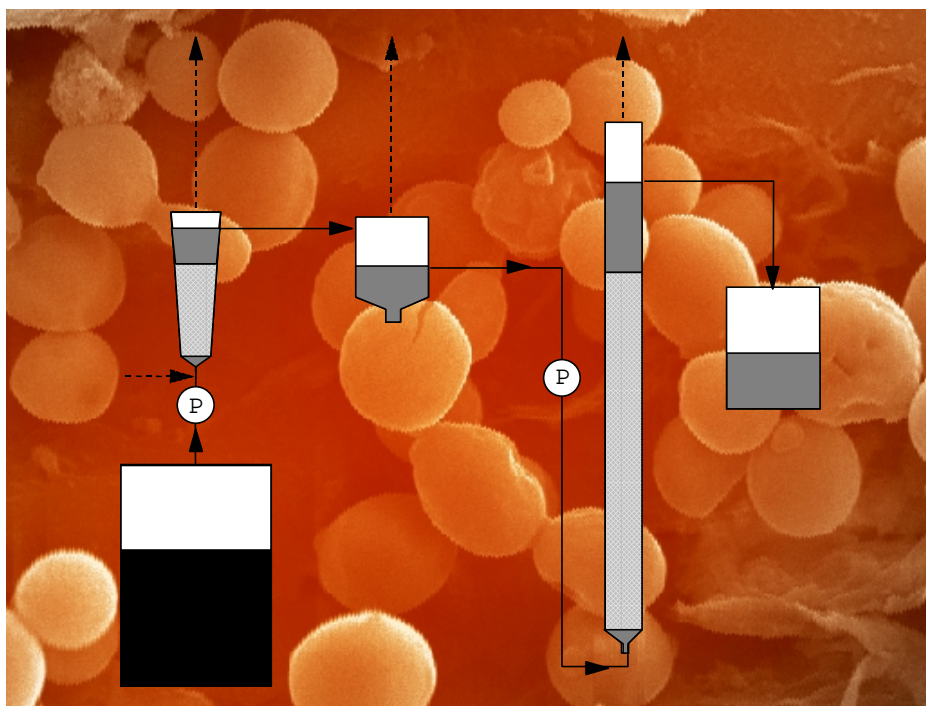


Ilkka Virkajärvi

Feasibility of continuous main fermentation of beer using immobilized yeast



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Feasibility of continuous main fermentation of beer using immobilized yeast

Ilkka Virkajärvi

VTT Biotechnology

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Abstract

Fermentation is the most time consuming step in the production of beer and therefore the effective use of fermentation vessels is a crucial element in brewing economy. One means of increasing the productivity of a batch process is to convert it to a continuous one. Experiments in continuous fermentation emerged during the 1950s and 1960s, but by the end of 1970s most of them had been closed down. Immobilization technique revitalised continuous fermentation research in the 1980s and led to industrial applications in the secondary fermentation and in the production of low-alcohol beers.

This work demonstrated that an immobilized, continuous main fermentation is a feasible process for production of lager beer. The immobilized main fermentation was stable for more than 14 months both in fermentation efficiency and in aroma compound formation. The formation of aroma compounds could be controlled by varying the composition and amount of gas feed into the first fermentation stage. The division of immobilized main fermentation into an aerobic and an anaerobic stage appeared to solve problems related to yeast growth and viability.

The carrier material affected the formation of flavour compounds in small-scale fermentations. Moreover the effect varied with the yeast strain used. The carrier affected the economy of immobilized fermentation: the carrier cost could be as high as one third of the investment. When a cheap carrier is used the investment cost for a continuous, immobilized process was estimated to be only about 70% of the investment cost of a batch process.

Preface

This work was carried out at VTT Biotechnology during the years 1995–2000. The work formed a part of a wider project of the Finnish malting and brewing industry aiming at continuous production of beer using immobilized yeast. Financial support was provided by the Finnish malting and brewing industry and Tekes, the National Technology Agency, which is gratefully acknowledged.

I am very grateful to Prof. Matti Linko, the former Laboratory Director, for his encouragement to write papers and this thesis. I also thank the present Research Director, Prof. Juha Ahvenainen, for providing excellent working facilities and the possibility to finalise this work. Prof. Katrina Nordström provided many valuable comments during the writing phase. Prof. Timo Korpela and Doc. Pekka Reinikainen I thank for the critical reading of the manuscript.

My special thanks go to my co-authors. Jukka Kronlöf, PhD and Esko Pajunen, MSc provided valuable viewpoints from practical brewing. Nana Lahtinen, MSc (née Pohjala), Katri Lindborg, MSc and Terhi Vauhkonen, MSc I thank for their pleasant co-operation, keen attitude and interesting discussions. Erna Storgårds, PhD I thank for continuously reminding that there exist more microorganisms than brewer's yeast – even in a brewery. To Silja Home, Dr. Sci. (Tech.) go my special thanks for her encouragement, continuous pressure to improve my writing and valuable discussions.

This work would not have been possible without the pleasant and occasionally humorous environment generated by all my colleagues at VTT Biotechnology, especially those of the former Brewery group. Hannele Virtanen MSc, Airi Hyrkäs, Marita Ikonen, Kari Lepistö, Arvi Vilpola and especially Eero Mattila have all helped throughout the years.

Lastly, I express my warmest thanks to Seija, Jussi and Juuso for letting me pile papers at home and for understanding my occasional absent-mindedness.

List of the original publications

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:

- I Virkajärvi, I. and Linko, M. 1999. Immobilization: A revolution in traditional brewing. *Naturwissenschaften*, Vol. 86, pp. 112–122.
- II Virkajärvi, I. and Kronlöf, J. 1998. Long-term stability of immobilized yeast columns in primary fermentation. *J. Am. Soc. Brew. Chem.*, Vol. 56, No. 2, pp. 70–75.
- III Virkajärvi, I., Lindborg, K., Kronlöf, J. and Pajunen, E. 1999. Effects of aeration on flavour compounds in immobilized primary fermentation. *Monatsschrift für Brauwissenschaft*, Vol. 52, No. 1/2, pp. 9–12, 25–28.
- IV Virkajärvi, I. 1999. Profiting from immobilized fermentation. *Proc. 5th Aviemore Conf. Malting, Brewing and Distilling*, Aviemore, 25–28 May 1998. London: Institute of Brewing. Pp. 290–293.
- V Virkajärvi, I. and Pohjala, N. 2000. Primary fermentation with immobilized yeast: some effects of carrier materials on the flavour of the beer. *J. Inst. Brew.*, Vol. 106, No. 5, pp. 311–318.
- VI Virkajärvi, I., Vauhkonen, T. and Storgårds, E. 2000. Control of microbial contamination in continuous primary fermentation by immobilized yeast. *J. Am. Soc. Brew. Chem.* Accepted for publication.

Some additional unpublished data are also presented.

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***Appendices of this publication are not included in the PDF version.
Please order the printed version to get the complete publication
(<http://otatrip.hut.fi/vtt/jure/index.html>)***

Abbreviations and terms

CSTR	continuously stirred tank reactor
DEAE	diamino diethyl
DMS	dimethyl sulphide
FAN	free amino nitrogen
GDC	granulated diamino diethyl modified cellulose carrier material (Spezyme [®] GDC 220)
HFCS	high fructose corn syrup
hl	hectolitre, a usual measure of volume within the brewing industry, equals 100 litres
PBR	packed bed reactor
VDK	vicinal diketones
°P	degrees of Plato (weight per cent of solids in wort)

Apparent degree of fermentation

$100 * (\text{original gravity} - \text{present gravity}) / \text{original gravity} \%$, measures the extent of fermentation

Attenuation limit

the maximum attainable degree of fermentation, a property of wort (and of the yeast strain used)

Green beer

beer after primary fermentation, which usually has high concentrations of vicinal diketones, also called young beer

Main fermentation

the first fermentation step in the production of lager beer, also called primary fermentation; most of the flavour compounds are formed in main fermentation

Primary fermentation

main fermentation

Secondary fermentation

the second fermentation step in the production of lager beer, also called lagering and maturation; the main purpose is to remove buttery off-flavour and its precursors to an acceptable level

Vicinal diketones

diacetyl and 2,3-pentanedione, which are responsible for buttery off-flavour. This flavour may be essential in some ales, as it is in some red wines.

1. Introduction

Brewing has changed from home brewing into very large-scale manufacturing. Most beer is brewed by large companies, but on the other hand, the number of small or very small breweries has increased in recent years. The competitive factor for these small breweries is not price, but beers that differ from mainstream beers. In recent years the tendency for globalisation has been evident, with brewing companies acquiring breweries all over the beer drinking world. This will make the market very competitive. Beer is a consumer product for which the image is very important. Another important factor in consumer products is the price. Only with a very efficient production and distribution chain can the brewery make this factor work its advantage. However, every step taken in cost reduction must preserve the flavour of the product.

Christensen (1997) showed that sometimes the market-leading, profitable and well-managed companies fail to see and react to a change that will eventually lead to loss of market dominance. Typically this change originally has a lower performance than existing technologies, but its faster rate of improvement rapidly changes the situation. Products of new technologies have features that customers value: cheaper, simpler, smaller and more convenient to use. Christensen (1997) called this event disruptive technological change. Examples in his book include companies in hard disk manufacturing, steel making, and excavator manufacturing, and thus the idea of disruptive technological change appears to be rather universal and may be extendable to the brewing industry.

Process biotechnology has advanced enormously by science-oriented and innovative brewmasters. They have published a great number of papers and patents and established even large-scale continuous fermentation units. However, by the late 1970s almost all of the continuous fermentation units had disappeared, leaving a bitter aftertaste of disappointment.

The present study deals with the challenges of continuous processes for brewing. It attacks the problems encountered in continuous main fermentation of beer and uses immobilization technology to solve these problems.

2. Beer fermentation and the brewing industry

2.1 Beer fermentation

All beers in the early days of brewing were produced using top fermenting yeast strains, but today lager beers, which are produced with bottom fermenting yeast – developed only about 150 years ago – dominate throughout the world. Below is a very short description of the brewing process for lager beer.

The first step of brewing is mashing in which malt is milled or ground and mixed with water. The enzymes of malt hydrolyse biopolymers: starch to mono-, di-, trisaccharides and dextrans, glucans to oligosaccharides, proteins to amino acids and peptides (Hough *et al.* 1971). A brewmaster uses a temperature profile to control the extent of these hydrolyses. Mashing takes about two hours. After mashing the insoluble fraction of malt, spent grains, is removed in either a lauter tun or a mash filter. Lautering normally lasts about 3–4 hours, although new designs of lauter tuns and mash filters have shortened the process to about 2 hours.

The next step is cooking, in which the wort is boiled for 1–2 hours. During the boiling, hops or hop products are added into the wort. Boiling ensures the asepticity needed, precipitates protein-polyphenol complexes, solubilises and isomerises hop components, removes certain off-flavours and brings the wort to desired gravity (Hough *et al.* 1971). Enzymes are inactivated during the boiling. The precipitated material is removed usually by a whirlpool (a wort cyclone), which again takes about one hour. Then the wort is cooled to fermentation temperature, aerated and pitched, usually in the transfer line to the fermenter (Hough *et al.* 1971). One fermenter may receive one or more batches of wort. Which of these batches are pitched and aerated are particular practices of a brewery. This is a description of all malt brewing, but other sources of fermentable sugars can be used: unmalted barley or cereal starch, which are added into the mash tun, or sugars and syrups, which are added into the wort kettle. The addition of hydrolytic enzymes in mashing may become necessary with the use of unmalted barley or starch.

The lager beer fermentation is divided into two phases: the main fermentation and the secondary fermentation. The main fermentation lasts from 6 to 10 days and temperatures used are between 7 and 15°C. Temperature profiles may also be used. During the main fermentation most of the flavour compounds are formed. At the end of the main fermentation the beer is cooled down to about 4°C and most of the yeast is separated from the beer. The secondary fermentation can be performed in the same vessel as the main fermentation or the beer can be transferred into a second vessel. The main objective of the secondary fermentation is to remove diacetyl, which causes an off-flavour in lager beer. The secondary fermentation normally lasts between one and two weeks, but even six-week times have been reported by brewing companies. The latter time was common before the lagering temperature was increased from 0°C to 10–15°C (Linko and Enari 1967).

Finally, the beer is stabilised by cooling it down to 0°C or even lower and by maintaining this temperature for up to 3 days. Different stabilising agents (i.e., silica gels, polyvinylpolypyrrolines, tannins) may be used. Yeast and protein-polyphenol complexes precipitate and these are then filtered off. Carbonating and packaging ends the production.

2.2 Beer flavour

Beer is a complex aqueous solution containing CO₂, ethyl alcohol, several inorganic salts and about 800 organic compounds (Hardwick 1995a). The flavour of beer must be preserved despite changes in the process. The flavour of beer is determined by the raw materials used, by the process and by the yeast. The compounds produced by yeast during the fermentation exert the greatest impact on the palate and on smell. Alcohols, esters, organic acids, carbonyl compounds and sulphur-containing compounds are the most important flavour compounds formed by yeast.

Ethyl alcohol has the highest concentration of alcohols in beer and it has an impact on the flavour of beer. Of the higher alcohols 3-methyl butanol (isoamyl alcohol) and 2-methyl butanol (amyl alcohol) also have an impact on the flavour. Other higher alcohols affect the flavour of beer through their cumulative effect as they seldom exist in concentrations exceeding their individual taste thresholds

(Meilgaard and Peppard 1986). Alcohols have taste thresholds approximately 10 times higher than esters and approximately 1000 times higher than carbonyl compounds, so despite their high concentration their impact on flavour is not the most important.

Esters are important flavour compounds in beer. Ethyl acetate, 3-methyl butyl acetate (isoamyl acetate), ethyl hexanoate (ethyl caproate), ethyl octanoate (ethyl caprylate) and 2-phenyl acetate are the major esters found in beer (Dufour and Malcorps 1994). According to Dufour and Malcorps (1994) 3-methyl butyl acetate was above its taste threshold in 90% of the European lagers tested, whereas ethyl acetate exceeded its taste threshold in less than 50% of the beers tested.

Beer is slightly acidic. Carbonic acid and organic acids are responsible for a positive taste effect, mild tartness, in beer (Hardwick 1995a). The organic acids are all essentially by-products excreted by yeast. Acetic, capric, caproic and caprylic acids are among the most flavour-active acids in beer.

Of the carbonyl compounds, acetaldehyde and diacetyl are the most important. Acetaldehyde may exceed its taste threshold during the active phase of fermentation, but normally in the later phase of fermentation it is reduced to ethanol (Angelino 1991). Diacetyl is the key flavour compound in secondary fermentation. The buttery off-flavour in beer caused by diacetyl is removed during the secondary fermentation.

Sulphur-containing compounds come mainly from malt, but hops may also have sulphur residues (Hardwick 1995a). The yeast-derived sulphur-containing compounds include hydrogen sulphide and sulphur dioxide. Normally these are removed from the beer during the fermentation, but in some cases this may not occur (poor yeast condition or slow fermentation) and off-flavour of rotten egg or burnt match may be detected (Angelino 1991).

2.3 Brewing industry

In the early days of brewing each household probably made its own beer, but soon some started to produce more than their own consumption and thus selling

to others started. An important turning point in brewing was the appearance of cooling equipment, because only this invention made it possible to produce lager beer all year around. Louis Pasteur elucidated the importance of yeast to the fermentation process in the mid-1800s. The use of pure cultures in brewing started with Emil Hansen in the 1880s.

Since the Second World War the brewing industry has followed the same pattern as in other branches of process industry: larger production units and fewer companies. Table 1 below shows the development of brewing companies, number of production units and total annual production of beer in the U.S.A. between 1936 and 1989 (data from Hardwick 1995b). It can be seen that the average annual production from one production unit has increased over 50-fold in 53 years.

Table 1. Developments in the brewing industry in the U.S.A. between 1936 and 1989. The original data (Hardwick 1995b) have been converted to litres (31 gallon barrel = 177 litres).

Year	Number of brewing companies	Number of brewing plants	Million litres sold	Million litres/plant
1936	750	750	5 300	7
1947	404	465	10 300	22
1958	211	252	10 400	41
1967	124	170	13 700	81
1975	57	120	18 500	154
1982	35	87	22 800	262
1989	26	65	23 200	357

The same tendency towards larger units/companies is valid today all over the world. In the UK, consolidation has been very strong and already in 1991 six companies produced 75% of the total amount of beer (Boulton 1991). Since then consolidation has continued in the U.K., elsewhere in Europe and in South America (Anon. 1999, Anon 2000a, Anon. 2000b, Anon. 2000c). Changes have also taken place in the Nordic countries. But brewing is and will be from now on

an international industry. This will increase pressure to reduce the production costs.

2.4 Productivity of beer fermentation

Traditional batch fermentation starts with filling a fermentation vessel with aerated, pitched wort. At the end of fermentation the vessel is cooled down, emptied, washed and sanitised. These operations, without the fermentation phase take 1 to 2 days. This down time diminishes the productivity of the fermentation vessel. A typical operation cycle for the main fermentation might be 1 day filling, 7–9 days fermentation and 1 day of emptying and washing. A graph of ethanol formation during beer fermentation is presented in Figure 1. From this it can be seen that the productivity when defined as ethanol formation rate per volume ($\text{g h}^{-1} \text{dm}^{-3}$) is low at the beginning, increases rapidly, reaches a maximum and then rapidly decreases again. The average productivity of the fermentation cycle is about 30% of the maximum productivity.

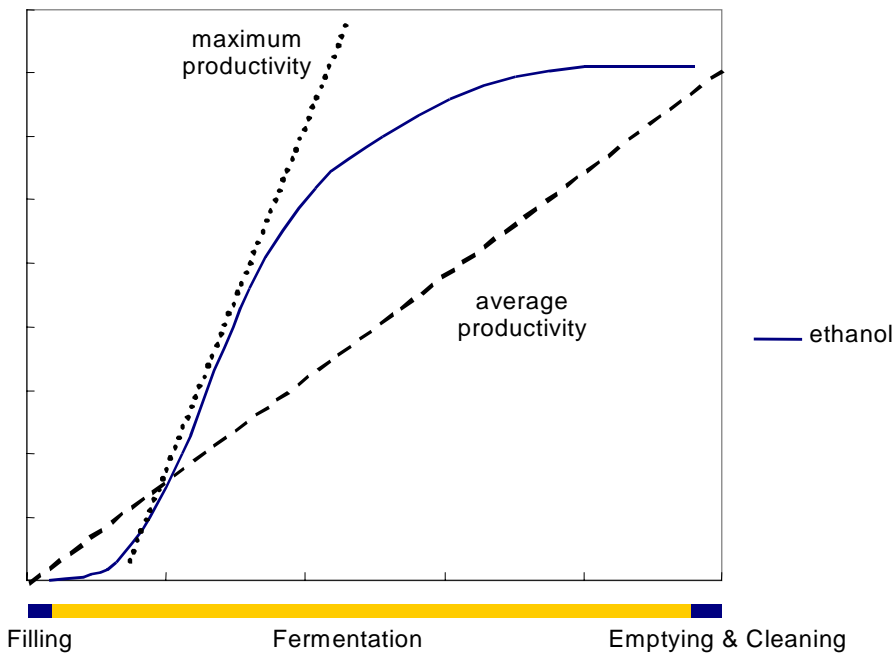


Figure 1. The productivity of batch fermentation.

The fermentation efficiency can be increased for example by high gravity brewing, by increasing fermenter size (Boulton 1991), by increasing fermentation temperature (Linko and Enari 1967) and by one step cleaning-in-place procedures (Dirksen 1998).

3. Continuous beer fermentation

One way to increase the productivity of beer fermentation is continuous operation. In continuous operation the productivity of a fermentation vessel (or rather, of a reactor) remains constant over time. In the optimal case the productivity is the maximum of the batch fermentation when measured as the mass of ethanol produced per reactor volume per unit time. The productivity of the vessel is further enhanced because the down time is minimised.

Below is a review of continuous fermentation processes. The first part (3.1) covers processes prior to the emerging of immobilization technology. The second part (3.2) deals with immobilized processes. The aim of the present review is to identify the advantages of continuous fermentation and the reasons for not adopting or closing down the continuous fermentation processes.

3.1 Continuous, non-immobilized processes

The level of enthusiasm for continuous fermentation can be read from Ricketts (1971), which was written when the first commercial continuous processes had been in operation for some years. According to Ricketts (1971) stirred continuous fermentation offers the following advantages

- approximately 25% lower hop rate
- reduced labour costs
- lesser beer losses
- elimination of duty-paid beer in the pitching yeast in collected wort
- collection of all CO₂ in 100% pure condition
- reduced building and vessel costs by virtue of shorter fermentation times.

Very similar advantages of continuous fermentation were reported by Steward (1974) and Smith (1991). Additional costs of stirred continuous fermentation included electrical stirring and cooling costs (Ricketts 1971). A 30% reduction in hop rates, reduction of beer losses from 1.5% to 0.7% and marginal savings in labour and detergent cleaning in the production of ale were reported (Seddon

1976). The fermentation time was reduced from three days to 4 hours and warm conditioning from three days to zero (Seddon 1976).

The economic advantages of continuous systems were claimed to be such that it is "inevitable that the future of the British fermentation systems lies in vertical conical-bottom vessels and continuous processes" (Ricketts 1971). Two major factors influencing the decision of New Zealand Breweries Limited to employ a continuous fermentation system in early the 1950s were the restrictive Government-imposed building regulations and excise paid by pitched wort (so the brewing company paid taxes on the amount of beer in processing) (Davies 1988). Other factors included higher vessel utilisation and lower costs. Fortuitous coincidences occurred: the need for brewery expansion at the moment when the new technology was there and the ability to fabricate steel (Kennedy 1996).

In the following section early attempts at continuous non-immobilized fermentation will be described. The next section will describe a number of larger scale experiments, through which the advantages and disadvantages of continuous non-immobilized fermentation are elucidated.

3.1.1 Early attempts

Delbrück (1892) used a very high concentration of yeast within a porous pot. Wort flowed and diffused through this pot and was fermented in 4 hours at 30°C. A continuous fermentation process was patented in France 1899 (Barbet) and in England 1905 (Barbet). A semi-continuous system, consisting of six closed fermentation tanks was suggested in 1906 (Schalk). In operation the first was filled with wort and inoculated, then it was allowed to ferment between 18 and 48 hours. After this period, half of the contents were transferred to a second tank in the series and both were filled up with fresh wort. The division of the contents of the lastly filled tank was continued until all the tanks were filled. At this time the first tank was emptied and cleaned. Contaminations limited the use of this process to one week, although in theory it could be run indefinitely. The Schalk process was improved to a more continuous one (Wellhoener 1954). This process used six tanks interconnected with pipes, of which the first three were held at 10°C and the last three at 0°C. Fresh wort was added daily into the first

vessel and corresponding amount of beer was removed from the last vessel. The residence time was 18 days in the first three vessels and 9 days in the last three vessels. The beer was reported to be of normal quality. The amount of yeast in the system was doubled in 28 days and the yeast had become rather granular after 28 days of operation (Hlavacek *et al.* 1959). The experiments above were not fully continuous and did not succeed. Because of their short operative life they did not offer any advantages over batch processes.

A fully continuous stirred system was patented in 1906 (van Rijn). The process had six vessels in series, such that each subsequent vessel was situated lower than the previous one. Wort flowed into the first vessel and fermenting wort overflowed into the following vessel. van Rijn must be credited for two reasons. Firstly, the stirrer was equipped with rubber strips to remove precipitates and dead cells from the walls and base of the vessel. Secondly, temperature control was achieved by circulating water through hollow shafts of the stirrers.

3.1.2 Large scale attempts

The Watney Process is a large-scale fermentation process, which was installed in several British breweries in capacities ranging from 8.2 to 34.3 million litres per year (Hough and Button 1972). But according to Maule (1986) the Watney system has been installed by 1970 in four breweries with a maximum capacity of 1700 million litres per year. The beers produced over many years were identical in flavour to batch produced beers. Bishop (1970b) stated that Watney Mann Ltd. produced two of the company's major brands by batch and by continuous fermentation and sent them out unblended, and to "the best of their knowledge the public has never commented on any differences". One of these installations operated at the Mortlake brewery between 1960 and 1975 and the continuously fermented beer was used interchangeably with batch beer (Whitewar 1991). The process is illustrated in Figure 2.

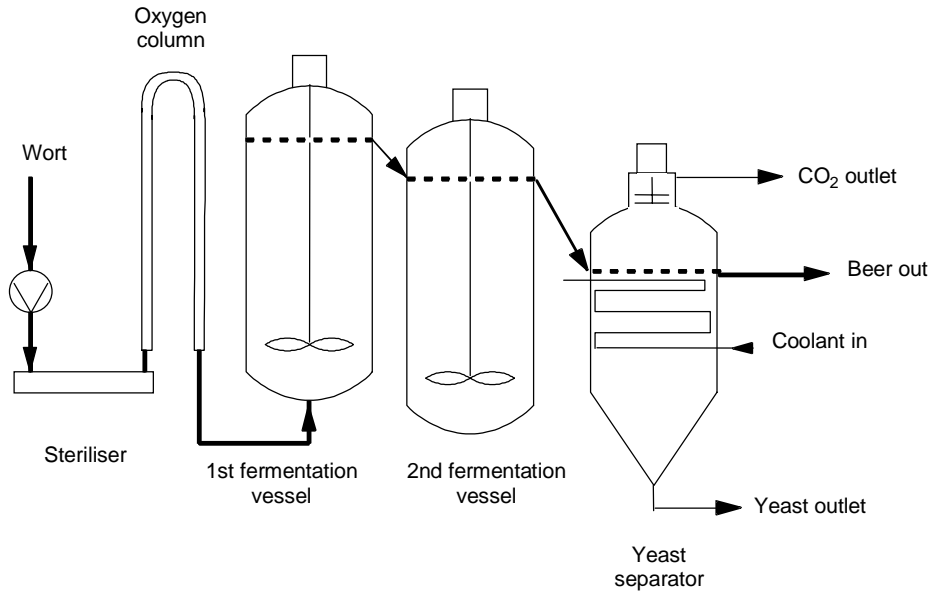


Figure 2. The Watney process.

The Watney process the output could be varied tenfold by controlling temperatures and flow rates (Bishop 1970a). A rather similar system to the above in 3-litre scale was used for continuous fermentation in two days, producing a beer that was comparable to conventional lager beer (Okabe *et al.* 1994).

The Tower Fermenter (Royston 1960) also known as A.P.V. Tower, is a continuous fermentation process which has been used in commercial scale in Britain, in the Netherlands and in Spain. In Spain, lager beer was produced from 1966 onwards and in Britain pale ales were produced. In both cases the quality of beer was satisfactory. The longest reported runs were 18 months (Shore 1986).

The first A.P.V. Tower fermenter (see Figure 3) in commercial use (by Bass at the Burton brewery) was 1.06 m in diameter and had a beer depth of 7.6 m with an output of 10 million litres per year. The second Tower fermenter at Bass was larger, 1.83 m in diameter with 70 million litres per year output (Seddon 1976). In Spain at La Cervecera del Norte brewery the whole process (wort production and fermentation) was continuous (Anon. 1967). The brewery went into

production in May 1966 and had a designed output capacity of 36 million litres per year. It had five A.P.V. Tower fermenters followed by four conditioning vessels, two yeast settling vessels and beer cellar facilities. The investment costs are reported to have been 60% of those of a comparable batch brewery, the extract losses were decreased by 50%, fuel and power costs were said to be 50% of those of the batch brewery and an additional financial advantage came from billing practice (Anon. 1967).

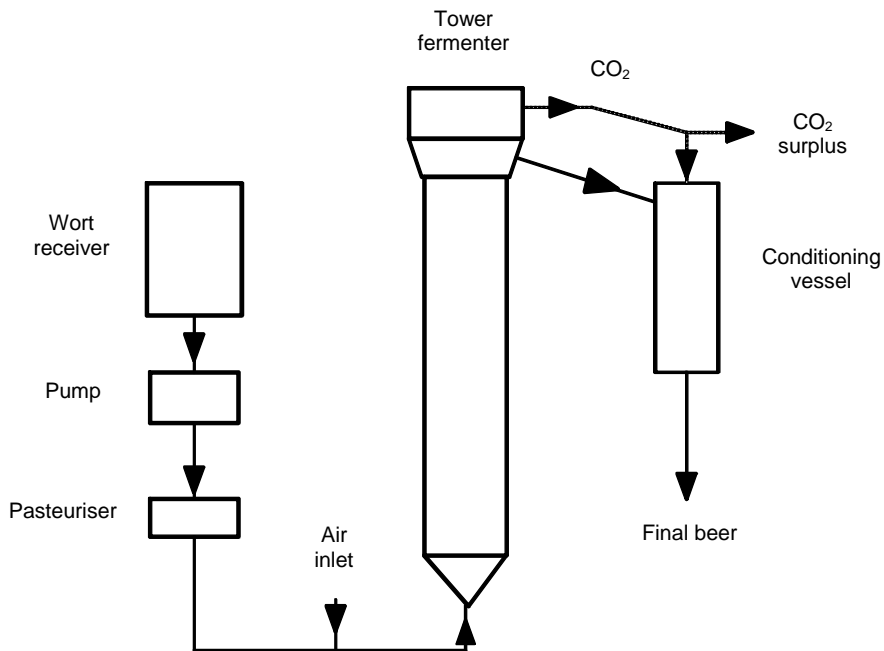


Figure 3. The A.P.V. Tower fermenter.

The start-up of A.P.V. Tower began with laboratory-grown yeast under mild aeration and slow addition of wort until the vessel was full. Slow continuous addition of wort was continued until after about one week the full rate was achieved, giving a residence time of 4–8 h.

The system was closed, i.e. virtually no yeast flowed out from the system, which lead to a higher nitrogen content of the outflowing beer Steward (1974). A beer with a normal nitrogen content could be produced by promoting yeast growth by aeration (Ault *et al.* 1969). Flavour matching was possible in lager beer

production (den Blanken 1974). The A.P.V. Tower fermenter could also be slowed down or even shut down for up to four days (Seddon 1976).

Morton Coutts of Dominion Breweries of New Zealand patented a continuous fermentation system (Dominion Breweries Ltd. 1956). The following description of the Coutts' process at Dominion Breweries Ltd. is taken from Dunbar *et al.* (1988). The process consists of three continuously stirred tank reactors (Figure 4) in cascade and employs a flocculent lager yeast strain. A beer with ca 5.5 % alcohol (1.054 original gravity) was produced with a 45 h residence time. After boiling, the wort is rapidly cooled to 0°C and trub is removed by sedimentation. Dilution to fermentation gravity is carried out on line to the fermentation systems. The Hold Up Vessel (HUV) offers some form of microbial control providing an environment of low pH (< 4.5) and an alcohol content of >2.0% w/v. The HUV comprises approximately 6% of the total volume of the system. The flow into HUV consists of wort and recycled flow from the second vessel (CF1) in the ratio of 1:1. Additionally, yeast is recycled from the yeast separator (YS) to achieve control of the fermentation rate through the amount of yeast in suspension. The HUV is continuously aerated. This aeration is very important for control of growth and ester formation. Fermentation vessels CF1 and CF2 comprise 66% and 22% of the system volume, respectively. The flow from CF1 to CF2 is by gravity via a balance line. Fermentation is at a maximum in CF1 and yeast growth is continued in CF1. The Yeast Separator (YS) and the Yeast Washer (YW) are conical in shape and the highly flocculent yeast is separated from the beer by gravity. Surplus yeast is washed in counter current flow and the mixture of beer/deaerated water is used to adjust the original gravity of the green beer, thus minimising extract losses. The amount of yeast produced is similar to that produced in batch fermentation. The rate of production could be altered by changing the wort flow into the system. The total residence time can be varied between 36 and 97 hours.

The market in New Zealand was without competition, so the market for continuously fermented beer was assured (Hough and Button 1972). The Palmerston North Brewery of New Zealand Breweries Limited was the world's first brewery to rely totally on continuous fermentation (Anon. 1987). The Palmerston North brewery (12 million litres per year) was "very cost effective, producing a good single brand of beer successfully and efficiently" (Anon. 1987), but in 1985 it was closed down, because instead of upgrading it was

decided for economical reasons to move the production to other breweries of the company.

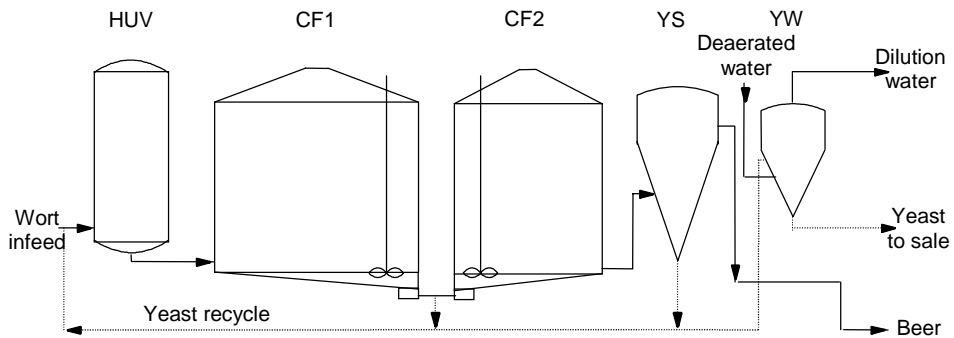


Figure 4. The Coutts process (redrawn from Stratton et al. 1994).

Geiger and Compton patented a rather similar process in 1957 (Geiger and Compton 1957). The beer produced using this continuous two-vessel system was judged by a taste panel data to "show a most gratifying similarity" to batch produced beer (Geiger 1961). For a short time in the USA a process that resembled the Coutts' process in fermentation was in operation. The Fort Worth was a fully continuous process (mashing, lautering, boiling and fermentation) and produced beer "essentially indistinguishable" from batch beer (Williamson and Brady 1965). The process was already closed by 1972 (Hough and Button 1972).

3.1.3 Reasons for failure of continuous, non-immobilized fermentations

By the end of the 1970s most of the continuous systems had been closed down, the famous exception being the Coutts' process in New Zealand (Portno 1978). Smith (1991) listed the disadvantages of continuous fermentation as inflexibility in the output rate or in changing the beer type, the high standard of hygiene needed, possibility of yeast mutation, extra procedures needed in diacetyl reduction, lack of control over degree of attenuation, need for highly skilled supervision, the large amount of ancillary equipment needed, the need for extremely flocculent yeast, the need for a separate vessel for excise declaration and the need for unpitched wort storage. Similar reasons for closing down the

Watney Mann Mortlake continuous fermentation were given: wort storage requirements, duty on-costs, difficulties in changing from one product to another and the cost of technical support (Whitair 1991). The problems of yeast mutation and the need for skilful guidance in continuous processes were also noted (Tenney 1985).

In another early review of fermentation Steward (1977) had already listed difficulty of maintaining production scale hygiene, yeast mutation, killer yeasts, flavour-matching problems, and inflexibility for production of several beers. Steward (1977) was one of the few authors to emphasise the differences in yeast metabolism: “Continuous fermentation as practised cannot reproduce the full cycle of changing metabolic patterns which characterises batch fermentation”, thus leading to differences in flavour of the beer compared to batch produced beer. The economics were claimed to be on the side of large-scale batch systems (Steward 1977). Harris and Irvine (1978) compared the operative and the capital costs for a semi-automatically controlled and a fully automatically controlled batch production of ale in dual purpose vessels. Further, the comparison with a continuous fermentation was made. They found that continuous production had the highest cost per unit.

Two peculiar reasons for implementing continuous fermentation in New Zealand were the government restriction on building and the taxation (Davies, 1988). Removing the building restriction naturally removed this advantage. In the U.K. the wort-based excise system was a powerful reason for retaining the batch system (Boulton 1991). Now the taxation system has been changed to an alcohol-based system (Anon. 1999).

Mutation of the yeast in a continuous process was feared by many brewers. The formation of mutants of bottom fermenting yeasts during a laboratory scale continuous fermentation of beer was studied (Thorne 1968). Over a run time of 9 months approximately half of the cells had mutated, losing flocculation, reducing the fermentative capacity, changing the growth rate and producing undesirable flavours. Others found no clear-cut evidence of mutants during 6 months of operation of an A.P.V. Tower fermenter (Ault *et al.* 1969). This may be a reflection of differences between bottom fermenting *S. carlsbergensis* (Thorne 1968) and top fermenting *S. cerevisiae* (Ault *et al.* 1969). No mutants in continuous fermentation in stirred tanks were detected (Bishop 1970a). In the

Coutts system no confirmed mutations in the Coutts system (Dunbar *et al.* 1988, Davies 1988) were reported.

Contamination was another threat that was feared to affect continuous brewing more than batch brewing. Gram-negative, anaerobic or facultative aerobes could grow in stored wort and produce celery-like off-flavours (Ault 1965). The principal strains were *Aerobacter aerogenes* and *A. cloacae*, with some intermediate strains. Lactic acid bacteria could grow in the A.P.V. Tower and were impossible to be removed (Ault 1965). Deliberate contamination of stirred continuous fermentation led to a constant number of *Obesumbacterium proteus*, *Acetobacter*, wild *Saccharomyces* or *Torulopsis* yeast in the vessel (Hough and Rudin 1959). In all these cases the rate of beer production decreased because the flow rate had to be adjusted in order to obtain fully fermented beer. Wild yeast contamination was the most dangerous contaminant in the Coutts system (Davies 1988).

In some designs there was no substrate gradient in a one vessel system, i.e. glucose was continuously present in the fermenting beer, which may have caused some problems. Maltose utilisation may be limited by the presence of glucose. Too high ester concentrations may be caused by limited yeast growth during fermentation. The flocculation capacity can be lost more easily.

Although the Coutts system can be operated with different outputs (Dunbar *et al.* 1988) inflexibility and a greater degree of brand segmentation affected the decision of New Zealand Breweries Limited to abandon continuous fermentation (Davies 1988).

The low number of suitable yeast strains limited the applicability of the A.P.V. Tower Fermenter (Hudson 1986). Even the Coutts systems uses a very flocculent yeast strain, although with the use of centrifuges in yeast separation and recycling should also facilitate the use of a non-flocculent yeast strain (Davies 1988). In the A.P.V. Tower the yeast growth was severely restricted (Hudson 1986), which was advantageous for yield, but made flavour matching difficult. The stirred fermentation (Watney Mann process) was deliberately designed so that the yeast growth was the same per unit carbohydrate fermented as in the batch process. Slightly higher yeast growth in a stirred continuous system compared to batch fermentation was reported (Maule 1973). 40% of dead

cells in a pilot scale A.P.V. Tower fermentation were found (Woodward 1967). In stirred fermentations the proportion of living cells was satisfactory (Bishop 1970), although variations in proportions of dead cells in stirred continuous fermentations were reported, leading to a lower productivity (Portno 1968a, b).

An economic analysis showed that the Coutts system had 20 to 42 % higher capital costs over batch fermentation in cylindro-conical vessels (Davies 1988). At a discussion panel of continuous fermentation at the same Convention Warren (1988) reported that in "economic terms ... there was benefit from neither one nor the other", i.e. continuous nor batch fermentation. Continuous fermentation over batch fermentation was favoured in economic terms when the brewery output was about 60 million litres per year (7000 brl/week) or more (Royston 1970), although this was challenged by MacDonald *et al.* (1984). They claimed that continuous fermentation failed to reach the designed output due to the inflexibility of continuous fermentation in the face of changes in demand and long start-up periods (MacDonald *et al.* 1984). The anticipated savings were not made in practice. Finished beer storage in practice was large in order to meet peak demands, labour was needed on a round-the-clock basis, which increased the payroll costs, CO₂ was not oxygen-free in the A.P.V. Tower fermentation, the energy consumption was not reduced due to batchwise wort production and microbial contaminations eliminated the savings in cleaning costs (MacDonald *et al.* 1984).

It is clear that the problems encountered in continuous fermentation are very many and varied, but there was no single reason to abandon it. Some of the problems were met in one system, but not in an other. Moreover, in New Zealand beer has been produced and still is produced by continuous fermentation (Dominion Breweries). Although continuous wort production processes were developed, only a very few totally continuous breweries were built. The Fort Worth (Williamson and Brady 1965), La Cervecera del Norte brewery (Anon. 1967) and the Centri Brew Process (Schöffel and Deublein 1980) should be mentioned as examples of totally continuous processes. Inflexibility with regard to the production rate and the changes between beers was surely one decisive factor at least with the A.P.V. Tower, and changes in yeast flocculation capacity may have caused problems in some stirred tank systems.

The knowledge amassed during experimenting in continuous fermentation led to improvements in batch processing (Hough and Button 1972). The research and development of continuous fermentation led to the survival of the batch process: batch processes became more logically arranged (see Table 2) (Hough and Button 1972).

Table 2. Advantages of continuous processes and corresponding developments in modern batch methods over traditional techniques (Hough and Button 1972).

Advantages of continuous fermentation	Counter developments in batch fermentation
Efficient use of plant Uses smallest amount of space	Specialised equipment for unit operations Optimum flow patterns to use plant efficiently Large number of batches passing through each line of equipment each day
Even demand on services and continuous flow of product	Parallel lines of equipment working in regular sequence
Cleaning minimised	Rapid, automatic in-place cleaning
Lower manpower requirements	Consoles giving remote control Partial or complete automation
More consistent product	Rapid batch plant on fixed programme and raw materials gives virtually same result

3.2 Continuous, immobilized processes

Immobilization means limiting free movement, e.g., enzymes or cells are bound to a defined space. Immobilization as a tool for mankind has been used for a long time in producing vinegar (Mitchell 1926), but the first technical papers of

immobilized enzymes in a laboratory scale appeared in the 1950s (Levin *et al.* 1964). The first immobilized enzyme process in industrial scale was the production of L-amino acids in 1969 (Sato and Tosa 1993). In 1971 a definition of an immobilized cell system was reached at the first Enzyme Engineering Conference: immobilized cells are "physically confined or localised in a certain defined region of space with retention of their catalytic activity – if possible or even necessary – their viability which can be used repeatedly and continuously" (Godia *et al.* 1987).

Immobilized cells were used for the first time in industrial scale in 1973 for the production of L-aspartic acid from fumaric acid (Shibatani 1996). This process used non-living *Escherichia coli* cells, so it could be regarded as an immobilized enzyme process. Since then a few industrial scale processes using immobilized cells have been reported. The use of viable (living) cells is less frequent, if production of vinegar and wastewater treatment are not included. On the other hand, higher cells, especially animal cells, need to be immobilized. Animal cells can produce many pharmaceutical products. Factor VIII (antihæmophilic factor for the treatment and diagnosis of hæmophilia) has been produced by immobilized cells in a continuously operated system since the 1980s (Wandrey 1996).

Immobilization technology has also affected the food and beverage industry. Immobilization of an enzyme, glucose isomerase, changed the production of high-fructose corn syrup (HFCS). Immobilized continuous production technology together with an increase in raw sugar prices made the HFCS economically feasible (Pedersen, 1993). The wide and rapid acceptance of HFCS by manufacturers of soft drinks, baking, confectionery and canned food changed the sweetener market as well as the raw sugar market. The production of HFCS was estimated to increase from 1 million tons to about 9 million tons between 1975 and 1995 (Pedersen 1993). The immobilization was a disruptive technology change for some sweetener companies when HFCS replaced artificial sweeteners.

There exist many ways to immobilize cells (or enzymes), but these can be divided into four categories, all of which have their own characteristics: cross-linking, adsorption, entrapment and retaining behind a membrane barrier. Of these, entrapment in different kinds of alginate polymers is the most widely

reported method. One operational difference between entrapment and adsorption on a solid support is the resistance of mass transfer by the entrapping polymer that is absent in the case of adsorption.

The effects that are due to the immobilization method and the effects of a changed microbial physiology must be differentiated when assessing the feasibility of immobilization technology. The direct effects of immobilization are very difficult to conclude from the literature. The reported effects vary: in one paper an increased element is found, while in another the same element is decreased. Another factor, which must be taken into account, is the reactor design, e.g., whether the reactor is a packed bed, an expanded bed, a fluidised bed or a loop bed. All of these designs have their advantages and disadvantages (Kronlöf 1994). For a general review see, e.g., Dervakos and Webb (1991), Groboillot *et al.* (1994) and for brewing applications see Masschelein *et al.* (1994) and Moll and Duteurtre (1996). The fundamentals of immobilized cell technology for brewing were described by Pilkinton *et al.* (1997). Norton and D'Amore (1994) reviewed the literature on implications of immobilization for brewing applications. Recent reviews of immobilized beer fermentations include Mensour *et al.* (1997) and Masschelein and Vandenbussche (1999).

The brewing industry has been interested in immobilization technology since the its emergence. The number of papers in which this technology has been used to produce beer continuously began to increase dramatically after the introduction of alginate as a carrier by White and Portno (1978). Thus continuous fermentation was seen as an attractive process. In 1991 Ryder and Masschelein (1991) identified properties of immobilized yeast systems which affect brewing (Table 3). These could be taken as an indication that immobilization technology can solve at least some of the problems previously encountered in continuous fermentation.

In the following some pioneering fermentations using immobilized yeast are presented. Some larger scale processes are then described. These examples together with (I) present how immobilization technique is used within the brewing industry, along with the problems and possibilities.

Table 3. Properties and influences of modern immobilized cell systems (Ryder and Masschelein 1991).

Property	Influence
Volumetric productivity	increased
Operation	continuous
Biomass separation	eased
Gas-liquid transfer	enhanced
Process design	simple
Substrate utilisation	enhanced
Yeast strains	indifferent
Hygiene	manageable
Scale-up	predictable
Product	consistent

3.2.1 The Bio-Brew Bioreactor

The immobilization method of Berdelle-Hilge (1966) for enzymes was developed to suit brewing with immobilized yeast (Narziss and Hellich 1971). Their bioreactor (Bio-Brew) was very simple: a mixture of kieselguhr and yeast in a kieselguhr filter through which wort was passed. The process is presented in Figure 5 (Narziss and Hellich 1972). The residence time was only 2.5 hours, but because of the high concentration of vicinal diketones in the green beer a maturation period was needed. Furthermore, addition of viable yeast was necessary. The start up, reduction of vicinal diketones and subsequent cold lagering increased the production time to 7 days.

There were also some other problems with Bio-Brew. The bioreactor had a lifetime of only 7 to 10 days before clogging. The amino nitrogen consumption was reduced, probably because of limited growth, leading to lower amounts of higher alcohols and esters and too high pH of the final beer. Furthermore, the

yeast viability at the exit of the reactor was decreased. However, probably the most serious problem was the high amount of α -acetolactate in the green beer (Narziss 1997). Decreased foam stability was noted by Dembowski (1992).

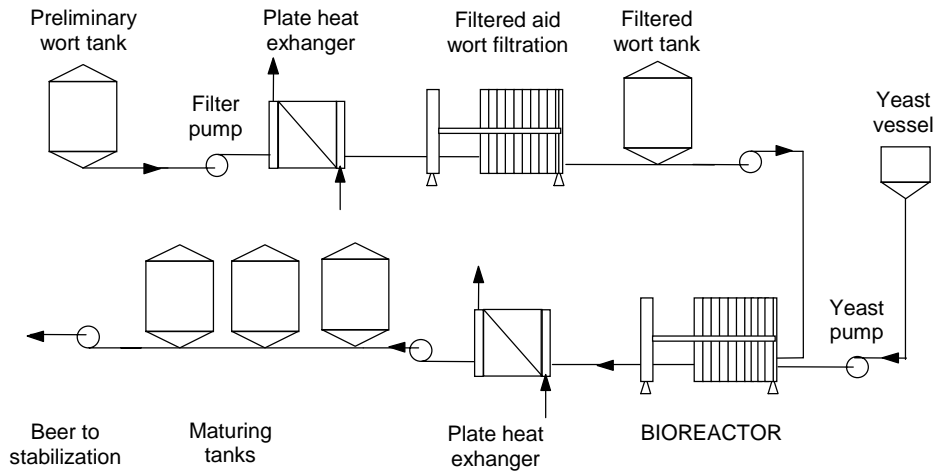


Figure 5. The Bio-Brew process (redrawn from Narziss and Hellich 1972).

Dembowski *et al.* (1993) developed the Bio-Brew further by optimising flow through the kieselguhr yeast bed and adding a cooling plate inside the reactor. This led to a slower decrease in yeast viability. Installing an aerobic phase in front of the filter led to improved sensory quality of beer and to better stability of the Bio-Brew (Dembowski *et al.* 1993), but the concentrations of low molecular weight nitrogenous substances in the green beer still remained too high. Overall the Bio-Brew experiments were not successful.

The very high VDK values of the Bio-Brew experiments still have an effect. Although in many of the later experiments the high VDK-concentrations are absent, the question of very high VDK-concentrations is still raised. The fact that in the Coutts process, yeast does not produce more diacetyl than in batch fermentation provided that the yeast and wort are kept the same (Dunbar *et al.* 1988), has not removed the doubt.

3.2.2 The continuous main fermentation system developed by Baker and Kirsop

The system of Narziss and Hellich (1971) was improved by Baker and Kirsop (1973). They were the first to report the heat treatment of beer for rapid conversion of diacetyl precursors to diacetyl and the subsequent removal of diacetyl by immobilized yeast. This concept is the foundation on which the industrial scale continuous secondary fermentation (maturation) processes are built today. Their system consisted of a yeast plug (formed with kieselguhr) in a tubular reactor for main fermentation and a heating unit, cooling coil and a smaller reactor for secondary fermentation (Figure 6). The system was gradually blocked and about 45 times the reactor volume of beer could be produced. Another problem was changing flavour of the beer. The beer from a plug fermenter resembled all-malt ale more than malt-adjunct ale (Pollock 1973).

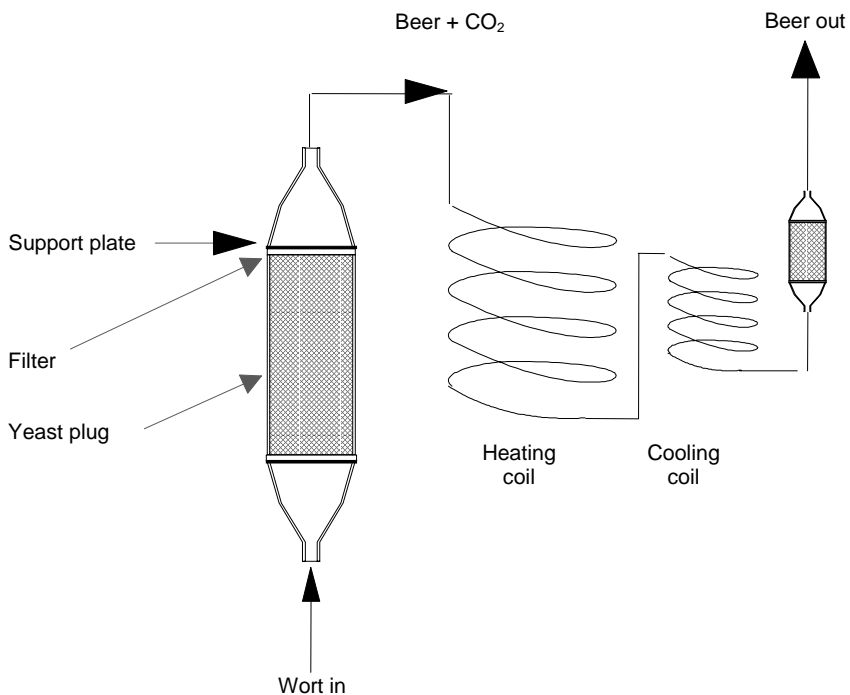


Figure 6. Continuous primary fermentation combined with heat treatment and secondary fermentation (redrawn from Baker and Kirsop 1973).

3.2.3 Alginate as carrier for brewing

Alginate carriers for brewing were introduced by White and Portno (1978). They immobilized yeast in calcium alginate flocs and found that the rates of fermentation and yeast growth were unaffected by the immobilization method used. They used a 5 litre laboratory tower fermenter to produce beer which had comparable amounts of flavour compounds to those in batch fermented beer. Contamination did not pose a problem in these experiments. The formed gel had no tendency to entrap bacteria. If wort containing bacteria was fed into the reactor, subsequent injection of sterile wort resulted in a rapid washout of the bacteria. The operation period was 7 months (White and Portno 1978). The level of ethyl acetate dropped to approximately 60 % during the last 6 months of operation.

3.2.4 Use of immobilized yeast in batch fermentation

Immobilized yeast in batch fermentation was reported by Hsu and Bernstein (1985). Their process minimised investments in new equipment making use of a slightly modified conventional fermentation vessel. However, the beer produced was not equal to the control beer. This was probably due to the immobilization method using alginate beads implying unsuitability of alginate for continuous brewing.

The authors immobilized yeast in alginate beads and modified a conventional fermenting vessel with two screens so that the beads were held in the vessel. Wort was pumped into the fermenting vessel and the fermentation proceeded to completion batchwise in the vessel. The whole process from wort production to bottling of beer took only seven days. The beer thus produced had slightly lower pH than the control beer and lower bitterness, probably due to adsorption of isohumulones to the alginate beads. Although the physical and chemical compositions of the beers produced in the bioreactor and in a traditional process showed very little difference, the quantities of flavour compounds and flavour precursors were lower in the former due to limited growth of yeast (Hsu and Bernstein 1985). The advantages of the process included low investment in equipment, simple clarification of wort, no wort storage and no need for an

extensive maturation process in contrast to the process of Narziss and Hellich (1971).

3.2.5 Process development at Kirin Brewery Company Ltd., Japan

The research scientists at Kirin Brewery Company published their process in 1985 (Nakanishi *et al.* 1985). The process was developed further over the years and the description below is taken from Yamauchi *et al.* (1994a). The process consisted of three bioreactors for rapid lager beer fermentation (Figure 7). The wort was sterilised prior to entering the first reactor, which was an aerated continuously stirred tank (CSTR) for yeast growth. The yeast was then removed with a centrifuge. The beer was fed into two packed bed reactors in series, where the main fermentation was completed. The next step was the conversion of α -acetolactate into diacetyl and partly directly to acetoin in heat treatment. This heat treatment concept resembled that of Baker and Kirsop (1973). Finally, the beer was matured in another packed bed reactor with immobilized yeast. The total residence time varied between 72 and 96 hours.

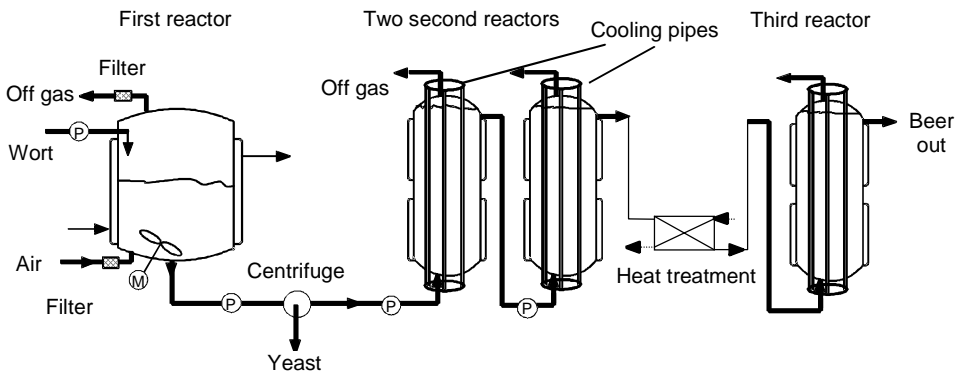


Figure 7. The Kirin process (redrawn from Yamauchi *et al.* 1994a).

The immobilization method firstly used was entrapment in alginate beads (Onaka *et al.* 1985), but it was abandoned because of decreasing fermenting capacity, insufficient mechanical strength and swelling of the carrier leading to plugging of the bioreactor and thus preventing long term operation. Other disadvantages attributed to alginate beads were heat lability and poor

regenerating properties for repeated use (Yamauchi *et al.* 1994a). Aseptic filling of the reactors was also impossible with alginate beads (Inoue 1995). Therefore, alginate was replaced by porous glass beads developed by Kirin.

The reason for dividing the process into different units was to separate the different phases of fermentation. The steriliser assured asepticity of the wort. In the first bioreactor yeast growth, pH reduction and fusel alcohol formation occurred. Out-flowing yeast in the fermenting beer was reduced below a certain level by centrifugation. In the second reactors the yeast was in stationary (non-growing) state for formation of ethanol and esters. The heat treatment converted α -acetolactate into acetoin and diacetyl. The third bioreactor contained yeast in a stationary state for VDK reduction.

Kirin investigated primary fermentation in bioreactors containing 0.5 m³ and 10 m³ of carrier. In the larger bioreactors an additional cooling device was necessary to maintain an even radial temperature distribution within the carrier. Other problems encountered in scaling up were decreased fermentation capacity per volume with increasing bioreactor size, and channelling (Yamauchi *et al.* 1994b). The reduced fermentation efficiency was attributed to higher flow rates with increasing reactor size (Inoue 1995). Continuous operation of the system at 3.6 million litres per year for more than 6 months was possible (Yamauchi and Kashihara 1995).

The product was sensorily acceptable lager beer, which however differed in some characteristics from conventional batch beer. It had a higher alcohol content and less fusel alcohols than the batch beer. It contained the same total amount of organic acids as the batch beer, but the pattern was different: it had less acetic acid and more succinic acid. The beer also contained more sulphite and it had higher bitterness (Yamauchi *et al.* 1995a). Restricted yeast growth was claimed to be the main reason for some of the differences. A series of papers described the formation and control of different aroma compounds in this process (Yamauchi *et al.* 1995a, Yamauchi *et al.* 1995b, and Yamauchi *et al.* 1995c).

The Kirin Brewing Company set up a small commercial scale production unit using the described process on the island Saipan, producing 185 000 litres per year. The brewing proved to be short lived. Reasons for the economic failure

were low demand and the limited number of products (Inoue 1995). The output could not be run at one fifth of the designed output without deterioration of yeast fermentative activity (Inoue 1995). Other operational disadvantages included slow start up of the system (2 weeks), high energy costs because of the heat treatment before the third bioreactor and beer losses with the centrifuged yeast (Inoue 1995).

3.2.6 Process development at Labatt Breweries of Canada

A research group at Labatt Breweries of Canada used κ -carrageenan beads containing yeast in a draft tube fluidised bed reactor (Norton *et al.* 1994, Mensour *et al.* 1995, Pilkington *et al.* 1999). The use of small bead size (0.2 to 1.4 mm) together with a fluidised bed design was claimed to solve problems such as insufficient amino acid consumption leading to an unbalanced flavour profile. Most of the improvements in beer quality were attributed to a better mass transfer. The yeast growth was controlled by air and carbon dioxide feeds into the bioreactor (Mensour *et al.* 1995). One advantage of κ -carrageenan as the carrier material is its density, which is close to that of water, thus minimising the energy for fluidisation.

The bioreactor (Figure 8) had a volume of 50 litres. The air proportion varied between 2 and 5 % in the feed of gas mixture, the rest was CO₂. The residence time was 20 hours. Although a perfect match to the traditionally produced control was not achieved, the immobilized cell product was judged by a taste panel to be acceptable and similar to the conventionally produced beer (Mensour *et al.* 1995, Mensour *et al.* 1996).

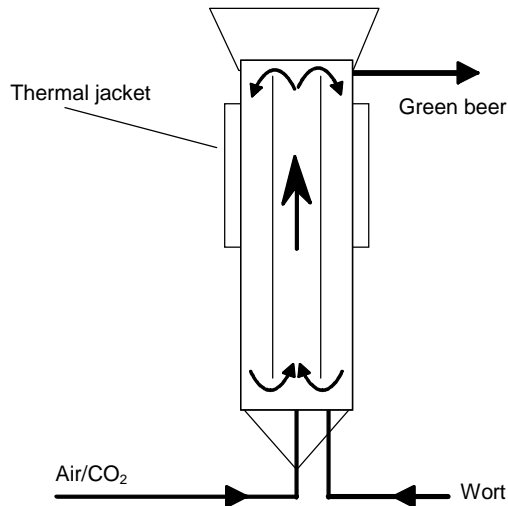


Figure 8. The Labatt reactor (redrawn from Mensour *et al.* 1995).

3.2.7 Process development at Meura Delta, Belgium

The unsuitability of Ca-alginate beads in a packed bed reactor (Masschelein *et al.* 1985) was solved by the company Meura Delta by using a different type of carrier and a loop reactor. They developed a tubular matrix of sintered silicon carbide. The matrix was 900 mm long, 25 mm in diameter and had 19 channels, each 2.5 mm in diameter. The pore size of the matrix varied from 30 μm near the outer surfaces to 150 μm in the core of the material (van de Winkel *et al.* 1993). A number of these matrices can be installed into a loop bioreactor. These reactors have been used for producing alcohol-free beer (Van de Winkel *et al.* 1995), secondary fermentation and for primary fermentation (Andries *et al.* 1995).

For the main fermentation of lager beer two similar bioreactors were used in series (Figure 9). Wort was continuously fed into the first bioreactor through silicone tubing that allowed control of aeration. A pump circulated the fermenting beer and facilitated the cooling in an external heat exchanger. The beer from the top of the first bioreactor was pumped to the top of the second. From the second reactor the green beer was pumped into a beer vessel to await the final treatment. The first bioreactor was operated at an apparent attenuation of 40% and the final attenuation was reached in the second bioreactor. The

residence time was 22 h per bioreactor. The aeration was arranged by diffusion through plastic tubing in the circulation loop. The productivity reported for one matrix at 15°C was 6.6 hl beer per year at 12°Plato and 9.1 hl per year at 16°Plato. The productivity per matrix means that for an annual output of 100 million litres over 100 000 matrices would be needed.

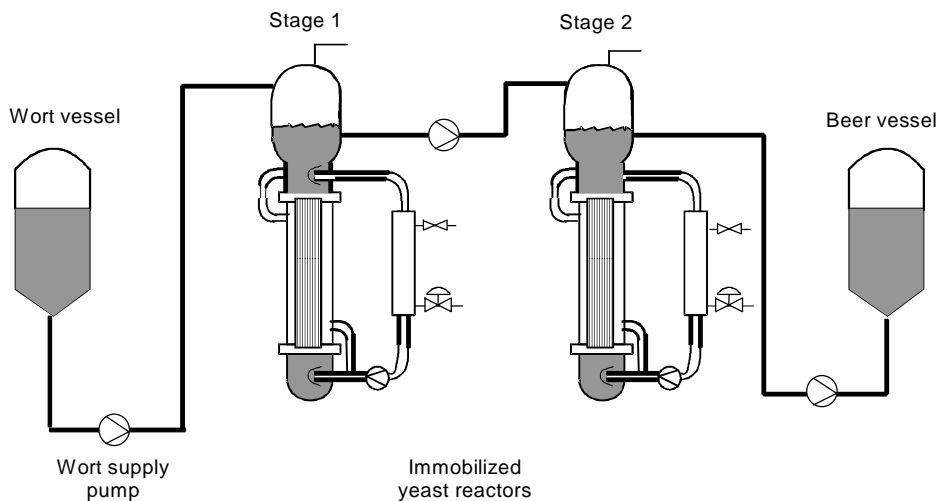


Figure 9. The Meura Delta system for lager beer production (redrawn from Andries *et al.* 1995).

The beer quality was said to resemble that of conventional batch fermented beer, although the amount of higher alcohols was somewhat lower and the amounts of esters were higher. The latter phenomenon can be controlled by aeration. The utilisation of amino acids was similar to that in conventional batch fermentation.

The system was adjusted for production of top fermenting beer in semi-industrial scale (Andries *et al.* 1997a). For this application the second immobilized yeast loop bioreactor was replaced by a cylindro-conical tank with free cells. This tank was equipped with an external circulation loop. Thus the system was a combination of immobilized and free cell stages. The immobilized bioreactor supplied the second stage continuously with free, viable cells. The beer was similar to that produced traditionally. Improved productivity and decreased investment costs compared with the totally immobilized system were the claimed advantages. At least the Aibel brewery is using the Meura Delta system

with 500 matrices in the production of top fermented beer (Mensour *et al.* 1997, Anon. 1997). In Canada at least one pub brewery has been using the Meura Delta system (Rajotte 1998). A group in Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) found the use of (Wackerbauer *et al.* 1996b) the loop reactor of Meura Delta to be relatively easy, whereas Tata *et al.* (1999) reported somewhat contradictory results.

3.2.8 Process development at Sapporo Breweries Ltd., Japan

Researchers of Sapporo Breweries Ltd. compared different carrier materials for a fluidised-bed reactor and found that a non-hollow chitosan bead was the best material. For this material the optimum bead size was found to be 1.0 mm on the basis of yeast cell density (max $5.0 \cdot 10^9$ cell ml⁻¹ beads) and fluidisation velocity (Shindo *et al.* 1994). A similar reactor was also used for secondary fermentation (Shindo *et al.* 1993).

The fermentation was carried out at 11°C with a feed rate of 40 ml h⁻¹ using 11°P wort. The fermentation system could be run for 900 hours without any damage to beads or decline in the fermentation efficiency. The recycling rate was 300 times higher than the feed rate and was chosen to remove CO₂ bubbles from the fermenting beer. The wort was treated with glucoamylase to increase the amount glucose, which led to increased acetate ester concentration (Shindo *et al.* 1992). The beer produced in this way was similar in flavour profile to conventionally produced beer (Shindo *et al.* 1994).

The process was scaled up to 80 litres in a repeated batch mode using non-porous (non-hollow) chitosan beads (Unemoto *et al.* 1998). 50 days of operation was reached, during which 18 batches were produced. The beer produced in this way had an acceptable and well-balanced flavour profile similar to that of the traditionally fermented product (Maeba *et al.* 2000).

3.2.9 Semi-industrial main fermentation at Hartwall Plc, Finland

Hartwall's semi-industrial continuous main fermentation unit is largely based on the system of (II) and has a capacity of 200 000 litres per year (600 litres per

day) (Kronl f and Virkaj rvi 1999). Later the unit was extended to include a continuous secondary fermentation unit with the same capacity (Kronl f *et al.* 2000). The yeast is removed from the green beer before the heat treatment section of the secondary fermentation by cross-flow filtration. Flavour formation was typical for lager beer (Table 4). The authors stated that it was fully possible to produce good beer in less than two days with this system in normal fermentation temperatures.

Table 4. Flavour compounds in beer produced by continuous, immobilized fermentation in both the main and secondary fermentation (adapted from Kronl f et al. 2000).

Beer analyses	Continuously produced	Conventionally produced
Ethyl acetate (mg l ⁻¹)	23.2	17.2
3-methyl butyl acetate (mg l ⁻¹)	1.0	1.3
Total amyl alcohols (mg l ⁻¹)	41.0	41.9
Total diacetyl (mg l ⁻¹)	0.02	0.01

3.2.10 Some other relevant experiments in immobilized main fermentation

An international research group has described a fixed bed reactor system with a circulation loop for main fermentation (Andersen *et al.* 1999, Pajunen *et al.* 2000a). The larger pilot unit has a bed volume of 1000 litres and an output of 50 l h⁻¹ (Figure 10). The residence time of liquid was between 20 and 30 hours (Pajunen *et al.* 2000b). The circulation loop is reported to handle CO₂ removal and heat control efficiently.

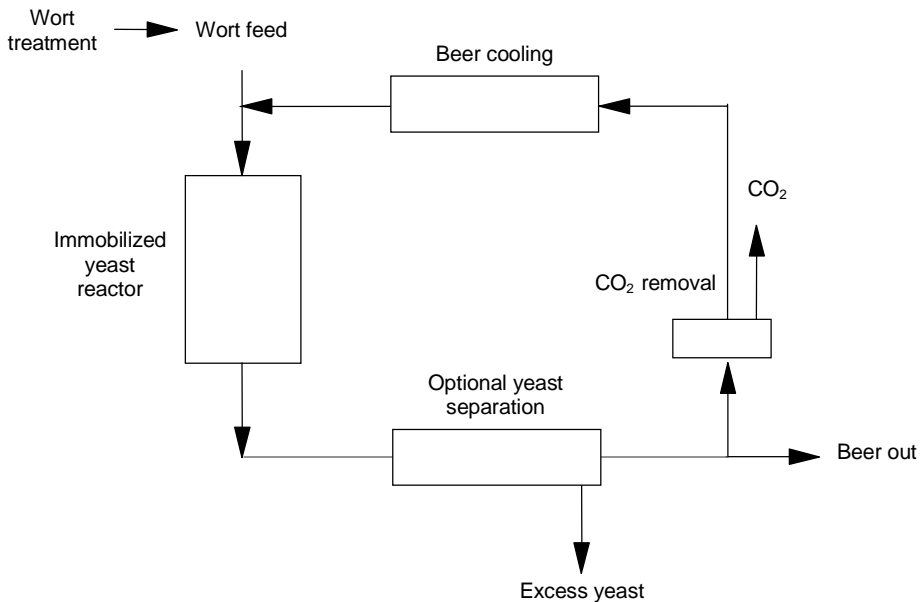


Figure 10. The fixed bed with a circulation loop, described by of Pajunen *et al.* (2000a) (adapted).

The system was able to produce stout with close enough quality match over a period of 8 weeks.

Pectate-immobilized yeast in a gas lift reactor was used by Smogrovicova *et al.* (1997). The VDK in this beer was at the same level as in traditionally produced beer. In a subsequent study (Smogrovicova and Domey 1999) the beer produced using Ca-pectate immobilized yeast was found by taste testers to be comparable with traditionally produced beer.

Two different systems for immobilized main fermentation was evaluated (Tata *et al.* 1999). A fluidised bed reactor with yeast immobilized on a porous glass bead carrier produced beer with quite acceptable flavour, albeit different from the conventionally produced beer. The sintered silicon carbide loop reactor system did not produce a completely fermented product with the same residence time as the fluidised bed reactor system. Tata *et al.* (1999) ended their paper by stating that immobilized yeast systems have advantages such as "stable and

consistent operation over a long period of time, with minimal operator intervention".

Researchers at VTT, Finland, were already experimenting with continuous fermentation in the 1960s (Mäkinen 1966). In 1984 immobilized fermentation experiments with secondary fermentation started (Kronlöf 1994). These investigations led to industrial applications at Sinebrychoff's Helsinki brewery in 1990 and later at Sinebrychoff's Kerava brewery (Pajunen and Grönqvist 1994) and at Hartwall Plc's Lahti brewery (Hyttinen *et al.* 1995). These applications differed slightly in carrier material and process design.

The research at VTT continued to the main fermentation. The DEAE-cellulose carrier that had been successful for secondary fermentation was also tested for main fermentation (Kronlöf *et al.* 1989). Because of technical difficulties, such as clogging of the reactors, DEAE-cellulose was later replaced by porous glass beads (Linko and Kronlöf 1991). With porous glass the flavour formation was also satisfactory and stable. The bioreactors used were either 1.6 litre tubular or 5 litre conical vessel (Kronlöf *et al.* 1989).

3.2.11 Flavour and economy

Ester formation could be controlled by the supply of oxygen. A feed mixture of air and nitrogen or carbon dioxide (2.5–5% air) was fed to a prereactor at a rate of 0.1 gas volume per carrier volume per minute. This was found to lead to a good balance of flavour compounds, to good yeast viability and to sufficient uptake of free amino nitrogen (Kronlöf and Linko 1992).

The flavour of beer is an important factor in evaluating the feasibility of immobilized primary fermentation. Some reported esters and higher alcohols in beers fermented by immobilized yeast and beers fermented conventionally are compared in Figure 11. The differences in esters and higher alcohols between beers from immobilized yeast fermentations and conventional batch fermentations are in w/w-%. The concentrations do not match exactly. However, and more importantly, there is no tendency with immobilized yeast fermented beers to be either lower or higher. Figure 11 is so arranged that the mixing power decreases from left to right (from fluidised bed reactors to packed bed

reactors). Interestingly, the relative amount of esters appears to be higher on right side of the figure. Higher alcohol were unaffected by increased superficial velocity in batch fermentation by calcium alginate immobilized yeast (Cop *et al.* 1989). The consumption of amino acids was increased, however.

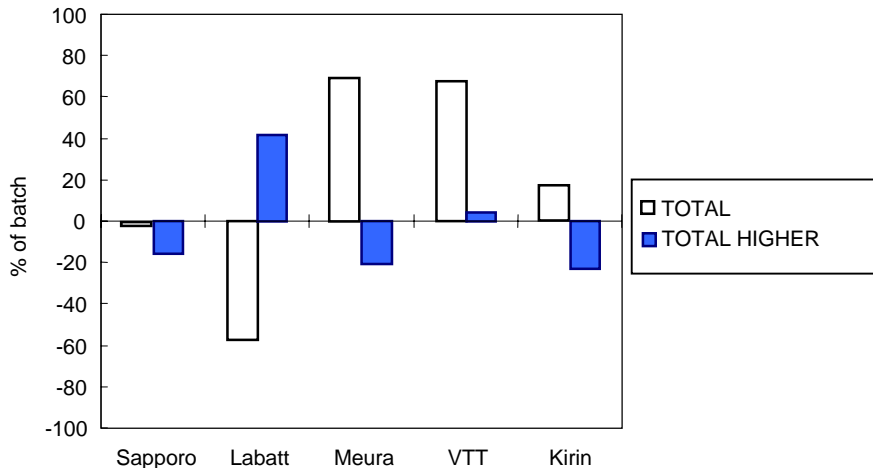


Figure 11. The differences (%) in total esters and total higher alcohol concentrations between immobilized yeast fermented and conventionally fermented beers in w/w per cent (data from Shindo *et al.* 1994, Mensour *et al.* 1997, Andries *et al.* 1995, Linko and Kronlöf 1991, Yamauchi and Kashihara 1995).

The economic analysis of immobilized yeast fermentations is more difficult and only a few published cases are available. Savings in the order of 1 DEM hl⁻¹ using immobilized secondary fermentation were estimated by Pajunen *et al.* (1991). These savings included savings on capital (only 10 to 20% capital investment of the conventional process), decreased beer losses and less interest on beer in lagering. A similar cost analysis for secondary fermentation, with savings of 1.6 DEM hl⁻¹ in a 150 million litres per year brewery is given by Borremans (1997). The total investment cost is given to be 43% of the conventional cost and the running costs were only 1/3 of those of the conventional process. Forschungsintitut für Technologie der Brauerei und Mälzerei der VLB Berlin (Wackerbauer *et al.* 1996a) did not find brewing with immobilized yeast to be economically feasible at the time of writing.

In October 1995 the European Brewery Convention (EBC) arranged a symposium "Immobilized Yeast Applications in the Brewing Industry" (Anon. 1995) in Espoo, Finland, where it was stated that "...primary and secondary fermentation using immobilized yeast leading to beer of sound flavour and other characteristics is a realistic technical possibility".

4. The aim of this study

The aim of the present study was to investigate the feasibility of continuous main fermentation of beer using immobilized yeast. The specific aims were

- 1) long term operation,
- 2) control of flavour formation,
- 3) microbial control and
- 4) economics.

This study forms part of a larger research project which full-scale continuous fermentation processes for brewing industry are developed. As a result of this project two Finnish breweries have installed industrial scale processes for rapid secondary fermentation of beer.

5. Materials and methods

Materials and analytical methods are presented in detail in papers II, III, IV and VI.

5.1 The state of the art in 1998 (I)

This paper reviewed the state of the art of immobilized processes in the brewing industry as published in the literature and by personal communications from investigators working in the field.

5.2 Microbial and flavour stability (II)

Long term stability was investigated in a long run (442 days) using a porous glass carrier (Siran) in a 20 litre scale pilot reactor system. The yeast strain used was VTT A-85072. After the first 137 days the system was used to model the formation of aroma compounds (III). These modelling experiments lasted for 182 days. After this period the original gas feeds were reintroduced and a new set of aroma compounds analyses was performed. The wort was obtained from a brewery and autoclaved prior to use. Microbial purity was analysed for the presence of wild yeasts and aerobic bacteria. The aroma compounds (ethyl acetate, 3-methyl butanol, 2-methyl butanol, propanol, ethyl caproate, 2-methyl propanol, acetaldehyde, 3-methyl butyl acetate) were analysed with a Perkin-Elmer Autosystem XL gas chromatograph, Perkin-Elmer head space auto-sampler (HS40) and FID detector. A 5 cm³ sample was incubated 90 min at 55°C in a 20 cm³ glass vial. n-Butanol was used as internal standard. The injector and detector temperatures were 225°C and 250°C, respectively. Helium was used as carrier gas at a flow rate of 1 cm³/min and nitrogen as make-up gas at 40 cm³/min. The column was PE-5, 1 µm film, 50 m. The temperature program used was: initially 40°C, then 12°C/min to 100°C with a 3.5 min hold at 100°C, thereafter 12°C/min to 150°C, hold for 5.0 min at 150°C.

5.3 Control of flavour (III and V)

During the long run used to analyse the long-term stability the effects of gas feeds on aroma compounds were studied. The possibility to control the formation of flavour compounds by changing the gas feeds into the prereactor was studied using an experimental design and response surface method. The gas feed rates were changed according to a central composite experimental design. Each different gas feed mixture was used for 14 days and then the aroma compounds were analysed by head-space gas chromatography. The aroma compounds were modelled into response surfaces using a commercial programme (Modde 3.0, Umetri Ab, Uppsala, Sweden).

Smaller scale reactors (0.8 litres of carrier material) were used with three different carriers and with four different yeast strains (VTT A-93110, VTT A-93102, VTT A-66024, VTT A-63015) to investigate the effects of carrier materials on flavour compounds (V). The carriers used were porous glass (trade name Siran, Schott Engineering GmbH, Mainz, Germany), DEAE-cellulose-based carrier (GDC, Cultor Ltd, Kantvik, Finland) and kieselguhr-based cylindrical carrier (Celite Corporation, Lompoc, CA, USA).

5.4 Effects of a contaminating bacterium (VI)

The effects of a contaminant (*Klebsiella terrigena*) were investigated in small scale (0.8 litres) reactors. The yeast strain was VTT A-85072. The carriers used were porous glass (from Schott Engineering GmbH, Mainz, Germany), beech wood chips (Rettenmayer & Söhne, Ellwangen-Holzmühle, Germany) and aspen chips (Tapvei, Kortteinen, Finland). The wood chips were pre-treated by boiling for 4 hours in water and then for 1h in 10 % (vol/vol) ethanol and finally for 1 hour in water. Two similar reactors were filled with the same carrier and one reactor was deliberately contaminated. The effects of the contaminant on yeast viability were analysed by methylene blue staining. DMS and biogenic amines were analysed by head-space gas chromatography and total nitroso compounds were analysed by liquid chromatography (Izquierdo-Pulido *et al.* 1993).

5.5 Economics (IV)

Economic analysis was performed by designing two new breweries on a greenfield with an annual production of 100 million litres (1 million hectolitres). The reference brewery used batch main fermentation and continuous immobilized secondary fermentation, whereas the continuous brewery used immobilized fermentations for both main and secondary fermentation. The beer had 4.5 % alcohol by volume and high gravity brewing was used with an original gravity of 15°P. Only the fermentation part of the brewery was taken into consideration, as much of the other equipment would be the same in the conventional brewery as in the continuous brewery. The total residence time in the continuous fermentation system was 72 hours.

Typical engineering calculations for investment and operation costs were performed in co-operation with the Laboratory of Plant Design at Helsinki University of Technology.

6. Results

6.1 Long term stability (II)

6.1.1 The apparent degree of attenuation

The apparent degree of attenuation remained high and very near the measured attenuation limit of the wort throughout the whole operation of 442 days (Figure 12). The changes after the prereactor can be explained partly by the movement of the glass beads in the prereactor. From time to time in the prereactor some of the glass beads rose and then fell back. This movement removed non-bound yeast from the prereactor, causing changes in the total amount of yeast in the prereactor. A similar kind of movement of the glass beads was seen in smaller scale (0.8 dm³) and larger scale (100 dm³) experiments. The changes in attenuation after the buffer tank can be partly explained by removal of excess yeast from the buffer tank. The removal was carried out ca every fortnight.

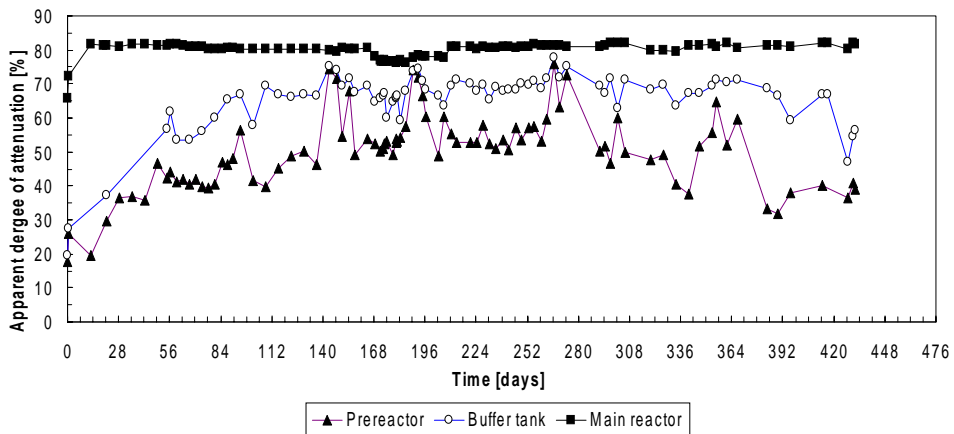


Figure 12. The apparent degree of attenuation in the outflows from the prereactor, the buffer tank and the main reactor (II).

6.1.2 Asepticity

No harmful microbial contamination and no wild yeast contamination were detected during the operation. The microbial counts for aerobic bacteria detected are shown in Figure 13. Although a low number of contaminants was detected in the samples taken from the system no colonisation occurred. This means that although main fermentation is more sensitive to contamination than secondary fermentation (Haikara *et al.* 1997) normal aseptic procedures are sufficient for main fermentation.

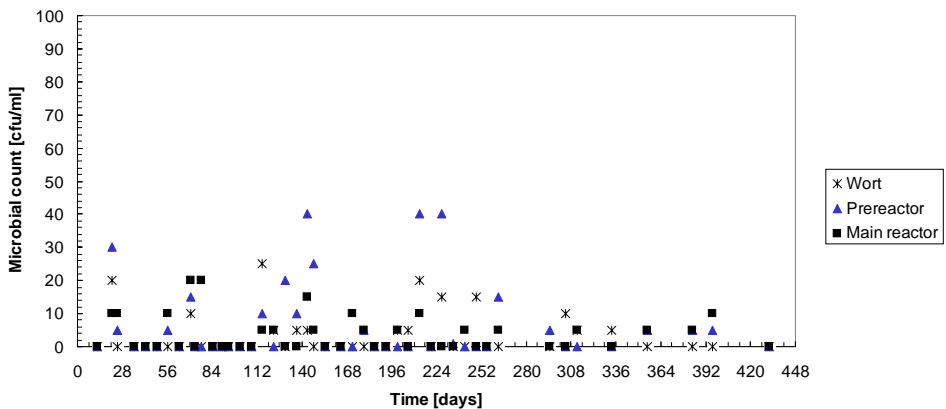


Figure 13. The aerobe counts detected in the immobilized yeast reactor system (II).

6.2 Flavour stability (II)

During the first phase of the run (137 days) the measured aroma compounds remained stable (Figure 14). This set of measurements is named S1. The only trends observed were decreases in propanol and acetaldehyde. 3-Methyl butyl acetate increased in the outflow of the prereactor, but was stable in the outflow from the main reactor (Figure 14).

After the first 137 days the system was used to model the formation of aroma compounds (II). These modelling experiments lasted for 182 days. After this period the original gas feeds were reintroduced and a new set of aroma compound analyses was performed.

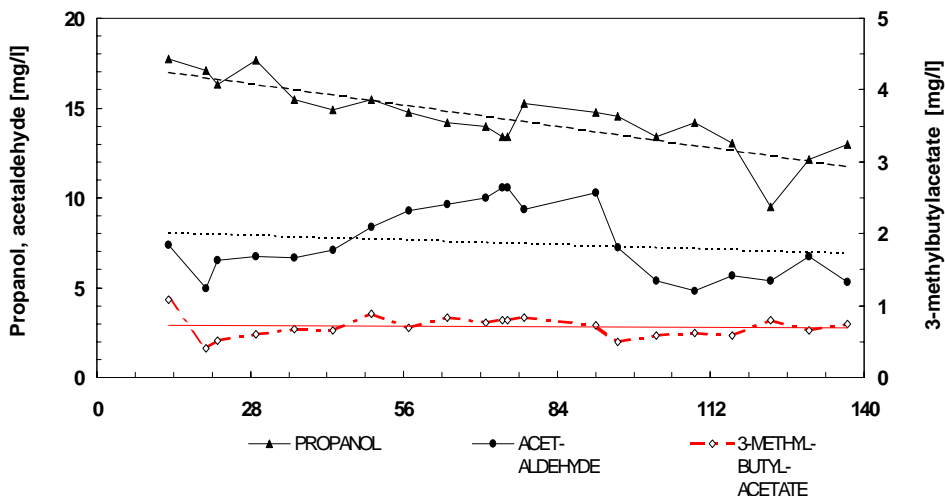


Figure 14. Propanol, acetaldehyde and 3-methyl butyl acetate concentrations during the first 137 days of operation in the outflow from the main reactor (linear trends included) (II).

The average values of this set (S2) are shown in Figure 15 combined with the data S1. It is clear that ethyl acetate and acetaldehyde values in the second set (S2) were lower. By contrast, concentrations of fusel alcohols were higher in the second set. These changes in higher alcohols are probably due to the increased yeast concentrations in the reactors and could be compensated by higher flow rates. Once, for a short time, the system was run at 750 ml/h (50% increase in capacity) and the concentrations of 3-methyl butanol, 2-methyl butanol, 2-methyl propanol, propanol and ethyl acetate were lower compared with those at 500 ml/h (Table 5).

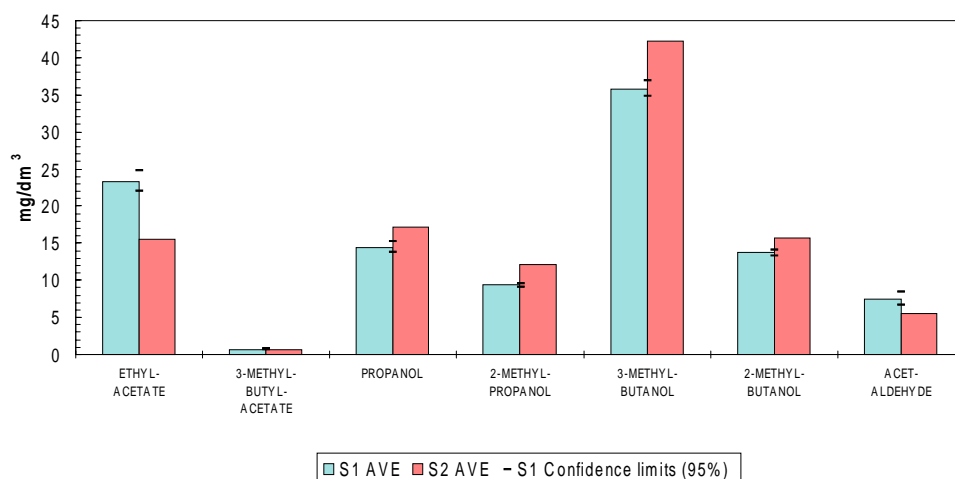


Figure 15. Comparison of the two sets of aroma compound analyses. S1 is the average value for the first 137 days and S2 is the average value for days 378 to 442 (II).

Table 5. Concentrations of the aroma compounds at two different flow rates [mg dm^{-3}] (II).

Flow rate	500 ml h ⁻¹	750 ml h ⁻¹
Ethyl acetate	22.3	15.0
3-Methyl butyl acetate	1.1	0.5
Propanol	16.6	15.6
2-Methyl propanol	12.5	10.7
3-Methyl butanol	45.3	38.7
3-Methyl butanol	18.2	14.6
Acetaldehyde	4.3	5.3

The aroma profile of beer from the immobilized fermentation was compared to a commercial Finnish lager produced with the same yeast and wort. This beer was matured, whereas beer from immobilized fermentation was not. A Finnish brewery can have fermentation vessels of hundreds of thousands of litres and the production of exactly the same beer in regard to aroma compounds in 20 litre scale reactors is challenging. High gravity brewing was used in the industry,

whereas the wort was diluted to 11°P and autoclaved for the continuous fermentation. For comparison, the results have been adjusted to the same original gravity and the commercial lager has been adjusted to 100% (Figure 16). The statistically calculated 95% confidence limits (n=16) for the commercial lager are included. The most serious deviation is in the concentration of 3-methyl butyl acetate. The beer produced by immobilized yeast had less than 50% of the 3-methyl butyl acetate of the conventionally produced beer. The differences in acetaldehyde or in particular in propanol were not so serious in regard to their taste thresholds.

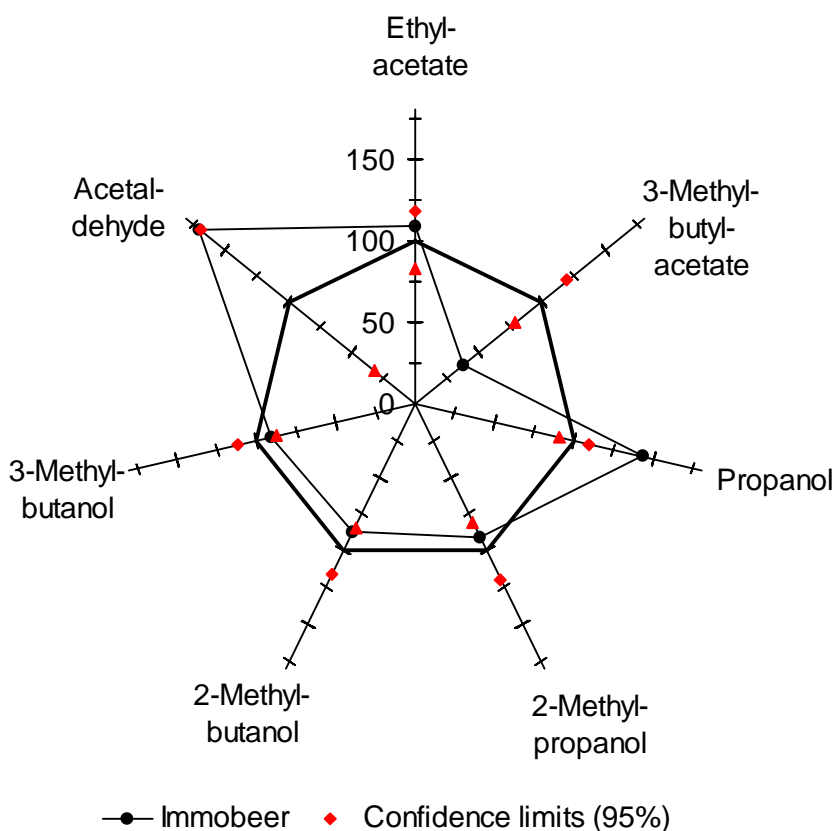


Figure 16. Comparison of aroma compounds with a commercial lager beer. Values in % of those of the matured, commercial beer. The 95% confidence limits for beer produced by immobilized yeast are indicated (II).

6.3 Control of flavour (III)

After performing the experimental design with different gas feed mixtures as described in III, the results were analysed using the response surface part of the Modde 3.0 programme (Umetri Ab, Uppsala, Sweden). For the modelling the concentrations of higher alcohols were summed and this sum was used. After removing one outlier (experiment number 14) a second order model (Figure 17) had $R^2 = 0.92$ and $Q^2=0.55$. R^2 is the goodness of fit (R^2 less than 0.8 indicates poor modelling) and Q^2 is goodness of prediction ($Q^2 > 0.5$ indicates good modelling).

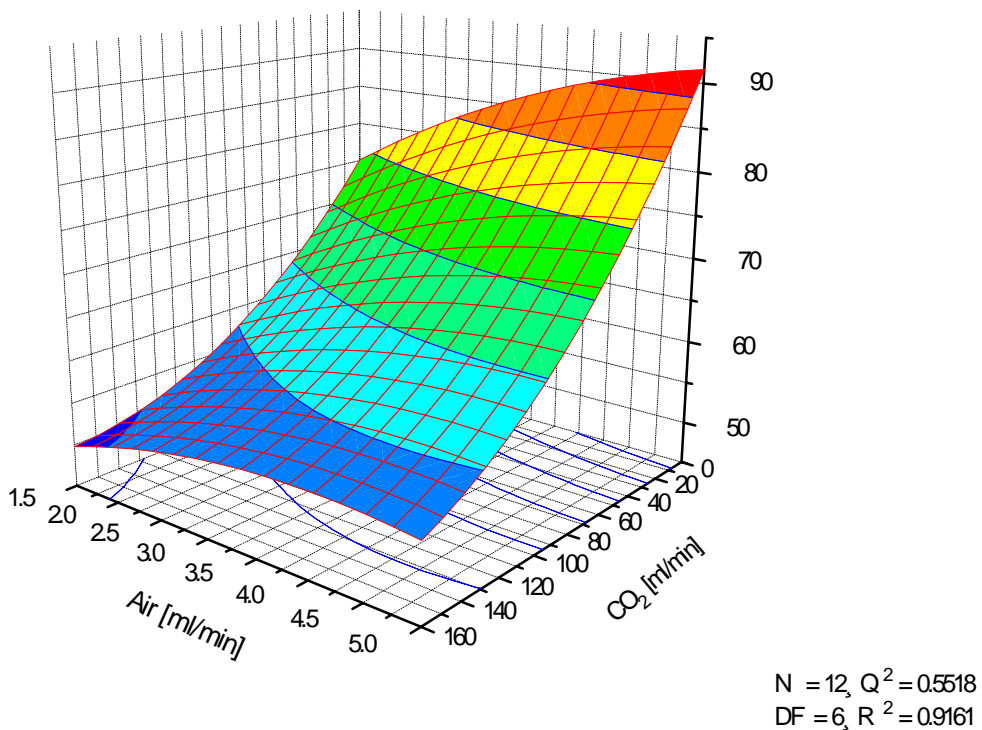


Figure 17. The second order response surface for higher alcohols (III).

The confidence intervals for the second order coefficients for the terms air*air, CO₂*CO₂ and air*CO₂ were so large that the simplification of the model to a linear one was justified (Figure 18). The goodness of fit was reduced from 0.92 to 0.90, but the goodness of prediction increased from 0.55 to 0.82.

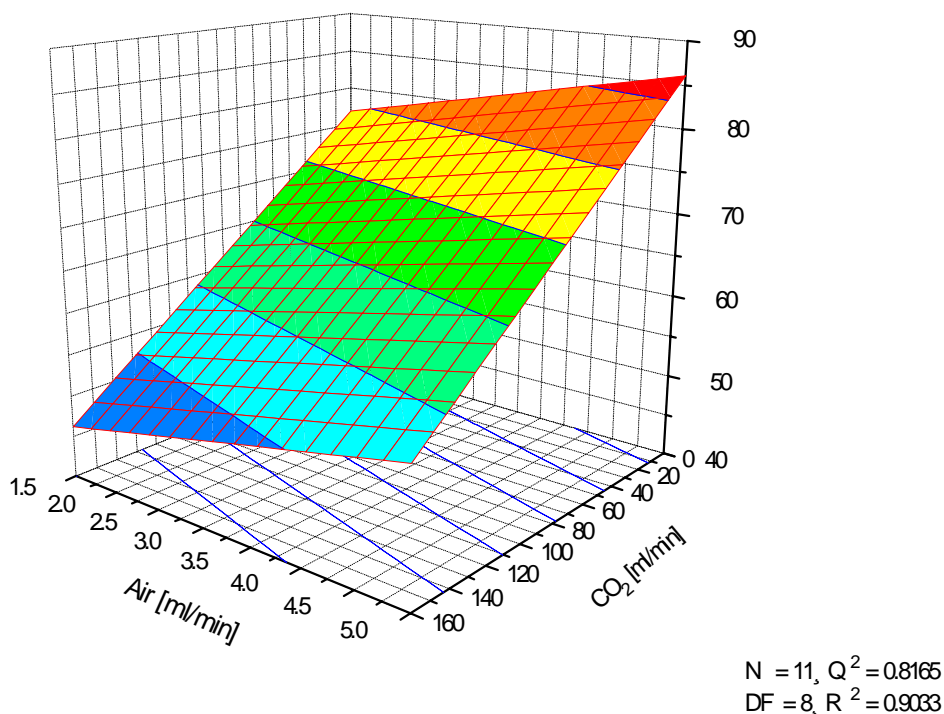


Figure 18. The linear response surface for higher alcohols leaving the prereactor (III).

Both models show that with a low CO₂ feed rate and high air feed rate the maximum amount of higher alcohols is formed in the prereactor. In a batch fermentation high aeration has a similar effect. Kahler *et al.* (1965) also noticed that higher aeration led to increased levels of higher alcohols and acetaldehyde in a continuous fermentation. It should be noticed that the high value for the sum of higher alcohols is almost double the low value in the studied range.

6.4 The effects of carrier material on the flavour of beer (V)

The fermentation performance was not affected by the choice of the carrier. With one yeast strain the apparent degree of attenuation remained low for the first 40 days with both Siran and GDC carriers. The carrier material had an effect on the flavour compounds, but the effect varied with yeast strains used. The statistical differences found in the concentrations of fusel alcohols are presented in Table 6 (V).

Table 6. The statistical differences found in the concentrations of fusel alcohols (V).

	Pair compared	Significance	Higher values
Propanol	Siran A110 vs. GDC A110	*	Siran
	Siran A24 vs. GDC A24	*	Siran
	Siran A15 vs. Celite A15	*	Siran
3-Methyl butanol	Siran A24 vs. GDC A24	*	GDC
	Siran A102 vs. GDC A102	*	GDC
2-Methyl butanol	Siran A110 vs. GDC A110	*	GDC

*** = $p < 0.01$ (very significant). ** = $0.01 < p < 0.05$ (significant). * = $0.05 < p$ (almost significant)
 A110 = VTT A-93110, A102 = VTT A-93102, A24 = VTT A-66024, A15 = VTT A-63015.

Some of the effects may be explained by the differences in the immobilization method, surface adsorption or adsorption onto a porous material, and by the direct effects of the carrier, for example differences in adsorption of wort components onto the carrier.

With strongly flocculent yeasts, higher amounts of propanol were produced with Siran as carrier than with GDC or Celite as carrier. Both a flocculent yeast strain and one non-flocculent strain produced higher amounts of 3-methyl butanol when using GDC than when using Siran. With a genetically modified, very

strongly flocculent strain 2-methyl butanol was also higher when using GDC than when using Siran. The beers produced using Siran carrier had higher concentrations of 3-methyl butyl acetate than beers produced with other carrier materials. Ethyl acetate was higher in the beer produced using Celite carrier than in the beer produced using Siran.

Both the yeast strain and the carrier affected concentrations of total diacetyl, but only the carrier had an effect on the concentrations of total pentanedione. With Siran both total diacetyl and total pentanedione concentration were lower than with GDC carrier. With Siran, lower total diacetyl concentrations were found compared with Celite. No difference was found in total pentanedione concentrations between Siran and Celite carriers. An interesting phenomenon is higher pH of the young beers produced using Siran with many strains, although the consumption of FAN was higher using Siran (see Figure 19).

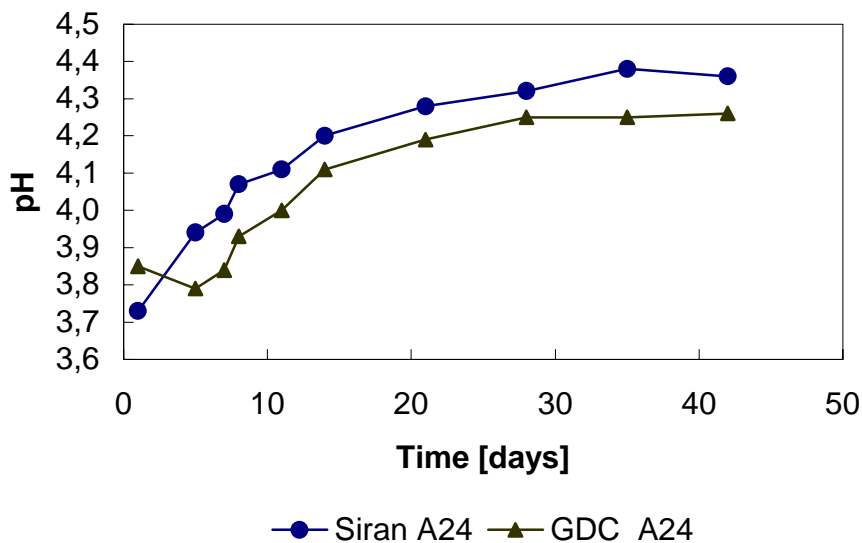


Figure 19. pH of beer from the outflow from the reactor. A24 = VTT A-66024 (V).

6.5 Contamination (VI)

With all carriers the deliberately introduced contaminant, *Klebsiella terrigena*, was able to survive and colonise the reactors. *K. terrigena* was detected in the outflowing green beer 6–11 days after the contamination. Within three weeks the level of *K. terrigena* stabilised, reaching 10^5 cfu/ml in the Siran reactor, 10^5 – 10^6 cfu/ml in the beech wood reactors and 10^4 – 10^5 cfu/ml in the aspen reactor (Figure 20).

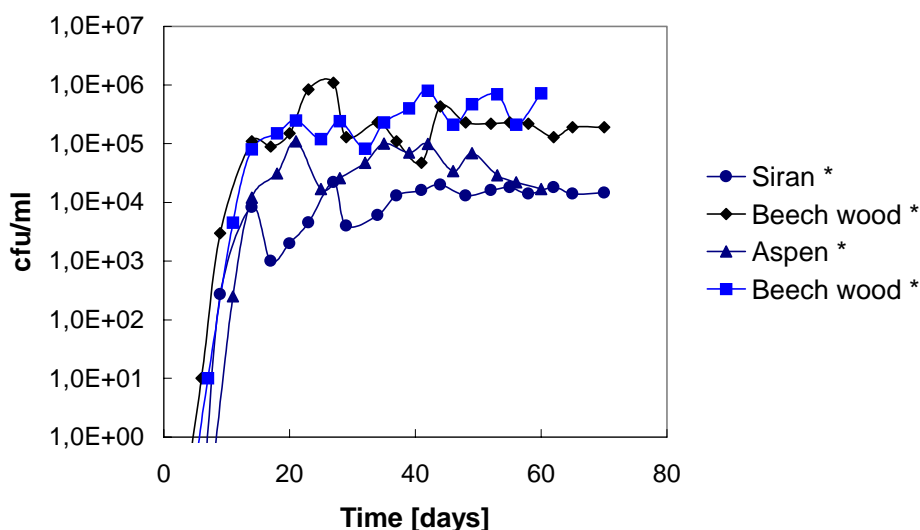


Figure 20. Amount of *K. terrigena* in the outflowing green beer from the contaminated reactors (VI).

The pH values of the green beers from the contaminated reactors were higher than those of the beers from non-contaminated reactors (VI). The differences were small but consistent with all the carrier materials used. The detection time for this difference was ca two weeks.

The experiments were run in small-scale reactors in which aeration of the wort was through the tubing only. This possibly contributed to the decrease in viability of the outflowing yeast, but the decrease appeared to be more pronounced in beers from contaminated reactors. At the end of the experiments, samples from the carrier were taken from the top part and from the bottom part

of the reactor and these were analysed for the contaminant and for yeast viability. In 7 of the 8 points analysed, the viability of the yeast was lower in the contaminated reactor.

K. terrigena increased the concentration of dimethyl sulphide (DMS) in the green beers. Three weeks after the contamination the taste threshold (50 µg/l) was exceeded. The carrier material appeared to affect the maximum concentration of DMS in the outflowing beer.

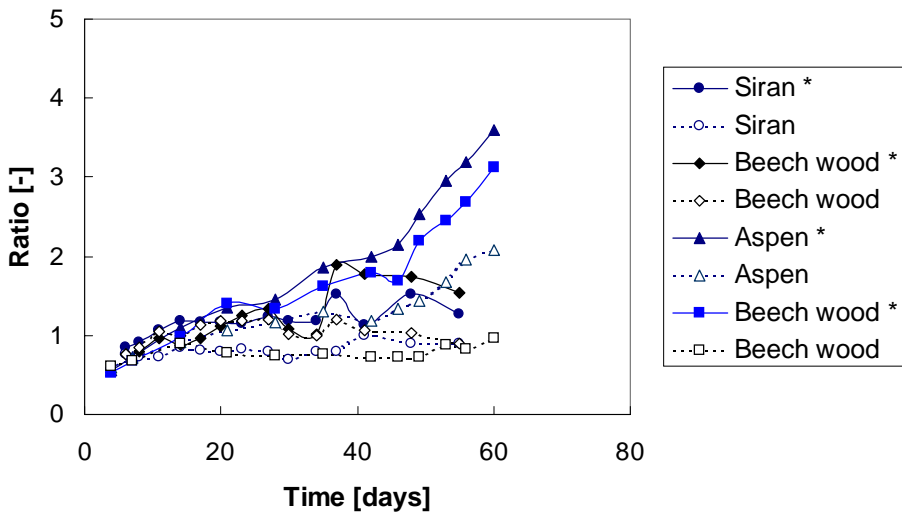


Figure 21. The ratio of total diacetyl to total pentanedione in the green beer. * indicates a contaminated reactor; no star indicates a control reactor (VI).

The fastest detection method for the contaminant was plate counting, because the changes in the chemical composition of the beer were only detected later. The slight elevation in the pH of the green beer or the change in the ratio between total diacetyl and total pentanedione (Figure 21) may be used as alarms, but both require a good knowledge of the process. This contaminant did not produce nitrosoamines and the concentration of biogenic amines was also low. The concentration of cadaverine was increased in the beer outflowing from the contaminated bioreactors.

6.6 Economics (IV)

The process for continuous, immobilized main fermentation is presented in Figure 22. The annual production was 100 million litres. The wort was produced batchwise and stored in tanks. The main fermentation was divided into two reactors, of which the first was aerated with a mixture of air and carbon dioxide.

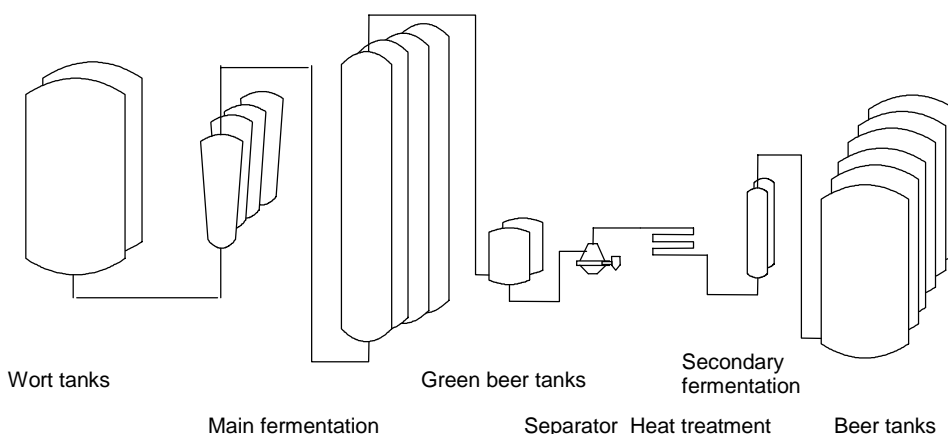


Figure 22. The immobilized continuous main fermentation process.

After the main fermentation, yeast was separated with a centrifuge and the young beer was heat treated and pumped to secondary fermentation reactors. The flow rate was $11.4 \text{ m}^3 \text{ h}^{-1}$. In the compared batch process the main fermentation takes 9 days and the secondary fermentation was an identical immobilized fermentation process.

The total investment cost for the immobilized process was 102% of the batch process with a carrier cost of 4000 Euro m^{-3} (7 549 000 and 7 386 000 Euro, respectively). If the carrier cost (2 412 000 Euro) was excluded the investment cost for the immobilized process was 70% of that of the batch process. Thus the carrier cost was significant for the economic feasibility. The price used in the calculation was a quotation from a manufacturer. The floor requirement of the immobilized process was estimated to be 660 m^2 compared to 770 m^2 for the batch process. This implies about 15% savings in the building cost. The personnel cost was estimated to be the same for both processes, with 7.5 workers.

Operating costs for the immobilized process are 66 Euro per m³ of beer (6 385 000 Euro per year) and for the batch process 63 Euro per m³ of beer. The main differences were the renewals of the carrier and the higher consumption of electricity in the immobilized process.

The cost of the carrier material forms a very significant part of the investment (Figure 23). The price used for the material was a quotation from a manufacturer. The price of a cheap carrier may be below 0.3 Euro l⁻¹.

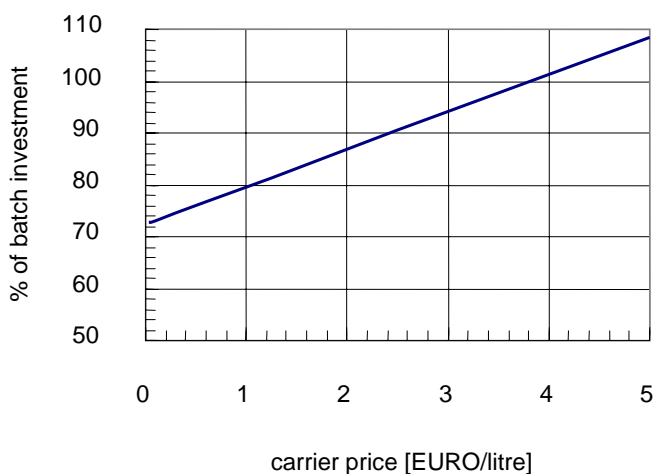


Figure 23. The effect of carrier price on the total investment cost as a percentage of the investment cost of a batch process (IV).

The electricity consumption of pumps was calculated from the rated power multiplied by the running time per year. This will be an overestimate, because at normal workload electric motors use less than the rated amount of electricity. CIP costs of water and chemicals lead to savings for the continuous process. Extra income was estimated to originate from lower losses of beer: there are less tank bottoms. Each transfer from a tank is estimated to cause a 1% loss of beer and each change of carrier results in a 1.5% loss of the reactor volume of beer. It was estimated that in a batch process 2% of the fermentable extract is used for yeast growth, compared to only 1.5% in an immobilized process.

When these extras are taken into account the total manufacturing cost for processes are: batch 63 Euro m⁻³ and immobilized 64 Euro m⁻³ when the carrier price is 4 Euro l⁻¹ (Table 7). If the carrier price is 0.3 Euro l⁻¹ the manufacturing costs for the immobilized process are 1% less than those of the batch process. With the annual world production of ca 1.3·10⁸ m³ (1 300 million hectolitres) this indicates savings in the order of 40 million Euros per year in the manufacturing costs.

Table 7. Manufacturing costs when savings are included (carrier price 4 Euro/litre) (IV).

Extra income	Immobilized			Batch	
	1000 Euro/a	Euro/m ³	% ¹⁾	Euro/m ³	%
decreased losses in transfers	155	1.6			
less growth of yeast	67	0.7			
Manufacturing cost		63.8	101	63.3	100
Carrier price 0.3 Euro/litre		63.0	99		

¹⁾% of the cost of the batch process

The feasibility of two cheap carriers was investigated (Linko *et al.* 1997) and found that either aspen or beech wood chips can be used as a carrier material. The use of these cheap carriers material will favour the economics of the immobilized process. The use of wood chips as a carrier in secondary fermentation is patented (Oy Panimolaboratorio – Bryggerielaboratorium Ab 1998).

7. Discussion

Continuous, non-immobilized fermentation failed to fulfil the requirements specified by Portno (1978):

- The system must maintain a high concentration of yeast to allow rapid fermentation.
- The system must be capable of using a wide range of yeasts.
- The system must be capable of producing a range of beers.
- The system must maintain a fermentation gradient
- The system must allow controlled growth of yeast at levels up to that of a batch system.
- The system must have a facility for control of growth rate to maintain a young and vigorous culture.
- The system must provide oxygen in controlled amounts in zones of high substrate and low product concentration.

In the present study the feasibility of continuous, immobilized fermentation was investigated. The process was demonstrated to be capable of fermenting lager beer in two days and of producing good lager beer for a long period of time. The formation of aroma compounds was stable for long operative periods. Changing the gas feed provided a means to control the formation of aroma compounds. Contamination did not pose a serious hazard to the system. Long fermentation periods were possible without contamination. The detection of a contamination was possible before any serious off-flavours or chemical changes occurred in the green beer.

7.1 Long term stability

The long operation period of 14 months (II) with stable attenuation demonstrated that the immobilized process is capable of functioning for operative times. An efficiency loss after 3 months of operation in large-scale pilot reactor, 10 000 litres of porous glass beads, was reported (Yamauchi *et al.* 1994a). Kirin's system could not maintain its operational stability for more than 6 months (Yamauchi and Kashihara 1995). 6 months of stable operation for lager and ale production with a Meura Delta system consisting of an immobilized and a free

cell stage was reported (Andries *et al.* 1997b). The long operation time is essential for economical reasons, although it is not sufficient in itself, as the reported long operation times of the A.P.V. Tower demonstrated (Shore 1986).

The formation of flavour compounds was stable for long operative times (II). The first period lasted 137 days and the second period 123 days. The experimental phase between these constant gas feed periods did not cause significant permanent changes in the formation of aroma compounds. In the literature, aroma compounds from such long runs have seldom been reported. 3-methyl butyl acetate decreased slowly during six weeks of operation in a 100 litre scale PBR system (Kronlöf *et al.* 1995). The mixing caused by gas feed may increase the stability of the system used in this study. Oxygen transfer from the gas phase into the liquid phase and finally to yeast may occur in a large part of the prereactor.

7.2 Control of flavour

The use of controlled gas feed to control the formation of aroma compounds was demonstrated in (III).

Yamauchi *et al.* (1995a) used air sparging at $0.017 \text{ dm}^3 \text{ dm}^{-3} \text{ min}^{-1}$ (vvm) into the first reactor (CSTR). By changing the ratio of the extract consumption in CSTR and PBR from 1:1 to 1:2 they obtained beer with a richer flavour. Acetate esters were produced in greater amounts in PBR than in CSTR. Mensour *et al.* (1995) used air proportions between 2 and 5 % in the gas feed to achieve an acceptable flavour profile. Andries *et al.* (1995) produced beer with higher ester concentration in a loop reactor than in batch fermentation. The aeration was 9.7 μg oxygen per minute in to the first reactor, which corresponds approximately to 2.5% vol air. They claimed that the ester concentration could be controlled by adjusting aeration. Shindo *et al.* (1992) used another approach to increase the low ester content of beer produced by immobilized yeast in batch process. They increased the glucose concentration of wort by treating it with glucoamylase. The 3-methyl butyl acetate concentration was more than doubled when the glucose concentration of the wort increased from 0.9% to 8.9% (Shindo *et al.* 1992). The results were similar when the treatment was applied in a continuous fluidised bed reactor (Shindo *et al.* 1994).

The oxygen requirement of yeast is a strain dependent factor. The oxygen transfer rate from the gas phase into the liquid phase is complex phenomenon, to which the reactor design, PBR, CSTR or air lift, influences significantly. Also the rate and the oxygen concentration effects on it.

7.3 The effect of carrier material on flavour

The combination of yeast strain and carrier material affected the flavour of beer (V). Very few papers report direct effects of carrier material on the flavour of beer. Linko and Kronlöf (1991) found that with alginate beads the amounts of fusel alcohols were low, but that with GDC the amounts of fusel alcohols were the same as in the batch process. Many different carriers were compared and tested for impact on beer flavour by soaking the carriers in beer Ruthaut *et al.* (1993). This way some components from carriers were extracted into beers. A process using immobilized yeast was not able to produce a similar aroma compound profile to a batch process (see Figure 11). Interestingly, the differences were not unidirectional. Andries *et al.* (1995) produced less higher alcohols in continuous immobilized fermentation than in batch fermentation, whereas Mensour *et al.* (1997) found more higher alcohols in beer produced by immobilized yeast.

Higher concentrations of 2-methyl butanol were found (V) with the GDC carrier than with Siran carrier, but only when using the strongly flocculent strain. This was in agreement with the results of Linko and Kronlöf (1991) with the GDC carrier: 3-methyl butanol and 2-methyl butanol concentrations were higher than with porous glass carrier (Kirin Bioceramics). They also found differences in 2-methyl propanol and propanol, in contrast to our results (V). Concentrations of propanol and 3-methyl butyl acetate were lower and ethyl acetate was higher with Celite compared to Siran. More esters in continuous immobilized fermentation than in batch fermentation are reported by Andries *et al.* (1995). In this work (V), differences were found in the concentration of 3-methyl butyl acetate: the use of Siran resulted in higher concentrations of 3-methyl butyl acetate than when using GDC.

The results indicate that the choice of carrier material cannot be based exclusively on literature data, but that the combination of the yeast strain and the

carrier will affect the flavour of beer produced. The origin of found differences might well lie in the changes of the yeast cell wall when cell reacts with the surface. These changes can and probably are strain specific. The final optimisation for a particular flavour may include changes in wort quality (e.g., FAN), aeration, temperature etc.

7.4 Contamination

The primary fermentation systems are much more sensitive to contamination than the less nutritious secondary fermentation systems (Kronlöf and Haikara 1991). Moreover, the contaminating organisms of primary fermentation and secondary fermentation reactors differ distinctively (Haikara *et al.* 1997, Kronlöf *et al.* 2000). Gram negative bacteria, such as *Obesumbacterium proteus*, other enterobacteria or acetic acid bacteria are the most common contaminants in immobilized yeast reactors used for primary fermentation. Besides bacteria, wild yeasts may contaminate both primary and secondary fermentation systems (Kronlöf and Haikara 1991, Haikara and Kronlöf 1995, Haikara *et al.* 1997, Kronlöf *et al.* 2000).

K. terrigena was able to colonise the reactors and survive in the process conditions. The use of beech wood chips resulted in the highest concentration of *K. terrigena* in the outflowing beer. Aspen chips and Siran supported lower concentrations. When comparing the figures for pH, DMS and *K. terrigena* in the outflowing green beers it was observed that the fastest detection method for *K. terrigena* was plate counting. Increased concentrations of DMS were detected in the green beer only on day 20, whereas *K. terrigena* was detected by plate counting after approximately one week. The change in pH of the green beer was seen after approximately two weeks. Another possibility is to follow the concentrations of vicinal diketones and especially the ratio between total pentanedione and total diacetyl. If this ratio starts to increase over a pre-set value or shows a stable increasing trend, this could be taken as an indicator of contamination. The reason for the increase in the ratio is unclear, but it could be a consequence of the reduced viability of the yeast.

Biogenic amines were not produced during the experiments in amounts that would be harmful to health. *K. terrigena* caused elevated concentrations of

cadaverine, but no changes in the concentrations of other measured biogenic amines were observed. Nitrosoamines were not detected at the end of the experiments.

7.5 Economics

The main economic advantages of continuous, immobilized fermentation are based on the possibilities to use very short fermentation times and to diminish the non-productive time. Using a very cheap carrier material, the investment cost was estimated to be ca. 70 % of that of a batch fermentation at an annual output of 100 million litres (IV). The smaller floor area requirement of the immobilized process implied 15% savings in the building cost. The running costs of the immobilized process were estimated to be 5% higher than those of the batch process. The Kirin Brewing Company Ltd (Table 8) has estimated rather similar costs (Inoue 1995) for the investment using their own carrier (Bioceramics, porous glass beads). Contrary to results in (IV), Inoue (1995) calculated the running costs to be 1.8 fold and the yield of beer to be lower. This may be a reflection of their experiences from the small scale Boca Boca brewery on Saipan.

Table 8. Estimated profit gained by immobilized yeast systems in a 100 million litre brewery (adapted from Inoue 1995). Both main fermentation and secondary fermentation were continuous, immobilized processes.

	Batch [%]	Immobilized [%]
Investment	100	58
Running cost	100	176
Space required	100	44
Yield of beer		lower

7.6 Compendium

Using the immobilization technique a high concentration of yeast can be maintained in the reactor. A change in the flow rate will not affect the amount of yeast in the system, so the output can be varied. The high concentration of yeast facilitates short fermentation times without an increase in temperature. With the systems presented in this study the main fermentation lasted about 50 hours (II, III and IV). In the early attempts at continuous fermentation very short fermentation times were reported (down to 4 hours), but in many of these experiments the fermentation temperature was elevated. Yamauchi *et al.* (1995a) reported a 1 day fermentation time for their system of CSTR and PBR. Meura Delta system with an inverted structure – a loop reactor followed by a CSTR – was also able to ferment in 1 day (Andries *et al.* 1997a). Residence times between 20 and 24 hours gave full attenuation in a fluidised bed reactor (Mensour *et al.* 1996). The slow start-up encountered in the A.P.V. Tower fermenter is abolished by using immobilization technology.

With immobilization technique the yeast strain is not limited to those with suitable flocculation characteristics, but any production strain can be used. In the papers II, III, V and VI five different strains were used. None of them posed any difficulties, although their flocculation characteristics were different. The use of a normal production strain in an immobilized fermentation process is reported by many authors, e.g., van de Winkel *et al.* (1993), Smogrovicova *et al.* (1997) whereas others simply describe the strain as brewer's yeast, e.g., Yamauchi *et al.* (1995a), Maeba *et al.* (2000).

Maintaining a fermentation gradient is possible either by the use of multiple vessels or by using packed bed type reactors. By controlling the amount of oxygen entering the system the yeast growth together with formation of aroma compounds can be controlled. When introducing air into wort just before the reactor, oxygen is introduced into high substrate and low product concentration.

Although, all the requirements of Portno (1978) can be fulfilled by using immobilization technique, there exist hindrances for the application of immobilized main fermentation. The total production of beer in the world is increasing slowly, but the increase is limited to very few countries: China, Russia, Vietnam, India. Elsewhere the production capacity is in excess (Western

Europe, USA). The amount of money tied up in the existing fermentation vessels alone could be in the order of 3 000 000 000 Euro (unit cost 0.16 million Euro, unit size 4000 hl, cycle time 18 days, the world production 1 300 million hectolitres). The lifetime of a fermenter can be 25 to 30 years. The capital tied up in the conventional fermenters is a factor against the immobilized main fermentation. Batchwise wort production diminishes the economic advantages of continuous main fermentation, as more tanks for wort storage are needed in the process. Different beers can be produced by changing the type of wort (Andries *et al.* 1997b), without any change in the yeast.

The image of the product is a factor that cannot be ignored when considering any major process changes in the beer production. There will always be individuals and organisations that prefer to stay with traditional methods and materials. The beer tasting guru Michael Jackson wrote (1992): "immobile yeast might be the technique of the future – and I probably won't like that, either", although he did not find any faults in continuously fermented Dominion Kiwi Lager. Fortunately, Meilgaard (2000) provided an example that new types of beer may be successful on the market.

8. Summary and conclusions

This work demonstrated that an immobilized, continuous main fermentation is a feasible process for production of lager beer. Recent larger pilot units confirm the attractiveness of continuous fermentation to the brewing industry. The immobilization technique as such has solved some problems encountered in continuous, non-immobilized processes in the 1960s.

The division of main fermentation by immobilized yeast into two stages, of which the first is an aerobic and the second an anaerobic stage, has facilitated the maintenance of yeast viability and vitality so that long operation periods are possible. In this work an operative period of over 14 months was found to be stable both in attenuation and in flavour formation. The controlled gas feed rates provided a tool to control the formation of flavour compounds. This control is achieved through controlling the growth-limiting substrate, oxygen, and its distribution in the first stage of the process. An interesting finding in this work was that not only has the carrier material an effect on the flavour of beer, but that this influence is strain dependent. The combination of carrier and yeast strain had an effect on the flavour of beer.

Although economic evaluation indicates that a continuous fermentation process using immobilized yeast has economic advantages over a batch process, these advantages should be confirmed in larger scale units than those used in this work. A key finding of this work was that the feasibility of wood chips as a cheap carrier material. These chips proved to be as good as porous glass beads, kieselguhr based or DEAE-cellulose based carriers, which are about 100-fold more expensive.

Contamination is a hazard, but not more so in continuous, immobilized fermentation than in batch fermentation. Detection by the plate counting method proved to be rapid enough to avoid the release of beer of inferior quality. In the future more rapid detection methods will probably be developed and these will provide even better protection. If a method to analyse the physiological state of yeast should emerge, this would give the brewmaster increased assurance, and the acceptability of continuous, immobilized fermentation would increase further.

Can immobilized fermentation cause a technological leap or a discontinuity in fermentation performance; can this technique be a disruptive change in the brewing industry? This question is still unanswered, but immobilization technique has elements of a disruptive technological change. Immobilized fermentation is developing rapidly and good lager beer can be already produced by continuous, immobilized fermentation.

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Title Feasibility of continuous main fermentation of beer using immobilized yeast			
Abstract <p>Fermentation is the most time consuming step in the production of beer and therefore the effective use of fermentation vessels is a crucial element in brewing economy. One means of increasing the productivity of a batch process is to convert it to a continuous one. Experiments in continuous fermentation emerged during the 1950s and 1960s, but by the end of 1970s most of them had been closed down. Immobilization technique revitalised continuous fermentation research in the 1980s and led to industrial applications in the secondary fermentation and in the production of low-alcohol beers.</p> <p>This work demonstrated that an immobilized, continuous main fermentation is a feasible process for production of lager beer. The immobilized main fermentation was stable for more than 14 months both in fermentation efficiency and in aroma compound formation. The formation of aroma compounds could be controlled by varying the composition and amount of gas feed into the first fermentation stage. The division of immobilized main fermentation into an aerobic and an anaerobic stage appeared to solve problems related to yeast growth and viability.</p> <p>The carrier material affected the formation of flavour compounds in small-scale fermentations. Moreover the effect varied with the yeast strain used. The carrier affected the economy of immobilized fermentation: the carrier cost could be as high as one third of the investment. When a cheap carrier is used the investment cost for a continuous, immobilized process was estimated to be only about 70% of the investment cost of a batch process.</p>			
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