New tools for management of beverage filling hygiene

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Background

Bacteria in aquatic environments are predominantly not in a free floating planktonic stage, but have a tendency to attach to surfaces, and form multi-species communities called biofilms. Aggregated in a biofilm, microorganisms have several advantages: they have a higher resistance to biocides and washing chemicals, nutrients are easier to access, and the creation of micro-niches (like anaerobic sites within a aerobic environment) facilitate the growth of organisms which normally could not grow outside the biofilm. The formation of a biofilm is a complex process, since attachment mechanisms vary between different bacterial species and surface properties and environmental factors have an influence on the attachment.

Although some general concepts can be applied to the attachment of cells and formation of biofilms, much species-specific behaviour exist. The primary attachment may be aided by structures extending from the cell surface, like fimbriae, flagella and pili. Surface type may have an effect on the use of pili, since Watnick et al. (1999) showed that *Vibrio cholerae* used different types of pili when attaching to biotic or abiotic surfaces. Also outer-membrane proteins may have a role in attachment (O'Toole et al. 2000, Dunne 2002). Cell surface hydrophobicity is regarded to predict bacterial adhesion to some extent. Generally hydrophobic cells are more adherent than hydrophilic ones, and most bacteria preferentially adhere to hydrophobic surfaces, but hydrophobicity and adherence do not always correlate (Pedersen 1990, Husmark and Rönner 1992, Carpentier and Cerf 1993, Neu 1996, Scheuerman 1998, O'Toole et al. 2000, Frank 2001, Dunne 2002). In addition, the electric charge of the surface and cell has an influence on the attachment. Most bacteria and surfaces are negatively charged, but there are exceptions, like *Actinomyces naeslundii*, whose rod-shaped cells have polarity in them (Poortinga et al. 2000).

It is assumed that most microbes become first irreversibly attached to a surface. If the environment and possible signalling molecules favour surface-attached growth, then the cells attach irreversibly by means of specific molecular mechanisms. The attachment is called irreversible when cells require mechanical force to be removed (Carpentier and Cerf 1993). After attachment microbes start to produce extracellular polymeric substances (EPS), often referred to as slime. With these substances they anchor to each other and the surface.

Open food and beverage contact surfaces do not normally provide a solid-liquid interface for microbial attachment, and true biofilms do not therefore form very often. Nevertheless, surfaces become soiled by drifting organic matter and microbes (Whitehead et al. 2008). Bottling or canning is the most critical phase in the beverage industry, because the product is open for microbes coming from surfaces, air and the environment. Because of a broad spectrum of products, there are lots of nutrients available. Increasing product range has in itself been shown to increase microbiological

risks in filling (Storgårds et al. 2006). Cleaning causes costs to the beverage industry in terms of production stoppages and consumption of energy, water, chemicals and working hours, which is why long runs between cleaning and short cleaning programs are economically favourable.

Coatings reducing microbial attachment

Process surface hygiene has traditionally been managed by controlling the roughness of stainless steel. Since microbial attachment is an unwanted process in many applications, material sciences have for some time worked with the aim of developing non-stick coatings, to which microbes would attach less than to for instance stainless steel. Photocatalytic titanium dioxide (TiO_2) coatings have been shown to be able to reduce microbial attachment. TiO_2-coatings become hydrophilic after exposure to short wave UV-irradiation (UVA), after which the sheeting water carriers the dirt away. Photocatalytic reactions also kill cells directly, so these coatings have a dual mechanism. The precise action of photocatalytic coatings on microbes is still not completely clear. Most attachment studies have been made with a single bacterial species, which does not reflect actual process conditions. The reductions in microbial numbers have varied from rather insignificant (10 %) to significant (90-99 %) (Li and Logan 2005, Raulio et al. 2006, Allion et al. 2007, Storgårds et al. 2007). Development of coating properties is an evolving field, and one way to improve their action against microbial attachment may be doping with nitrogen to increase their visible light activity.

Many metals, like copper and silver, have antimicrobial or antibacterial properties. Even though these metals are not typical components of stainless steel, different antimicrobial steels have been developed, the action of which is based on controlled dissolution and interaction of these metals with bacteria. To obtain bactericidal activity in weak UV-light, TiO_2 films with very small concentrations (e.g. 0.02 µg cm^{-2}) of antibacterial metals like copper or silver have also been developed (Sunada et al. 2003, Storgårds et al. 2007). The advantage of silver is that it forms an even precipitation layer on steel, does not weaken its corrosion tolerance, and is not harmful to humans. On the other hand, silver ions in waste waters may disturb the action of biological waste water treatment plants.

A third promising group of coatings to reduce microbial attachment or facilitate their removal are low energy, hydrophobic coatings (Tsibouklis et al. 1999, Parkin and Palgrave 2005). Because they are hydrophobic, water forms almost spherical droplets on them, carrying the dirt away. This is the biological action used by lotus plants (Marmur et al. 2004). These coatings work best in an inclined position, which is why they could be applicable to splash areas, where inclined surfaces are used. Like with photocatalytic coatings, the action of low energy coatings is not yet fully understood, and there are varying results of their actions against bacterial attachment (Liu and Zhao 2005, Tsibouklis et al. 1999, 2000).

Practical experiences of functional coatings

The action of functional coatings has been studied in laboratory and process studies in recent projects coordinated by the Finnish malting and brewing industry (Figure 1). The antimicrobial

function of the photocatalytic coatings varied. It seems that TiO_2 coatings may be more active against bacteria than eukaryotic yeasts and moulds, also noted by Kühn et al. (2003). In laboratory studies TiO_2 coatings, where antibacterial silver had been added (TiO_2+Ag), were shown to significantly decrease attached microbial numbers, but the same effect was not seen with TiO_2 coatings without added silver. In brewery filling hall process studies TiO_2 and TiO_2+Ag coatings caused 1-4 log reductions in attached microbial numbers in some places after 3-6 months of installing the coatings. The results were, however, not statistically significant, and in most places no effect of the coatings on attached microbes could be seen. There are many experimental variables on filling machines affecting results, like cleaning procedures and changes in the product range. In addition, as the number of sample coupons is low because of practical reasons, statistically significant differences are hard to observe. Material characterizations at the end of the study showed that TiO_2-coatings endured process conditions in dry places, but in wet places damaged areas were formed on the coatings. Silver had mostly dissolved from the coatings during one year in production conditions, meaning that a more long-lasting way to incorporate silver to the coatings should be found.

Also various hydrophobic low energy coatings have been studied in these projects. Low energy coatings were shown in laboratory studies to significantly reduce the area coverage of microbes, but no effect on live microbial numbers was seen in process studies. In addition to decreasing microbial attachment (non-stick), coatings may also improve process hygiene by being easy-to-clean. In laboratory washing studies low energy coatings were slightly better washable than stainless steel. Low energy coatings did not, however, yet endure process conditions, because parts of them had peeled off during one-year process studies. In addition, they had changed from hydrophobic to hydrophilic. In any case, the novel coatings will probably require regular regeneration – it just has to be economically feasible.

There may be several reasons for the varying functionality of the coatings. The amount of UVA light is critical for the efficacy of photocatalytic coatings, and is a limiting factor in process conditions. In laboratory studies different UVA exposures were used, but so far in process conditions it has not been possible to control the amount of light. Also the chemical form of TiO_2 influences the action of coatings, and material development may improve the activity of coatings. Inclined installation enabling water and dirt to roll off the surface is central for the action of low energy coatings. Possibly the 10° inclination used in process studies was not sufficient for the optimal functioning of the coatings. It was also noted that amorphous silica precipitates may form on the surfaces in process conditions, and they probably also affect the functionality of the new materials. Laboratory washing tests for artificial and process-formed precipitates showed that different detergents vary on their actions on different dirt, which favours alternating detergents in washes.

Physical disinfection during production

Coatings decreasing microbial attachment may help to control the hygiene during process, without stops needed for washes. Another possible means to control microbial numbers during production

are physical disinfection means. The germicidal action of UVC-irradiation has been known for long (Sharp 1939), and UVC disinfection is utilized e.g. in disinfection of drinking water (Hoyer 1998, Orava 2003, Jungfer et al. 2007). UVC has also been used successfully in combination with air rinsing for disinfection of empty cans prior to filling (Tapani et al. 2009). UVC inactivates microorganisms through breaking chemical bonds in their DNA chains. In laboratory studies UVC irradiation killed even 99.999 % of the microbes on surfaces, when the dose was high enough. Wet surfaces needed higher doses than dry surfaces. Biofilm, cell aggregates and dirt decrease penetration of UVC, so it is most suitable for prevention of microbial attachment and biofilm formation, and not in removing already formed biofilms.

Process air is part of brewery hygiene. Aerosols formed in washes and microbes removed from surfaces by air currents float in the air, and act as contaminants after settling down to surfaces again. Air ionization has been shown to have potential for killing microbes in the air, but the phenomenon is not totally understood, and results are partly contradictory (Krueger et al. 1957, Phillips et al. 1964, Mäkelä et al. 1979, Rosenthal et al. 1979, Marin et al. 1989, Gabbay et al. 1990, Seo et al. 2001, Grinshpun et al. 2005). The effect of air ions can be different on different microbial species, and several other environmental factors like aerosol size may also affect the action of ions. Low concentrations of ions are naturally present in the air, but effective numbers can be produced by ionisators. In laboratory studies, approximately 10 000 negative ions cm^{-3} and $>10^6$ positive ions cm^{-3} were produced in the air. This so called active oxygen worked best against gram negative bacteria, but did not affect gram positive bacteria and yeasts. Ionisation may also produce ozone, which is why safety issues have to be considered when installing ionisators.

Future

The novel means have potential in improvement of brewery hygiene, but at least part of them need more development in order to really function in process conditions. The activity and durability of functional coatings needs to be improved. The action of UVC irradiation and air ionisation was confirmed in laboratory studies, but it would be useful to have information of their functioning in process conditions. It is worthwhile to perform both controlled laboratory studies and process studies, because conditions in them may be quite different. In most natural and industrial environments multispecies microbial communities are found rather than single species. Starvation and production of protective extracellular polymeric substances (slime) are common among these communities leading to a stronger stress response and increased resistance against chemical and physical disinfection (Figure 2). Therefore, results obtained in controlled laboratory conditions can never be directly applied to industrial environments. Laboratory experiments on the other hand are essential to obtain comparable results in standardised conditions.

Summary

Hygiene and cleaning are of paramount importance to the beverage industry, but they also cause considerable costs in terms of production interruptions and consumption of energy, water, chemicals and working hours. At the same time environmental demands urge for reduction in water

consumption and the use of more environmentally friendly chemicals. With the help of novel functional coatings the attachment of microbes on process surfaces can possibly be reduced, or their removal facilitated. Another means to improve process hygiene could be physical disinfection means. VTT Technical Research Centre of Finland and Tampere University of Technology have in close co-operation with the brewing industry and providers of coating and disinfection technology, applied new coating techniques to process surfaces, as well as UVC disinfection and air ions as physical disinfection methods during production. In addition, the aim has been to adapt cleaning chemicals to function along with new surfaces and disinfection methods. Different photocatalytic TiO_2 coatings and hydrophobic low energy coatings have been studied in laboratory and process studies. Microbiological results from photocatalytic coatings have been variable, and it seems that they may be more active against bacteria as compared to eukaryotic yeasts and moulds. Coatings supplemented with Ag ions reduced numbers of attached microbes significantly in laboratory studies, and this effect was also seen in some process tests. Low energy coatings reduced microbial coverage significantly in laboratory studies, but similar effect on attached microbial numbers was not seen in process studies. In laboratory washing studies low energy coatings were washed slightly more efficiently than stainless steel. UVC killed 99.999% of microbes on the surfaces when the dose was high enough in laboratory experiments. Air ions worked most efficiently on gram negative microbes. These novel means have potential for the management of brewery hygiene, but at least functional coatings need more development in order to make them withstand process conditions and be really functional there.

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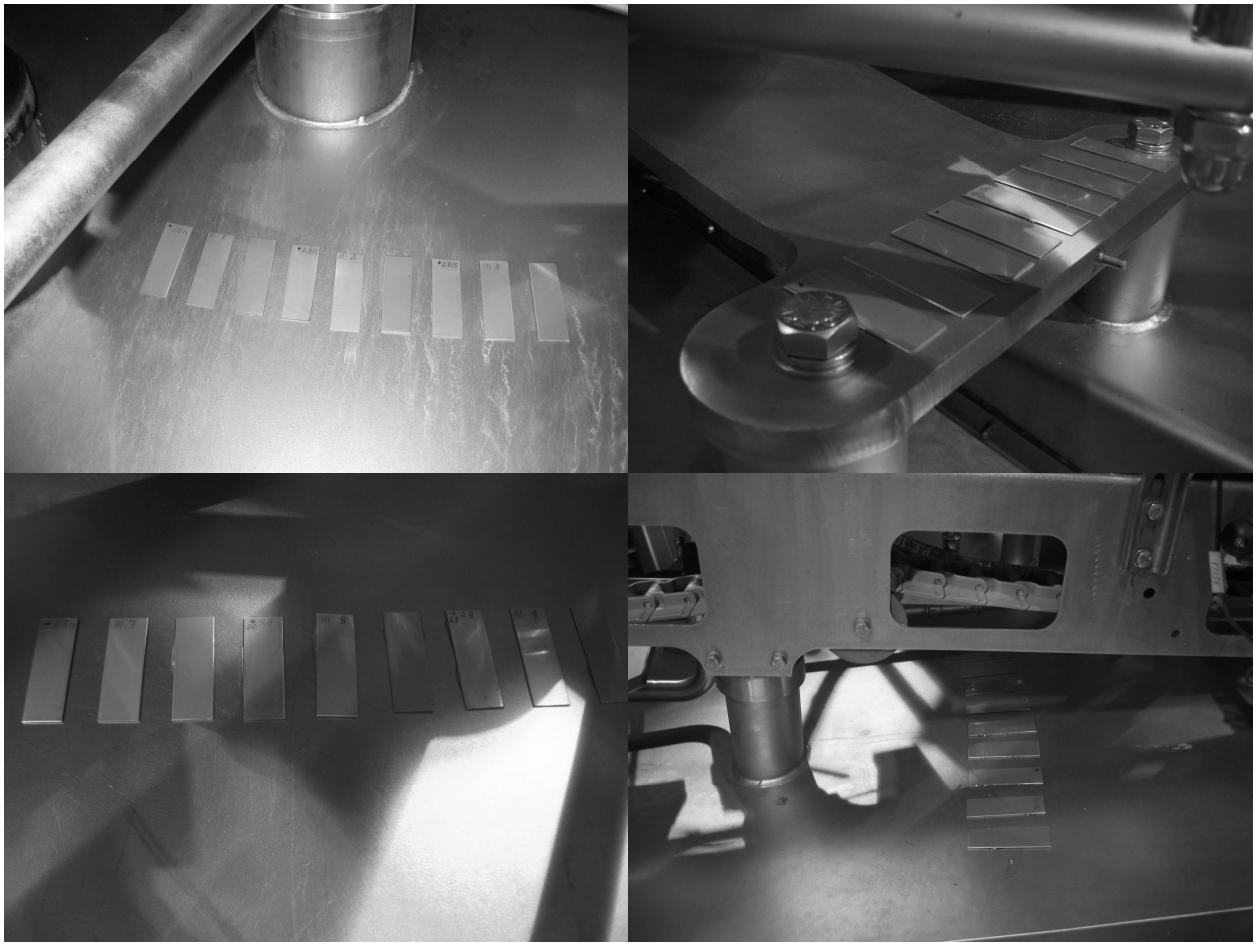

Figure 1. Sample coupons on filling machines.

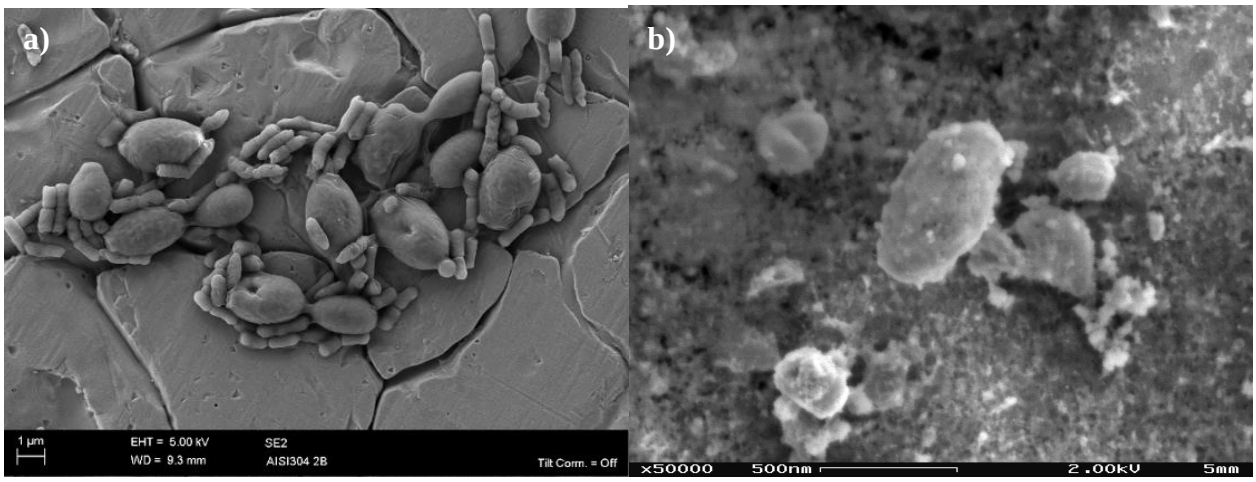

Figure 2. Microbes on stainless steel surface a) in the laboratory and b) on a sample coupon from a one-year process study.