moraxella* sp., *Klebsiella oxytoca* och *Bacillus pumilus*. Det är också intressant att lägga märke till att 24 utav de 25 vidarekarakteriserade stammarna isolerats under sommaren. De flesta var Gram-negativa bakterier (23), vilket stämmer överens med att Gram-negativa är mera motståndskraftiga mot desinficeringsmedel än vad Gram-positiva bakterier är.

APPENDIX 5: DAIRYNET – HYGIENIAN HALLINTA POHJOISMAIDEN MEIJEREISSÄ

ABSTRAKTI

Nordic Innovation Centren (aiemmin Nordic Industrial Fund) rahoittaman kolmannen pohjoismaista meijerihygieniaa käsittelevän projektin ”DairyNET – Hygiene control in dairy environment, P00027” tutkimustyö keskittyi raakamaidon, prosessipintojen prosessivesien ilman ja lopputuotteiden hygienia- ja laatuasioihin. Projektissa toukokuun 2001 ja lokakuun 2004 välisenä aikana tehtyyn työhön ovat osallistuneet pohjoismaiset meijerit Arla Foods, Milko, Nordurmjolk, Norrmejerier, Skåne mejerier, TINE ja Valio Oy sekä tutkimuslaitokset BioCentrum-DTU, Danish Technological Institute (DTI), Icelandic Fisheries Laboratories (IFL), Royal Institute of Technology (KTH), MATFORSK, SIK, Akureyrin yliopisto ja VTT Biotekniikka kuten myös kemikaali-, reagenssi- ja laitevalmistajat Finnsonic Oy, JohnsonDiversey, Lagafors Fabriks AB, Mjöll, TetraPak Nordic Processing and Orion Diagnostica Oy. Dosentti Gun Wirtanen VTT Biotekniikasta on toiminut projektin koordinaattorina. Oddur Gunnarsson Nordic Innovation Centrestä toimi tämän projektin erikoisasiantuntijana. Tutkimustyöt raportoitiin pohjoismaisessa DairyNET-yhteistyöprojektissa, ja niiden pohjalta projektikokouksissa käytiin avointa keskustelua, ja näin ollen saavutettiin huomattavia synergiaetuja. Projektituloksien perusteella joitain uusia hygieniaan vaikuttavia muutoksia on tehty meijeriprosesseihin. Yhteenvetona kaikkien viiden kansallisen projektin sekä pohjoismaisen meijeriyhteistyön tuloksista voidaan todeta seuraavaa:

- Esimerkkinä uudesta prosessihygieniaa indikoivasta määrityksestä voidaan pitää pesuaineiden ja desinfiointiaineiden kemikaalijäämien määrittämistä pinnoilta. Mikrobiologisilla menetelmillä saatu tulos ”ei kasvua” saattaa johtaa prosessihygienian virheelliseen tulkintaan etenkin mikäli vain kokonaismikrobimääriä on mitattu. **Ensimmäinen synergiatehtävä** kemikaalijäämien määrittämisestä tuotantotiloista valittiin toteutettavaksi, koska normaalisti meijereillä ei ole käytössä testimenetelmiä kemikaalijäämien määrittämiseen pinnoilta loppuhuuhtelun jälkeen. Valobakteerin valontuoton vähenemiseen perustuvan menetelmän tulokset osoittivat menetelmän toimivan jäämien määrittämiseen sekä nestemäisistä näytteistä että pintanäytteistä. **Toisen synergiatehtävän** tavoitteena oli kehittää avointen

tuotantolaitteiden pesemiseen vertailumenetelmä. Vaikeasti puhdistuvia ja hygienian kannalta kriittisiä kohtia saadaan määritettyä UV-valon avulla. Tässä työssä osoitettiin, että kohdat, joiden tiedettiin olevan hygieenisen laitesuunnittelun mukaan ongelmallisia, saatiin visualisoitua. Näiden tunnetusti vaikeasti puhdistuvien kohtien visualisointi on tärkeää, koska se mahdollistaa ongelma-alueiden osoittamisen monimutkaisemmissakin laitteissa, joissa vaaditaan erityistä tarkkaavaisuutta laitteelle määritetyn puhdistusohjelman toteuttamisessa. On myös pohdittu, saastuttavatko kaikki juustoloiden ilmasta löydetyt homeet juustoja. **Kolmannessa synergia-tehtävässä** käytettiin selektiivistä kasvatusalustaa, Creatine sucrose dichloran -agaria (CREAD), rinnakkain yleisagarin kanssa ilmavälitteisten homeiden eristämiseen. Tavoitteena oli selvittää, voidaanko CREAD-agaria käyttää haitallisten homeiden nopeaan määritykseen juustoloissa. CREAD-agarilla kasvavat homeet ovat samoja lajeja, joita löydetään juustoista.

- Norjalaisten määräysten mukaan raakamaidon bakteeritaso ennen pastörointia ei saa ylittää 300 000/ml. Tilalta tulevassa maidossa tulisi olla bakteereja alle 100 000/ml. Bakteerimäärään pääasiallisesti vaikuttavat tekijät ovat hygienia sekä maidon ikä ja varastointihistoria. Tämän projektin tarkoituksena oli tutkia meijereissä ennen pastörointiyksikköä olevien kohteiden hygieniaa ja löytää yhteyksiä puhdistuksen, hygieenisen kunnon ja raakamaidon laadun välille. Viidessä norjalaismeijerissä käytiin tarkastamassa puhdistusohjelmat ja prosessirutiinit sekä laadunvalvonnasta saadut tulokset. Tämän kartoituksen mukaan paras hygieniataso saavutettiin meijereissä, joissa oli käytössä perinteiset puhdistusohjelmat. Yhtenä syynä tähän oli luultavasti desinfioivien liuosten orgaaninen saastuminen. Yksikään meijereistä ei pystynyt osoittamaan yhteyttä tilalta tulleen raakamaidon, meijerin raakamaitotankissa olevan maidon ja pastöroidun tuotteen mikrobitason välillä.

- ***Listeria monocytogenes*** on merkittävä elintarvikevälitteinen patogeeni. Sen alkuperäisen lähteen ja **kontaminaatioreittien** määrittämisellä voi olla ratkaiseva merkitys elintarviketeollisuudessa. Tämä islantilaisessa meijerissä tehty tutkimus kohdistettiin *L. monocytogeneksen* esiintymisen ja leviämisen selvittämiseen koko tuotantoketjusta. Yli 1 400 näytettä kerättiin vuoden aikana mahdollisen vuodenaikavaihtelun selvillesaamiseksi. *L. monocytogenes* -positiivisia tuotenäytteitä (n = 200) ja prosessilinja-näytteitä (n = 250) ei löydetty. Talvella kerätyistä maitonäytteistä 13 osoittautui positiiviseksi, ja

kesällä otetuista näytteistä ei löytynyt *L. monocytogenestä* (molemmissa sarjoissa n = 459). Nämä tulokset viittaavat siihen, että tutkimuksessa mukana olleen alueen raakamaitojen *L. monocytogenes* -esiintymät olisivat riippuvaisia vuodenajasta. *L. mono-cytogeneksen* alkuperän selvittämiseksi kerättiin lisänäytteinä yhdeltä positiivisia tuloksia antaneelta tilalta näytteet jokaisesta lypsävästä lehmästä (n = 20) sekä 30 lisänäytettä navetan ympäristöstä ja varastotankeista. Tulosten mukaan yksi lehmä sekä kahdeksan navettaympäristöstä otettua näytettä olivat positiivisia *L. monocytogeneksen* suhteen. Tilalta eristetyt *Listeria*-kannat olivat kaikki samaa riboryhmää ja pulssityyppiä.

- Ruotsalaisessa projektissa **neljästä juustolasta analysoitiin ilmasta, vedestä ja juustoista** Gram-negatiivisia bakteereita, hiivoja ja homeita. Tulokset osoittivat vesipisteiden, kuten letkujen, suuttimien ja hanojen, puutteellisesta hygieniasta ja kunnossapidosta aiheutuvan vesivälitteisen kontaminaatiovaaran tuotantolaitoksella. Yleisimmät vesipisteistä löydetyt mikrobit olivat bakteereita, kuten esim. *Acinteobacter* spp. ja *Pseudomonas* spp.*,* hiivoja, kuten esim. *Candida* spp. ja *Rhodotorula* spp., sekä homeita, kuten esim. *Phoma* spp., *Fusarium* spp. ja *Acremonium* spp. *Penicillium* spp. saatiin harvoin eristettyä vedestä. Ilmanäytteenottoa voidaan käyttää ilmavälitteisille kontaminaatioille alttiiden kohtien osoittamiseen prosessilinjalta. Muutaman juustonäytteen analysointi viittaa siihen, että ensisijainen homekontaminaatiolähde on ilma ja lisäksi vesi on lähteenä *Geotrichum* spp. ja *Trichoderma* spp. -kontaminaatioille.

- Bioaerosoli voidaan määritellä erittäin pieniä kiinteitä tai nestemäisiä hiukkasia sekä mikrobeja sisältäväksi seokseksi. Biologisesta ja mikrobiologisesta materiaalista saattaa tulla ilmavälitteistä usealla tavalla, esim. ihmisten liikkeiden, niistämisen ja aivastelun johdosta. Home-bioaerosolien on havaittu olevan hyvin riippuvaisia sääolosuhteista. Ruotsalaistyöpanostuksen yhtenä osana pohjoismaiseen DairyNET-projektiin tehtiin **ilmavälitteisiä mikrobihiukkasia** ja ilman kokonais-hiukkasmäärää koskeva **hygieniakartoitus** neljässä eri puolilla Ruotsia sijaitsevissa juustoloissa. Mikrobiologinen määritys tehtiin kolmella eri ilmanäytteenottolaitteella, ja bakteereiden, hiivojen ja homeiden kasvatukseen käytettiin erilaisia kasvatusalustoja. Yleisesti ottaen testitulokset viittasivat siihen, että valvotuimmat alueet ovat asianmukaisessa kunnossa. Mikrobiologisten määritysten tehokkuuden parantamiseksi sopivia kasvatusalusta- ja

määrityslaiteyhdistelmiä tulisi käyttää. Suomalais-tutkimuksessa tutkittiin **Herajoen meijerin** ilmanlaatua. Taustatietoja tätä tutkimusta varten kartutettiin tutustumalla Herajoen meijerin ilman-suodatusjärjestelmään ja rakennuksen historiaan. Tämän työn tulosten tarkastelussa oli huomioitava vuodenaikavaihtelut sekä meijerissä käynnissä olleet rakennustyöt. Olemassa olevien suositusten mukaan ilman laatu tuntui olevan hyvä.

- Toisessa Herajoen meijerissä tehdyssä tutkimustyössä tarkoituksena oli **optimoida CIP-pesut ja tarkistaa kiertopesuvesien puhtaus.** Huomattiin, että ATP-menetelmä mahdollistaa veden puhtauden tutkimisen nopeasti. Optimoimalla CIP-pesut meijeri säästää vettä, kemikaaleja ja aikaa. Herajoen meijerissä tutkittiin myös pesutunnelissa puhdistettujen palautuvien maitolaatikkojen hygieniaa. Tulosten mukaan maitolaatikoiden pohjat olivat likaisimpia, ja pohjien pesutulokset olivat huonoimmat. Ultraäänipesurilla tehtävien pesujen optimoinnissa käytettiin apuna Taguchi-koesuunnittelumenetelmää. Keinotekoisesti liatuilla maitolaatikonpaloilla pilottimittakaavassa tehdyt tutkimukset osoittivat ultraäänipesumenetelmän soveltuvan palautuvien maitolaatikoiden puhdistukseen ja pärjäävän myös vertailussa pesutunnelimenetelmälle. Palautuvien muovilaatikoiden puhdis–tusta tutkivassa työssä todennettiin kolmen viljelymenetelmän, perinteisen viljelyn, Petrifilmin ja **DryCultin,** soveltuvuus kokonaisbakteeritason määrittämiseen. DryCult® TPC on käytännöllinen vaihtoehto, jos testit pitää tehdä kentällä.

- Levylämmönvaihtimet ovat laajalti käytössä meijereissä hyödykkeiden ja tuotteiden lämmitys-, jäähdytys- ja regenerointitarkoituksiin. Jälkikontaminaation riski on yhteydessä levyissä tapahtuviin vuotoihin. Joskus laitteeseen muodostuu väsymishalkeamia, kulumia tai ruostetta, ja niiden seurauksena aiheutuu levyn läpi meneviä reikiä. Markkinoille tuli jokunen vuosi sitten meijerikäyttöön tarkoitettuja tuplalevypakoilla varustettuja lämmönvaihtimia, joissa levyt ovat vain puristettu yhteen levylämmönvaihtimen kehikossa olevien ruuvien avulla. Saksalaisinstituutissa tehdyissä tutkimuksissa tarkasteltiin, esiintyykö vuotoja, kun yksi kerros tuplalevypakasta rei'itetään. Testitulokset osoittivat, että reikä yhdessä tuplalevypakan kerroksessa aiheutti vuodon, joka oli havaittavissa visuaalisella tarkastelulla. Kokemukset tuplalevylämmönvaihtimien käytöstä meijereissä ovat kuitenkin vielä melko vähäisiä.

- Juustonvalmistus on osittain avoimessa tilassa tapahtuvaa tuotantoa, jossa valmistettava tuote on alttiina monille mahdollisille kontaminaatioille. **Prosessilaitteet** ovat merkittäviä **mikrobikontaminaatioiden** lähteitä. Kriittisiksi kohdiksi todettiin liukuhihnojen alapuolet ja telat. Tulosten mukaan juustomuottien pesulaitteen loppuhuuhteluvesi oli kontaminoitunut mikrobeilla. Kontaminoitumisen ehkäisemiseksi tulee kehittää juusto-muottien pesulaitteille tarkastus- ja puhdistusohjelmat. Puhtausarvioinnin tulisi sisältää seuraavat asiat: kriittisten alueiden ja kohtien määrittäminen, siivousryhmään kuulumattoman puolueettoman henkilön tekemät päivittäiset tarkastukset, dokumentointi, puhdistuksen tehokkuuden auditointi yleiseltä pohjalta, vaihtelevista tuloksista seuraavat toimenpiteet ja palaute siivousryhmälle.

- Desinfiointi vaaditaan niihin elintarviketehtaan toimintokohtiin, joissa kosteat pinnat tarjoavat suotuisat kasvuolosuhteet mikrobeille. Tehokkaiden desinfiointi-aineiden käyttö minimoi tuotteen kontaminoitumisriskiä, pidentää tuotteen säilyvyysaikaa ja vähentää ruokavälitteisten sairauksien vaaraa. Desinfiointi-aineiden pidennetty vaikutusaika pinnoilla parantaa mikrobisidistä tehoa, mutta ylipitkä vaikutusaika johtaa usein mikrobien vastustuskyvyn lisääntymiseen käytettyä desinfiointiainetta kohtaan. Islannin projektissa **pienin mikrobi-kasvua estävä desinfiointiainekonsentraatio** määritettiin 140 meijeri-ympäristöstä eristetyllä mikrobikannalla ja neljällä desinfiointi-aineella. Eristetyillä ja tyypitetyillä kannoilla tehdyt kokeet osoittivat yleisesti, että vastustuskykyisimpien kantojen jakaantumisajat pienenivät kaksin- tai kolminkertaisesti desinfiointiainekonsentraatioiden kasvaessa kun taas herkillä kannoilla havaittiin pienempiä vähenemiä. Myös Norjan projektissa tutki-mukset keskittyivät elintarviketeollisuudessa esiintyviin **resistensseihin mikrobeihin**. On pohdittu, aiheuttaako desinfiointiaineiden väärinkäyttö valikoitumista ja edesauttaako se desinfiointiaineille resistenttien mikrobien ilmaantumista prosessiympäristöön. Mikrobit ovat selviytyneet meijereissä kemikaalikäsittelyistä, kuten esimerkiksi sumutusdesinfioinnista ja jalkine-desinfiointialtaista. Bakteereja eristettiin noin 75 % tutkituista jalkine-desinfiointialtaista, ja yksikään käytetyistä desinfiointiaineista ei pystynyt täysin tuhoamaan mikrobipopulaatiota. Tego-desinfiointiainetta sisältäneistä jalkinedesinfiointialtaista eristetyt *Serratia marcescens* -kannat olivat vastus-tuskykyisiä suositetulle käyttökonsentraatiolle. Prosessiympäristöstä eristetyt *Methylobacterium* sp., *Rhodococcus erythropolis* ja *Rhodotorula mucilaginosa* -kannat olivat myös vastustuskykyisiä useille desinfiointi-aineille.

- Yksityiskohtaiset neuvot ja ohjeet hygieenisestä laitesuunnittelusta ovat usean yhtenäistetyn eurooppalaisen standardin ja ohjeen aiheena. Viisi esimerkkiä huonoista laitesuunnitteluratkaisuista (leijupetireaktori, tuplapakkalevylämmönvaihdin, juustonviipalointikone, pussituslaite ja muovipullojen täyttölaite) osoittavat, että käytännöllistä tietoa prosesseista ja prosessiolosuhteista tarvitaan, jotta pystytään tekemään hygieenisiä rakennelmia. Kuitenkin koko tuotantolaitoksen hygieenistä rakentamista ja kunnossapitoa koskevia tutkimuksia on toistaiseksi riittämättömästi. **Integroitu lähestyminen hygieeniseen rakentamiseen** on saatu tuloksena systemaattisesta lähestymisestä hygieeniseen tehdasrakentamiseen pienistä osista ja prosessilinjojen välisistä yhteyksistä koko tuotantolaitoksen hygieniavaatimusten saavuttamiseksi. Järjestelmä perustuu asianmukaisiin määritelmiin ja joka hierarkiatasoon liittyvien mahdollisten ongelmatyyppien identifiointiin. Tämä lähestymismalli tullaan julkaisemaan European Hygienic Engineering and Design Group (EHEDG) -ohjesarjassa.

23 JOHTOPÄÄTÖKSET

23.1 RAAKAMAIDON LAATU

Viidestä tutkitusta norjalaismeijeristä kolme käytti pesuissa emästä ja happoja ja pesun jälkeiseen desinfiointiin kuumaa vettä. Loput kaksi meijeriä pesivät emäksellä ja desinfioivat peretikkahappopohjaisilla happamilla desinfiointiaineilla. Prosessilaitteita ja pesuasemia tarkasteltiin visuaalisesti UV-valossa, ja niistä otettiin mikrobiologisia näytteitä. Lisäksi puhdistusohjelmia ja prosessirutiineja sekä laadunvalvonnan tuloksia tarkasteltiin. Näytteenottomenetelmät, näytteenottokohtien valinta ja mikrobiston tutkiminen mukautuivat projektin aikana saatujen kokemusten mukaan. Tämän kartoituksen mukaan paras hygieniataso saavutettiin meijereissä, joissa oli käytössä perinteiset puhdistusohjelmat. Visuaalinen tarkastelu osoitti, että jokaisessa meijerissä oli kohtia, joiden hygienia ei ollut tyydyttävä, mutta mikrobiologiset näytteet ja visualinen tarkastelu eivät paljastaneet kaikille meijereille yleisiä ongelma-alueita. Tärkeitä tarkkailupisteitä ovat puhdistusaineet, tiivisteet ja hygieenisen laitesuunnittelun kannalta hankalat alueet, kuten pesusuuttimet, miesluukun kannen ympäristö, anturit, ilmaseparaattorien kannet ja säiliöautot. Laitteistoista pesun ja desinfioinnin jälkeen määritetty mikrobisto kuului yleisiin raakamaidon ja pastöroidun maidon pilaajabakteerilajistoihin. Yksikään meijereistä ei pystynyt osoittamaan yhteyttä tilalta tulleen raakamaidon, meijerin raakamaitotankissa olevan maidon ja pastöroidun tuotteen mikrobitason välillä.

23.2 *LISTERIAN* KONTAMINAATIOREITIT

Tämä projekti keskittyi pohjoismaiden meijerihygieniaan, ja Islannissa tehdyt tutkimukset kohdistettiin *Listerian* kontaminaatioreittien selvittämiseen. *L. monocytogenes* voi välittyä raakamaitotuotteiden tai kontaminoituneiden meijerituotteiden kulutuksen yhteydessä. Meijeriteollisuuden tilanne on hieman erilainen verrattaessa muihin elintarvikesektoreihin, sillä maito käy läpi pastörointiprosessin. Näin ollen voidaan olettaa, että meijerituotteiden kontaminoituminen *Listerialla* on peräisin prosessilinjasta. Tässä kartoituksessa 450 meijerin ympäristöstä, prosessilaitteista ja valikoiduista meijerituotteista otetuista näytteistä ei löytynyt *Listeria*-positiivisia näytteitä. *L. monocytogenes* löydettiin 13 raakamaitonäytteestä (1,5 %), mikä on samalla tasolla aiemmin Länsi-Euroopassa ja Amerikassa raportoitujen tulosten kanssa. Raakamaidossa

olleen *L. monocytogeneksen* alkuperän selvittämiseksi kerättiin lisänäytteinä kaikkina kolmena talvinäytteenottokerralla positiivisia tuloksia antaneelta tilalta näytteet jokaisesta lypsävästä lehmästä sekä näytteitä navetan ympäristöstä. Kaikki tässä tutkimuksessa saadut ribotyypit olivat samoja kirjastossa olevien *L. monocytogenes* kuvioiden kanssa yhtä lukuun ottamatta, joka oli *L. seligeeri*. Nämä tutkimukset varmentavat, että *L. monocytogenes* voi vakiinnuttaa paikkansa ja pysyä paikallaan pitkiä ajanjaksoja, mikä tuntuu olevan osoitus laitoksen sisäisen kannan muodostumisesta. Lisäksi oli mielenkiintoista huomata, että raakamaidon *L. monocytogenes* -esiintymät näyttivät olevan riippuvaisia vuodenajoista. Tämä ilmiö voi johtua siitä, että navetan ympäristöstä ja säiliöistä tulee kontaminaatioita talviaikaan, kun eläimiä pidetään suhteellisen lähekkäin pitkiä aikoja.

23.3 ILMAN LAADUN ARVIOINTI

Elintarviketeollisuudessa yleisimmin käytettyjä rutiinimenetelmiä ilmanlaadun valvontaan ovat laskeumamaljat ja ilmakeräimet, jotka perustuvat mikrobien keräykseen törmäys- tai linkoamismenetelmällä. Seurannan tehokkuus on riippuvainen laajalti valvontamenetelmistä, ja ilmakeräimet ovat osoittautuneet tarkemmiksi näytteenottomenetelmiksi. On olemassa kahdentyyppisiä laitteita, joissa ilma ja ilman mikrobit törmäävät kiinteään alustaan: rakokeräimiä ja seulakeräimiä. Ilmamikrobien keräyslaite MAS-100 (microbial air sampler) on törmäysperiaatteella toimiva laite, joka imee vaaka- tai pystysuorassa asennossa olevan rei'itetyn suuttimen läpi ilmaa normaalikokoisella petrimaljalla olevan agarin pinnalle. Reuterin linkokeräin RCS (Reuter Centrifugal Sampler) on helppokäyttöinen, mutta kaikkien hyvin pienikokoisten elävien hiukkasten elpyminen ei ole todennäköistä. Suodatusmenetelmät eivät kerää kaikkia kasvutilassa olevia soluja talteen elinkelpoisessa muodossa, koska keräyksen aikana tapahtuva kuivuminen rasittaa soluja. Keräysaika vaikuttaa kuivumiseen ja on siten kriittinen tekijä herkkien mikrobikantojen elpymisen kannalta. Gelatiinivaahdosta valmistetut gelatiinisuodatinkalvot on suunniteltu suojelemaan kasvutilassa olevia mikrobeja suodatuksenaikaisen kuivumisen inaktivoivalta vaikutukselta ja siten parantamaan menetelmän luotettavuutta. Kaikilla ehdotetuilla valvontamenetelmillä on rajoituksensa. On myös huomioitava, että eri keräysmenetelmillä saadut ilmavälitteisten mikrobien määrät eivät ole suoraan vertailukelpoisia keskenään mutta ne todennäköisimmin näyttävät

samansuuntaisia trendejä. Mikrobivalvonnan tehostamiseksi tulisi käyttää sopivia yhdistelmiä kasvatusalustoista ja näytteenottolaitteista.

Neljän ruotsalaismeijerin tulokset osoittavat, että valvotuimmat ympäristöt ovat asianmukaisemmassa kunnossa. Kontrolloidumman ympäristön saavuttamiseksi juustoloissa valmistajien täytyy luoda laatuvaatimukset ohjearvoineen tuotannolle, varastointi- ja pakkausalueille. Vanhat ilmastointijärjestelmät tulee ajanmukaistaa, jotta vaadittava ilmanlaatu saavutetaan. Oikeaoppisesti asennetut järjestelmät ylipaineistuksineen ja ilmavirtoineen ovat tarpeen tuotantoalueilla, joissa pidetään kontaminaatioille alttiina olevia tuotteita ilman suojaavia pakkauksia. Tällaisissa tuotantovaiheissa HEPA-suodattimilla varustetut täsmäkohteet saattaisivat parantaa tuotantotoimintoja. Ympäristön valvonnan pitäisi perustua riskinarviointiin. Suomalaisessa osaprojektissa tutkittiin Herajoen meijerin ilman laatua vuonna 2001, jolloin tuotantotiloissa tehtiin korjaus- ja rakennustöitä. Tässä tutkimuksessa hiiva- ja homemäärien tasot olivat kutakuinkin samat käytettäessä RCS- ja MAS-ilmakeräimiä. RCS-ilmakeräin on käyttökelpoinen laite meijerin ilman mikrobiologisen laadun määritykseen, vaikkakaan sitä ei suositella ensisijaiseksi laitteeksi hiivojen ja homeiden määritykseen. Osoitettiin, että jakamalla tuotantoalue ja pitämällä kriittiset osastot ylipaineistettuna voitiin välttyä viereisistä tiloista tulevilta kontaminaatioilta.

23.4 HYGIENIA MEIJERIPROSESSEISSA

Tehtäessä hygieniakartoitusta suositellaan näytteenottosuunnitelman laatimista vedelle, ilmalle, pinnoille ja juustolle. On kohtalokasta valita näytteenottokohdat sattumanvaraisesti ilman mahdollisen ongelmapaikkojen arviointia. Mikrobikantojen keräys ja sitä seuraava tyypitys paljastavat kriittiset mikrobilajit ja niiden lähteen laitoksessa. Nämä tulokset havainnollistivat vesivälitteisen kontaminaation voivan tapahtua tuotantolaitoksessa vesilähteiden, kuten letkujen, suuttimien ja hanojen, riittämättömän hygienian ja kunnossapidon johdosta. Neuvoja näytteenottoon ja vesiletkujen kunnossapitoon niin, että veden laatu pysyy samantasoisena koko letkun läpi tullessaan, annetaan 25. joulukuuta 2003 päivätyssä Ruotsin vesiasetuksessa. Tuotteeseen liittyvä näytteenotto tulisi tehdä, mikäli veden epäillään aiheuttavan ongelmia tuotannossa. Tehtävät analyysit tulisi valita tuotteesta saatujen kokemusten mukaisten ongelmatyyppien mukaan. Näistä ympäristöistä löydettäviä mikrobeja ovat todennä-

köisimmin bakteerit, kuten *Acinteobacter* spp. ja *Pseudomonas* spp., hiivat, kuten *Candida* spp. ja *Rhodotorula* spp., ja homeet, kuten *Phoma* spp. ja *Fusarium* spp. *Penicillium* spp. eristettiin harvoin vedestä.

Paikat, joissa kontaminaatiotasot olivat koholla, saatiin määritettyä ottamalla näytteitä vesipisteistä. Kahdessa tehtaassa havaittiin useita kontaminoituneita vesipisteitä, joista otettiin vettä pesuihin. Huomattiin myös, että ilmanäytteenottoa voidaan käyttää osoittamaan prosessikohtia, joilla on osuutta huonoon hygieniaan. CIP-veden puhtautta voidaan testata ATP-menetelmällä, jossa kaikissa orgaanisissa aineissa oleva ATP reagoi ja näyttää nopeasti, onko vesi puhdasta vai ei. Perinteinen viljely voi osoittaa veden olevan likaista eli veden mikrobitaso on korkea, mutta tietyistä kemikaalijäämistä aiheutuvien häiriötekijöiden vuoksi ATP-menetelmä voi osoittaa saman vesinäytteen olevan puhdasta. Joissain tapauksissa ATP-menetelmä voi osoittaa, että vesi ei ole puhdasta, mutta perinteinen viljely osoittaa veden olevan hyvin puhdasta. Tämäkin johtuu siitä, että kemikaalit vaikuttavat ATP reaktioon.

Puolikovien juustojen homekontaminaatiot ovat ajoittain merkittävä laatuongelma, ja siten sillä on taloudellisia vaikutuksia juustontuottajille. Juustoloiden sisäympäristössä tehdyissä hygieniakartoituksissa löydetyistä homeista suurin osa ei aiheuttanut juustotuotteiden kontaminaatio-ongelmia. Homeiden eristämiseen koitettiin valikoivaa CREAD-agaria yhdessä yleisagarin kanssa, jotta saataisiin tietoa ympäristössä olevista homeista ja näiden homeiden kyvystä kasvaa juuston pinnalla. Kannattaa testata tunnistettujen kriittisten lajien kasvu CREAD-agarilla, jotta tietää, kuinka homekasvua tulee tulkita. Kasvukyky juustossa tai juustoagarilla, kuten CREADilla, kylmässä varastointilämpötilassa on yksi tärkeä juustotuotteita pilaavien kriittisten lajien valintaperuste.

Synergiatehtävän tulokset osoittivat, että on mahdollista kehittää UV-valoon perustuva testiohjelma avointen laitteiden pintojen puhtauden raportoinnin ohjeeksi. Havainnollistaminen tehtiin käyttämällä vertailulevyjä, joissa oli huonoa hygieenistä laitesuunnittelua edustavia osasia. Nämä vertailulevyt liattiin piimällä ja pestiin kevyellä pesuohjelmalla, jolloin hygieenisen laitesuunnittelun kannalta tunnetusti ongelmalliset kohdat saatiin havainnollistettua. Näiden tunnetusti vaikeasti puhdistuvien kohtien visualisointi on tärkeää, koska se mahdollistaa ongelma-alueiden osoittamisen monimutkaisemmissakin laitteissa. Yleisesti hyväksytyn testimenetelmän käyttö tulee parantamaan uusien laitteiden

muotoilua johtuen laitevalmistajien välisestä kilpailusta. UV-lampulla oli helppo havaita ruostumattomalla teräksellä olleet jäämät. Muovimateriaalit olivat itsestään fluoresoivia, mutta kontaminaatiot oli mahdollista havaita, koska niistä lähti erilaista valoa. UV-lampulla saatiin välittömästi tulokset puhdistuksen tehokkuudesta. Heti puhdistuksen jälkeen tehtävä arviointi on ensisijaisen tärkeää muutaman päivän kuluttua tulevien laboratoriotulosten perusteella käytävälle keskustelulle.

Todettiin, etteivät juustomuotit puhdistu tyydyttävän tasoisiksi käytössä olleella puhdistusohjelmalla. Kontaminaation estämiseksi on luotava tarkastus- ja puhdistusohjelmat juustomuottien pesulaitteelle. Viimeisestä huuhteluvedestä otetusta näytteestä tehdyllä analyysillä on hyvä osoittaa ohjelmien olevan kohdallaan ja toimivia. Myös liukuhihnojen puhdistuksen tiedetään olevan ongelmallista. Lähes 20 % näytteistä sisälsi hiivaa ja hometta, kun taas Gram-negatiivisten bakteereiden esiintyminen oli harvinaisempaa. Kriittiset pisteet, joista kontaminaatiota löydettiin, olivat liukuhihnan alapuoli ja telat. Arla Foodsilla esitelty arviointiohjelma sisältää kriittisten alueiden ja kohtien määrittämisen, siivousryhmään kuulumattoman puolueettoman henkilön tekemät päivittäiset tarkastukset, dokumentoinnin, puhdistuksen tehokkuuden auditoinnin yleiseltä pohjalta, vaihtelevista tuloksista seuraavat toimenpiteet ja palautteen siivousryhmälle.

Pestyistä palautuvista kuljetuslaatikoista saadut tulokset osoittivat, että voidaan määritellä sekä laatikoiden visuaalinen että mikrobiologinen hygienialuokitus. Pesutulosten parantamiseksi puhdistusparametrit tulisi pitää vakiotasolla. Kun prosessissa tehtiin mikrobiologista valvontaa, pesäkkeitä muodostavien yksiköiden määrät pesuvedessä olivat 80–300 PMY/ml. Ultraäänipesumenetelmä soveltuu palautuvien muovilaatikoiden puhdistukseen ja on huomionarvoinen menetelmä verrattaessa pesutunnelimenetelmään. DryCult® TPC on käytännöllinen vaihtoehto, jos mikrobiologiset testit on tehtävä kenttä-olosuhteissa.

23.5 HYGIEENINEN LAITESUUNNITTELU TEOLLISUUSESIMERKEISSÄ

Haluttu tavoite integroidun lähestymistavan toteutuksessa on välttää vaaroja, joita muuten voisi aiheutua. Nämä vaarat voivat olla mikrobiologisia, kemiallisia tai elintarvikkeen luonteeseen liittyviä. Toissijaisena tavoitteena on minimoida

takaisinvetojen, oikeuskanteiden, huonon maineen ja hyvän yrityskuvan romuttumisen riskiä. Kolmanneksi, integroidun lähestymistavan toteutuksessa toivomme vähentävämme muita turvallisuuteen liittymättömiä ongelmia, kuten haitallisia ympäristövaikutuksia, liiallisia kustannuksia ja resurssien, kuten veden, kemikaalien ja energian, ylenmääräistä käyttöä. Lopuksi, integroidun lähestymisen pitäisi todennäköisimmin johtaa viimeistellyn laitesuunnittelun taloudellisuuden maksimointiin. EHEDG-ohjesarjaan tulevaa yleisohjetta ”Hygienic systems integration” (integroitu lähestyminen hygieeniseen rakentamiseen) ovat olleet tekemässä Roland Cocker (Cocker Consulting), Stefan Åkesson (TetraPak), Alan Friis (BioCentrum-DTU), Paul Bartels (A & F Netherlands), Hans Hoogland (Unilever), Gerd Klimmeck (Johnson Diversey), Hans Oosterom (DSM Bakery Ingredients) ja Jeff Wilkinson (PGA).

Tulokset ovat osoittaneet, että bakteereiden kiinnittyminen tai irtoaminen ei tunnu olevan riippuvainen pinnan topografiasta silloin, kun R_a-arvot ovat hyvin pieniä (< 0,8 μm). Tämä saattaa johtaa oletukseen, että huippusileät pinnat eivät tarjoa hygieniaetua. Kuitenkaan hygienialla ei tarkoiteta pelkästään bakteereita ja likaa vaan myös pinnan vastustuskykyä tuotannossa käytettyjä kemikaaleja vastaan. Uuden tanskalaisen terästeollisuudelle suunnatun kilpailukeskuksen tarkoituksena on tehostaa ruostumattoman terästeollisuuden kilpailukykyä esimerkiksi elintarvikeprosesseissa. Sileät pinnat ovat selvästi kalliimpia, ja tulostemme mukaan ei ole syytä vaihtaa yleistä pinnankarheuden suositusta R_a < 0,8 μm. Erittäin sileiksi kiillotetuista pinnoista saattaa olla hyötyä esimerkiksi putkistoissa, joissa kulkee tuotteita, joita ei enää kuumenneta.

Hygieenisen laitesuunnittelun vaatimukset perustuvat EHEDG- ja FDA-ohjeiden yhdistelmään. Nämä ohjeet ovat itsessään erinomaisia, mutta hygieenistä laitesuunnittelua ei voi oppia pelkkien teoriatietojen avulla. Kyetäkseen suunnittelemaan laitteen tiettyyn erikoistarkoitukseen on tärkeää, että suunnittelijalla on yksityiskohtaista käytännön tietoa olosuhteista, jossa laitetta käytetään. Useat elintarvikeyritykset ovat sulkevat aina vain enemmän tilojaan vierailijoilta, joten elintarvikeprosessilaitteiden suunnittelijat saavat yhä vähemmän palautetta. Suunnittelijoiden on aina vain vaikeampi saada aiheesta oikeaa käytännöllistä ymmärtämystä, joka on välttämätöntä, jotta he pystytään vaadittavaan kehitykseen uusien optimaalisten laitteiden suunnittelussa. Tässä julkaisussa esitetään viisi esimerkkilaitetta (leijupetireaktori, tuplapakkalevy-lämmönvaihdin, juustonviipalointilaite, pussituskone ja muovipullon täyttölaite),

joissa on tyypillisiä hygieenisiä laitesuunnitteluongelmia. Arvioinnissa muotoilun vaikutus on arvioitu seuraaviin alueisiin liittyen: tuotteen kanssa kosketuksissa olevat pinnat, lähellä tuotekontaktia olevat pinnat ja tuotantoympäristö.

Tuplapakkalevylämmönvaihtimien käyttö on osoittanut, että tarvitaan lisäpanostusta laitoksen suunnittelussa, toiminnassa ja ennaltaehkäisevässä kunnossapidossa. Tarvitaan useampia levyjä, koska kaksiseinäisten levyjen lämmitystehot ovat huonommat kuin yksiseinäisten. Aiheutuvat ongelmat ovat joko painehäviöt levylämmönvaihtimessa tai hidas virtaus kanavissa ja hidastunut virtaus saattaa aiheuttaa puhdistuvuusongelmia. Kapeita kanavia voidaan myös käyttää parantamaan lämmönsiirtoa, mutta kuumilla käyttöalueilla kerrostumat saattavat aiheuttaa nopeasti tukoksia. Levylämmönvaihtimen huuhtelua tulisi myös välttää, koska vaarana on, että kapillaarivoimat saattavat imeä nestemäisen tuotteen ja/tai pesuainejäämiä vuotavaan kennoon ja aiheuttaa ruostumista tai mikrobikasvua. Siksi suositellaan suojakuorien laittamista levylämmönvaihtajaan. Jos lika on kuivunut levyjen väliin, on erittäin todennäköistä, että myös toinen levy ruostuu puhki. Siksi suositellaan säännöllistä painetestiä vuotoja aiheuttavien pienten halkeamien löytämiseksi. Tuplapakkalevylämmönvaihtimen käyttöä suositellaan vain, jos prosessin asettamia vaatimuksia ei voida muuten ratkaista.

23.6 DESINFIOINTIAINEET – JÄÄMÄT JA RESISTANSSI

Desinfioinnin tarkoituksena on vähentää mikrobien määrää elintarvikkeiden kanssa kosketuksissa olevilta pinnoilta ja siten välttää raaka-aineiden ja tuotteiden kontaminoituminen patogeeneilla ja pilaajaorganismeilla. Kun desinfiointiprosessi pettää, johtuu se useissa tapauksissa liian laimean desinfiointiainepitoisuuden käytöstä, lämpötilasta, vaikutusajasta tai virheestä pesuprosessissa, jolloin desinfioitavalle pinnalle on jäänyt likaa. Itiöt sekä jotkut vastustuskykyiset kasvutilassa olevat bakteerit voivat selvitä desinfioinnista, koska ne ovat muodostaneet resistanssin käytettävää desinfiointiainetta vastaan. Ensimmäisessä synergiatehtävässä käytetty kemikaalijäämien arviointimenetelmä perustuu viiden minuutin kontaktiajan jälkeen mitattavaan valobakteerin tuottamaan valomäärään. Tutkimusten mukaan tuotannon kannalta ensisijaisen tärkeille pinnoille oli jäänyt kemikaalijäämiä. Valobakteerimenetelmän tulokset voidaan luokitella inhibition perusteella seuraaviin jäämä-

tasoihin: selviä jäämiä, kohtalainen tulos ja ei jäämiä. Menetelmä tarjoaa täten käytännöllisen vaihtoehdon pinnoilla olevien kemikaalijäämien testaamiseen.

Useat laboratoriotutkimukset ovat osoittaneet, että pintaan kiinnittyneet solut ovat vastustuskykyisempiä desinfiointikäsittelyille kuin liuoksessa olevat solut. Tämä on johtanut erityyppisten rasitustestien, esimerkiksi pinnalle kuivattujen solujen ja testipinnalle rakennetun tai kasvatetun biofilmin käyttöön perustuvien testien, kehittämiseen. Jotkut prosessista eristetyt bakteerit pysyvät alkuperäistilassa, kun taas toiset sopeutuvat käytettyyn desinfiointiaineeseen. Ruostumattomalla teräspinnalla tehdyt biofilmin kasvatuskokeet viittasivat siihen, että *Sphingomonas* sp. -kannoilla on paljon nopeampi alkukiinnittymisvaihe kuin muilla kannoilla. Islantilaisessa hygieniakartoituksessa 140 kantaa eristettiin kesä- ja talviaikaan meijeriympäristöstä. Oli mielenkiintoista huomata, että jotkut kannat olivat laajavaikutteisesti vastustuskykyisiä desinfiointiaineille. Näistä kannoista monet tunnistettiin *Psedomonas putidaksi, Moraxellaksi*, *Klebsiella oxytocaksi* ja *Bacillus pumilukseksi.* On myös mielenkiintoista huomata, että 24 näistä 25 pidemmälle luokitellusta kannasta oli eristetty kesäaikaan. Useimmat näistä kannoista olivat Gram-negatiivisia (23), mikä korreloi hyvin siihen, että Gram-negatiiviset bakteerit ovat vastustuskykyisempiä desinfiointiaineille kuin Gram-positiiviset bakteerit.

APPENDIX 6: DAIRYNET –ÞRIFA STJÓRN Í NORRÆNUM MJÓLKURBÚUM

SAMANTEKT

Í þriðja norræna rannsóknarverkefninu Nordic dairy hygiene project P00027 "DairyNET-Hygine control in dairy environment", sem Norræna nýsköpunarmiðstöðin (áður Norræni iðnþróunarsjóðurinn) fjármagnar, hefur áhersla einkum verið lögð á að kanna hreinlæti og gæði hrámjólkur, vinnsluflata, vinnsluvatns, lofts og lokaafurða. Rannsóknirnar hafa farið fram í norrænu mjólkurvinnslustöðvunum: Arla Foods, Milko, Norðurmjólk, Norrmejerier, Skåne mejerier, TINE og Valio Ltd., ásamt rannsóknastofnunum BioCentrum-DTU, Danish Technological Institute (DTI), Rannsóknastofnun fiskiðnaðarins (IFL), Royal Institute of Technology (KTH), MATFORSK, SIK, Háskólanum á Akureyri og VTT Biotechnology. Loks má geta að eftirfarandi efna- og tækjaframleiðendur tóku einnig þátt í verkefninu: Finnsonic Oy, JohnsonDiversey, Lagafors Fabriks AB, Mjöll, TetraPak Nordic Processing og Orion Diagnostica Oy frá maí 2001 til október 2004. Gun Wirtanen dósent, frá VTT Biotechnology er verkefnisstjóri. Oddur Gunnarsson sá um yfirstjórn verkefnisins fyrir hönd Norrænu nýsköpunarmiðstöðvarinnar. Niðurstöður hinna ýmsu rannsókna voru birtar á vegum norræna DairyNET-verkefnisins í þeim tilgangi að örva umræður á verkefnafundum. Nýjar hreinlætisaðferðir, sem byggja á niðurstöðum úr rannsóknarverkefninu, hafa verið teknir upp í mjólkurvinnslustöðvunum. Draga má niðurstöður úr rannsóknarvinnu í hverju norrænu þátttökulandanna, sem og norræna netverkefninu, saman á eftirfarandi hátt:

- Dæmi um nýja aðferð til að meta virkni þrifa er að mæla efnaleifar þvotta- og sótthreinsiefna á vinnsluyfirborði. Mælingar sem sýna engan örveruvöxt geta leitt til mistúlkunar á hreinlæti við vinnslu sérstaklega ef einungis heildarfjöldi baktería er mældur. **Fyrsta samræmda verkefnið** um mælingar á efnaleifum á matvælavinnslusvæðum var valið þar sem slíkar mælingar eru yfirleitt ekki framkvæmdar eftir skolun. Niðurstöður ljóshindrunar aðferðarinnar með ljósgefandi bakteríur sýna að hægt er að nota hana til að mæla efnaleifar bæði í vökva og á yfirborði. Markmið **samræmdaverkefnis tvö** var að gera viðmiðunarraðferð (reference method) fyrir hreinsun opinna vinnslukerfa. Útfjólublátt ljós reynist vel til að finna

mikilvæga staði (“hot spots” eða “critical points”). Þessi vinna sýndi að hægt var að sjá þá staði sem vitað er að eru vandamál m.t.t. þrifavænnar hönnunar. Það er mikilvægt að sjá þessa erfiðu staði því það gerir kleift að staðsetja vandamálasvæði á flóknari vinnslutækjum, þar sem sérstakrar athygli er þörf við þrifaferlið. Það hefur einnig verið rætt hvort allir myglusveppir sem finnast í lofti í ostagerð mengi osta eða ekki. Í **þriðja samræmda verkefninu** var valætið, Creatine sucrose dichloran agar (CREAD), ásamt hefðbundnu æti, notað til að einangra myglu í lofti í ostagerðum. Þetta var gert til að finna út hvort hægt sé að nota CREAD æti til hraðvirkari greininga á skaðlegum mygluvexti í ostagerð. Mygla sem vex á CREAD æti eru tegundir sem finnast á osti.

- Samkvæmt norskum reglugerðum þá má líftala í hrámjólk fyrir gerilsneyðingu ekki fara yfir 300.000 gerlastök per ml. Í mjólk frá kúabúum skal líftala ekki vera yfir 100.000 gerlastök per ml. Helstu þættir sem hafa áhrif á fjölda baktería eru hreinlæti, aldur og geymsla mjólkurinnar. Markmið norska verkefnisins var að kanna hreinlæti á svæðum fyrir **gerilsneyðingu í mjólkurvinnslustöðvum** til að kanna samband milli þrifa, hreinlætisástands og gæði hrámjólkur fyrir vinnslu. Fimm norskar mjólkurvinnslustöðvar voru heimsóttar til að kanna þrifa- og vinnsluaðferðir og niðurstöður gæðastjórnunnar. Niðurstöður þessarar könnunar voru að mjólkurvinnslustöðvar sem notuðu hefðbundnar þrifaaðferðir skiluðu besta árangrinum hvað varðar hreinlæti. Ein ástæða þess var trúlega lífræn mengun sótthreinsivökvanna. Engin mjólkurvinnslustöðvanna gat sýnt fram á tengsl á milli bakteríufjölda í hrámjólk frá búum og í geymslutönkum mjólkurvinnslustöðvanna og bakteríufjölda í gerilsneyddri lokaafurð.

- *Listeria monocytogenes* er fæðuborinn sýkill og því afar mikilvægt að þekkja uppruna hans og smitleiðir í matvælaiðnaði. Í íslensku rannsókninni var athyglinni beint að **tíðni og útbreiðslu *L. monocytogenes* í allri vinnslulínu mjólkurvinnslustöðvar** á Íslandi. Meira en 1.400 sýni voru tekin yfir eitt ár til að hægt væri að athuga hvort hægt væri að sjá breytingu eftir árstíma. Engin jákvæð *L. monocytogenes* sýni fundust í afurðunum (n = 200) né í vinnslulínum (n = 250). Þrettán jákvæð sýni fundust í sýnum sem tekin voru að vetrarlagi, en engin í sýnum sem tekin voru að sumarlagi (n = 459 vetur og sumar). Þessar niðurstöður gefa til kynna að um árstíðabundinn mun sé að ræða á tíðni *L. monocytogenes* í hrámjólk frá þessum svæðum.

Vegna rekjanleika *L. monocytogenes* voru sýni tekin úr öllum mjólkurkúm (n = 20) á einu búanna sem hafði verið jákvætt, ásamt 30 sýnum úr umhverfi og vothey. Niðurstöður leiddu í ljós að ein kýr og átta umhverfissýni sýni reyndust jákvæð fyrir *L. monocytogenes,* stofnarnir sem voru einangraðir frá sama kúabúi tilheyrðu öll sama ribohóp og pulsohóp (greining með Ribotyping og PFGE).

- Í sænska hluta verkefnisins var **loft, vatn, og ostur** í fjórum ostagerðum kannað m.t.t. Gram neikvæðra baktería, gersveppa og myglu. Niðurstöðurnar sýna fram á möguleika þess að mengun geti borist í matvælavinnslur með vinnsluvatni, vegna ófullnægjandi hreinlætis, ónógs viðhalds vatnsleiðslna eins og slanga, stúta og krana. Þær örverur sem oftast fundust í vatni voru t.d. *Acinteobacter* spp. og *Pseudomonas* spp.*,* gersveppir eins og. *Candida* spp. og *Rhodotorula* spp. ásamt myglusveppum eins og t.d.. *Phoma* spp., *Fusarium* spp. og *Acremonium* spp. *Penicillium* spp. voru hins vegar sjaldan einangraðir úr vatni. Loftsýni er hægt að nota til að finna staði í vinnslulínunni sem eru í hættu vegna mengunar sem berst með lofti. Þau ostasýni sem voru rannsökuð benda til þess að loft sé aðallega orsakavaldur að myglumengun á osti og auk þess að vatn sé uppspretta mengunar af völdum *Geotrichum* spp. og *Trichoderma* spp.

- Lífrænan loftúða (bioaerosol) er hægt að skilgreina sem lausn sem inniheldur örsmáar fastar eða fljótandi agnir og örverur. Það eru nokkrar leiðir til að lífræn efni og örverur berist með lofti, t.d. með hreyfingu fólks, af völdum hnerra eða hósta. Það hefur sýnt sig að lífrænn loftúði sem inniheldur myglusveppi er mjög háður veðurskilyrðum. Hluti af framlagi Svíþjóðar til Norræna DairyNET verkefnisins var framkvæmd hreinlætiskönnunar sem tók til loftborinna örvera og á **heildaragnafjölda í andrúmslofti í fjórum ostagerðum** á mismunandi stöðum **í Svíþjóð**. Örverufræðilegar mælingar voru gerðar með þremur mismunandi tegundum af loftsíunartækjum og mismunandi æti voru notuð til að meta vöxt baktería, gersveppa og myglu. Almennt benda niðurstöðurnar til að ákjósanlegt væri ef hægt væri að stjórna umhverfisaðstæðum betur. Til að auka gagn örverufræðilegs eftirlits þarf að finna viðeigandi samsetningu á æti og mælibúnaði til að nota. Í finnskri rannsókn voru gæði **lofts í Herajoki mjólkurvinnslunni** könnuð. Í undirbúningsskyni fyrir rannsóknina voru m.a. loftræstikerfi mjólkurvinnslunar athuguð, svo og saga húsnæðisins. Í

svona rannsókn þarf að taka tillit til árstíðabreytinga og þá skiptir saga byggingarframkvæmda í mjólkurvinnslunni máli þegar lagt er mat á niðurstöðurnar. Gæði loftsins virtust í þessu tilfelli góð, a.m.k. miðað við heimildir sem til eru.

- Í annarri rannsókn sem gerð var í Herajoki mjólkurvinnslunni var tilgangurinn að hámarka árangur þrifa í lokuðu kerfi (CIP) og að athuga hreinleika **CIP-vatns** í lokuðum kerfum. Sýnt var fram á að ATP ljósmæling nýtist sem fljótvirk aðferð til að kanna hreinlæti vatns. Með því að hámarka áhrif þrifa í lokuðum kerfum mun mjólkurvinnslan spara vatnsnotkun, hreinsiefni og tíma. Þá var **hreinlæti endurnýtanlegra plastkassa, sem þvegnir eru í þvottagöngum**, einnig metið í Herajoki mjólkurvinnslunni. Niðurstöðurnar sýndu að botn kassanna reyndist óhreinasti hluti þeirra, með verstu þrifaniðurstöðurnar. DOE aðferð (Design of Experiment, Taguchi method) var notuð við uppsetningu á tilrauninni til að hámarka þvottaaðferðina sem byggir á últrahljóðbylgjum (ultrasonic). Hljóðbylgju-þvottaaðferðin nýtist við að hreinsa endurnýtanlega plastkassa og stenst samanburð við þvott í göngum, en sýnt var fram á þetta með því að láta útbúin óhreinindi í kassa á tilraunarskala. Hagkvæmni þriggja ræktunaraðferða, þ.e. **hefðbundinnar ræktunnar**, **Petrifilm** og **DryCult® TPC til að fylgjast með heildarörverufjölda** var einnig sannreynd við hreinsun plastkassanna. DryCult® TPC er hentug aðferð, ef það þarf að framkvæma prófið á staðnum.

- **Plötuvarmaskiptar** eru mikið notaðir í mjólkuriðnaði til hitunar, kælingar og varmaskipta milli hrávöru og fullunninnar vöru. Hætta á endurmengun er tengd leka í gegnum plöturnar. Það getur komið gat á plöturanr t.d. vegna málmþreytu eða tæringar. Fyrir nokkrum árum komu varmaskiptar á markað fyrir mjólkurvinnslur sem höfðu tvöfaldar plötur, þar sem plötunum er pressað saman með skrúfum í varmaskipta rammann. Prófanir voru gerðar á þýskri rannsóknastofnun til að kanna hvort leki yrði ef annað lagið á tvöföldu plötunum var gatað. Kom í ljós að ef hola var gerð í annað lag plötunnar olli það leka sem mátti greina með berum augum. Hins vegar er enn tiltölulega lítil reynsla komin á notkun tvöfaldra varmaskipta í mjólkurvinnslu.

- Framleiðsla á ostum fer að hluta til fram í opinni vinnslulínu þar sem möguleikar eru á mengun lokaafurðarinnar. **Tækjabúnaður er mikilvæg uppspretta örverumengunnar.** Áhættustaði er m.a. að finna undir færiböndum og keflum. Niðurstöður rannsóknarinnar sýndu að vatn sem notað var til lokahreinsunnar ostamóta var mengað af örverum. Til að koma í veg fyrir örverumengun þyrfti að setja skýrar verklagsreglur um eftirlit og þrif á vélinni sem þrífur ostamótin. Mat á þrifum þarf að vera eftirfarandi: Skilgreining á áhættusvæðum, dagleg úttekt óháðs aðila, sem ekki tilheyrir hreinlætishóp, skráning, reglulegt eftirlit á áhrifum þrifa, aðgerðir þegar frávik finnast og svörun/endurgjöf til hreinlætishóps.

- Þörf er á að sótthreinsa matvælavinnslu þar sem blautt yfirborð skapar ákjósanlegar aðstæður fyrir vöxt örvera. Notkun árangursríkra sótthreinsiefna dregur úr líkum á að varan mengist, eykur geymsluþol vörunnar, og minnkar líkur á matarsýkingum. Langvarandi notkun sótthreinsiefna á slíka fleti eykur áhrif sótthreinsunarinnar, en of mikil notkun slíkra efna getur valdið því að bakteríurnar myndi þol gegn þeim sótthreinsiefnum sem notuð eru. Í íslenska verkefninu var notuð **MIC (minimum inhibitory concentration)** aðferðin til að ákvarða lágmarks styrk fjögurra þvotta-/sótthreinsiefna til hindrunar, skoðaðir voru 140 bakteríustofnar sem voru einangraðir úr umhverfi mjólkurvinnslu. Stofnarnir sem voru einangraðir og greindir sýndu að almennt voru þolnari stofnar með tvöfalt til þrefalt minni kynslóðatíma með auknum styrk sótthreinsiefna, en þeir stofnar sem voru ekki eins þolnir voru lengur að fjölga sér. Í norska verkefninu var **athyglinni einnig beint að tíðni þolinna örvera í matvælaiðnaði**. Vangaveltur hafa verið um það hvort misnotkun sótthreinsiefna hafi orðið til þess að val hafi orðið og fram hafi komið örverur í matvælavinnslur sem eru ónæmar fyrir sótthreinsiefnum. Örverur hafa lifað af efnameðferð svo sem þokuúðun og notkun sótthreinsimotta í mjólkurvinnslum. Bakteríur voru einangraðar í um 75% þeirra sótthreinsimotta sem kannaðar voru og ekkert þeirra sótthreinsiefna sem notuð voru náðu að koma algjörlega í veg fyrir að örverurnar lifðu af. *Serratia marcescens* sem einangruð var í sótthreinsimottum sem innihéldu Tego reyndust ónæmar fyrir þeim styrk sem ráðlagður var. *Methylobacterium* sp., *Rhodococcus erythropolis* og *Rhodotorula mucilaginosa*, sem einangraðar voru í vinnsluumhverfinu, reyndust einnig ónæmar fyrir nokkrum tegundum sótthreinsiefna.

- Ýtarlegar ráðleggingar og leiðbeiningar varðandi þrifavæna hönnun tækjabúnaðar í matvælavinnslum er efni margra leiðbeininga og samræmdra evrópskra staðla. Dæmin fimm um slæma hönnun m.t.t. þrifa (sótthreinsiböð án sírennslis (a static fluid bed), a DuoSafe varmaskiptir (PHE), ostaskeri, pokaáfyllir og plastflöskuáfylling) sýna glöggt fram á þörfina á hagnýtri þekkingu í vinnslu og á vinnsluaðstæðum til þess að hægt sé að vinna eftir kröfum um þrifavæna hönnun. Hingað til hafa fáar úttektir verið gerðar á þrifavænni hönnun varðandi byggingu og viðhaldi matvinnslustöðva í heild sinni. **Samræming á aðferðum við þrifavæna hönnun** er afrakstur skipulagðs starfs sem lýtur að hönnun einstakra hluta og vinnslulína til að uppfylla hreinlætiskröfur vinnslustöðva í heild sinni. Starfið/vinnan byggir á viðeigandi skilgreiningu og greiningu á þeim tegundum vandamála sem steðjað geta að á hverju stigi framleiðslunnar. Þessi nálgun mun verða birt í væntanlegum leiðbeiningum frá European Hygienic Engineering and Design Group (EHEDG).

23 ÁLYKTANIR

23.1 GÆÐI HRÁMJÓLKUR

Fimm norskar mjólkurvinnslustöðvar voru kannaðar. Þrjár af þeim notuðu basa (alkali) og sýru við þvott og síðan sótthreinsun með heitu vatni. Hinar tvær stöðvarnar notuðu basa til þvotta og sótthreinsuðu síðan með perediksýru. Vinnslutæki og hreinsunarstaðir voru könnuð með sjónmati undir útfjólubláu ljósi og örverusýni voru tekin. Þar að auki var lagt mat á þvotta- og vinnsluaðferðir og niðurstöður úr gæðaeftirliti. Aðferðir til sýnatöku, val á sýnatökustöðum og könnun á örveruflóru voru aðlagaðar á verkefnatímabilinu eftir því sem reynsla fékkst. Niðurstöðurnar í þessari könnun leiddu í ljós að þær mjólkurvinnslur sem notuðu hefðbundnar aðferðir við þrif náðu bestum árangri. Sjónrænt mat sýndi að í öllum mjólkurvinnslunum var að finna staði þar sem hreinlæti var ábótavant, en hvorki örverusýnataka né sjónrænt mat gátu sýnt fram á sameiginleg vandamálasvæði í öllum mjólkurvinnslunum. Miklvægt er í þessu sambandi að huga að hreingerningarlausnum, pakkningum og stöðum sem skapa vandkvæði vegna hönnunar, t.d. hreinsistútar/spíssar, í kringum mannop á tönkum, skynjarar, lok á loftskiptum og tankbílar. Örveruflóran eftir þvott og sótthreinsun reyndist mismunandi eftir vinnslustöðvum en yfirleitt var um að ræða algengar skemmdarörverur sem er að finna í hrámjólk og gerilsneyddri

mjólk. Engri af mjólkurvinnslunum tókst að sýna fram á tengsl örverufjölda í hrámjólk frá bæum og geymslutönkum í mjólkurvinnslunum og fjölda í gerilsneyddri afurð.

23.2 SMITLEIÐIR *LISTERIA*

Þetta verkefni kannaði hreinlæti í norrænum mjólkurvinnslustöðvum og á Íslandi beindist athyglin einkum að mengunarleiðum *Listeria*. *L. monocytogenes* getur smitast með neyslu afurða úr hrámjólk eða með menguðum mjólkurafurðum. Hvað þetta varðar er ástandið í mjólkuriðnaði nokkuð frábrugðið öðrum greinum matvælavinnslu, þar eð mjólk er gerilsneydd. Því er ekki líklegast að mjólkurvörur mengist af *Listeria* í framleiðsluferlinu. Í þessari rannsókn reyndist ekkert af þeim 450 sýnum sem tekin voru í vinnsluumhverfinu, framleiðslubúnaði, og þeim afurðum sem kannaðar voru vera *Listeria* jákvæð. *L. monocytogenes* fannst hins vegar í 13 sýnum af hrámjólk (1,5%), sem er sambærilegt við það sem gerist í V-Evrópulöndum og BNA. Til að hægt væri að rekja uppruna *L. monocytogenes* úr hrámjólk voru sýni tekin af hrámjólk frá öllum kúm og umhverfi á einum bæ sem var með jákvæð sýni í öllum þremur sýnatökunum að vetrarlagi. Í þessari könnun reyndust allir ribohópar samsvara *L. monocytogenes* munstri sem til er í gagnabanka, fyrir utan einn ribohóp sem var *L. seligeeri*. Það hefur verið staðfest að *L. monocytogenes* getur fest sig í umhverfi og verið viðvarandi í langan tíma, sem gefur til kynna að um hússtofn sé að ræða. Auk þess vekur það nokkra athygli að það virðist vera árstíðabundinn breytileiki varðandi tíðni *L. monocytogenes* í hrámjólk. Hugsanleg skýring á þessu er sú að hætta á mengun sé meiri yfir vetrartímann þegar kýr standa inni á fóðrum.

23.3 MAT Á GÆÐUM LOFTS

Í matvælaiðnaði byggja algengustu matsaðferðir á loftmengun á skálum sem standa opnar og virkri loftsöfnun með viðeigandi tækjabúnaði. Gagnsemi eftirlitsins fer að miklu leyti eftir þeim aðferðum sem notaðar eru og virk sýnataka hefur reynst vera árangursríkust. Tvær tegundir búnaðar sem draga í sig loft sem sest á yfirborð agars eru til: annars vegar með rist og hins vegar með síu. MAS-100 er sýnatökutæki sem sogar loft ýmist lárétt eða lóðrétt í gegnum gatasigti og á yfirborð agars í venjulegri ræktunarskál. Reuter Centrifugal Sampler (RCS sampler) er handhægur í notkun en nær þó ekki að sía mjög smáar lífrænar agnir. Síunaraðferðir ná reyndar ekki öllum örverum lifandi þar

sem sýnatökuaðferðin veldur álagi á frumurnar vegna þess þær þorna við sýnatökuna. Sýnatökutíminn hefur áhrif varðandi þornunina og er því mjög mikilvægur þáttur þegar verið er að safna sýnum af viðkæmum örverustofnum. Til þess að ráða bug á þessum vanda var hönnuð gelatínfilter himna úr gelatín froðu, sem á að koma í veg fyrir að örverur þorni upp við síun og gera þessa aðferð nokkuð áreiðanlegri fyrir vikið. Hver þessara sýnatökuaðferða er þó takmörkunum háð. Þess ber að geta að niðurstöður á fjölda í loftsýnum sem tekin eru með mismunandi aðferðum er ekki samanburðarhæfur, en flestar sýna þó trúlega svipaða tilhneigingu. Til að bæta árangur af örverueftirliti þarf að nota rétt æti og tæki til sýnatöku.

Niðurstöður rannsókna, sem gerðar voru í fjórum ostagerðum í Svíþjóð, benda til að ákjósanlegast sé að hafa eins mikla stjórn á öllum aðstæðum og kostur er. Til að ná betri stjórn á umhverfinu í ostagerðum verða framleiðendur að tilgreina nákvæmlega framleiðslu-, geymslu- og pökkunarsvæði fyrirtækisins. Gömul lofthreinsikerfi þarf að bæta til að auka gæði lofts. Þörf er á að hafa kerfi sem er rétt sett upp með yfirþrýsting og loftstreymi á opnum vinnslusvæðum þar sem afurðir viðkvæmar fyrir mengun eru geymdar. Á slíkum framleiðslusvæðum myndu HEPA síur bæta vinnuumhverfið. Umhverfisvöktunin á að byggjast á áhættumati. Í finnska undirverkefninu voru loftgæði í Herajoki mjókur-vinnslustöðinni rannsökuð árið 2001, þegar endurbætur og endurbygging átti sér stað á framleiðsluaðstöðunni. Í rannsókninni gáfu mælingar með RCS og MAS loftsýnatækjum svipaða niðurstöðu hvað varðar gersveppi og myglu. RCS er mjög nytsamlegt þegar mæla á magn örvera í lofti í mjólkurvinnslum, þó svo ekki sé mælt með því það sé eina aðferðin við mælingu á gersveppum og myglu. Rannsóknin leiddi í ljós að með því að skipta framleiðslusvæði upp í svæði og viðhalda yfirþrýstingi á lofti á viðkvæmum svæðum var hægt að koma í veg fyrir mengun frá samliggjandi svæðum.

23.4 HREINLÆTI Í MJÓLKURFRAMLEIÐSLU

Til að framkvæma könnun á hreinlæti er mælt með að til staðar sé sýnatöku-áætlun fyrir vatn, loft, yfirborðsfleti og ost. Mikilvægt er að sýnatökustaðir séu valdir af handahófi en ekki valdir vegna fyrirfram ákveðinna skoðanna á því hvar hugsanlegar hættur kunni að leynast. Sýnatakan og nánari greining örverustofnanna leiðir síðan í ljós hvaða tegundir eru mikilvægastar í vinnslunni og uppruna þeirra. Niðurstöðurnar sýndu fram á möguleikann á því að mengun

bærist með vatni vegna lélegs hreinlætis og viðhalds vatnsleiðslna, t.d. slangna, stúta og krana. Leiðbeiningar hvað þetta varðar er m.a. að finna í sænskum lögum um gæði vatns frá 25. desember 2003 (Swedish Water), þar sem t.d. er fjallað um hvernig taka skuli sýni og hvernig viðhalda eigi vatnslögnum til að vernda gæði vatnsins. Taka þarf sýni úr afurðum þegar grunur leikur á að vara sé skemmd af völdum mengunar í vatni. Framkvæma skal greiningu í samræmi við vandamálið sem upp kemur í viðkomandi afurð. Þær bakteríur sem einna líklegastar eru til að festa sig við þessar aðstæður eru *Acinteobacter* spp. og *Pseudomonas* spp.; gersveppir svo sem *Candida* spp. og *Rhodotorula* spp.; myglusveppir eins og *Phoma* spp. og *Fusarium* spp. Hins vegar var fátítt að *Penicillium* spp. fyndist í vatni.

Með sýnatöku úr vatni fundust staðir þar sem aukin mengun hafði átt sér stað. Í tveimur vinnslum var vatn mengað úr vatnslögnum sem notaðar voru við þrif Einnig kom í ljós að hægt er að nota sýnatöku á lofti til að finna framleiðslustaði þar sem er ófullnægjandi hreinlæti. Nota má ATP-aðferðina við mat á hreinleika CIP vatns, þar sem ATP hvarfast við allt lífrænt efni og sýnir á fljótvirkan hátt hvort vatnið sé hreint eða ekki. Hefðbundin ræktun getur aftur á móti sýnt fram á að vatn sé óhreint, þ.e. innihaldi mikið magn örvera, jafnvel þó ATP aðferðin hafi sýnt að vatnið sé hreint, en þetta getur stafað af efnaleifum. Í nokkrum tilvikum getur ATP-ljósmæling sýnt fram á vatn sé óhreint, en hefðbundin ræktun að vatnið sé mjög hreint. Skýringuna getur á sama hátt verið að finna í magni hreinsiefna í vatninu sem skekkja niðurstöður úr ATP-ljósmælingu.

Mengun af völdum myglu veldur öðru hverju vandamálum við framleiðslu á millihörðum ostum og getur því valdið ostaframleiðendum fjárhagslegu tjóni. Flestar af þeim sveppategundum sem fundust við hreinlætisúttektir innanhúss í ostagerðum reyndust hins vegar ekki valda mengun í osta afurðunum. Úttekt var gerð á valætinu CREAD, ásamt hefðbundnum ætum sem notuð eru til einangrunar á myglu, til að afla upplýsinga um myglu í framleiðsluumhverfinu og hvort þessar myglutegundir gætu vaxið á yfirborði ostanna. Það er ráðlagt að prófa vöxt mikilvægra tegunda á CREAD til að vita hvernig á að túlka mygluvöxtinn. Vaxtarmöguleikar á osti/osta-æti, t.d. CREAD við geymslu í kæli er mikilvæg vísbending um mikilvægar tegundir sem skemma ostaafurðir.

Niðurstöður samræmda verkefnisins sýndu að það er mögulegt að þróa mæliaðferð sem byggir á útfjólublárri ljósi til skrá virkni þrifa á opnum

yfirborðsflötum. Þetta var framkvæmt með því að notast við plötu, sem á voru svæði sem voru illa hönnuð m.t.t. þrifa, þessi plata var óhreinkuð með súrmjólk og síðan þrifin með "mildum" hreinsiaðferðum. Þetta sýndi að hægt var að sjá svæði sem vitað var að vour illa hönnuð m.t.t. þrifa. Það að sjá þekkt vandamála-svæði er mikilvægt til að hægt sé að finna þau í flóknum tækjum. Með því að hafa mæliaðferð sem er almennt viðurkennd gæti hönnun tækja batnað vegna samkeppni á milli tækjaframleiðenda. Auðvelt var að greina leifar á yfirborðs-flötum úr ryðfríu stáli með því að nota útfjólublátt ljós. Hlutir úr plasti reyndust sjálfkrafa flúrljómandi, en þó reyndist mögulegt að greina mengun þar sem hún gaf frá sér annars konar ljós. Útfjólubláa ljósið gaf samstundis niðurstöður á mati á þrifum. Betra er að fá slíkar niðurstöður eins fljótt og kostur er, frekar en að þurfa að bíða í nokkra daga eftir niðurstöðun mælinga frá rannsóknarstofu.

Í ljós kom að ostamót reyndust ekki fullnægjandi þrifin með þeim aðferðum sem notaðar voru. Til að koma í veg fyrir mengun verður að þróa aðferðir til eftirlits og þrifa á vélinni sem þrífur ostamótin. Ágæt leið til að athuga hvort að þrifa-ferlið sé í lagi er að taka sýni af síðasta skolvatninu og mæla það. Þrif á færiböndum er einnig vel þekkt vandamál. Um 20% sýnanna innihéldu ger-sveppi og myglu, en tíðni Gram neikvæðra baktería var lág. Áhættustaðir, þar sem mengun fannst, reyndust vera undir færiböndum og á keflum. Matsferli, sem sett var upp í Arla Foods felur í sér skilgreiningu á áhættusvæðum/stöðum, daglega úttekt af óháðum aðila sem ekki tilheyrir þrifahóp, skráningu, reglulegri endurskoðun á virkni þrifa og að gripið sé til aðgerða um leið og frávik koma í ljós. Þá er mikilvægt að þrifahópur fái svörun eða endurgjöf (feedback) á vinnu sína.

Niðurstöður úr rannsóknum á endurnýtanlegum plastkössum eftir þvott sýndu að hægt er ákvarða hreinlæti þeirra bæði sjónrænt og með örverumælingum. Til að bæta árangur þrifa þarf að viðhalda stöðugum kröfum (breytum) varðandi þrifin. Við örverueftirlit reyndist fjöldi þeirra í hreingerningarvökvanum vera á bilinu 80–300 CFU/ml. Últrahljóðbylgjuaðferðin er heppileg til að hreinsa endur-nýtanlega plastkassa og er eftirtektarverð aðferð borið saman við þvott í þvotta-göngum. DryCult® TPC-aðferðin er hagkvæm leið ef framkvæma á prófið á staðnum.

23.5 Þrifavæn hönnn á iðnaðarskala

Eitt af markmiðunum með því að koma á fót samræmdum aðferðum er að reyna að koma í veg fyrir þær hættur sem hugsanlega gætu skapast að öðrum kosti. Þær hættur geta t.d. verið af örverufræðilegum, efnafræðilegum eða eðlisfræðilegum toga, sem gætu leynst í neytendaafurðum. Annað markmið er að draga úr tilfellum þar sem fyrirtæki þurfa að innkalla vörur, draga úr lögsóknum, koma í veg fyrir vont orðspor og almennt að vörutegundir og fyrirtæki missi traust neytenda. Í þriðja lagi, með samræmingu er vonast til að einnig sé hægt að draga úr öðrum vandamálum sem þó teljast ekki beint öryggisatriði, s.s. eins og neikvæð umhverfisáhrif, ónauðsynlegan kostnað og ónauðsynleg not á vatni, efnum og orku. Að síðustu, samræming gerir það líklegra en ekki að hægt sé að gera hönnun eins hagkvæma og kostur er. Þeir sem eiga heiðurinn af vinnunni við væntanlegar leiðbeiningar EHEDG "Hygienic systems integration" eru: R. Cocker (Cocker Consulting), S. Åkesson (TetraPak), A.Friis (BioCentrum-DTU), P. Bartels (A & F Netherlands), H. Hoogland (Unilever), G. Klimmeck (Johnson Diversey), H. Oosterom (DSM Bakery Ingredients) og J. Wilkinson (PGA).

Rannsóknir sýna að viðloðun og hreinsun baktería er ekki háð áferð yfirborðsflata sem hafa lágt R_a-gildi (< 0.8 μm). Þetta gæti verið vísbending um að óvenju sléttir (ultra-smooth) yfirborðsfletir veiti ekki meira hreinlæti. Hreinlæti er hins vegar ekki einungis spurning um bakteríur og óhreinindi, heldur einnig mótstöðu yfirborðsflatarins gegn þeim efnum sem notuð eru í framleiðslunni. Tilgangur nýrrar danskrar rannsóknarmiðstöðvar sem þjónar ryðfría stáliðnaðinum er m.a. að efla samkeppnishæfni stálframleiðenda í matvælaiðnaði. Rennisléttir yfirborðsfletir eru dýrari í framleiðslu og rannsóknir okkar benda ekki til að breyta þurfi almennum viðmiðunargildunum Ra < 0.8 μm. Mjög sléttir yfirborðsfletir geta verið til bóta, t.d. við framleiðslu afurða í lokuðum leiðslum og eru ekki hitaðar fyrir neyslu.

Þær kröfur sem gerðar eru um þrifavæna hönnun búnaðar taka mið af ströngum viðmiðunarreglum sem settar eru fram af EHEDG og FDA. Þessar viðmiðunarreglur eru í sjálfu sér mjög góðar, en ekki er þó hægt að læra þrifavæna hönnun eingöngu af lestri bóka og viðmiðunarreglna. Nauðsynlegt er að sá sem ætlar að hanna slíkan búnað hafi staðgóða þekkingu á aðstæðum þar sem búnaðurinn er notaður. Flest matvælaframleiðslufyrirtæki eru farin að taka fyrir heimsóknir

gesta og gangandi, sem þýðir að þeir sem hanna tækjbúnað fyrir matvælavinnslur fá æ minni svörun/endurgjöf (feed back). Ein afleiðing af þessu er að það verður sífellt erfiðara fyrir hönnuði að afla sér nákvæmra, hagnýtra upplýsinga um aðstæður, sem þó er áríðandi ef hægt á að vera að fylgjast með þróun sem útheimtir sífellt betri lausnir varðandi hönnun. Í þessari bók eru tekin fimm dæmi um tæki/búnað (sótthreinsiböð án sírennslis (a static fluid bed), a DuoSafe varmaskiptir, ostaskeri, pokafyllir og plastflösku áfylling) þar sem dæmigerð vandkvæði hvað þrifavæna hönnun snertir hafa verið tekin til athugunar. Við athugunina á þessum tækjum voru eftirfarandi atriði sérstaklega skoðuð: snertifletir við afurð, fletir í nánasta umhverfi afurðar og framleiðsluumhverfið.

Notkun tvöfaldra varmaskipta hefur sýnt fram á að taka þarf tillit til ýmsra annarra þátta, s.s. við hönnun vinnslunnar, framleiðsluferla og til fyrirbyggjandi viðhalds. Þörf er á fleiri plötum, þar sem hitaleiðni tvöfaldra platna er verri en einfaldrar. Vandamál af þessum sökum er t.d. þrýstingsfall í varmaskiptinum eða minna flæði í hverri rás, sem aftur getur skapað vanda hvað þrif varðar. Þröngar rásir geta einnig verið notaðar til að bæta varmaskipti, en á heitum stöðum geta óhreinindi myndað fyrirstöðu og stíflu. Forðast skal að skola varmaskiptinn þar sem hárpípukraftar gætu sogað vökva úr afurðinni og/eða leifar hreinsiefna inn í hólf sem getur orsakað tæringu eða verið gróðrastía fyrir örverur. Það er því mælt með að bæta við hlífum á varmaskiptinn. Mikil hætta er á að tæring myndi göt einnig í seinni plötuna ef óhreinindi þorna á milli platna. Þess vegna er nauðsynlegt að þrýstingsprófa reglulega til að finna litlar sprungur sem valda leka. Eingöngu er mælt með notkun tvöfaldra varmaskipta þar sem öðrum vinnslulausnum verður ekki komið við.

23.6 Sótthreinsiefni – Efnaleifar og Þolni

Megintilgangur sótthreinsunar er að lágmarka fjölda örvera sem eru á yfirborðsflötum í matvælavinnslu og koma þannig í veg fyrir mengun hráefna og afurða af völdum sýkla og skemmdarörvera. Þegar sótthreinsun bregst er oftast hægt að finna skýringuna í því að of lágur styrkur af sótthreinsilausn hafi verið notaður, vitlaust hitastig valið, of stuttur tími notaður eða að ónógur þvottur hafi orðið til þess að leifar af óhreinindum hafi orðið eftir á yfirborðsflötunum. Gró og sumar tegundir þolinna örvera geta lifað sótthreinsun af, vegna þess að þær hafa byggt upp þol gegn þeim sótthreinsiefnum sem notuð eru. Aðferðin sem

notuð var til að meta efnaleifar (fyrsta samræmda verkefnið) byggði á því að meta ljómun frá photobacteria eftir aðeins 5 mínútna ræktun. Rannsóknir sýndu að efnaleifar leynast á yfirborðsflötum fyrir framleiðslu. Niðurstöður aðferðarinnar þar sem ljósgefandi bakteríur voru notaðar má flokka á eftirfarandi hátt, eftir því hversu hindrunin var mikil: mjög áberandi, í meðallagi og engar leifar. Aðferðin býður þ.a.l. upp á hagkvæma leið til að meta efnaleifar á yfirborðsflötum.

Ýmsar rannsóknir, sem gerðar hafa verið á rannsóknarstofum, hafa leitt í ljós að örverur sem loða við yfirborðsfleti eru þolnari gegn sótthreinsiaðgerðum heldur en stakar frumur. Þetta hefur orðið til þess að þróaðar hafa verið mælingar t.d. próf þar sem notast er við örverur sem þurrkaðar hafa verið á yfirborðsflötum, bakteríum sem hafa myndað biofilmu og biofilmur sem ræktaðar eru á tilraunaplötum. Sumar bakteríurnar sem eru einangraðar úr vinnslu viðhaldast á meðan aðrar mynda þol gegn sótthreinsiefninu sem notað er. Tilraunir með að rækta biofilmur á yfirborði úr ryðfríu stáli gefa til kynna að *Sphingomonas* sp. hafi miklu meiri tilhneigingu til viðloðunar snemma heldur en aðrar tegundir sem einangraðar voru. Í íslensku rannsókninni voru 140 stofnar einangraðir í mjólkurvinnslustöð, bæði að sumar- og vetrarlagi. Það var athyglisvert að sumir stofnarnir sem einangruðust reyndust hafa breiðvirkt þol gagnvart sótthreinsiefnum. Mörg þessara afbrigða voru greind, s.s. *Psedomonas putida, Moraxella*, *Klebsiella oxytoca* og *Bacillus pumilus.* Ennfremur er athyglisvert að 24 af 25 þessara stofna sem voru kannaðir nánar voru einagraðir að sumarlagi. Flestir þessara stofna voru Gram neikvæðir (23) sem samræmist vel því að Gram neikvæðar bakteríur eru yfirleitt þolnari gagnvart sótthreinsiefnum heldur en Gram jákvæðar bakteríur.

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APPENDIX 8: AIRBORNE VIABLE PARTICLES

Measurements of airborne viable particles have been performed at the four plants with three different active sampling devices; a slit-sampler FH3, a sieve-sampler MAS, and a centrifugal sampler RCS. These devices for the active sampling of airborne aerobic colony-forming-units (CFUs) have been compared earlier for their biological efficiency and evaluated as giving relatively comparable results (Ljungqvist & Reinmüller, 1998).

The media used for the collection and enumeration of CFUs have been:

- Cloramphenicol-Glycos-Yeast extract (CGY) – 9 cm agar plates – for the enumeration of yeasts and moulds in dairy products and food.
- Rose Bengal Agar (YM) – agar strips – for the enumeration of yeasts and moulds. Restricts the growth of *Rhizopus* and *Mucor* spp. that otherwise could overgrow the agar. Streptomycin is present to inhibit bacterial growth.
- Sabouraud-Dextros Agar (SDX) – agar strips – for the enumeration of yeasts and moulds, neutral pH with antibiotics to inhibit contaminant bacterial flora.
- Modified Creatine Sucrose Dichloran Agar (CREAD) – 9 cm agar plates – for distinguishing species of *Aspergillus* and *Penicillium*
- Drigalski Agar "Blue plate" – 9 cm agar plate – for the isolation and cultivation of Gram-negative bacteria.
- Tryptone-Soy-Agar (TSA) – 9 cm agar plates – for the general enumeration of aerobic microbes.

Measured concentrations are given as number of airborne colony forming units per cubic metre (CFU/m^3).

Table A8.1. Summary of microbiological results from plant A. Sampling was performed April 3–4, 2002.

Location/ Activity	Media	Number / m^3		Air sampler
		Bacteria	Yeast & moulds	
Close to vat	CGY		<1	FH3
			20	
			12	
	CGY		<3	MAS
			3	
			<3	
			15	
			<3	
			<3	
			<3	
			3	
Washing/ Rinsing	CGY		600	MAS
			<3	
Cheese out of mould	CGY		<1	FH3
			1	
			1	
			1	
	CGY		<3	MAS
			<3	
			3	
			3	
			<3	
			<3	
			<3	
			<3	
			6	
			8	
			<3	
			<3	
Packaging	CGY		3	FH3
	CGY		3	MAS

Table A8.2. Summary of microbiological results from plant B. Sampling was performed February 19, 2003.

Location/ Activity	Media	Number per m^3		Air sampler
		Bacteria	Yeast & moulds	
3 m above floor; general area	TGA	198		FH3
	Blue	2		FH3
	CGY		12	FH3
	CREAD		14	FH3
	YM		<6	RCS
			6	
Cheese forming area	TGA	142		FH3
		72		
		188		
		48		
	Blue	2, <2		FH3
	CGY		4	FH3
			2	
	CREAD		<2	FH3
			2	
	YM		<6	RCS
			<6	
			<6	
			<6	
			12	
Cheese out of mould	TGA	88		FH3
	Blue	<2		FH3
	CGY		2	FH3
	CREAD		<2	FH3
	YM		<6	RCS
			6	
Re-circulated supply air	TGA	26		FH3
	Blue	4		FH3
	CGY		4	FH3
	CREAD		<2	FH3
	YM		6	RCS

Table A8.3. Summary of microbiological results from the plants C. Sampling was performed January 19, 2003.

Location/ Activity	Media	Number per m^3		Air Sampler
		Bacteria	Yeast & moulds	
Close to vat	CGY		24	FH3
			96	
			24	
			134	
	YM		1	RCS
			38	
			1	
			75	
Cheese out of mould	CGY		122	FH3
			200	
			102	
			86	
	YM		94	RCS
			94	
			180	
			7	
Supply air	CGY		32	FH3
			20	
			34	
			39	
	YM		32	RCS
			14	
			32	
			14	

Table A8.4. Summary of microbiological results from the plants D. Sampling was performed March 13, 2003.

Location/ Activity	Media	Number per m^3		Air Sampler
		Bacteria	Yeast & moulds	
Cheese forming area	CREAD		500	FH3
	Blue	100		FH3
	SDX		500	RCS
			500	
			500	
Cheese out of mould	CREAD		500	FH3
			400	
			144	
	Blue	350		FH3
		20		
		<2		
	SDX		630	RCS
			540	
			290	
			500	
Weighing & transport	CREAD		500	FH3
			500	
	Blue	110		FH3
		500*		
	SDX		500	RCS
			500	
Supply air	CREAD*		64	FH3
			400	
	Blue	36		FH3
		150		
	SDX		<6	RCS
			100	

* Values as reported by the 1st reading before transport.

COMPARISONS OF MICROBIOLOGICAL RESULTS

The comparison of air sampling devices and of different media is based on summaries made in Tables A8.1 to A8.4. The Tables A8.5–A8.8 form the basis for the graphs in Figures 24–27. In the tables, the value 1.1 is used instead of the value 1 as logarithmic scales are used on the *y*-axis in graphs based on data from the tables. To simplify the graphs, mean values of results from the same locations and conditions (media and sampler, respectively) have occasionally been calculated.

Table A8.5. Plant A, comparison of results from 2 sampling devices and 1 media. Based on results from microbiological sampling performed April 3–4, 2002.

Location	FH3 CGY yeasts	FH3 CGY moulds	MAS CGY yeasts	MAS CGY moulds
Press vat	8	4	600	22
Press vat	18	2	18	2
Tiromat room	1.1	2	1.1	2

Table A8.6. Plant B, comparison of results from 2 sampling devices and 5 media. Based on results from microbiological sampling performed February 19, 2003.

Location	Result Number of CFU per m3						
	FH3 Total number bacteria	FH3 Gram negative bacteria	FH3 CGY yeasts	FH3 CGY moulds	RCS YM yeasts	RCS YM moulds	FH3 CREAD moulds
General area	198	2	6	6	1.1	1.1	14
Cheese to mould / Mean	107	2	4	1.1	2	1.1	1.1
Cheese to mould / Mean	118	1.1	1.1	2	6	1.1	2
Cheese out of mould / Mean	88	1.1	1.1	2	3	1.1	1.1
Supply air	26	4	1.1	4	6	1.1	1.1

Table A8.7. Plant C, comparison of results from 2 sampling devices and 2 media. Based on results from microbiological sampling performed January 19, 2003.

Sampling place in the process	FH3 CGY Yeasts	FH3 CGY Moulds	RCS YM Yeasts	RCS YM Moulds
Close to vat	2	22	1.1	1.1
Close to vat	86	10	25	13
Close to vat	20	4	1.1	1.1
Close to vat	130	4	75	1.1
Cheese out of mould	100	12	94	1.1
Cheese out of mould	180	28	94	1.1
Cheese out of mould	90	12	175	7
Cheese out of mould	74	12	7	1.1
Supply air	26	6	32	1.1
Supply air	20	20	7	7
Supply air	30	4	25	7
Supply air	30	9	7	7

Table A8.8. Plant D, comparison of results from 2 sampling devices and 3 media. Based on results from microbiological sampling performed March 13, 2003.

Sampling place in the process	FH3 Blue Gram negative	FH3 CREAD yeast	FH3 CREAD mould	RCS SDX yeast	RCS SDX mould
Mould filling	100	500	54	500	120
Cheese/mould sep.	350	200	25	600	30
Cheese/mould sep.	20	100	40	500	40
Cheese/mould sep.	1.1	100	25	270	20
Weighing/Transport	200	500	200	500	114
Weighing/Transport	110	500	200	500	120
Supply air	36	50	6	50	1.1
Supply air	150	300	20	800	40

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DAIRYNET – HYGIENE CONTROL IN NORDIC DAIRIES

Abstract

The research work in DairyNET – Hygiene control in dairy environment (P00027), which is the third Nordic project in dairy hygiene funded by the Nordic Innovation Centre (formerly the Nordic Industrial Fund), has focused on hygiene and quality issues relating to raw milk, process surfaces, equipment and utensils, process waters, air and final products e.g. semi-hard cheese as well as contamination routes for *Listeria* from the cow shed to that the products leave the dairy. The 3 synergy tasks common for all national projects dealt with detection of cleaning agent or disinfectant residues left on process surface before production start-up, visualisation of organic soil in problematic spots using a UV-light based method to pinpoint poor hygienic design and detection of fungal contamination on cheese using CREAD agar. The work was carried out in the Nordic dairies Arla Foods, Milko, Norðurmjólk, Norrmejerier, Skåne mejerier, TINE and Valio Ltd together with the research organisations BioCentrum-DTU, DTI, IFL, KTH, Matforsk, SIK, the University of Akureyri and VTT Biotechnology as well as the following suppliers of chemicals, reagents and equipment: Finnsonic Oy, JohnsonDiversey, Lagafors Fabriks AB, Mjöll, TetraPak Nordic Processing and Orion Diagnostica Oy from May 2001 to October 2004. New procedures in hygiene based on the project findings have been implemented in processing at the dairies.

Keywords
dairies, dairy products, hygiene, milk quality, bioaerosols, disinfection, air quality, microbial contamination, cleaning, *Listeria monocytogenes*

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