STELLINGEN

behorende bij het proefschrift
Flocculation of Brewers' yeast
- quantification, modelling and control -
door E.H. van Hamersveld

1. Zolang de hydrodynamische begrippen 'gently shaken' en 'gently mixed' niet zijn gekwantificeerd, kunnen de resultaten van verschillende flocculatie-assays niet met elkaar worden vergeleken.

   Amory, D.E., P.G. Rouxhet and J.P. Dufour, *J. Inst. Brew.*, 94 (2), 79-84, 1988.
   Kihn, J.C., C.L. Masy, M.M. Mestdagh and P.G. Rouxhet, *Can. J. Microbiol.*, 34, 779-781, 1988.

2. De brouwerij-industrie is voor het oplossen van het flocculatievraagstuk gebaat bij een multidisciplinaire benadering.

   De proefschriften

3. Het afleiden van de botsingssnelheid tussen gistcellen uit de omwentelingssnelheid van een schudkolf is overmoedig en kan leiden tot het trekken van verkeerde conclusies.

   Stratford, M. and M.H.J. Keenan, *Yeast*, 3, 201-206, 1987.

4. Bij het beschrijven van flocculatie van brouwersgist wordt het belang van vlokafbraak zwaar ondergewaardeerd en wordt ten onrechte vrijwel uitsluitend gesproken over vlokvormingssnelheid.

5. Voor de toekomstige brouwer is het zaak het 'leven in de brouwerij' te reduceren tot de vergistings- en lageringskuipen.

6. De huidige stand van het flocculatie-onderzoek is verder dan de uitspraak van Calleja doet vermoeden: 'When the big floc meets the small floc, the big floc becomes bigger and the small floc disappears'.

   Calleja, G.B., Bioadhesion II, Louvain-la-Neuve, 1993.

7. Gezien de verhouding inspanning/vergoeding is de te verwerven doctorstitel niet alleen de hoogste maar ook de duurste van het Nederlandse onderwijsstelsel.

8. Wanneer een promovendus over zijn promotieperiode spreekt en het getal '4' in de mond neemt, bedoelt hij meestal '5' of '6'.

Chemisch Weekblad, 1 april 1996.

9. Het is voor boeren niet meer mogelijk de koe bij de horens te vatten.

10. Het begrip 'reality tv' suggereert terecht dat de overige amusementsprogramma's niets met de realiteit van doen hebben.

11. Een automatische telefoonbeantwoorder is voor sommige gevallen geschikt als leugendetector.

12. Het publiekelijk uitbrengen van stemmen moet worden afgeraden om te voorkomen dat het ongewenste effect van 'schapen over een dam' optreedt.

13. Voor stellingen geldt: er gaan er 13 in een dozijn.

Delft, 4 juni 1996

Flocculation of brewers' yeast

- quantification, modelling and control -

E.H. van Hamersveld

front cover: Czech advertisement board

Flocculation of brewers' yeast

- quantification, modelling and control -

PROEFSCHRIFT

ter verkrijging van de graad doctor
aan de Technische Universiteit Delft,
op gezag van de Rector Magnificus Prof.ir. K.F. Wakker,
in het openbaar te verdedigen ten overstaan van een commissie,
door het College van Dekanen aangewezen,
op dinsdag 4 juni 1996 te 16.00 uur door

Everardus Hendrikus van HAMERSVELD

doctorandus in de scheikunde

geboren te Baarn

Dit proefschrift is goedgekeurd door de promotor: Prof.ir. K.Ch.A.M. Luyben

Samenstelling promotiecommissie:

Rector Magnificus (voorzitter)	
Prof.ir. K.Ch.A.M. Luyben (promotor)	Technische Universiteit Delft
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Dr.ir. H.Y. Steensma	Rijksuniversiteit Leiden
Dr. W.G. Iverson	Heineken Technical Services B.V.

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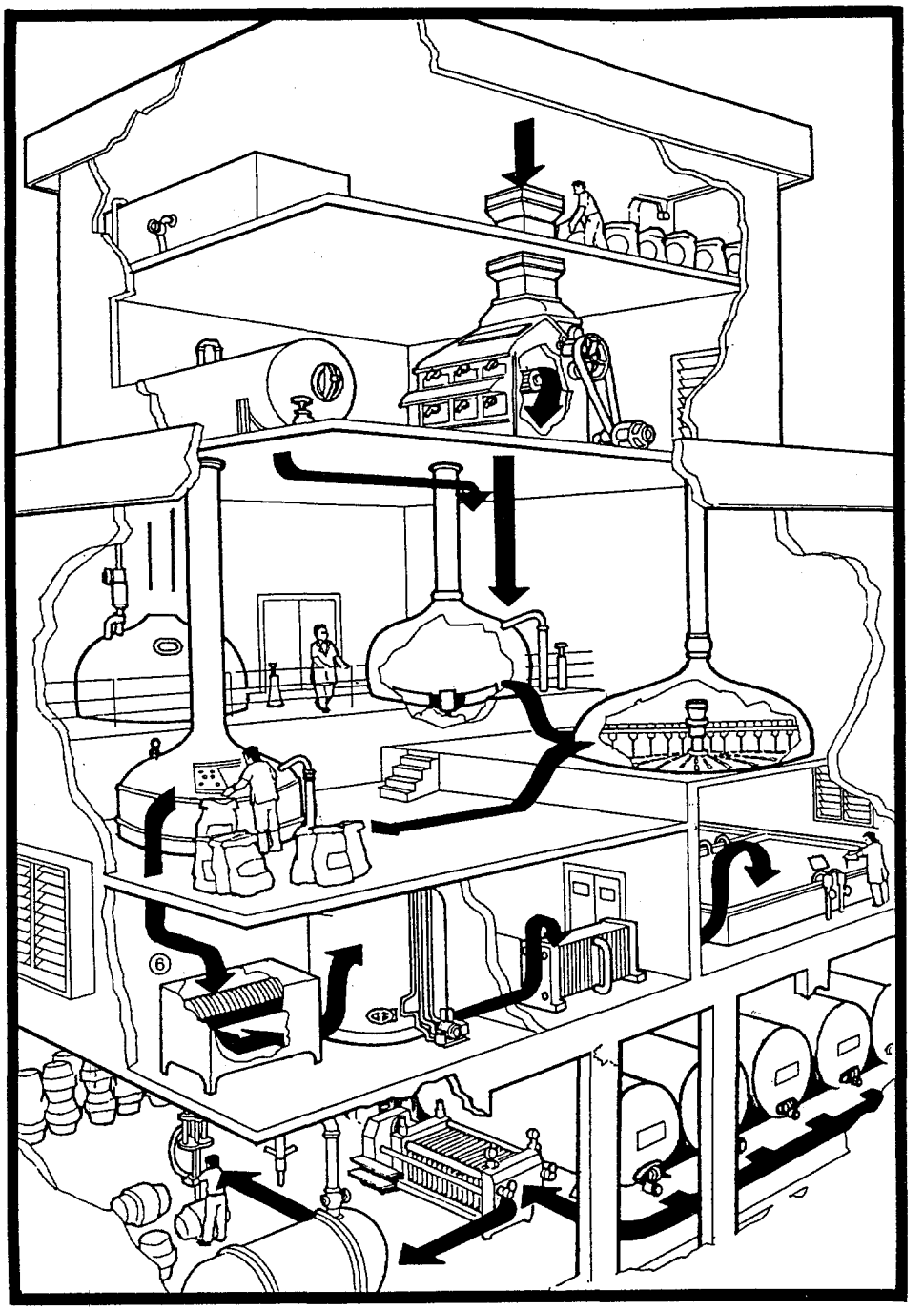
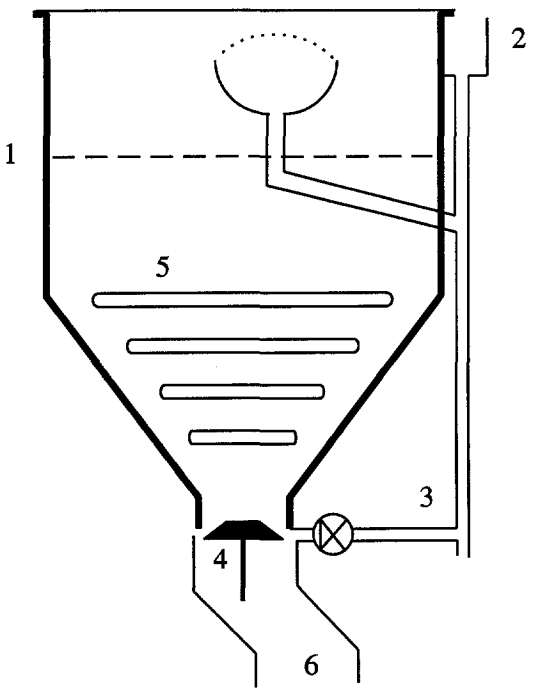
'Het is niet erg om dood te gaan,
het was tenslotte ook niet erg toen je nog niet bestond'

Dr. A.H. Heineken



Traditional brewing house (de bierbrouwerij, Landelijke Stichting Beroepsopleiding Levensmiddelenindustrie, Apeldoorn).

CONTENTS



A cylindroconical steep tank 1) grain level 2) overflow 3) dirty steep water discharge 4) valve 5) aeration piping 6) grain discharge.

The malting and brewing process ...

Steeping	- Barley that is stored in a silo is dropped into the steep tank containing water of 15 °C. After aeration during 12 h to 24 h the steeping is arrested by draining, then the so called air rest starts: the barley corn is left standing with a film of water in which oxygen can dissolve. This takes a few hours, whereafter again steeping is started followed by a second air rest. This process continues until the barley contains 42% of water by weight. Steeping takes about 2 days. - Steeping is carried out to moisture the barley. Enzymes are being formed that are necessary during later steps. During steeping the formation of the rootlets begins.

... to be continued on page 28 ...

CHAPTER I

Introduction

GENERAL

Brewers want to maintain a constant product quality, every brew must have the same taste. International brewers face an additional challenge, they want to produce a uniform beer worldwide. For this it is necessary to understand the process of brewing microbially as well as technologically. To cite the chief executive of Heineken, Karel Vuursteen: 'Brewing Heineken beer abroad is a technological feat in its own right. It is even more difficult than it sounds to take water and hops in Asia and turn them into a brew that tastes the same as beer made from water and hops in Europe' (Van de Krol, 1994). For this reason in the last century a lot of research and development has been done, in which especially the fermentation of the wort has received considerable attention.

Chronological review of important discoveries concerning the beer fermentation (Stewart and Russell, 1986; CBK, 1991).

1788 Richardson developed the saccharometer. With this apparatus it is possible to determine the sugar content of wort. It was one of the first steps to a more constant product quality.
1842 Yeast and fermentation techniques of Bavaria were smuggled to Czechoslovakia, leading to the development of pilsner beer in the town Pilzen. This led to a mondial spread of bottom fermentation techniques (lager beer). The formerly used top fermentation techniques were largely discarded except for the British Isles and some of its colonies.
1857 Pasteur proved the association of fermentation with living matter.
1876 Pasteur discovered the process which is now called pasteurisation. If a beer is heated shortly it can be preserved longer. During heating, yeast and all kinds of bacteria are killed. In this way the beer becomes free from micro-organisms.
1879 Von Linde developed the cooling machine, that plays an important role during the brewing of beer with bottom cropping yeast.
1880 Extensive use of steam to combat the 'ferments of disease', a phrase as used by Pasteur.
1883 Start of pure strain brewing by the Carlsberg Brewery. Emil Christian Hansen who studied brewing yeast at this company succeeded in isolating four strains of bottom fermenting yeast.
1897 Buchner initiated the unravelling of fermentation pathways, leading to the Embden-Meyerhof-Parnas pathway. The enzyme zymase is found to be responsible for the formation of carbon dioxide and ethanol from sugar.
1930 Development of the concept of cilindroconical fermentation tanks by the Swiss engineer L. Nathan.
1935 Wing stained vegetative cells of *Saccharomyces cerevisiae* and showed they were diploid. His study presented the possibility of breeding new yeast strains for specific purposes.
1960 Substantial interest for continuous fermentation arised in the brewing industry.

During the fermentation the wort from the wort kettles is turned into green beer by yeast. The sugars from the wort are converted to ethanol and carbon dioxide. Next to this yeast and flavour components are formed. At the end of the fermentation the main part of the yeast has to be removed, which is achieved by sedimentation. The sedimentation of the yeast is enhanced by the phenomenon that cells form flocs at the end of the beer fermentation. This phenomenon is called flocculation and plays a main role in the removal of the yeast. However, due to strain variability flocculation may be too strong or too weak. Both cases are unfavourable. To handle these problems more insight in the mechanisms of flocculation is needed. In this thesis the physical mechanisms of yeast flocculation are described and modelled. Next to this attention is payed to the quantification of yeast flocculation.

FERMENTATION OF WORT

The fermentation of wort is actually one of the last steps of the beer brewing process. The total process consists of nine process steps and is divided into two parts: the malting process and the brewing process (Figure 1). Nowadays only a few brewers have their own malt house. Most brewers leave the malting to the maltsters and get their malt from them. During the nine process steps the barley is turned into beer. On the first page of each Chapter of this thesis the most important operations of each step are mentioned and their main purpose is explained.

Beer consumption

The absolute top of beer consumption was in the middle ages (1300-1650) when some 400 l were consumed annually per head of population in Western Europe (CBK, 1991). The main reason for this was the low quality of drinking water. Nowadays in Germany, the country with the highest beer consumption in the world, the annual consumption is 138 l (CBK, 1994). In the Netherlands the annual consumption is 85 l.

Notwithstanding all the efforts being payed to the fermentation still a lot of unsolved problems remain. As stated by Hudson (1983), brewers have only limited control over yeast and fermentation. The means of control lie in altering the composition of wort through choice of grist materials and mashing conditions at brewing, and by sugar concentration, pitching rate and temperature adjustments in the fermentation tank. Especially during fermentation the possibilities for control are very limited. Only temperature and pressure seem to be suitable for this. It was expected that control would

be improved with the introduction of continuous fermentation in the '60s. However, until now continuous fermentation has not proven to be a commercial success. Batch fermentation is relatively simple and little attention is required during the process. Continuous fermentation on the other hand requires constant monitoring and control of flow rates, temperature gradients, yeast recycling and oxygen concentration levels (Stewart and Russell, 1986).

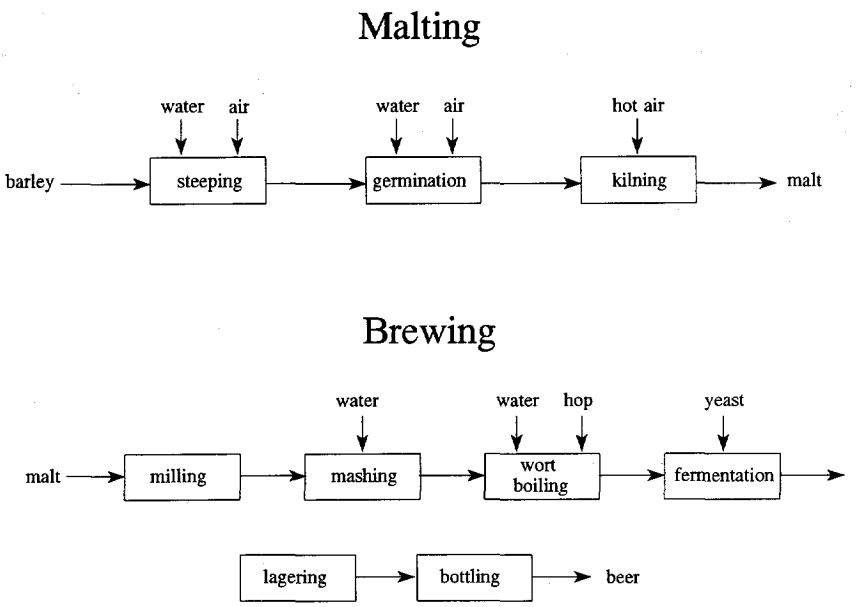


Figure 1. Schematic representation of the malting and brewing processes.

With respect to 'control' two items have to be distinguished:

i. production of a uniform beer in any fermentation configuration
ii. control of fermentation, in the sense that one is able to take action if something goes wrong during the process.

In both cases a good understanding of yeast metabolism and the influence of process conditions is necessary.

ad i The characteristic taste of beer is caused by many flavour components and ethanol. The following groups of substances are present: alcohols, esters, carbonyls, organic acids, sulphur compounds, amines, phenols and a large number of other compounds (Stewart and Russell, 1986). Whether or not these compounds are formed during fermentation and in which amount depends on yeast species, temperature, pH, type of malt, etc. To be able to produce a uniform beer the starting material (wort and yeast strain) and the process conditions (temperature course during fermentation, mixing, pressure etc.) should always be the same. An important aspect in this is the configuration of the fermentation tank. As was shown by Renger (1991) the height of the tank is an important parameter since it determines the liquid pressure in the tank, which is involved in the formation of flavour components. More insight in all the influences of these parameters (starting material as well as process conditions) on yeast metabolism will ultimately lead to a more constant beer quality.

ad ii One of the most troublesome situations encountered by the brewer is a 'hung' or 'stuck' fermentation (i.e. incomplete or too slow attenuation of the wort). The major cause for this problem is early sedimentation of the yeast leaving an unfinished product. The counterpart of this problem is the failure of the yeast to sediment caused by poor flocculation or failure of flocculation. This will lead to problems during the next process step, the lagering. High yeast concentrations will then influence the beer quality (e.g. yeast autolysis). Besides this, removal of the yeast, that has to be carried out after the lagering by filtration, will be troublesome. Increase of fermentation controllability should solve this problem. Therefore the mechanisms determining the last part of the fermentation

Carbon dioxide

Mixing in the fermentation tank is brought about by the carbon dioxide produced by the yeast itself. A lager fermentation carried out at a temperature around 9 °C reaches a maximum carbon dioxide production of 2.4 μmol CO_2 per kg medium per second. A fermentation tank with a volume of 400 m^3 will thus give an average carbon dioxide flow of 80 m^3/h. This gas production will cause fluid velocities up to 50 cm/s (Renger, 1991), sufficient to mix the tank contents.

In a tank of 400 m^3 a total amount of 16 000 kg carbon dioxide is produced. The produced carbon dioxide has to be collected, because 4% of carbon dioxide in air can cause asphyxiation (Hough, 1985).

Regarding the annual beer production a lot of carbon dioxide is produced. Roughly it can be calculated that the annual carbon dioxide production is 4.8 Megaton, which is 3% of the total exhaust of this gas (VROM).

process, at which flocculation, sedimentation and yeast removal take place, should be well understood.

COURSE OF FERMENTATION

After wort boiling the hopped wort is clarified, cooled and aerated (Appendix). The wort is pumped to a fermentation vessel and inoculated with yeast, whereafter the fermentation starts. Normally a batch fermentation of wort is inoculated to give a yeast concentration of 0.5-1.0 g/l (dry mass). The species most commonly used in lager fermentation is *Saccharomyces cerevisiae*; another well known species is *Saccharomyces carlsbergensis*. The main difference between these strains is that the latter is capable of fermenting the sugar melibiose whereas the first is not.

Table 1 Typical composition of a wort (Hough, 1985).

Component	concentration (g/l)
fructose	2.1
glucose	9.1
sucrose	2.3
maltose	52.4
maltotriose	12.8
non-fermentable carbohydrate	23.9
total nitrogen	0.8
total amino acid	1.95
total phenolic constituents	0.25
iso α acids	0.035
calcium ions	0.065

Hopped wort contains a wide range of components. The major ones are listed in Table 1. During fermentation sugars in the wort are being converted into mainly yeast, ethanol, carbon dioxide and heat. The overall equation looks as follows, taking only maltose and amino acid as a starting material:

100 g maltose + 0.5 g amino acid →
5 g yeast + 48.8 g ethanol + 46.8 g carbon dioxide + 209 kJ energy

The wort is aerated before the fermentation starts. The dissolved oxygen is needed for the formation of new cells. The growth limiting factor during fermentation is not exactly known. Recent studies have shown oxygen to be involved indirectly whereby the shortage of sterols and unsaturated fatty acids are most likely the actual factors. By this nutrient limitation the cell division will stop and the flocculence of the yeast cells will be induced (Straver et al., 1993a) (Figure 2).

The wort contains mainly maltose as a convertible sugar (Table 1). Maltose as well as maltotriose are enzymatically hydrolysed to glucose within the yeast cell. This glucose is converted to carbon dioxide and ethanol according to the following overall reaction

$$C_6H_{12}O_6 \rightarrow 2\ CO_2 + 2\ C_2H_5OH$$

The course of a typical fermentation is depicted in Figure 2. The sugar concentration (extract) is expressed as degrees Plato (1 °P is 10 g sugar per kg medium).

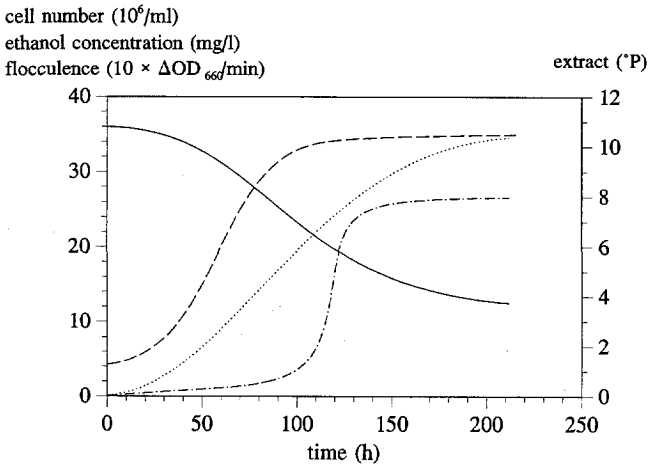


Figure 3. The course of a typical bottom fermentation at 10 °C. cell number (---), ethanol concentration (····), extract (—) and flocculence (-·-·-) (Chapter II).

At the end of the fermentation the fermentable sugars have been converted and the major part of the yeast is then removed by flocculation and subsequent sedimentation

of the yeast. The so-called green beer and remaining part of the yeast are subsequently transported to the lager cellars. In Figure 3 the desired course of the yeast amount is shown during fermentation and lagering.

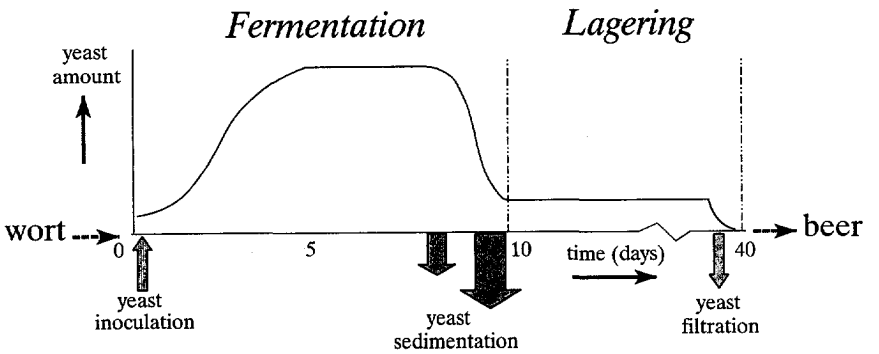


Figure 3. Course of yeast amount that is in suspension during the fermentation and lagering process.

FLOCCULATION

Sedimentation is the factor determining the removal of the yeast from the fermentation broth. In this process flocculation plays a main role. The last few decades flocculation of brewers' yeast has received considerable attention in literature, especially from a biochemical point of view (Speers et al., 1993). This resulted in quite some insight in the effects of environmental conditions on flocculation. However, the precise mechanisms remain to be unravelled.

Definitions

Defining flocculation is not easy, since it involves several consecutive mechanisms. In this thesis we will use the following three terms: flocculence, flocculability and flocculation. For flocculation to occur three conditions have to be met:

i) The cells must be flocculent.

i) The composition of the medium must be such as to allow binding between the flocculent cells; at least 10^{-8} mol/l calcium is necessary, the temperature must be between 5 °C and 50 °C (both conditions are strain dependent).
iii) The cells must come together in the liquid. This can only occur if velocity gradients are present in the liquid and if the cell concentration is high enough.

Flocculation will not necessarirly lead to sedimentation of the flocs formed. For sedimentation of the flocs the turbulence intensity or fluid velocities in the fluidum must be low enough.

Based on these conditions the terms can be described as follows,

Flocculence — The definition of flocculence is derived from Amory et al. (1988). Flocculence is the ability of cells to flocculate if all environmental conditions are favourable. The environmental conditions are: presence of the right inducer, temperature of the medium, sufficient cells to allow floc formation etc.

Flocculability — The definition of flocculability is derived from Kruyt (1949). Flocculability is the ability of yeast cells to flocculate in a given medium if the hydrodynamical conditions are favourable (sufficient collision rate and not too high breakup forces) and sufficient cells are present. To have a certain degree of flocculability, flocculence is a necessary condition.

Flocculation — Flocculation is the actual floc formation under the actual environmental conditions. For flocculation to occur flocculability is an essential condition. Flocculation will not unconditionally lead to sedimentation.

Sedimentation — Sedimentation is the actual settling of flocs. Sedimentation is only possible under favourable hydrodynamical conditions (low turbulence intensity or fluid velocities and not too large breakup forces). Flocculation is a necessary condition for sedimentation.

In Table 2 the conditions that have to be met to allow the above mentioned phenomena to occur are listed.

Table 2 Conditions for the occurrence of the mentioned processes (1: necessary, 0: not necessary).

	presence of the right genes; favourable growth conditions	favourable physiological conditions	favourable hydrodynamic conditions	fluid velocities lower than settling velocities of the flocs
Flocculence	1	0	0	0
Flocculability	1	1	0	0
Flocculation	1	1	1	0
Sedimentation	1	1	1	1

Quantification

Control of flocculation can only take place if a proper method to quantify this phenomenon is available. Measurement of the different aspects of flocculation characteristics could be an important tool for the brewing industry. However, hardly any brewer makes use of this tool, which could prevent problems caused by premature or hampered flocculation.

- Flocculence can be determined by the method as described by Smit et al. (1992). This off-line test gives the degree of flocculence of the cells.
- For a brewer, flocculability of the yeast is of more value because it reflects the ability of the yeast cells to form flocs in the beer. An advantage of flocculability is that it can be detected on-line.
- Quantification of flocculation will give direct information about the size of the flocs, which is important with respect to the settling velocity of the flocs. However, the quantification of flocculation is difficult because flocculation is easily disturbed by measurement. Yeast flocs are very weak and simply broken up during sampling.

Molecular aspects

Flocculation occurs in the absence of cell division. Initiation of flocculence coincides with an arrest of cell division (Straver et al., 1993a). The cell division stop is caused by nutrient limitation. At the onset of flocculence a sharp increase in hydrophobicity of the cells was observed (Straver et al., 1993a). This increase appeared to be due to the presence of fimbriae-like structures on the cell wall (Straver et al., 1994a). Apart from this, an agglutinin in the cell wall is also involved (Straver et al., 1994b). These results olny account for industrial yeast strains. It is very likely that a combination of these factors leads to the flocculence of the cells.

Genetic aspects

As part of the molecular mechanisms of yeast flocculation, also genetics play an important role in the onset of flocculence. The genetic base of flocculation was mentioned by many early researchers (Thorne, 1951; Roman et al., 1951; Gilliland, 1951). Nearly thirty genes are involved in the occurrence of flocculation (reviewed in Teunissen and Steensma, 1995c). Some of these are regulatory genes. The sequences of the dominant genes FLO1 and FLO5 suggest, however, they both might encode cell wall-located, lectin-like flocculins. The genes have been localized to chromosome I (Russell et al., 1980; Teunissen et al., 1993) and VIII (Teunissen et al., 1995b) respectively. There is a strong correlation between the onset of flocculation and the transcription of FLO1 or FLO5-like genes in brewers' strains. Whether these gene products can be linked to the fimbriae-like structures described by Straver et al. (1994a) requires further study.

Heat production during fermentation

Apart from yeast, ethanol and carbon dioxide also heat is produced during the fermentation of beer. The heat production is 2.1 MJ per kg maltose for a batch fermentation (400 m^3) carried out at 9 °C. Since this takes about 200 h an average cooling capacity, of 116 kW is needed, while at the maximum sugar conversion a capacity of 200 kW is required.

Physiological aspects

The genetically controlled onset of flocculence and the appearance of fimbriae-like structures on the cell wall does not automatically lead to floc formation. To reach a

certain flocculability of the yeast the medium in which the cells are suspended has to meet several conditions.
To start, calcium is absolutely necessary for flocculation of yeast cells (Lindquist, 1953). However, threshold amounts of only 10^{-8} mol/l are reported to be sufficient to initiate flocculation (Taylor and Orton, 1975). Secondly the pH of the medium must be within the range pH 2 to pH 8 (Calleja, 1987). A recent article from Straver and Kijne (1995) describes the relation between pH and the adherence of flocculent yeast cells to latex beads. Temperature also plays a role, a too high or too low temperature will lead to deflocculation. The temperature effect is strain dependent and is also influenced by the composition of the medium. The temperature at which the flocs will completely disperse can be up to 60 °C (Taylor and Orton, 1975; Mill, 1964) for different yeast strains. Flocculation can also be inhibited by specific compounds such as mannose and α-methyl-mannopyranoside (Smit et al., 1992).

The explanation of the mentioned effects is rather difficult. The most probable explanation is the blocking of the active sites of the molecules involved in the adhesion process. This can be caused by protonation in case of pH effect or by specific adhesion of other molecules in case of inhibition by sugars. The temperature effect might be explained from the fact that the spacial orientation of the involved molecules on the cell wall is such that adhesion of two cells cannot take place. If the structure of the molecules ("fimbriae") on the cell wall of flocculent cells is known and if the role of an agglutinin which is probably involved in the flocculation process is elucidated, the effects of medium on flocculability of the cells can be explained more precisely.

Physical aspects

Flocculability of the yeast cells will not lead to actual floc formation without the occurrence of sufficient cell-cell encounters caused by velocity gradients in the liquid.

The physical process of flocculation/aggregation has been studied extensively especially in case of nonbiological materials. For example in the chemical industry aggregation is used in the production of solid products such as ceramics and catalysts. In these cases physicochemical interaction between particles is described by the DLVO theory and more attention is being payed to the particle formation itself.

In case of bioadhesion the physicochemical interaction is far more complex, since the molecules involved are mostly complex and sometimes their composition is even unknown, which makes application of the DLVO theory impossible. Bioadhesion is applied in wastewater treatment and the biochemical industry. In wastewater treatment the removal of active sludge from treated water is carried out by flocculation and subsequent sedimentation. These processes are mostly carried out in equipment especially designed for these purposes. Flocculation takes place in a flocculator where the conditions are optimal for floc formation. Occasionally so-called flocculants or coagulants are added to the suspension in order to improve the flocculability. The sedimentation of the flocs formed takes place in sedimentation basins, that have their own specifications for optimal settling of the flocs formed in the flocculator. For proper design of a flocculator much research was needed for the flocculation process itself (Ives, 1975). These studies give quite some insight in the flocculation process.

An example with a biological material is the production of proteins from vegetable or genetically engineered sources (Ayazi Shamlou et al., 1994). Removal of these proteins from the mother liquor is difficult, since the density difference between the proteins and the medium is less than 10%. This makes removal by, for example, high speed centrifugation difficult and expensive. Aggregation of the proteins to large and dense aggregates can solve this problem. In the last decades considerable attention has been payed to this kind of removal processes (Bell et al., 1983; Ayazi Shamlou, 1993). Both flocculation in waste water treatment and flocculation in the biochemical industry are studied in technological departments; civil

Beer production

The beer brewing industry produces about 1.2×10^9 hectolitres per year worldwide (CBK, 1994). The two largest brewers in the world, Anheuser-Busch and Heineken have an annual production of 110×10^6 hl and 60×10^6 hl respectively (Delos, 1993; Heineken, 1994), which is about 15% of the total beer production in the world. Anheuser-Busch has merely twelve breweries, all located in the USA, while Heineken has got more than a hundred in about fifty countries. Another well-represented brewer in the world is Guiness owning fifteen breweries in foreign countries while in another thirty breweries Guiness is brewed under license.
In the middle ages every town had its own breweries and some cities even had over a hundred. The number of brewers has decreased from then on, for example, France had over 3500 brewers in those days and only twenty are left now. More precisely, 90% of the total beer amount in France is produced by only three brewers (Delos, 1993).

engineering and biochemical engineering, respectively. This might be the reason why the physical aspects of the process are emphasized.

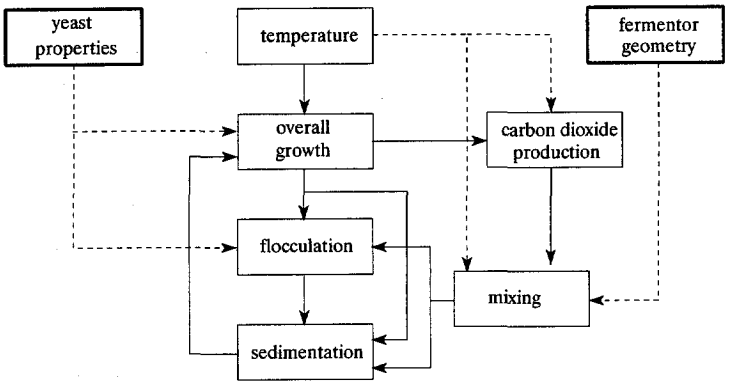


Figure 4. Schematic representation of flocculation and sedimentation.

In the case of yeast flocculation, research has mainly focused on the microbial aspects, next to the physicochemical interaction between the cells, which was proven to be very complicated. The fact that the exact composition of the cell wall is not known and the presence of relatively large molecules that also seem to be branched (Straver et al., 1994a) make the application of classical theories even more difficult. In contrast to flocculation in wastewater treatment or the biochemical industry, the quantitative physical mechanisms of flocculation are hardly mentioned in literature for the brewing industry.

Control

The process of flocculation and sedimentation at the end of the fermentation is complex. Lots of factors influence the process (Figure 4), which makes it difficult to control. Both, flocculation and sedimentation are carried out in the same vessel where the fermentation takes place. This makes it even more difficult to set the optimal conditions

for both processes at the same time. Apart from this there are hardly any possibilities to enhance the process. The addition of flocculants or polymers to improve flocculability of the cells is undesirable as well as the inhibition of flocculability by adding EDTA to the medium. A possibility would be the genetic control of flocculence, for example by regulation of the onset of flocculence by temperature. Nevertheless here public acceptance will determine the time frame of introduction. Therefore, mechanically influencing the process seems to be the best option for the time being.

AIM OF RESEARCH

The major aim of the total yeast flocculation project (of which a part is described in this thesis) was to improve the controllability of yeast flocculation and sedimentation in any fermentation configuration. This should lead to a more constant product quality. This goal has been pursued in a multidisciplinary project team. The characterization and isolation of molecules involved in flocculation were studied by M.H. Straver (Institute of Molecular Plant Sciences, Leiden University). Her part of the project resulted in the thesis entitled 'Molecular Mechanism of Yeast Flocculation' (Straver, 1993b). A.W.R.H. Teunissen (Institute of Molecular Plant Sciences, Leiden University) focused on the characterization and analysis of genes involved in flocculation via a molecular genetic approach. The results are described in the thesis 'Flocculation Genes of *Saccharomyces cerevisiae*' (Teunissen, 1995a). The coordination, application and testing of the experimental findings were performed in cooperation with a brewery. Finally the physicochemical interactions between the cells and the physical mechanism of yeast flocculation are described in this thesis.

The major aim of the study described in this thesis is improvement of the controllability of yeast flocculation by physically influencing the process. This aim is reached via the following steps:

- Physicochemical description of yeast flocculation.
- Development of a method to quantify flocculability of the yeast during fermentation.
- Determination of the physical conditions that have to be met to enable flocculation of the cells.

- Development of a model to describe the suspension / sedimentation of yeast during fermentation.
- Proposal for increasing controllability of yeast flocculation and sedimentation.

OUTLINE OF THE THESIS

In Chapter II the physicochemical interactions between yeast cells are discussed. Considerable attention has been payed to this subject in the past. However, the role of colloid science in yeast flocculation was still unclear. Speers et al. (1993) were the first to discuss the subject based on classical theory.

In Chapter III to Chapter V the quantification of flocculation in general is discussed and some new methods are presented.

In Chapter III a method to measure flocculability online is presented. The method is based on light extinction which makes it safe to operate and nondestructive. This method is both cheap and simple, testing on large scale should prove whether it will be applicable to the brewing industry. Quantification of the actual flocculation of yeast cells is difficult. Quite some parameters have to be controlled to allow measurement without disturbing the system.

In Chapter IV the quantification of flocculation in general is discussed, following the division of flocculation into the three subdefinitions. The effect of environmental conditions on the measurement is studied as well as different methods to be applied to the quantification of the three different phenomena.

In Chapter V a method to quantify flocculation is presented. The method is based on a combined light extinction/sedimentation technique, which was earlier used by Davis and Hunt (1986). Flocculation was expressed in terms of floc size and number of single cells.

In Chapter VI the physical mechanisms of flocculation are described. The developed model is based on particle movement by fluid flow that is in a dynamic equilibrium with floc breakup caused by pressure differences due to fluctuating fluid velocities and by velocity gradients over the floc surface.

In Chapter VII a fermentation model is presented. The model describes the amount of yeast that is in suspension during fermentation. The suspension of yeast depends on many factors such as degree of flocculability, fluid velocities in the vessel etc. The model is tested in a large scale brewery fermentation.

Finally in Chapter VIII the results are evaluated and a few methods to improve controllability are presented and discussed.

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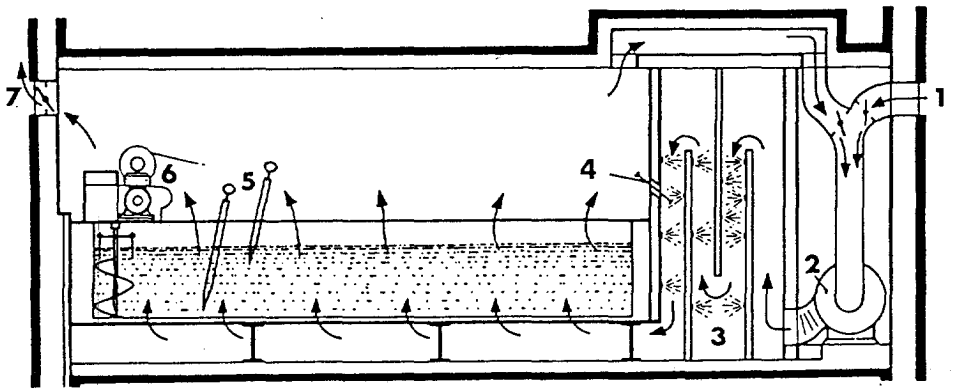

A Saladin box: 1) fresh air inlet 2) fan 3) water sprays 4) and 5) thermometers 6) helical-screw turner 7) valve for air recirculation.

... the malting and brewing process ...

germination	- For germination the grain is spread onto a perforated malting floor. The grain is conditioned with water saturated air to provide a temperature of 15 °C. To prevent the formed rootlets to mat together, a mechanical turner separates the germinating grains. The process normally takes 4-6 days. - During germination the starchy endosperm is broken down. Monitoring of the process is done by taking samples and examining the embryonic shoot. The process is stopped if this structure has grown 2/3 the length of the corn. In this step new enzymes are formed and the enzymes present are activated. Insoluble starch molecules and proteins are converted into soluble ones.

... to be continued on page 46 ...

CHAPTER II

How important is the physicochemical interaction in the flocculation of yeast?

Abstract

Yeast flocculation is considered to be ruled by colloid-chemical and biological interactions. The change in hydrophobicity, surface potential and surface structures and the appearance of lectins on the cell wall point in this direction. According the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory we calculated the net attraction energy between nonflocculent and flocculent yeast cells. There seems to be no difference between the secondary minima of the potential energy curves of both states of the cells, although most parameters used within the DLVO model change in a manner suggesting that flocculation is favoured when the cells become flocculent. Parallel to these calculations we measured the net attraction force between flocculent cells. This force was approximately 2000 times higher than the force calculated with the DLVO theory. From these findings it is concluded that the interaction between flocculent yeast cells has only a biological character. The changes in the physicochemical state of the cell wall must be ascribed to changes in its biological nature.

INTRODUCTION

Yeast flocculation has been studied for several decades; this has not, however, led to a comprehensive description of the flocculation mechanism (Stratford, 1992; Calleja, 1987). Flocculation is considered to be influenced by colloid-chemical and biological interactions (Kihn et al., 1988). The relevance of these interactions is still being discussed. Several research groups have reported that genes (Russell et al., 1980; Stratford, 1992; Teunissen et al., 1993), surface structures (Mozes et al., 1989) and lectins on the cell wall (Miki et al., 1982; Straver et al., 1994) might be responsible for yeast flocculation.

The importance of a colloid-chemical approach of flocculation has recently been emphasized by Speers et al. (1992). Several researchers have stressed the importance of physical parameters such as hydrophobicity and charge (Eddy & Rudin; 1958; Perera & Rose, 1976; Mozes et al., 1987). Amory et al. (1988) and Smit et al. (1992) found a direct relationship between cell surface hydrophobicity and the appearance of flocculence. The manifestation of flocculence correlated with an increase of hydrophobicity. The relationship between cell surface potential and flocculence is not as clear as that. Eddy and Rudin (1958) found no relationship between zeta potential and flocculation characteristics for a series of yeast strains. Amory et al. (1988) found a relationship between zeta potential and phosphate content of the outer cell wall. Perera and Rose (1976) found that the presence of carboxyl groups rather than phosphate is responsible for flocculation. However, Lawrence et al. (1989) found flocculation to be dependent on the zeta potential.

From these studies it cannot be concluded whether the observed changes in hydrophobicity and surface potential directly influence the flocculation process or are only an indication of biological changes in the cell wall, resulting in flocculence of the cells.

In this paper we discuss the importance of biological and physical interactions for the flocculation process in brewers' yeast. To achieve this the relevant parameters that change during a beer fermentation process and the net attraction force between

flocculating cells were measured. The measured interaction force was compared with theoretical calculations based on colloid-chemical theories.

THEORY

Yeast cells are charged particles with a diameter of approximately 7 μm; thus they resemble colloids. A comprehensive theory concerning the interaction energy between colloidal particles was developed by Derjaguin and Landau (1941), and Verwey and Overbeek (1948). This so-called DLVO theory combines the electrostatic (repulsive) energy with the attractive Van der Waals energy between spherical particles. The expression for the attractive energy (Φ_A) between spherical particles has been stated by Hamaker (1937):

$$\Phi_A = -\frac{A\ r}{12\ h} \tag{1}$$

where A is the Hamaker constant, r is the particle radius and h is the interparticle distance.

The repulsive energy (Φ_R) can be described by a formula derived by Derjaguin (1934):

$$\Phi_R = \frac{64\ \pi\ r\ n_0\ k\ T\ \Upsilon_0^2}{\kappa^2}\ e^{-\kappa\ h} \tag{2}$$

where n_0 is the ion number, k is the Boltzmann constant (1.38×10^{-23} J K^{-1}; Weast, 1985), T is the temperature , Υ_0 is a parameter given by

$$\Upsilon_0 = \tanh\left(\frac{z\ e\ \Psi_0}{4\ k\ T}\right) \tag{3}$$

where z is the valence, e is the elementary charge (1.6×10^{-19} C; Weast, 1985), Ψ_0 is the wall potential and κ is the reciprocal double layer thickness given by

$$\kappa = \left(\frac{e^2 \ N_A}{\varepsilon \ k \ T} \ 2 \ 10^3 \ I \right)^{\frac{1}{2}} \tag{4}$$

where $\varepsilon = \varepsilon_r \ \varepsilon_0$ in which ε_0 is the permittivity of a vacuum (8.85×10^{-12} F m^{-1}; Weast, 1985) and ε_r is the dielectric constant, N_A is the Avogadro constant (6×10^{23} mol^{-1}; Weast, 1985) and I is the ionic strength given by

$$I = \tfrac{1}{2} \sum_{i=1}^{i=n} z_i^2 \ M_i \tag{5}$$

where M_i is the ion concentration.

The total interaction energy (Φ_{net}) can now be defined as,

$$\Phi_{net} = \Phi_A + \Phi_R \tag{6}$$

During the fermentation process the parameters needed for calculating the total interaction energy were measured. We compared the total interaction energy (Φ_{net}) of the cells at the beginning of the fermentation when the cells are not flocculent, with the energy at the end of the fermentation when the cells are flocculent.

The calculated total interaction energy was then compared with a measured interaction energy. Hereto, the flocs were dispersed in a laminar shear field. The force needed for complete dispersion of the flocs was calculated from the required shear rate. Breakup of the flocs was determined with a Photometric Dispersion Analyser (Gregory, 1985).

EXPERIMENTAL

Fermentation

The fermentation was carried out under batch conditions in a stirred vessel with standard geometry as described by Beek and Mutzall (1986). The cells were grown in a medium prepared with freeze dried brewery wort (12 °P, where 1 °P is equal to 10 g of sugars per 1000 g of medium) at 9 °C. Before inoculation the medium was saturated with air and the headspace was filled with nitrogen gas. As an inoculum a flocculent strain of *Saccharomyces cerevisiae* (MPY-3) was pregrown on wort at 15 °C. After pitching, the initial cell number was 4×10^6 cells per ml. The fermentations were carried out under strictly anaerobic conditions, for oxygen is the limiting compound for cell division. To prevent oxygen introduction only oxygen tight Viton® tubes were used.

Analysis

Cell size and cell number were detected with a particle counter (Coulter Counter model Z_B, Coulter Electronics Ltd., Harpenden, UK). Before measurement the cells were suspended in physiological NaCl solution (0.9%) to yield concentrations of approximately 3×10^5 cells per ml.

The total sugar content (extract) was expressed as °P. The extract was quantified by measuring the density of the medium without the cells. With the following set of equations the extract is calculated from the density of the medium (Ruppert, 1986),

$$E_w = 199.07\ \rho^{20} + 0.20109\ E_0 - 198.74$$

$$E_0 = 187\ f^3 - 229.8\ f^2 + 259.17\ f - 0.0061$$

$$f = \frac{\rho_0^{20}}{\rho_{demi}^{20}} - 1$$

where E_w is the extract of the medium, ρ^{20} is the density of the medium at 20 °C, E_0 is the initial extract of the wort before pitching, f is the sugar fraction, ρ_0^{20} is the density of the wort before pitching at 20 °P and ρ_{demi}^{20} is the density of demineralized water at 20 °C.

The density measurements were carried out with a density meter (density meter DMA 48; Anton Paar K.G., Graz, Austria).
The ethanol concentration was measured by gaschromatography (Packard 437A) on a glas column (Haysep 1 m × 2 mm inner diameter).
The pH was measured with an Ingold electrode and an Applikon E561/3 pH meter.
Flocculence of the yeast cells was quantified by a flocculence assay according to the method described by Smit et al. (1992).
Zeta potential measurements were carried out with a Laser Z meter, model 501. The cells were suspended in a NaCl solution to a concentration of 10^6 cells per ml.
The hydrophobicity of the cells was determined by measuring contact angles as described by Smit et al. (1992).
The viscosity of the fluid was measured with a capillary viscosimeter (ASTM 68235).

During the fermentation the extract, pH, ethanol concentration, cell number, cell size and flocculence were measured every 24 hours until complete attenuation of the medium was obtainded. The zeta potential and the hydrophobicity were measured twice, once with nonflocculent cells between 20 and 70 hours after pitching of the wort and once with flocculent yeast cells at the end of the fermentation.

Floc strength measurement

Flocculation measurements were carried out under laminar conditions at different shear rates with a Photometric Dispersion Analyser (PDA-2000, Rank Brothers Ltd. , Bottisham, UK) (Gregory, 1985). The output of the apparatus corresponds to the floc size and thus state of flocculation of the suspension. By increasing the shear rate within the apparatus and comparing the output of the apparatus to that of nonflocculent cells. complete dispersion of the flocs at high shear rates was detected.

The flocculation was measured at the end of the fermentation at different shear rates. To check the independence of the PDA output with the shear rate this experiment was repeated with flocs, completely dispersed with ethylene-diamimne-tetra-acetic acid (EDTA).

RESULTS

During the fermentation process sugars are converted into ethanol, which causes several changes in the composition of the medium, implying possible changes in cell properties. In Figure 1a the development of cell number, flocculence and extract are shown. As can be seen the cells become flocculent 100 h after pitching of the wort. The change of parameters relevant to the DLVO theory is shown in Figure 1b. The observed increase in cell diameters has a direct influence on the attractive and repulsive energies. The pH decreased from approximately 4.8 to 3.8, which directly influences the zeta potential as was found earlier by Bowen et al. (1992). The zeta potential is also influenced by the ionic strength (Hiemenz, 1986). We found the zeta potential (according to the Smoluchowski theory (Hiemenz, 1986)) to be -30 mV for nonflocculent and -25 mV for flocculent cells measured at pH and ionic strength according to the medium.

Table 1. Values of parameters relevant to the physicochemical interactions of nonflocculent and flocculent yeast cells.
For calculation of the net attraction energy between two yeast cells according the DLVO-theory the next values are used for nonflocculent and flocculent cells.
The secondary minimum in the potential energy curve for nonflocculent cells is -15.05 kT. Each parameter has been changed to its value for flocculent cells. The results of this change are given and the proportional change in respect of the value for nonflocculent cells was calculated.
[a] This value is not used for the calculations presented in Figure 3.

parameter	nonflocculent cells	flocculent cells	Φ_{net} at secondary minimum (kT)	proportional change (%)
cell radius (μm)	3.25	3.75	-17.35	15.4
zeta potential (mV)	-30	-25	-15.96	6.1
ionic strength (mM) [33]	30.3	21.6	-12.35	-17.9
dielectric constant (-)	78.5	75.8	-15.12.	0.5
Hamaker constant (kT) [27,28]	0.8	1.6 [a]	-34.06	126.5

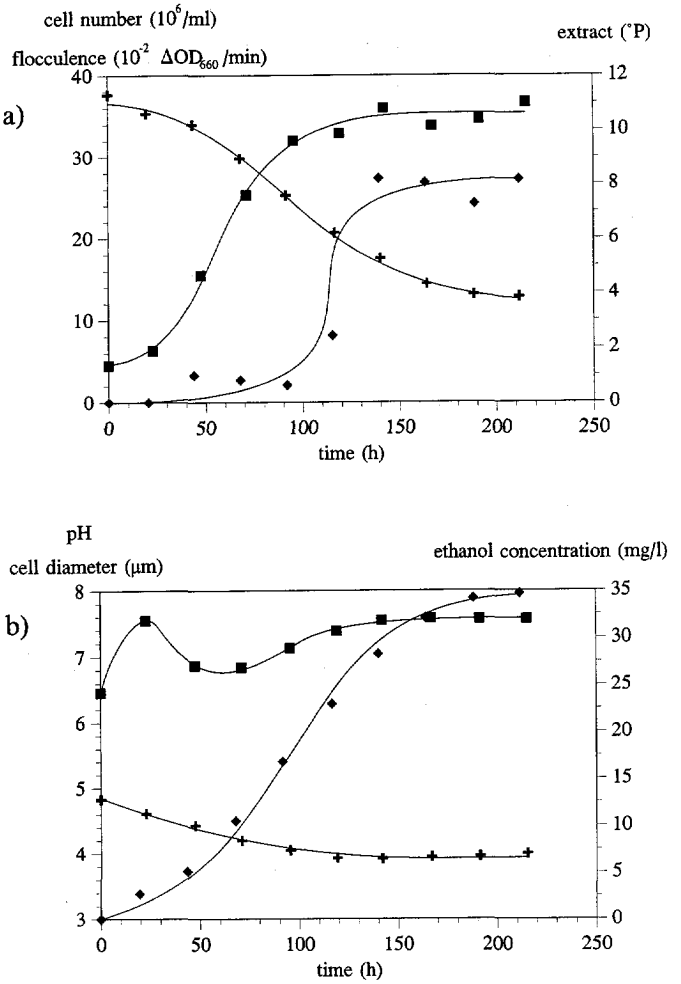

Figure 2. **a)** Cell number (■), flocculence (◆) and extract (+) as a function of time during a fermentation of *Saccharomyces cerevisiae*.
b) Parameters that directly or indirectly influence the colloid-chemical interaction between cells of *Saccharomyces cerevisiae* as a function of time. Cell diameter (■), pH (+), ethanol concentration (◆).

Furthermore the dielectric constant of the medium changes due to ethanol production. For wort the dielectric constant of water (78.5 (Weast, 1985)) was employed. The dielectric constant of ethanol is 24.3 (Weast, 1985). The dielectric constant of the

medium at the end of the fermentation was calculated by taking the weighted average of the dielectric constant of ethanol and water resulting in 75.8. The contact angles of nonflocculent and flocculent yeast were found to be 50° and 60°, respectively. The Hamaker constant was estimated from the results of Nir (1976). The ionic strength was calculated from the results of Mändl (1974). In Table 1 the values of parameters relevant to the physicochemical interactions of nonflocculent and flocculent yeast cells are presented. The values for nonflocculent cells were determined between 20 and 70 hours after pitching of the wort, the values for flocculent cells were determined at the end of the fermentation.

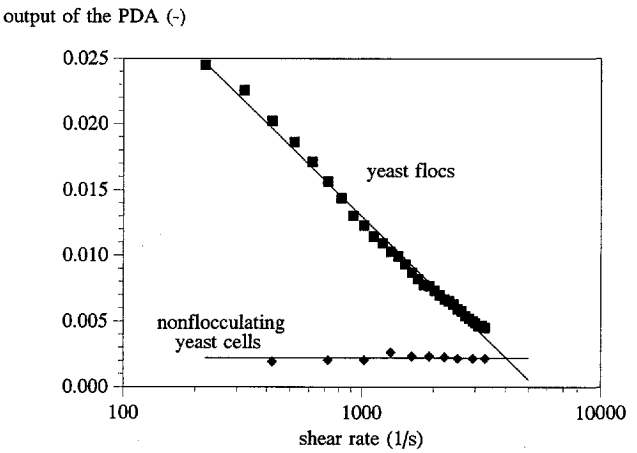

Figure 3. The state of flocculation as a function of the shear rate. The flocs are fully dispersed at a shear rate of 4000 s^{-1}.

To obtain an insight into the total interaction force between the cells a Photometric Dispersion Analyser (PDA) was used to measure the floc size at different shear rates. In Figure 2 the results of the experiments are shown. The output of the Photometric Dispersion Analyser depends on the size of the flocs. For nonflocculent cells the output of the apparatus is therefore independent of the shear rate. For yeast flocs the output decreases with increasing shear rate. The flocs become smaller until they are completely dispersed. To identify the shear rate at which complete dispersion was achieved the lines in Figure 2 were extrapolated and a value of 4000 s^{-1} was obtained.

DISCUSSION

During the fermentation, several parameters were subject to changes and influenced the net attraction energy between the cells. Below, the influence of each parameter on the attraction energy and repulsion energy is discussed.

The attraction energy Φ_A is dependent on the Hamaker constant and the radius of the cell. For microbial systems no exact values of the Hamaker constant are known. A theoretical estimation of the Hamaker constant for biological particles was made by Nir (1976), who found values between 0.24 and 1.46 kT. Lips and Jessup (1979) found a value of 0.75 kT for hydrocarbon layers and cell walls. For the calculations of the total attraction energy we assumed a value of 0.8 kT for nonflocculent and flocculent cells. Because it is almost impossible to translate the increase in hydrophobicity into a Hamaker constant, the increase in contact angle was neglected. Despite this, an increase in hydrophobicity would lead to an increase in attraction energy. The radius of the cells also increases during fermentation, as can be seen in Figure 1b, which also positively influences the attraction energy.

The repulsion energy Φ_R is dependent on the cell radius, the ionic strength, the cell wall potential and the dielectric constant of the medium. An increase in the cell radius (Figure 1b) will lead to an increase in the repulsion energy. The ionic strength decreases during the fermentation because of the biomass formation (Table 1), which has the same effect on the repulsion energy. The cell wall potential decreases during the fermentation, because of changing pH and ionic strength. This will lead to a decrease of the repulsion energy. For an ionic strength below 50 mM and a zeta potential above -40 mV the zeta potential is assumed to be equal to the wall potential mentioned in Equation 3 (Hiemenz, 1986). The dielectric constant of the medium decreases during the fermentation owing to the ethanol production, which leads to a decreasing repulsion energy.

The net attraction energy is calculated according to Equation 6. Figure 3 gives the potential energy curve for flocculent and nonflocculent cells. The parameters needed to compute the net attraction energy by Equations 2 and 1 for nonflocculent and flocculent cells are presented in Table 1, except the Hamaker constant which is held constant.

As can be seen from Figure 3 there is a very slight difference in the potential energy for flocculent and nonflocculent cells. The total attraction energy in the secondary minimum, where the flocculation is assumed to occur, is 15.0 kT for nonflocculent cells and 15.2 kT for flocculent cells.

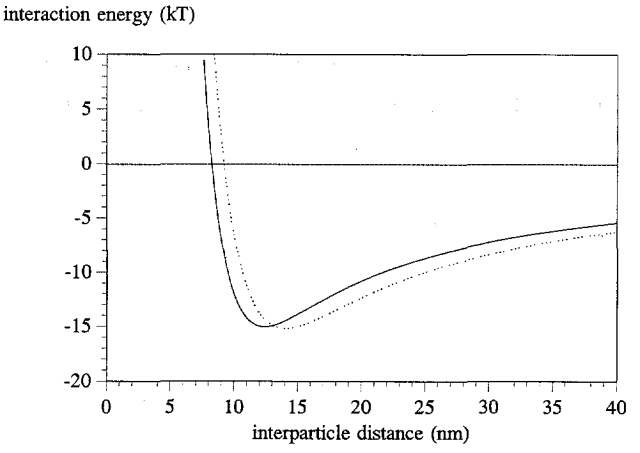

Figure 4. The net attraction energy between nonflocculent and flocculent cells of *Saccharomyces cerevisiae* as a function of the distance between the cells.

To show the sensitivity of the different parameters we first calculated the total interaction energy for nonflocculent cells and then changed each parameter to its value for flocculent cells. Subsequently the total interaction energy in the secondary minimum was computed; the results are shown in Table 1. To show the influence of the Hamaker constant it was multiplied by a factor of two. It can be seen that during the fermentation all parameters, except the ionic strength, change in such a way that flocculation is favoured. However, the net effect on the secondary minimum is only marginal.

The interaction energy given by Equations 1 and 2 can be translated into an interparticle force by taking the derivative of both Equations, $\delta\Phi = -F\,\delta(h)$, yielding,

$$F_A = -\frac{A\ r}{12\ h^2} \tag{8}$$

$$F_R = \frac{64\ \pi\ r\ n_0\ k\ T\ \Upsilon_0^2}{\kappa}\ e^{-\kappa\ h} \tag{9}$$

With this formula the total interparticle force between two flocculent yeast cells was calculated including the parameters mentioned in Table 1. The Hamaker constant was assumed to be 0.8 kT. The interparticle force was found to be 2.41×10^{-12} N.

The net attraction force between the cells was also directly measured. Goren (1971) derived an expression for calculating the force between two particles in laminar flow,

$$F = 6\ \pi\ \eta\ r^2\ \dot{\gamma}\ \sigma \tag{10}$$

where η is the viscosity of the fluid, r is the particle radius, $\dot{\gamma}$ is the shear rate and σ is a constant (in case of monodispersity $\sigma = 2.04$ (Goren, 1971)).

In Figure 2 it is shown that the flocs were fully dispersed at a shear rate of 4,000 s^{-1}. The attraction force between two flocculating cells was calculated and found to be 5.0×10^{-9} N. This is higher by a factor of 2000 than that predicted by the DLVO theory. Therefore it was concluded that for the attraction between flocculating cells the colloidal cell-cell interactions are unimportant.

If the interaction between cells was biochemical in nature, the number of bonds that holds two cells together could be estimated from the bond strength. Based on data from Bell (1978) and Poretz and Goldstein (1970) we estimated a bond strength of 2×10^{-10} N. This would imply approximately 25 bonds between two flocculating cells, which is not an unrealistic value.

will prevent close contact of the cells. Two cells can approach each other up to a distance of approximately 10 nm. However, recent investigations have shown that fibril-like structures appear on flocculating cells of *Saccharomyces cerevisiae* (Miki et al., 1982; Straver et al., 1994). These structures have a much smaller radius of curvature. Since the repulsion energy is more dependant on this radius than the attraction energy, small structures will receive less or no repulsion when approaching another surface. Therefore, these fibrils can bridge the repulsive barrier existing between yeast cells. (Van Loosdrecht et al., 1989).

CONCLUSIONS

The calculations based on the DLVO theory compared with the measurement of the actual interaction force show that physicochemical interactions play almost no role in the yeast flocculation process. The observed changes in hydrophobicity and surface potential of flocculent cells are therefore a reflection of the changing composition of the cell wall and do not induce flocculation by themselves.

Acknowledgements

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NOMENCLATURE

$\dot{\gamma}$	shear rate	s^{-1}
ε_0	permittivity of vacuum	$F\ m^{-1}$
ε_r	dielectric constant	-
η	viscosity of the fluid	$Pa\ s^{-1}$
κ	reciprocal double layer thickness	m^{-1}
ρ_0^{20}	density of the wort before pitching at 20 °P	$kg\ l^{-1}$
ρ^{20}	density of the medium at 20 °C	$kg\ l^{-1}$

ρ^{20}_{demi}	density of demineralized water at 20 °C	kg l^{-1}
σ	constant	-
Φ_A	attraction energy	J
Ψ_0	wall potential	V
Υ_0	parameter	-
A	Hamaker constant	J
e	elementary charge	C
E_0	initial extract of the wort before pitching	°P
E_w	extract of the medium	°P
f	sugar fraction	-
h	interparticle distance	m
I	ionic strength	M
k	Boltzmann constant	J K^{-1}
M_i	ion concentration	mol m^{-3}
n_0	ion number	m^{-3}
N_A	Avogadro constant	mol^{-1}
r	particle radius	m
T	temperature	K
z	valence	-

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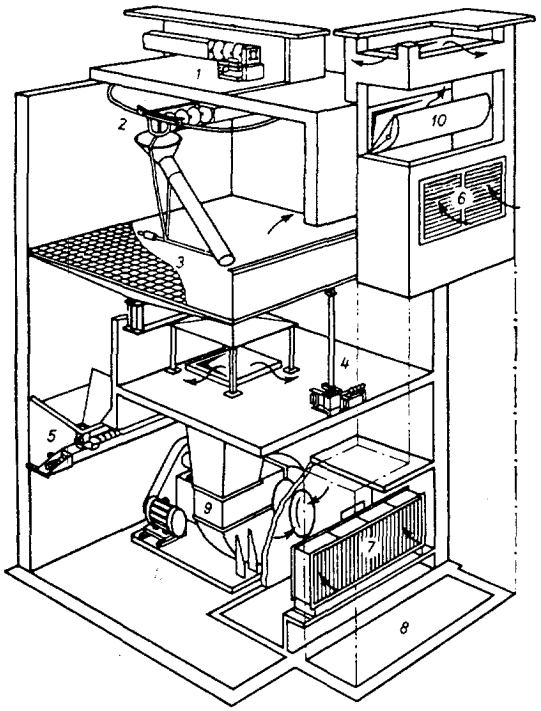


Kiln-house

1) malt corn from storage
2) malt corn feed
3) movable floor
4) driving-shaft to floor
5) malt transport
6) fresh air inlet
7) heat exchanger
8) air channel
9) fan
10) air release

... the malting and brewing process ...

kilning	- In a kiln the malt corns are dried. The rate of kilning determines the type of malt that will be obtained. A typical temperature trajectory (20 °C to 80 °C) for pilsner malts is applied in this process. Kilning is stopped at approximately 5% moisture, depending on the type of malt desired. The process takes about 24 hours. - Kilning is carried out to stop germination by drying out the malt grains. Next to this it determines the flavour and colour of the beer by following a typical temperature traject during the kilning process.

... to be continued on page 58 ...

CHAPTER III

On-line measurement of brewers' yeast flocculation during fermentation

Abstract

The separation of yeast at the end of a beer fermentation depends on the ability of the yeast to become flocculent, form flocs and sediment to the bottom of the fermenter. To monitor these processes an on-line method has been developed. With the instrument, a Photometric Dispersion Analyser, it is possible to determine flocculation on-line at the same conditions as in the fermenter.

INTRODUCTION

In the last two steps of the beer brewing process, fermentation and lagering, yeast plays a main role by forming ethanol and flavour components. After the fermentation process the major part of the yeast needs to be removed by sedimentation, while the yeast in suspension is needed for the lagering process. In this scope the feasibility to control the removal of the yeast with respect to the desired attenuation is important.

During the first stage of the beer fermentation yeast cells grow in number. Partway through the fermentation the cell division stops and the cells become flocculent that means they become able to form flocs. If the environmental conditions are optimal, flocs will be formed and settle out of the suspension.

Flocculation is defined by Stratford (1992) as 'the property of certain yeast strains to adhere into clumps, dispersible by EDTA or specific sugars, and the subsequent removal of these clumps from the medium'. This definition is a combination of earlier definitions (Stewart et al., 1976; Johnson et al., 1988). Flocculation must be distinguished from flocculence which is the tendency of a strain or a culture to form flocs under optimal environmental conditions (pH, Calcium concentration, temperature, state of agitation, initial cell number, etc.) (Masy et al., 1991). There are several tests to detect flocculation or flocculence. The first test widely used, was developed by Burns (1937). In this test a certain amount of yeast is suspended in tap water, the formed flocs sediment and after ten minutes the volume of the sedimented yeast is measured. The conditions during the test corresponded reasonably well with those of a fermentation. Therefore, this test can be regarded as a flocculation test. More recent methods use the sedimentation rate of the flocs measured by the change of the optical density (Miki et al., 1982; Smit et al., 1992). In these tests the flocculation is measured under optimal conditions (pH 4.5; 5 mM $CaCl_2$) and are therefore flocculence tests. Davis and Hunt (1986) used the same principle to measure flocculation, but their test was carried out with cells suspended in the growth medium.

A disadvantage of all these methods is the off-line character, making them less feasible for use in a process control strategy. Therefore we have studied the possibility of using

a Photometric Dispersion Analyser (PDA) for on-line quantification of flocculation of yeast during fermentation.

THEORY

A flowing suspension has certain optical properties which can be monitored by the PDA. For the measurement the suspension is pumped through a glass tube (diameter 1 - 3 mm). The tube is placed between a light source (λ = 850 nm) and a photodiode. The output of the photodiode consists of a steady state (dc) component and a varying (ac) part. The steady (average) voltage (V_{dc}) corresponds to the turbidity of the suspension according to the Lambert-Beer Law,

$$V_{dc} = V_{dc0}\, e^{-N\,C\,L} \tag{1}$$

where V_{dc0} is the incident light intensity, N is the number of particles, r is the particle radius, Q is the scattering coefficient, L is the length of the light path and C is the scattering cross section given by

$$C = \pi\, r^2\, Q \tag{2}$$

The scattering coefficient is mainly dependent on the size of the particles, for yeast cells or flocs (r > 4 μm) the coefficient is approximately 2 (Kerker, 1969).

The fluctuating signal is related to the variations in turbidity caused by the flow of suspension. As the fluctuations follow the Poisson distribution, the standard deviation about the mean value is equal to the square root of the average number of particles in the sample volume. The root mean square (rms) value of the ac-component represented by V_{rms} is therefore proportional to the standard deviation about the average number of particles. This leads to the following equation for Vrms (Gregory, 1985),

$$V_{rms} = V_{dc0}\, e^{-N C L} \left(\frac{N L}{A}\right)^{1/2} C \qquad (3)$$

in which A is the cross sectional area of the light beam.

The ratio of V_{rms} and V_{dc} has been shown to be the appropriate value to follow the state of aggregation/flocculation of particles (Gregory, 1985). The ratio value can be calculated by dividing V_{rms} by V_{dc} giving,

$$\frac{V_{rms}}{V_{dc}} = \left(\frac{N L}{A}\right)^{1/2} C \qquad (4)$$

When flocculation occurs the number of particles will decrease, but their scattering cross-section (C) will increase more strongly because of the loose packing of particles within a floc. Therefore, flocculation will cause a net increase in the ratio value.

MATERIALS AND METHODS

The fermentations were carried out in a stirred vessel (1.5 l) with standard geometry as described by Perry et al. (1984). The cells were grown in brewery wort (12°P) at 9°C. Before inoculation the medium was saturated with air and the headspace was filled with nitrogen gas. The inoculum of the used flocculent strain of *Saccharomyces cerevisiae* (MPY-3) was pregrown on wort at 15°C. After pitching the initial cell number was 4×10^6 cells/ml. All tubing used was made of Viton or glass to be sure that the fermentations were carried out under strictly anaerobic conditions.

Figure 1 gives a schematic representation of the experimental set-up. A glass capillary tube with a diameter of 1.5 mm was used for the measurements. The state of flocculation was monitored with a photometric dispersion analyser (PDA-2000, Rank Brothers Ltd., Bottisham, U.K.). During the whole fermentation the ratio of the Vrms and the Vdc was measured on-line at a shear rate of 455 s^{-1} in the tube.

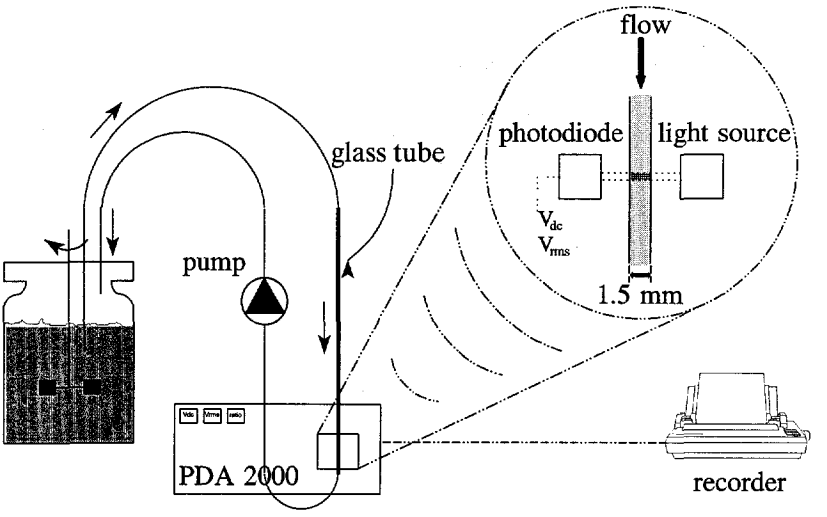


Figure 1. Experimental set-up for the PDA-measurements.

Besides the PDA-measurements, a test to monitor the flocculence of the yeast cells was carried out, using the method described by Smit et al. (1992). The cell size and number were detected with a particle counter (Elzone 280 PC).

RESULTS

The characteristics of standard batch fermentation are shown in Figure 2. After inoculation of the medium the cells divide until a maximum of circa 33 $\times$ 10^6 cells per ml is achieved. At this point, 100 h after inoculation, the cells become flocculent as can be seen from the results of the flocculence test. When the cell division stops the cells continue to grow to a size of circa 7.5 μm. In the course of the fermentation sugars in the wort are being converted into ethanol.

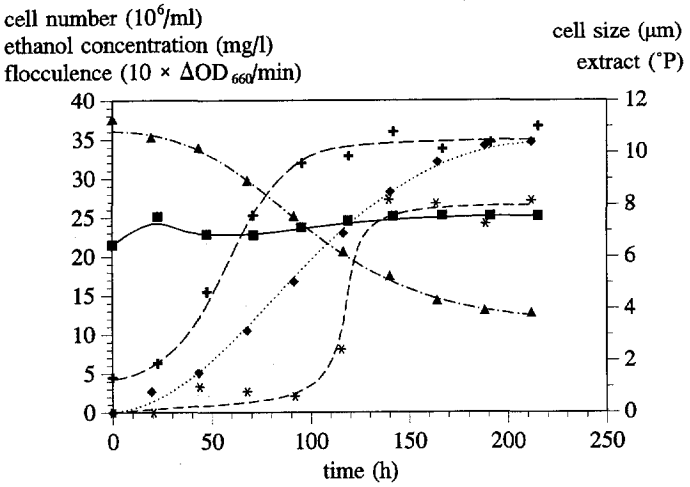


Figure 2. Change of characteristic parameters during a fermentation of wort. (cell size (■), cell number (+), ethanol concentration (◆), extract (▲), flocculence (*))

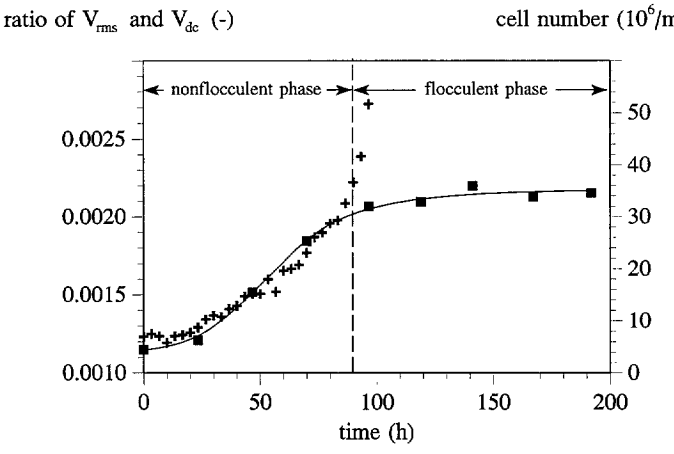
During the first part of the fermentation the ratio value shows a small increase. With Equation 4 it can be calculated that this increase is mainly due to the increase of cell number, the results in Figure 3a confirm this. After 100 h the number of cells becomes constant, only the cell size increases from 100 h until 140 h. This causes an increase of the ratio value of approximately 15%, which is negligible compared to the increase caused by flocculation (Figure 3b).

In Figure 3b the off-line flocculence test and the on-line PDA measurement are compared to each other. The ratio value increases by a factor of about 17 from the moment the cells start to flocculate until a constant value is reached after about 160 h (Figure 3b). The flocculence test however shows a maximum at only 140 h.

DISCUSSION

During the fermentation process yeast cells become flocculent and start to flocculate, which will lead to sedimentation of yeast flocs. Several methods are used to measure the ability to flocculate (flocculence), however, these are all off-line methods. Here an on-line method has been developed.

a)



b)

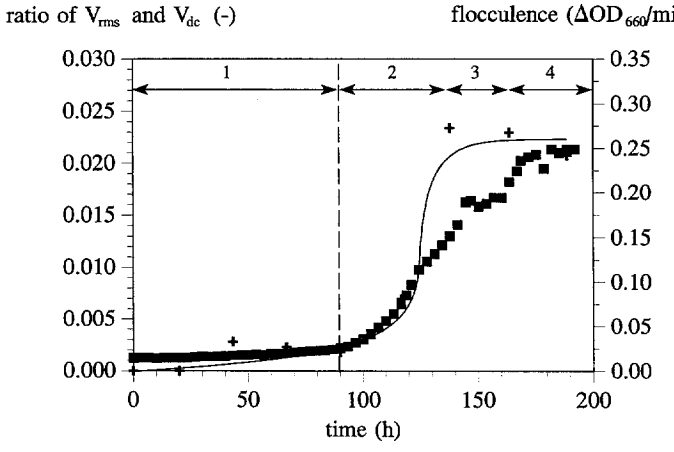


Figure 4. **a)** Comparison between the number of yeast cells (■) as determined by a particle counter and the output of the PDA (+).
b) Comparison between flocculence (■) and flocculation (+) of brewer's yeast during a fermentation. 1: growth phase, 2: increase in flocculence, 3: increase in flocculation, 4: steady state

Floc formation depends on many conditions as agitation (Stratford & Keenan, 1987), temperature (Kamada & Murata, 1984), initial number of particles, surrounding medium etc.. In the reported off-line assays to monitor flocculence or flocculation (Burns, 1937; Davis and Hunt, 1986; Miki et al., 1982; Smit et al., 1992) the agitation of the yeast suspension remains undefined. In this way the degree of flocculation is very hard to quantify and can be influenced easily by experimental conditions. Most of these methods are based on dynamics of floc settling. In the early phase of flocculation the formed flocs are very small which makes it difficult to determine the onset of the flocculation process.

In the course of the growth phase of the fermentation process the output of the apparatus only depends on the number of cells, because the size of the cells hardly changes and flocculation will not occur. This makes the employed method suitable of detecting the increase of cell number during the first part of the fermentation.

At the moment the cells start to form flocs the cell number is constant but the total number of particles and the size of the particles will change strongly. This will immediately lead to a change in the output of the PDA. In this way the onset of flocculation can be detected in an early state and flocculation can be followed during the remainder of the fermentation.

Within the PDA the yeast suspension flows through a tube, in this tube the flow conditions are laminar and the shear stress on the yeast flocs can be regulated by the flow rate. In this way the 'agitation' is well defined. Because the shear rate can be regulated, it is possible to monitor the flocculation at similar shear rate as in the fermenter. For this, shear rate within the PDA can be adapted to that in the fermenter any time by increasing or decreasing the flow rate within the measuring tube.

Because the PDA measurement is on-line without a need to separate the cells from the medium (as in most off-line tests), the flocculation can be monitored under the actual conditions (temperature, medium composition, etc.) giving the best information to a control strategy of the flocculation process.

CONCLUSIONS

The PDA is a reliable instrument for on-line monitoring of flocculation. The apparatus makes it possible to (i) follow the cell division during the growth phase of the fermentation (ii) detect the onset of flocculation and (iii) monitor the flocculation during the second phase of the fermentation.

Acknowledgements

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NOMENCLATURE

A	cross sectional area of the light beam	m^2
C	scattering cross section	m^2
L	length of the light path	m
N	number of particles	-
Q	scattering coefficient	-
r	particle radius	m
V_{dc}	light intensity	V
V_{dc0}	incident light intensity	V
V_{rms}	root mean square value of the light intensity	V

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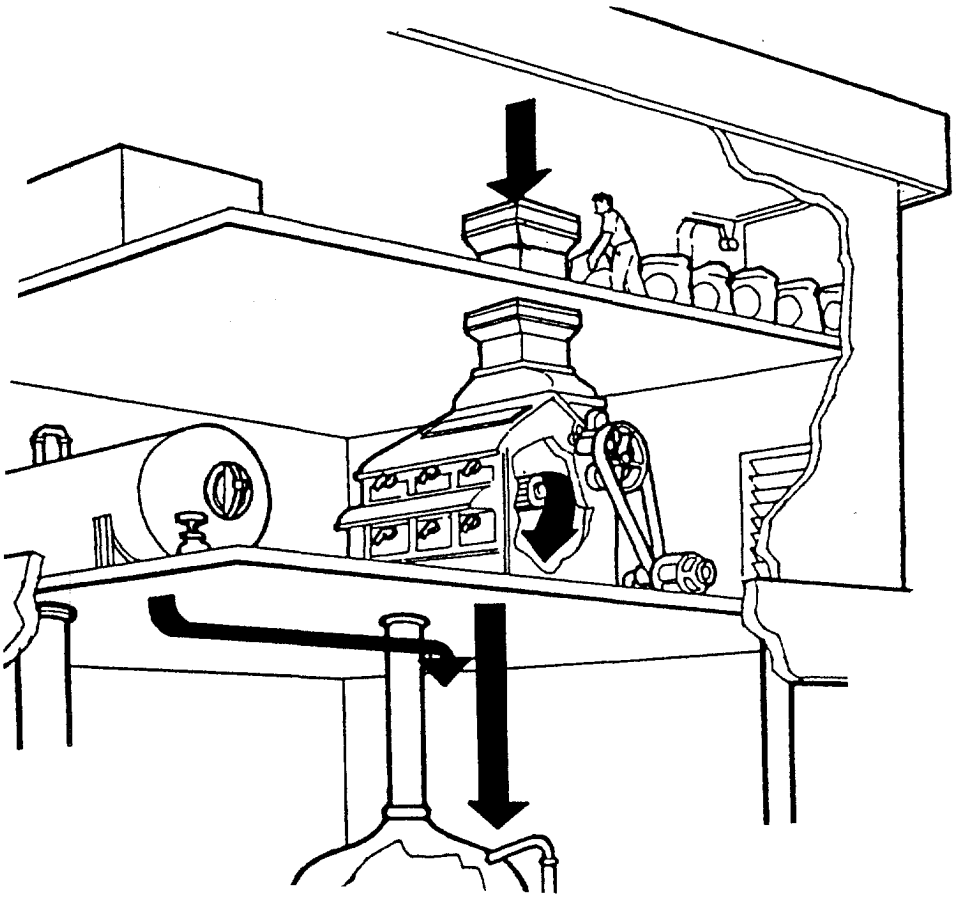

... the malting and brewing process ...

milling	- Milling is carried out in a 4 or 6-roll mill. To keep the husk intact some brewers spray the malt with water. Another method is to subject it to steam just before it enters the mill. - The objective of milling is crushing of the malt to produce grist. It is important that the husk is kept intact, for it is needed to form a permeable filter during wort recovery from the mash.

... to be continued on page 92 ...

CHAPTER IV

On the measurement of the flocculation characteristics of brewers' yeast

Abstract

The capacity of certain yeast strains to flocculate is important to the brewing industry. So is the determination of the flocculation characteristics of a yeast strain. In this study we subdivided the flocculation characteristics into three phenomena. A proposal for the most suitable method to quantify each phenomenon is given. For this, four parameters (bond strength, floc size, settling rate and number of single cells) that serve as a measure to these phenomena have been studied. Next to this, attention is payed to the influence of environmental conditions (temperature, calcium concentration, pH and the hydrodynamic conditions during the test) on the result of the test. During this part of the study the flocculence of the yeast cells was constant, so the effect of the yeast on the results of the test is excluded. It turned out that the temperature of the medium and the hydrodynamic conditions during the test most strongly influence floc formation. Next to this, medium viscosity is important if the flocculation characteristics are quantified via settling experiments.

INTRODUCTION

In the brewing process yeast flocculation is used to remove yeast from green beer at the end of the fermentation. Therefore, it is of prime concern for a brewer to select a yeast strain with the appropriate flocculation characteristics. Weakly flocculating yeast will lead to problems during lagering and subsequent filtration of the beer. On the other hand a strongly flocculating yeast strain will lead to early sedimentation of yeast while complete attenuation has not yet been reached. Consequently it is important to be able to detect the flocculation characteristics of a yeast strain and to translate this to brewing conditions. Speers et al. (1992a) noticed that many methods to measure the flocculation characteristics of yeast can be found in literature. Unfortunately in the literature contradictions are present due to the various types of empirical methods to quantify yeast flocculation (Calleja, 1987). Next to this, it is mostly unclear how the measured parameter has to be interpreted.

The method used mostly, is derived from Burns (1937). Hereby a fixed number of yeast cells are suspended in tap water. The flocs formed settle and after ten minutes the amount of settled yeast is measured. Since Burns introduced this test, many variants on this test were developed. Helm et al. (1953) substituted the tap water with a pH 4 acetate buffer containing some calcium. Instead of the amount of settled yeast Mill (1964a) measured the turbidity decline of a flocculating yeast suspension in a colorimeter. The most recent flocculation test based on the settling characteristics of yeast flocs was developed by Smit et al. (1992).

Next to these tests, which are mainly based on the settling velocity of yeast flocs, other tests were developed. Taylor and Orton (1975) measured the 'melting point' of yeast flocs to qualify yeast flocculation. This test is based on measuring the turbidity of a yeast suspension at different temperatures. The 'melting point' is the temperature at which complete dispersion is reached. Eddy (1955) dispersed the flocs by adding sugars (mannose and maltose) to the yeast suspension. In this case the critical sugar concentration is the 'melting point' or 'flocculation value'. Stahl et al. (1983) used EDTA instead of sugar to disperse the flocs. Stewart and Russell (1981) noted the need for a standard flocculation test that, however, has not been developed until now.

The large number of tests to quantify flocculation characteristics is not surprising, if one regards the definition of flocculation (Stratford, 1992): "the property of certain yeast strains to adhere into clumps, dispersive by EDTA or specific sugars, and the subsequent removal of these clumps from the medium". We propose to define flocculation according to the factors involved in the process. For this, the definition consists of three parts representing the factors involved in the adhesion of yeast cells into flocs. For flocculation to occur three conditions have to be met:

i) The cells must be flocculent.

i) The composition of the medium must be such as to allow binding between the flocculent cells: at least 10^{-8} mol/l calcium is necessary, the temperature must be between 5 °C and 50 °C (both conditions are strain dependent).

iii) The cells must come together in the liquid. This can only occur if velocity gradients are present in the liquid and if the cell concentration is high enough.

Based on these conditions the definitions are as follows,

- Flocculence was defined by Amory et al. (1988): "Flocculence is the ability of a yeast strain to form flocs under optimal environmental conditions (presence of the right inducer, optimal temperature and sufficient cell material to allow the flocs to be formed)".
- The definition of flocculability is derived from Kruyt (1949). Flocculability is the ability of a yeast strain to flocculate in a given medium under favourable environmental conditions (the right interactions between cells and flocs).
- Finally, flocculation is the actual floc formation under the actual environmental conditions.

Translating the phenomena mentioned in Table 1 in terms of the size of yeast flocs at a certain moment during fermentation, the following can be said. Flocculence corresponds to the maximum size the flocs will reach under optimal conditions. Flocculability corresponds to the maximum size the flocs can reach in the wort/beer (or other given medium). Finally, flocculation reflects to the actual size that the flocs will have under brewing conditions.

Table 1 The way environmental conditions have to be chosen to characterize the phenomena, flocculence, flocculability and flocculation of yeast cells.

environmental conditions	phenomenon		
	flocculence	flocculability	flocculation
medium (composition, properties)	optimal	given	given
hydrodynamic conditions; yeast amount	optimal	optimal	given

In this report we want to present four parameters to quantify the flocculability of yeast cells in beer. We measured the bond strength of the yeast cells, the size of the flocs, the settling rate of the flocs and the number of single cells. To investigate the suitability of the parameters to be a measure to flocculability, the environmental conditions (medium, yeast concentration and hydrodynamic conditions) were varied. The parameters mentioned can also be used to serve as a measure for the flocculence and flocculation characteristics of a yeast suspension by choosing the right conditions.

MATERIALS AND METHODS

A matrix of experiments was carried out to quantify the flocculability of yeast cells. Four parameters were investigated on their suitability to be a measure for flocculability. To achieve this aim the effect of environmental conditions, medium as well as hydrodynamic conditions, on the flocculability of yeast cells was studied. The experiments were carried out in beer with flocculent yeast cells. To exclude the effect of the yeast on the results, the flocculence of the yeast was constant during all experiments. The matrix of experiments is given in Table 2.

Environmental conditions

medium and cells

Medium and yeast cells were obtained from a standard beer fermentation as described earlier (Van Hamersveld et al., 1994). A total of six fermentations, with identical growth conditions, was carried out to provide medium and cells for the experiments. The reproducibility of the experiments was high. The average medium composition of the

fermentation was as follows (the relative error is given between brackets): pH 4.0 (±5%), calcium concentration: 1.2 mol/m^3, extract: 3.8 °P (±3%), ethanol concentration: 35 g/l (±3%), viscosity: 2.3 mPa s (±5%), temperature: 9 °C. The viscosity of the medium was measured with an Ostwald viscosimeter. So the medium was constant for all experiments except those experiments whereby pH and calcium were changed. In that particular case the medium from the fermentation was used as starting material.

Table 2 The experiments described in this paper are enumerated. Four parameters as measure for the flocculability of yeast cells were examined by varying the environmental conditions between the mentioned values. In case of measuring settling rate and number of single cells the flow conditions were turbulent. In the other cases the flow conditions were laminar.

	variation of the environmental condition			
	medium			hydrodynamics
measured parameter	pH	calcium concentration (mM)	temperature (°C)	shear rate (s^{-1})
bond strength	4 - 7	1 - 13	2 - 28	-
floc size	4 - 6.8	1 - 13	2 - 28	200 - 3500
settling rate	3.5 - 8.3	1 - 12	3 - 25	25 - 500
number of single cells	3.5 - 8	1 - 12	3 - 25	100 - 500

The used cells of the pure strain *Saccharomyces cerevisiae* (MPY-3) were flocculent. The pureness was checked by DNA fingerprinting. The dead cell percentage was determined using methylene blue and did not exceed 5%. The variation of this percentage between the experiments was less than 1%. The cell concentration after each fermentation was 35 × 10^6 cells per ml (±10%). Flocculence was measured according the method of Smit et al. (1992) and had a value of 0.25 ΔOD_{660}/min (±15%) during all experiments. The diameter of the yeast cells (needed to calculate the bond strength between the cells) was measured by a particle counter (Elzone PC 280).

The experiments were carried out 'in situ' after the fermentation. This was done to maintain aseptic conditions during the experiments to minimize the chance of contamination. The contamination by bacteries was checked after each experiment by microscope, no contamination was found in this way. The experiments were done in a

fully attenuated medium, so growth did not occur during the experiments, this was verified by counting the cells again after the experiment. In case of the experiments at different cell concentrations the cells were separated from the medium by centrifuge. After this, the cells were resuspended in the medium to reach the desired concentration. This was done at a constant temperature (9 °C) under anaerobic conditions.

The experiments were carried in a medium without fermentable sugars, so no cell division could occur during the experiments. The experiments lasted a few hours. If possible the conditions were returned to the starting condition after the last experiment, in this way it was checked whether any changes had occurred during the experiment, which was not so. Next to this, the flocculence of the cells was also measured and did not change during the experiments.

The effect of medium on the flocculability of the cells was examined by changing the pH, calcium concentration and temperature of the medium. The pH was adjusted by adding a 1 M NaOH solution. The calcium concentration was adjusted by adding a 1 M $CaCl_2$ solution. Wort and beer contain a calcium concentration of approximately 1 mol/m^3 (Mändl, 1974) so the calcium concentration could only be varied above this value. The temperature was adjusted with an element placed in the vessel.

Hydrodynamics

The hydrodynamic condition forms one of the factors determining the flocculation of brewers' yeast. The liquid flow within a vessel or tank can either be laminar or be turbulent. In this study both cases have been examined. In laminar flow the fluid slips as parallel planes over each other (Figure 1). The tension caused by this flow is called shear stress. The shear stress (τ_s) within the fluid can be calculated by dividing the force (F) acting on the planes by the area (A) of the planes ($\tau_s = F/A$). The shear stress will determine the formation of the yeast flocs and is therefore an important factor during the flocculation of yeast cells. However, the hydrodynamic conditions of a system are mostly expressed in terms of shear rate ($\dot{\gamma}$) which is defined as the velocity gradient within the fluid ($\dot{\gamma} = dv_x/dy$). The shear stress can be calculated from the shear rate and the viscosity (η) of the fluid ($\tau_s = \dot{\gamma}\ \eta$).

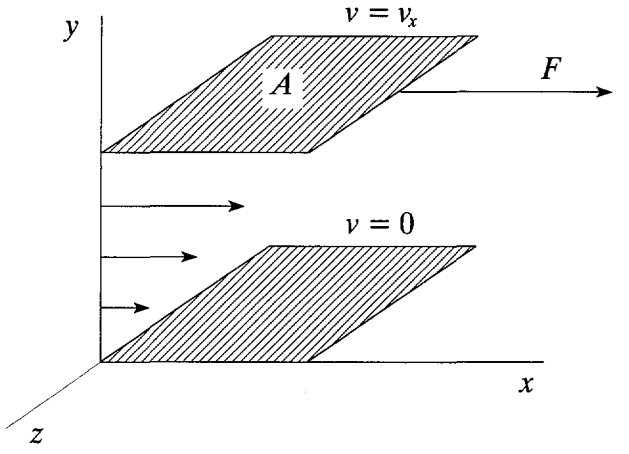

Figure 1. Schematic representation of laminar liquid flow. The velocity gradient (shear rate) can be calculated by dividing the velocity of the liquid in the x-direction by the distance between the planes in the y-direction ($\dot{\gamma} = dv_x/dy$). Shear stresses acting on the flocs depend on shear rate and viscosity of the liquid ($\tau_s = \dot{\gamma}\ \eta$).

In turbulent flow the fluid motion is random in space and in time. For this it is difficult to define the shear rate, which is needed to calculate the shear stress. Camp and Stein (1943) succeeded in deriving an equation for shear rate in turbulent flow, which depends on the power input into the system (see Appendix 1).

The floc size and bond strength measurements were carried out under laminar flow conditions. The settling rate measurements and the measurement of the number of single cells were performed under turbulent flow conditions. The yeast suspension was exposed to laminar flow by circulating the suspension through a capillary tube. The maximum Reynolds number during the experiments was 350. Turbulent flow arises at Reynolds numbers above 2000 (Beek and Muttzall, 1986). The turbulent conditions were obtained in a 2 l stirred vessel of standard geometry equipped with a Rushton turbine (Beek and Muttzall, 1986).

Parameters to quantify flocculability

Bond strength

The bond strength of the yeast cells can be determined by dispersing the flocs in a laminar flow field. By means of determination of the shear rate (γ) at which dispersion of the flocs is achieved the bond strength (F) can be calculated (Goren, 1971),

$$F = \frac{3}{2} \pi d_c^2 \eta \dot{\gamma} \sigma \qquad (1)$$

where η is the viscosity of the medium, d_c is the diameter of the yeast cells, which was 7.2 μm. The distribution of the cell sizes was lognormal with a standard deviation of 0.15. The shape factor (σ) had a value of 2.04.

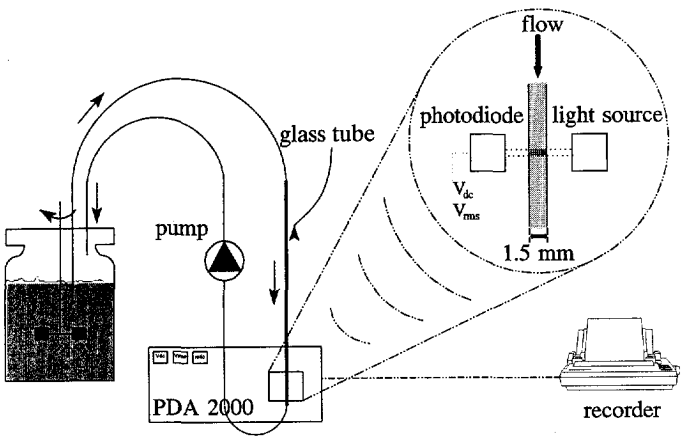

Figure 2. The experimental setup for the PDA measurements. Bond strength of the yeast cells and the size of the yeast flocs were measured with this setup.

For detection of the shear rate at which dispersion of the yeast flocs is achieved a Photometric Dispersion Analyser (PDA) was used (Figure 2). The yeast suspension was monitored by pumping it through a capillary tube placed between a light source

(λ = 850 nm) and a photodiode. The tube had an internal diameter of 1.0 mm and was made of glass. The PDA measures two properties of the yeast suspension flowing through the tube, the turbidity of the suspension and the turbidity fluctuations caused by the flow (Gregory, 1985). The output of the PDA is expressed in terms of the ratio of these two signals and is approximately linear with the size of the flocs within the suspension (see Appendix 2).

The 'dispersion shear rate' was determined by increasing the shear rate within the tube and comparing the output (signal ratio) of the PDA with the signal ratio of a nonflocculating yeast suspension with the same number of yeast cells (Van Hamersveld et al., 1994). The nonflocculating suspension was prepared by adding EDTA to the medium (complexes with calcium). By means of the 'dispersion shear rate' and the results of the viscosity measurements the bond strength could be calculated (Equation 1). The bond strength measurements were carried out at various medium conditions.

Floc size

Floc size measurements were carried out with the same setup as described above. The tube used during these experiments had a diameter of 1.5 mm.

During the measurements the medium and hydrodynamic conditions were varied as described above (Table 2). The hydrodynamic conditions were varied by changing the flow rate through the measuring tube. To prevent turbulent flow within the tube the Reynolds number was kept below 2000. During the experiments the shear rate was varied between 200 and 3000 s^{-1}. To provide constant hydrodynamic conditions within the vessel the stirrer speed was kept constantly at 150 rpm during the measurements. The floc size was expressed in terms of signal ratio (Appendix 2).

Settling rate

The settling rate of the yeast flocs in a quiet medium was measured with the setup as shown in Figure 3. A 2 l stirred vessel was modified by installation of a tube on the vessel. A spectrophotometer was fixed around the tube to measure the optical density (OD_{660}) of the yeast suspension in the tube (λ = 660 nm). Inside the tube a plunger could be operated to move the yeast suspension in front of the spectrophotometer. When the suspension reaches the quiet zone within the tube, the yeast flocs start to settle. The

settling of the yeast flocs causes a decline of the OD_{660}. During the settling measurement the tube was closed by a plug to prevent mixing in the tube. A recorder registered the signal.

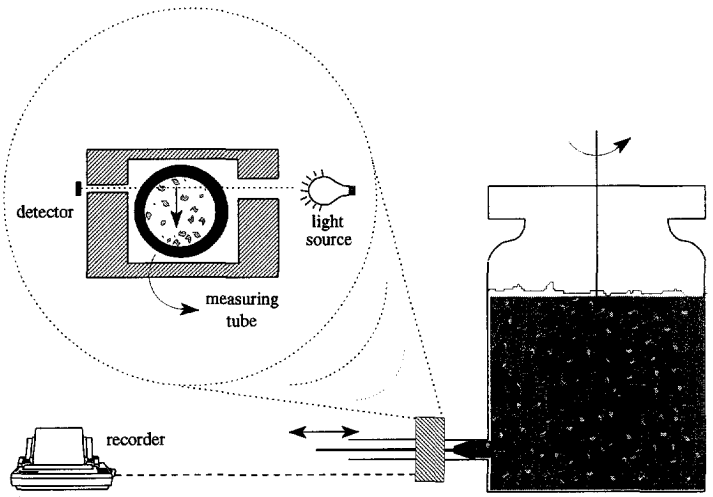

Figure 3. Experimental setup for measurement of the settling rate of yeast flocs and the number of single cells in a flocculating yeast suspension.

Figure 4 represents an example of the recorder signal. From the recorder signal a decline of the OD_{660} could be derived by determining the steepest slope of the curve. This slope can be taken as a measure for the settling rate of the flocs (Mill, 1964a). The settling rate is therefore expressed in terms of OD_{660} decline in time.

Number of single cells

With the setup as described above it was also possible to detect the number of single cells (or very small flocs). During the settling of the yeast flocs the single yeast cells will remain in suspension due to their low settling rate compared to the flocs. If all flocs are settled or at least passed the light beam, the OD_{660} decline will stop as can be seen in Figure 4. The value of the OD_{660} at that point corresponds to the number of single cells. A sample from the fermentation broth was analysed under a microscope to check for the appearance of single cells.

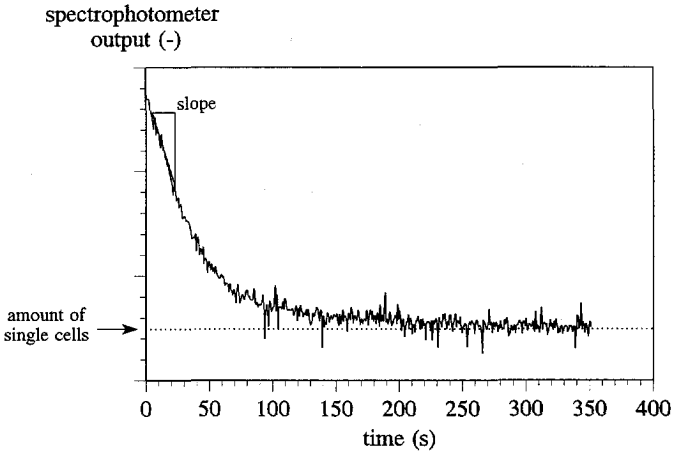

Figure 4. Example of a recorder output during measurement of settling rate and number of single cells. The slope of the curve represents the settling rate, while the plateau corresponds to the number of single cells.

The apparatus was calibrated with single cells suspended in an EDTA solution to prevent flocculation. The OD_{660} was linear with the number of cells in suspension. The number of single cells is expressed in terms of the fraction of the total number of yeast cells.

RESULTS AND DISCUSSION

The results of the experiments are discussed below. First the results on varying environmental conditions (medium, hydrodynamics) are evaluated. Secondly the parameters determined to quantify the flocculation characteristics are discussed. In the last part a proposal to standardize flocculation assays is given. The flocculating yeast suspensions contained flocs and single cells, which was confirmed by analysis with a microscope.

Environmental conditions: Medium

pH

Increasing pH leads to a small decrease in bond strength and floc size, as is shown in respectively Figure 5 and Figure 6. The settling rate of the flocs shows an increase if the pH changes from 3.5 to 4.5 (Figure 7). At higher pH the settling rate decreases again. The effect of pH on the number of single cells is shown in Figure 8. The number of single cells increases with increasing pH within the range pH 4.5 to pH 8.

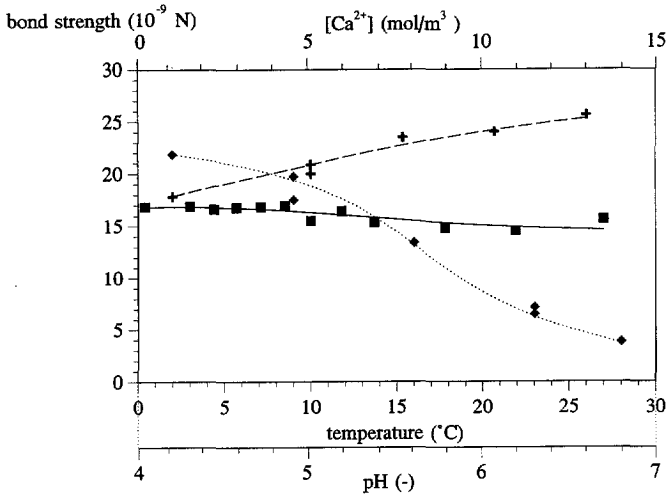

Figure 5. The bond strength between yeast cells as determined by dispersing the flocs in a high shear field. The composition of the medium is varied: pH (■), calcium concentration (+) and temperature (♦).

The results from the settling rate of the flocs confirm the findings of Mill (1964b) and Smit et al. (1992). Both authors quantified the flocculence of yeast cells by quantification of the settling velocity of the flocs. Both carried out their test in a 50 mol/m^3 Sodium Acetate buffer containing 0.1% $CaCl_2$. Apparently the effect of pH in beer as examined in this study is similar to that in an artificial medium.

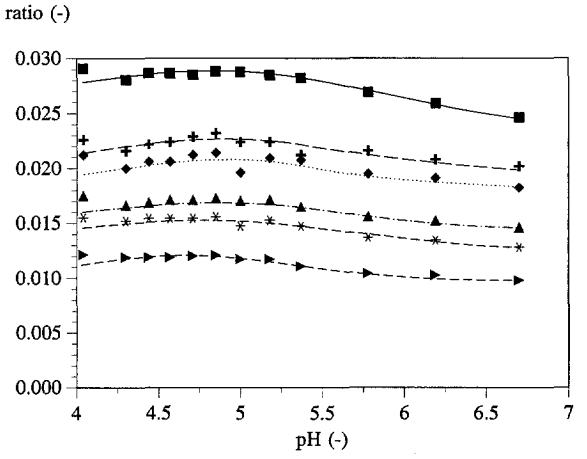

Figure 6. Floc sizes as a function of the pH of beer measured at various shear rates in the tube. The size of the yeast flocs is expressed in terms of ratio (see Appendix 2). $\dot{\gamma}$ = 250 s^{-1} (■), 500 s^{-1} (+), 700 s^{-1} (♦), 950 s^{-1} (▲), 1100 s^{-1} (*) and 1400 s^{-1} (►).

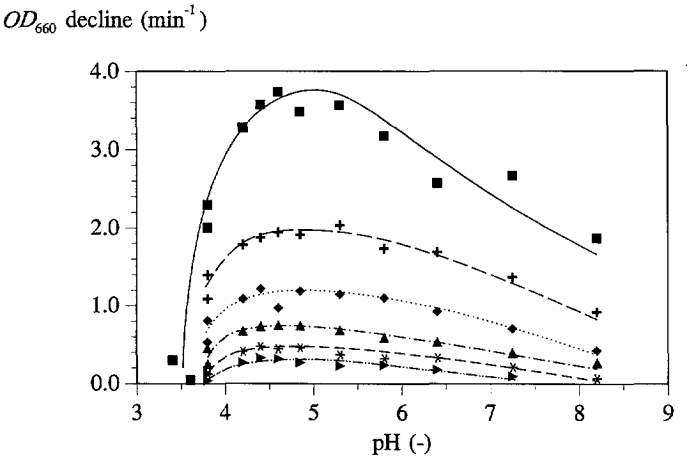

Figure 7. Settling rates of yeast flocs as a function of the pH of beer at various hydrodynamic conditions. The settling rate is expressed in terms of OD_{660} decline. The hydrodynamic conditions are expressed in terms of stirrer speed (N). N = 100 rpm (■), 200 rpm (+), 300 rpm (♦), 400 rpm (▲), 500 rpm (*) and 600 rpm (►). For translation to shear rates, see Appendix 1.

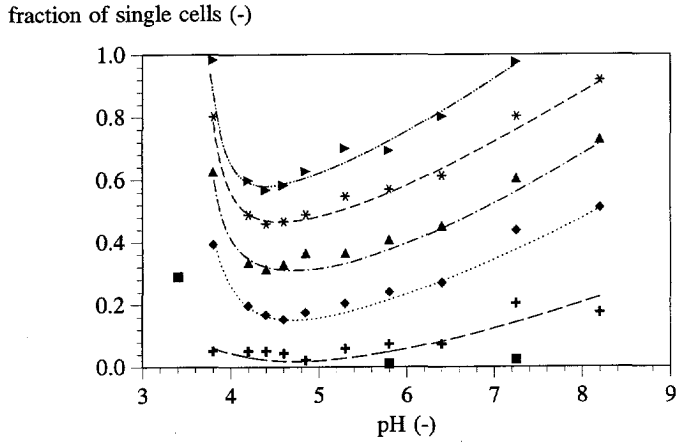

Figure 8. Relation between the number of single cells and the pH of beer, measured at various hydrodynamic conditions, which are expressed in terms of stirrer speed (N). N = 100 rpm (■), 200 rpm (+), 300 rpm (♦), 400 rpm (▲), 500 rpm (*) and 600 rpm (►). For translation to shear rates, see Appendix 1.

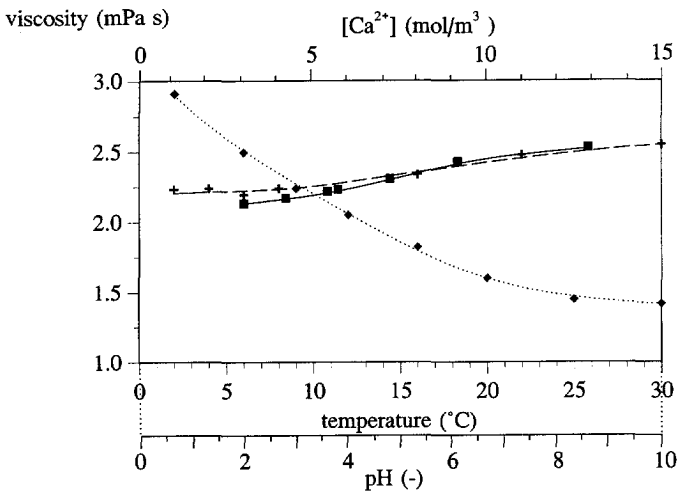

Figure 9. Relation between the viscosity of beer and pH (■), calcium concentration (+) and temperature (♦).

The results of the bond strength (Figure 5) and floc size measurements (Figure 6) show a smaller decrease as expected from the settling measurements in the same pH range. The difference can be explained from the increase of the viscosity in the pH range as is shown in Figure 9. The viscosity increases about 20% between pH 2 and pH 8. According to Stokes' equation the settling velocity of particles is inversely proportional to the viscosity, which explains the relatively large decrease in settling velocity as the pH increases. If the settling velocity is taken as a measure to determine flocculability, the influence of viscosity must be allowed for.

Calcium concentration

Beer and wort contain approximately 1 mol/m^3 (Mändl, 1974). The effect of adding calcium to beer is relatively low this can be explained from the fact that the largest increase in flocculability of yeast cells is between a calcium concentration of 0 to 1 mol/m^3 (Amory et al., 1988). Above 1 mol/m^3 the increase in flocculability was reported to be almost zero (Masy et al., 1991).

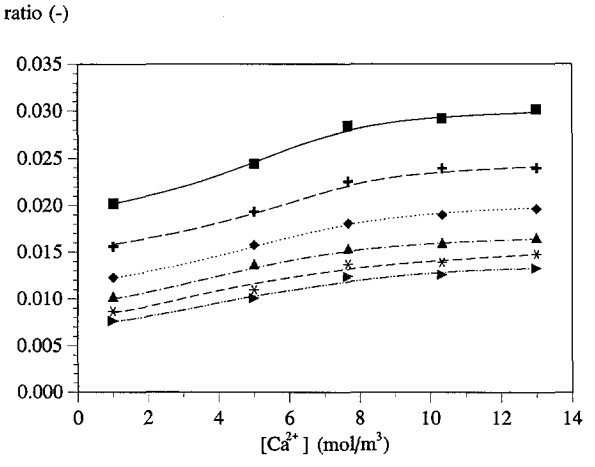

Figure 10. Floc sizes as a function of the calcium concentration in beer measured at various shear rates in the tube. The size of the yeast flocs is expressed in terms of ratio (see Appendix 2). $\dot{\gamma}$ = 400 s^{-1} (■), 700 s^{-1} (+), 1000 s^{-1} (♦), 1300 s^{-1} (▲), 1600 s^{-1} (*) and 1900 s^{-1} (►).

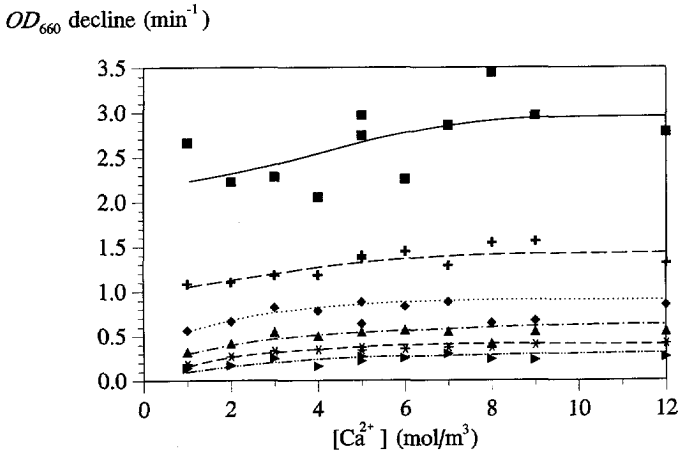

Figure 11. Settling rates of yeast flocs as a function of the calcium concentration in beer at various hydrodynamic conditions. The settling rate is expressed in terms of OD_{660} decline. The hydrodynamic conditions are expressed in terms of stirrer speed (N). N = 100 rpm (■), 200 rpm (+), 300 rpm (♦), 400 rpm (▲), 500 rpm (*) and 600 rpm (►). For translation to shear rates, see Appendix 1.

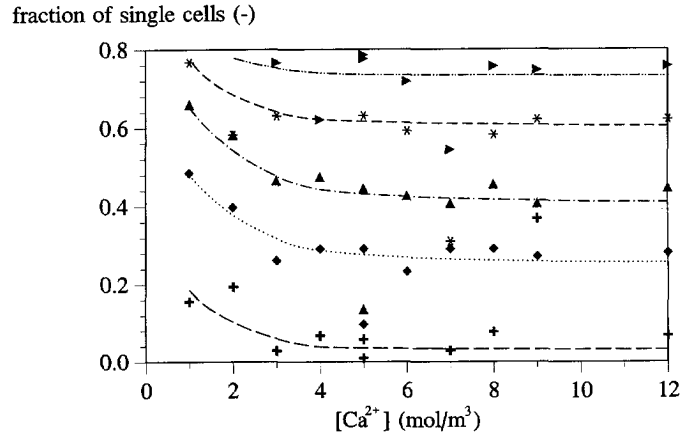

Figure 12. Relation between the number of single cells and the calcium concentration of beer, measured at various hydrodynamic conditions, which are expressed in terms of stirrer speed (N). N = 100 rpm (■), 200 rpm (+), 300 rpm (♦), 400 rpm (▲), 500 rpm (*) and 600 rpm (►). For translation to shear rates, see Appendix 1.

Figure 5 and Figure 10 show that the bond strength between the yeast cells and the size of the yeast flocs increases if the calcium concentration is increased above 1 mol/m^3. The settling velocity shows a smaller increase within the same concentration range (Figure 11). This apparent discrepancy can again be explained from the viscosity of the medium (Figure 9). The viscosity increases with increasing calcium concentration yielding a lower settling velocity of the yeast flocs. The number of single cells decreases with increasing calcium concentration (Figure 12). These results correspond to the results obtained from the measurement of bond strength (Figure 5) between the cells at the same calcium concentration.

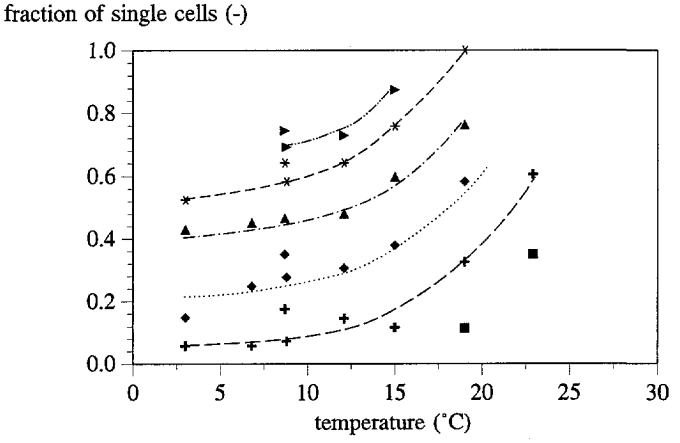

Figure 13. Relation between the number of single cells and the temperature of beer, measured at various hydrodynamic conditions, which are expressed in terms of stirrer speed (N). N = 100 rpm (■), 200 rpm (+), 300 rpm (♦), 400 rpm (▲), 500 rpm (∗) and 600 rpm (▸). For translation to shear rates, see Appendix 1.

Temperature

The effect of medium temperature on the flocculability of yeast cells in beer is rather strong. At a medium temperature of 30 °C complete dispersion of the yeast flocs into single cells was achieved. Figure 5 shows that the bond strength decreases with a factor of more than 5 if the medium temperature raises from 2 °C to 28 °C. The effect of temperature on the fraction of single cells corresponds to the effect of temperature on bond strength of the yeast cells. Figure 13 shows that the fraction of single cells increases strongly with increasing medium temperature.

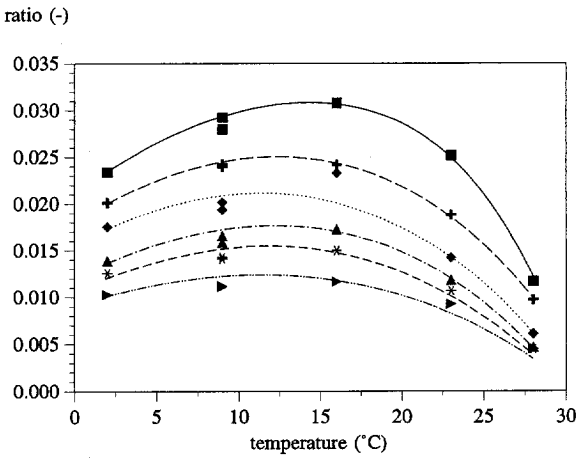

Figure 14. Floc sizes as a function of the temperature of beer measured at various shear rates in the tube. The size of the yeast flocs is expressed in terms of ratio (see Appendix 2). $\dot{\gamma}$ = 250 s^{-1} (■), 500 s^{-1} (+), 700 s^{-1} (♦), 950 s^{-1} (▲), 1100 s^{-1} (*) and 1400 s^{-1} (►).

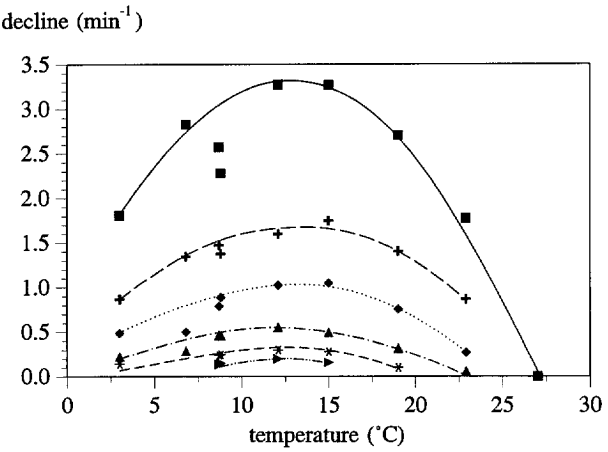

Figure 15. Settling rates of yeast flocs as a function of the medium composition at various hydrodynamic conditions expressed in terms of stirrer speed (N). The settling rate is expressed in terms of OD_{660} decline. N = 100 rpm (■), 200 rpm (+), 300 rpm (♦), 400 rpm (▲), 500 rpm (*) and 600 rpm (►). For translation to shear rates, see Appendix 1.

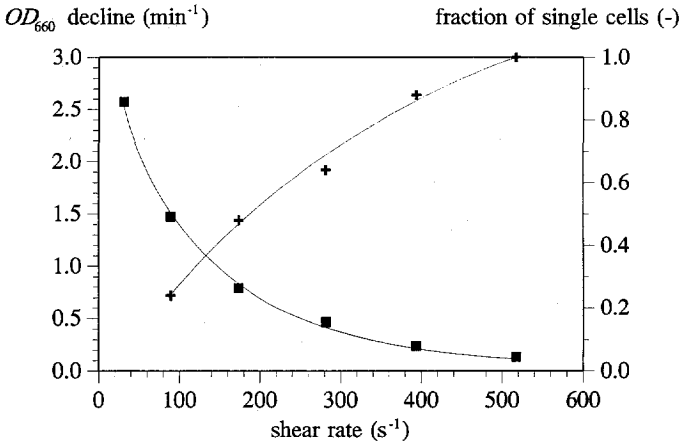

Figure 16. The influence of turbulent flow conditions on the settling rate of yeast flocs (■) and the number of single cells (+) within a stirred vessel. Yeast was suspended in beer. The settling rate is expressed in terms of OD_{660} decline.

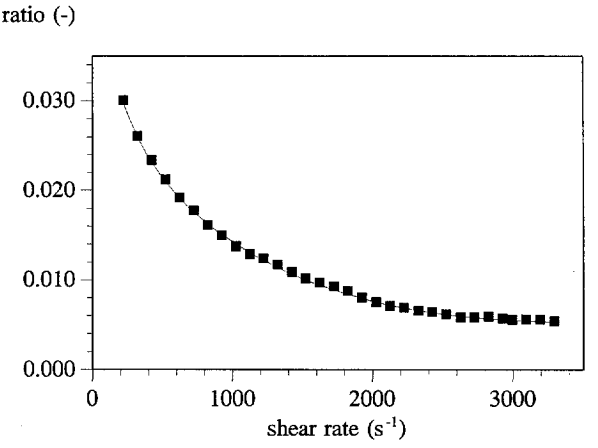

Figure 17. The relation between floc size and laminar flow conditions in a tube, as measured in beer. The size of the yeast flocs is expressed in terms of ratio (see Appendix 2).

The size of the flocs (Figure 14) and the settling rate of the flocs (Figure 15) show an increase if the medium temperature increases from 3 °C to 15 °C, while the bond strength decreases slightly. From this small decrease in bond strength one would expect the floc size also to decrease. Again the discrepancy can be explained from the viscosity effect. The viscosity decreases strongly with increasing temperature (Figure 9). This leads to a lower shear stress (Figure 1) at the same shear rate, yielding bigger flocs. Because of this increase in floc size and decrease in viscosity the settling rate of the flocs increases if the temperature is raised from 3 °C to 15 °C.

Above a temperature of 15 °C, the bond strength (Figure 5), floc size (Figure 14) and settling rate (Figure 15) of the flocs decreases considerably. Due to the decreasing bond strength the number of single cells shows a sharp increase above a temperature of 15 °C (Figure 13). These results are important for the brewing industry, because temperature is often used as a control parameter for flocculation. The effect of temperature on flocculation characteristics is strongly medium dependent as was shown by Taylor and Orton (1975). Mill (1964b), who found the same pH effect in an artificial medium as was found in this study in beer, reported a dissociation temperature of 60 °C for the yeast flocs in the medium as described above. This value is much higher than the dissociation temperature in beer as was found in this study, which is about 30 °C. It again indicates the strong influence of medium composition on flocculability. However, strain variability may also play a role here.

Environmental conditions: Hydrodynamics

In search of favourable hydrodynamic conditions during a flocculation assay and to show the effect of hydrodynamics on flocculation of yeast, we have examined the effect of flow conditions on floc size, settling rate and number of single cells.

The effect of hydrodynamic conditions is comparable to the temperature effect so far that at sufficient high shear rates it is possible to achieve complete and reversible dispersion of the cells without changing the flocculence of the yeast cells. Under turbulent flow conditions complete dispersion of the flocs is achieved at a shear rate of approximately 500 s^{-1} (Figure 16). In Figure 17 the results of the floc size measurements under laminar flow conditions are shown. Here complete dispersion of the flocs is

attained at a shear rate above 3000 s^{-1}. The discrepancy can be explained from the shear distribution in a stirred vessel compared to that in a tube. In a stirred vessel the maximum shear rate is about ten times the average value (de Boer, 1987). Because the floc forming time is large compared to the mixing time, the residence times in regions with low shear will be too short for the flocs to reach the equilibrium floc size. In laminar tube flow the shear rate varies from zero at the tube axis to a maximum of 1.5 times the average value at the tube wall (Gregory, 1981). In laminar tube flow the suspension is hardly mixed, and the effect described above will not occur.

Parameter evaluation

Four parameters (bond strength between the cells, the size of the yeast flocs, the settling rate of the flocs and the number of single cells) were investigated to serve as a measure to the flocculability of yeast cells. Hereafter the suitability of these parameters to serve as a measure for flocculence, flocculability and flocculation characteristics of a yeast suspension is discussed.

Flocculence

Almost all flocculence tests in literature are based on the settling velocity of the flocs. For measuring the degree of flocculence this is not the best choice. The settling of yeast flocs depends on many factors and the results might therefore lead to wrong conclusions. This is most clearly shown by the effect of temperature on the flocculability of yeast cells in beer. While the bond strength shows a decrease with increasing temperature (Figure 5), the settling rate increases if the temperature is raised from 2 °C to 15 °C (Figure 15). This apparent contradiction is caused by the decrease of viscosity that will lead to higher settling rates at the same or somewhat smaller floc sizes.

The increase of the floc size if the medium temperature is raised from 2 °C to 15 °C can also be explained from the viscosity decrease in the same temperature trajectory. At the same shear rate a lower viscosity will lead to a lower shear stress ($\dot{\gamma}$) on the flocs, resulting in larger floc sizes.

Measuring bond strength between the cells is to be considered the best way to quantify flocculence. Moreover this type of measurement is independent of hydrodynamic

conditions, so if the medium is chosen to be optimal the outcome of the measurements will only be determined by the state of the cells. The measurement of the number of single cells, done earlier by Stratford and Keenan (1988) will also lead to good results, provided that the hydrodynamic conditions and the initial cell number are chosen optimally. As shown in this study the relation between number of single cells and temperature is similar to the results from the bond strength measurements.

Flocculability

For quantification of flocculability measuring of floc size or the settling rate of the flocs is suitable. The hydrodynamic conditions have to be chosen accurately, since it will strongly influence the formation of the flocs and by that the outcome of the assay.

Measuring bond strength is also suitable to quantify flocculability, not in the least because the effect of hydrodynamic conditions is out of the question. For this, dispersion of the flocs has to be carried out under well defined fluid forces to be able to exactly calculate the bond strength. Next to this, the point of complete dispersion has to be measured accurately.

Flocculation

For quantification of flocculation the margins are broader than for the characterization of flocculence and flocculability. Measuring the bond strength is of little use to determine the flocculation characteristics. Nevertheless, if more insight in the flocculation of yeast as a dynamic process is obtained, bond strength measurements can be used to predict floc sizes at defined hydrodynamic conditions.

Regarding the interest of a brewer, floc size and even more settling rate of the flocs are the best choices as a parameter to quantify the flocculation of yeast. Determination of floc formation by settling rate measurement under given environmental conditions will yield information, which can be translated directly into terms of removal rate of yeast from the medium. In this context the measurement of the number of single cells is a valuable addition to the flocculation assay, because it will determine the removal efficiency.

Proposal for standardization of flocculation assays

Parameters

Table 3 gives a summary of the applicability of the methods to quantify the different phenomena discussed above. As shown, bond strength measurement is the best method for quantification of flocculence of yeast cells. Floc size measurement is the best way to determine the flocculability of the cells, and finally determination of the settling rate is most suitable to quantify yeast flocculation.

Table 3 Survey of the parameters which can be used to quantify the subdivided phenomena of yeast flocculation and their applicability to serve as a measure for the mentioned phenomena.

parameter to be measured	phenomenon		
	flocculence	flocculability	flocculation
bond strength	++	±	-
floc size	+/±	++	+/++
settling rate	±	+	++
number of single cells	+/++	+	+

For development of a method to quantify the phenomena as mentioned above, the environmental conditions have to be considered apart from the parameter measured. In case of flocculence, medium, amount of yeast and hydrodynamic conditions have to be chosen optimally. For the quantification of flocculability the hydrodynamic conditions and amount of yeast need to be favourable. In case of quantification of flocculation all environmental conditions are given, so flocculation assay development can be reduced by choosing the right parameter to be measured.

Medium

The composition of the medium is only important for the determination of the flocculence of the yeast cells. Almost all flocculence tests reported in literature are carried out in a pH 4.5 Sodium Acetate buffer (Helm et al., 1953; Jayatissa and Rose, 1976, Mill, 1964a; Smit et al., 1992). Next to this, the calcium concentration is around 1 mol/m^3 in most cases. Unfortunately, hardly any author mentioned the temperature at which the test was carried out, while it influences the measurements to a large extend as shown before. Considering the results presented in this study and the growth

temperature during fermentation, a temperature around 15 °C is desirable. So an optimal medium for usage within the flocculence assay can be a pH 4.5 NaAc buffer with a calcium concentration of at least 1 mol/m^3 and a temperature of 15 °C.

Hydrodynamics

The influence of hydrodynamic effects on flocculation is underestimated in literature. This study shows that, yeast flocs are very sensitive to shear. Stratford and Keenan (1987) were the first to note the importance of hydrodynamic conditions prior and during flocculation assays. Only a few authors mentioned the effect of agitation on flocculation of yeast (Brohan and Mcloughlin, 1984; Calleja, 1987; Kihn et al., 1988; Speers et al. 1992b). Despite the awareness of the effect hydrodynamic conditions have on flocculation the conditions are still uncontrolled in most cases and undefined in all cases.

Stratford and Keenan (1987) reported a 'threshold' agitation for flocculation to occur at 40 rpm. Unfortunately the experiments were carried out in an orbital shaker, which makes it almost impossible to determine the more uniform quantity, shear rate. This makes comparison between results from literature impossible. The hydrodynamic conditions during the test of Smit et al. (1992) are also undefined.

The favourable hydrodynamic conditions must be to such an extent to initiate flocculation of the cells. Too low shear rates will prevent floc formation, while a too high shear rate causes disruption of the flocs and makes comparison between different yeast strains and/or media difficult. In view of the results obtained in this study, the favourable value for the shear rate to be used within the flocculence and flocculability assay should be chosen between 50 s^{-1} and 250 s^{-1}. The values count for both laminar and turbulent liquid flow.

Yeast amount

Next to medium and hydrodynamics also the number of yeast cells plays a role in the flocculation process. In this study the influence of cell number was not examined, however the importance of yeast concentration on flocculation was already shown by Stratford & Keenan (1987). For quantification of flocculence the amount of yeast has to be chosen optimally. A low cell concentration will lead to a very high floc formation

time, while on the other hand too many cells will lead to a situation were flocs can't be discerned from each other which makes the measurement of the involved parameters impossible. We propose to apply a cell number between 15×10^6 and 60×10^6 cells per ml within the concerned assays.

CONCLUSIONS

The flocculation process of yeast cells into flocs can be quantified in various ways, measuring under various environmental conditions and determining different parameters to serve as a measure for this phenomenon. By making a differentiation of the definition of the flocculation process into three phenomena, the choice for the proper parameter to be measured can be simplified. A subdivision into the next phenomena is made, flocculence, flocculability and flocculation. The definitions are based on the way the environmental conditions (medium composition, number of yeast cells and hydrodynamic conditions) are chosen.

Regarding the strong influence of the environmental conditions (especially the temperature of the medium and the hydrodynamic conditions) on the result of a flocculation assay they have to be chosen and set accurately. In this study four parameters were studied to serve as a measure for the three phenomena as mentioned above. It is shown that bond strength between yeast cells is the most suitable parameter to serve as a measure for flocculence. The measurement is independent of hydrodynamic conditions. So by choosing the optimal medium and an optimal number of cells this assay is most accurate. For determining the flocculability of yeast cells, floc size measurement turned out to be the best choice. In this case the amount of yeast must such as to achieve sufficient cell-cell encounters, necessary for floc formation. The hydrodynamic conditions have to be chosen favourable too. Regarding the interest of a brewer, settling rate measurement combined with the determination of the number of single cells is the best proposal for the flocculation assay. With this test both removal rate and removal efficiency of yeast from beer can be predicted.

Acknowledgements

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APPENDIX 1

Calculation of hydrodynamic parameters

A well-known parameter to characterise the hydrodynamic conditions is the shear rate within the fluid. Within flocculation studies it is therefore often used as a parameter in models that describe floc sizes (Potanin, 1993; Tambo and Hozumi, 1979) or flocculation rates (Cleasby, 1984; Parker et al., 1972; Speers et al., 1992b).

In laminar tube flow the shear rate varies linearly from zero at the tube axis to a maximum value at the wall. The average shear rate ($\dot{\gamma}$) can be calculated from the flow rate (Gregory, 1981) (Q),

$$\dot{\gamma} = \frac{64\ Q}{3\ \pi\ d^3} \tag{1}$$

where d is the internal diameter of the tube.

In case of turbulent flow, the equation for shear rate was derived by Camp and Stein (1943),

$$\dot{\gamma} = \sqrt{\frac{\varepsilon\ \rho_l}{\eta}} \tag{2}$$

where ε is the power input per mass and ρ_l is the density of the medium.

The specific power input in a stirred vessel depends on the stirrer speed (Beek and Muttzall, 1986) (N),

$$\varepsilon = \frac{Po\ N^3\ D_{Rush}^5}{V_f} \tag{3}$$

where Po is the power number, D_{Rush} is the diameter of the Rushton turbine and V_f is the volume of the vessel. The power number depends on the Reynolds number and can be found in a table (Janssen and Warmoeskerken, 1987). Under turbulent flow conditions the power number is approximately 5.

Equation 2 shows that the shear rate depends on the viscosity of the fluid at turbulent flow conditions. Because the stirrer speed was constant for a series of experiments while the viscosity changed we will express the hydrodynamic conditions in terms of stirrer speed. Table 4 gives a list of stirrer speeds, the corresponding specific power input and an average shear rate for the 2 l vessel used in the experiments.

Table 4 Relation between stirrer speed, power input and shear rate in a 2 l stirred vessel containing beer. The viscosity was 2.3 mPa s and the temperature was 9 °C.

stirrer speed (rpm)	specific power input (mW/kg)	shear rate (s^{-1})
100	2.8	35
200	23	100
300	77	180
400	180	280
500	360	390
600	620	520

APPENDIX 2

The relation between PDA output (signal ratio) and floc size

The Photometric Dispersion Analyser measures the turbidity of a flowing suspension. A glass tube is placed between a light source and a photodiode in order to measure turbidity and turbidity fluctuations caused by the flowing of the suspension. The output of the apparatus consists of two components an alternating voltage (ac) and a steady voltage (dc). The ratio of these two signals is defined as follows (Gregory, 1985),

$$\text{ratio} = \left(\frac{N_f L}{A}\right)^{½} \frac{\pi}{4} d_f^2 Q_{sca} \tag{4}$$

where N_f is the number of flocs, L is the length of light path, A is the cross sectional area of the light beam d_f is the floc diameter and Q_{sca} is the scattering coefficient.

If the cells start to flocculate the floc diameter increases whereas the floc number decreases, which causes a net increase in the signal ratio. To quantify the change of the signal ratio when the cells start to flocculate, the change in floc number in relation to the change in floc diameter needs to be known. This relation can be derived from the theory of fractals. It is widely accepted that yeast flocs can be described by this theory (Davis and Hunt, 1986; Fontana et al., 1991; Logan and Wilkinson, 1991),

$$N_f = N_c \left(\frac{d_f}{d_c}\right)^{-D} \tag{5}$$

where N_c is the total number of yeast cells, d_f is the floc size d_c the size of a yeast cell and D is the fractal dimension of a floc.

If Equation (4) and (5) are combined, the following relation for the signal ratio can be found,

$$\text{ratio} = \frac{\pi\ N_c^{½}\ L^{½}\ d_c^{½D}\ Q}{4\ A^{½}}\ d_f^{2-½D} \sim d_f^{2-½D} \tag{6}$$

The first term of the signal ratio is constant, so the signal ratio is only influenced by the floc size. This derivation is based on the assumption that the suspension is monodisperse however from literature it is known that a yeast suspension is polydisperse (Davis and Hunt, 1986). To get the appropriate relation between signal ratio and floc size a size distribution $f(x)$ is introduced. A suitable size distribution for flocs is a lognormal distribution (Gregory, 1985),

$$f(x) = \frac{\exp\left(-½\left(\frac{\ln x - \ln d_f}{s}\right)^2\right)}{\sqrt{2\pi}\ s\ x} \tag{7}$$

where s is the standard deviation of the size distribution.

Equation (6) can now be rewritten to give,

$$\text{ratio} \sim \left(\int_0^\infty x^{4-D}\ f(x)\ \mathrm{d}x\right)^{½} \tag{8}$$

The combination of Equation (7) and (8) has no closed form solution. The integral can be solved numerically. In Figure 18 the results of such solutions are plotted against the average floc size for different size distributions. The relation between floc size and signal ratio appears to be almost linear.

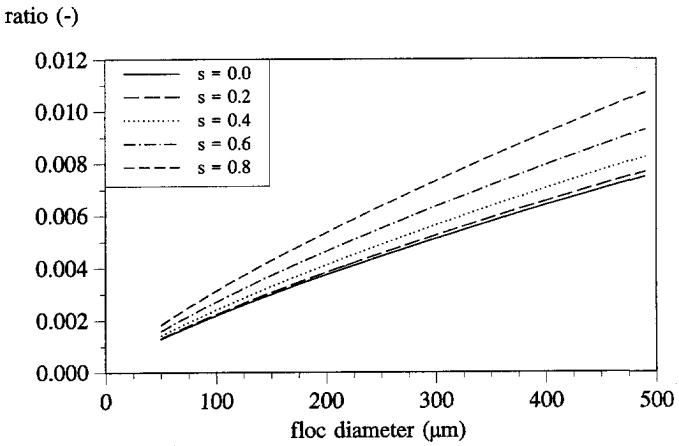

Figure 18. Relation between the output of the PDA (ratio) and the average size of yeast flocs, for different size distributions.

NOMENCLATURE

ε	specific power input	W/kg
$\dot{\gamma}$	shear rate	s^{-1}
η	viscosity	Pa s
λ	wave length of light	m
ρ_l	density of the medium	kg/m^3
σ	shape factor	-
τ_s	shear stress	Pa
A	cross sectional area of the light beam	m^2
d_c	cell diameter	m
d_f	floc diameter	m
D_{Rush}	Rushton turbine diameter	m
f	size distribution	-
F	bond strength	N
L	length of the light path	m

N	stirrer speed	s^{-1}
$N_{c,tot}$	total cell number per unit of volume	m^{-3}
N_f	floc number per unit of volume	m^{-3}
OD_{660}	optical density of the suspension at a wavelength of 660 nm	-
Po	power number	-
Q	flow rate	m^3/s
Q_{sca}	scattering coefficient	-
r	tube radius	m
s	standard deviation of ln d_p of a lognormal size distribution	-
V_f	fermenter volume	m^3

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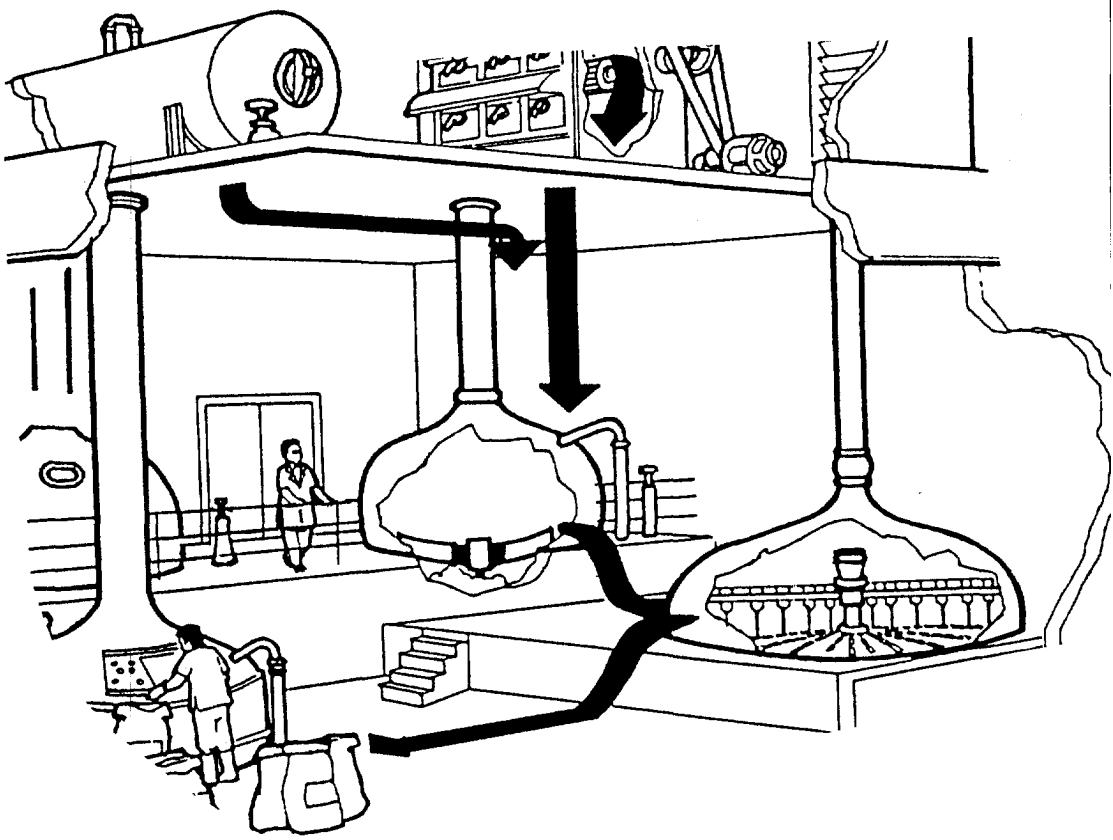

... the malting and brewing process ...

mashing	- In the upper part of the mash tun the grist is sprayed with hot water and mixed to obtain a thick porridge. The mash then falls into the tun that is partly filled with hot water (70 °C). The mash water around the particles dissolves extractable material and drains through the bed, filtered by the husks. The water has become sweet wort and can be drained from the mash tun. - Mashing is carried out to produce fermentable sugars and non fermentable dextrins. The enzymes α-amylase and ß-amylase degrade amylose and amylopectin into sugars.

... to be continued on page 122 ...

CHAPTER V

Quantification of brewers' yeast flocculation in a stirred tank

Abstract

Quantification of brewers' yeast flocculation under defined conditions will help to understand the physical mechanisms of the flocculation process. Flocculation was quantified by measuring the size of the yeast flocs and the number of single cells. For this a method to measure floc size and number of single cells 'in situ' was developed. In this way it was possible to quantify the actual flocculation during the fermentation, without influencing flocculation. In view of the time constants for flocculation and mixing the method can be used to predict flocculation behaviour on full scale.

Next to this the effects of three physical parameters (floc strength, fluid shear and yeast cell concentration), on flocculation during a beer fermentation, will be treated. Increasing floc strength results in larger flocs and lower number of single cells. If the fluid shear is increased the size of the flocs decreases, the number of single cells remains constant at approximately 10% of the total cell amount present. The cell concentration also influences flocculation, a reduction of 50% in cell concentration leads to a decrease of about 25% in floc size. The number of single cells decreases linearly proportional to the cell concentration. This means that at settling of the yeast at full scale the number of single cells decreases. From the results of the study an estimation of flocculation on full scale is made.

The results of this study will be used in a model for yeast flocculation. With respect to full scale fermentation the effect of cell concentration will play an important role, for flocculation and sedimentation will occur simultaneously leading to a quasi steady state between these phenomena.

INTRODUCTION

One of the steps in the beer brewing process is the fermentation of wort. In this step ethanol, carbon dioxide and flavour compounds are formed. At the end of the fermentation the main part of the yeast has to be removed. The remaining part is needed for e.g. rest conversions of off-flavours as acetaldehyde and diacetyl, to take place in the next process step, the lagering. One of the advantages of brewers' yeast is the ability of the cells to form flocs under certain environmental conditions. This property of the yeast facilitates its removal by sedimentation at the end of the beer fermentation.

To provide a constant product quality, control of the amount of yeast in suspension during fermentation and subsequent lagering is of prime concern to a brewer. To be able to control the amount of yeast in suspension, the conditions at which sedimentation of the yeast takes place must be known. Knowledge of the factors involved in both flocculation and sedimentation will be necessary for this. In this study we will focus of the factors influencing flocculation. The conditions for sedimentation will be subject of a separate study. To enable flocculation of the yeast, the yeast suspension has to meet the following conditions:

1) The yeast cells must be flocculent (flocculence is the ability of yeast cells to form flocs if all environmental conditions are favourable (Amory et al., 1988)).
2) The physiological conditions must be favourable (presence of sufficient calcium, favourable temperature and pH of the medium, etc.), to enable a certain bond strength between the cells.
3) The hydrodynamical conditions must be favourable (sufficient collision rate and not too large breakup forces).
4) The amount of yeast in suspension must be sufficient to satisfy the number of collisions necessary to form flocs.

The factors as mentioned above (flocculence, bond strength, hydrodynamics, yeast cell concentration) are not constant during a full scale fermentation.

- Partway through the fermentation the yeast cells become flocculent. After initiation the flocculence of the cells increases rapidly.
- From the moment the cells become flocculent, the flocculability, which represents the actual floc strength under favourable environmental conditions also increases. The pH

and sugar concentration of the medium, that both change during fermentation, are factors influencing the flocculability (floc strength) of the cells (Smit et al., 1992).

- The third factor, the hydrodynamics, which determines the fluid shear, depends on the carbon dioxide production. This gas, produced by the yeast, forms bubbles that rise and by that cause a turbulent fluid motion in the vessel. The carbon dioxide production rate is not constant during fermentation. At the start of the fermentation the production rate increases due to growth of the yeast. Partway through the fermentation, the carbon dioxide production rate reaches its maximum. From then the carbon dioxide production starts to decrease.
- The last factor is the amount of yeast in suspension. From the moment the cells become flocculent the number of yeast cells in suspension is constant. At the end when the yeast starts to settle the amount of yeast in suspension decreases.

In this study we will quantify the effect of bond strength, fluid shear and cell concentration on the flocculation of yeast. This will be done by determining the floc size distribution in a stirred vessel under defined hydrodynamic conditions. The effect of yeast concentration and agitation were earlier determined by Kihn et al. (1988) and Stratford et al (1988). However, the experiments were carried out under undefined hydrodynamic conditions and Kihn et al. (1988) only obtained qualitative results. Here the effects of the mentioned parameters will be quantified under conditions as occurring during full scale fermentation.

Determination of floc size is complicate in turbulent flow. Because of the large velocities within the vessel, simply taking pictures of the particles is impossible. Separation of the particles from the medium seems also a possibility. However, yeast flocs have a weak structure, therefore, sampling will easily damage the flocs. To avoid these problems a method for quantification of flocculation in turbulent flow, by which the floc size distribution can be measured was developed. This was achieved with a modified vessel with an 'in situ' sampling device. A part of the suspension could be separated gently to a quiet zone where the settling of the flocs was monitored by light extinction. By modelling this settling process the floc size distribution could be derived from the settling curve.

THEORY

Light extinction techniques are often used to quantify the amount of biomass in a suspension. The technique is both simple and powerful. In yeast flocculation almost all methods to determine flocculation are based on a light extinction technique (Calleja, 1987; Speers et al., 1992a; Stratford, 1992) or light extinction in combination with sedimentation (Davis and Hunt, 1986). Here we used the latter method. By modelling the optical density decline caused by settling of the flocs and comparing the modelling results to the experimental optical density decline, the floc size distribution could be determined. Hereafter we will explain how the optical density decline is modelled.

Optical density

The optical density is linearly proportional to the turbidity of the suspension. The turbidity of the suspension is proportional to the particles concentration and the scattering cross section of the particles. Because of settling of the flocs the number of particles in the light beam will decrease. The particle concentration depends on the settling rate of the flocs, which on its turn depends on the floc size, which is one of the input parameters of the model (Figure 1).

The optical density as a function of time at a wave length of 660 nm ($OD_{660}(t)$) can be described by the next formula,

$$OD_{660}(t) = OD_{660,0} + \tau(t)\ L\ K \qquad (1)$$

where $OD_{660,0}$ is the optical density of the fluid without particles, $\tau(t)$ is the turbidity, L is the length of the light path and K is a calibration factor.

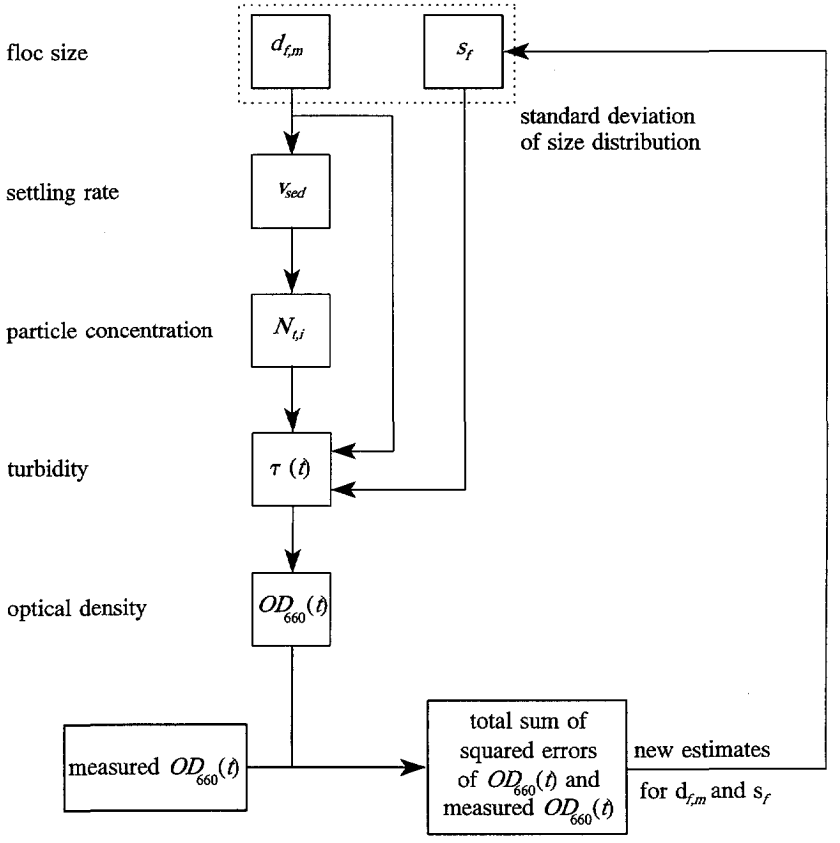

Figure 1. Schematic representation of calculation procedure for the determination of the floc size distribution.

Turbidity

A flocculating yeast suspension contains flocs and single cells (Miki et al., 1982a and 1982b), which makes the total size distribution of a flocculating yeast suspension bimodal. Therefore the relation describing turbidity contains two terms one describing the turbidity of the cells and one describing the turbidity of the flocs. In case of flocs, a size distribution is defined which is divided into n size classes. The turbidity as a function of time is defined as,

$$\tau(t) = N_s(t)\ C(d_c) + N_f(t) \sum_{i}^{n} S(d_{f,i})\ C(d_{f,i}) \qquad (2)$$

where N_s is the number of single cells, d_c is the cell diameter, N_f is the number of flocs, $d_{f,i}$ is the floc diameter of size class 'i', S is the fraction of the total number of flocs within a size class and C is the scattering cross section of a particle, which is defined as,

$$C(d_p) = \frac{\pi}{4}\ d_p^2\ Q_{sca} \qquad (3)$$

where d_p is the particle (cell or floc) diameter and Q_{sca} is the scattering coefficient, which equals 2 in case of a particle diameter above 5 µm (Kerker, 1969).

The fraction ($S(d_i)$) of the total particle number in size class 'i', with a range Δd, can be calculated by integration of the size distribution ($f(d)$), in this case a lognormal distribution is assumed,

$$S(d_i) = \int_{d-½\Delta d}^{d+½\Delta d} f(d_{f,i})\,\mathrm{d}\,d$$

$$\int f(d_{f,i})\,\mathrm{d}\,d = \cdot ½\ \mathrm{erf}\ \frac{\left(\dfrac{\ln d_{f,i} - \ln d_{f,m}}{s_f}\right)}{\sqrt{2}} \qquad (4)$$

where $d_{f,m}$ is the average floc diameter or median diameter and s_f is the standard deviation of the size distribution.

Particle concentration

The number of particles in the light beam as a function of time depends on the settling rate of the particles (Figure 1). For the measurements were carried out in a curved tube (Figure 2), the particle number as a function of time has to be corrected for the curvature of the tube leading to the next equation, describing the number of particles at height 'h_L' in the tube,

$$N_{t,i} = N_{0,i} \sqrt{\frac{r^2 - (h_L + v_{sed,i}\, t)^2}{r^2 - h_L^2}} \qquad for \quad v_{sed,i} < r - h_L$$

$$N_{t,i} = 0 \qquad for \quad v_{sed,i} > r - h_L \tag{5}$$

where $N_{t,i}$ is the particle number of size class 'i' at height 'h_L' in the pipe at t = t s, $N_{0,i}$ is the particle number of size class 'i' at height 'h_L' in pipe at t = 0 s, r is the radius of the pipe (7.4 mm), v_{sed} is the settling velocity of the particles and h_L is the height of light path within the pipe (2.7 mm).

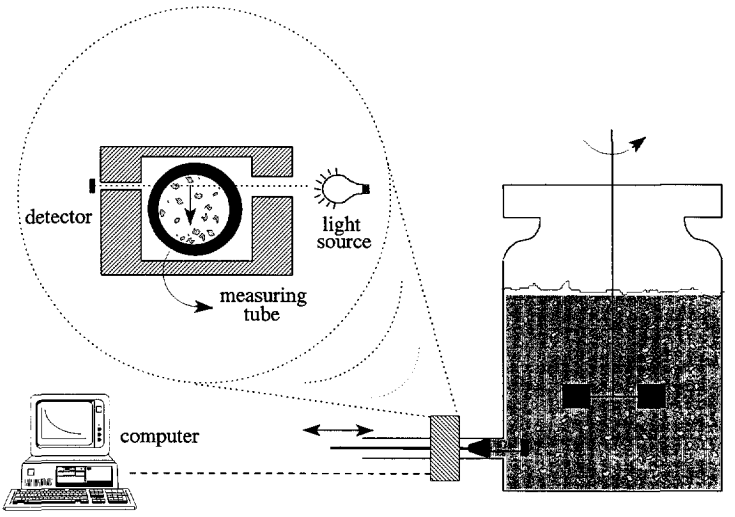

Figure 2. Experimental setup for 'in situ' measurement of the size of yeast flocs formed in turbulent flow.

At t = 0 s the number of particles can be calculated, for this the total cell number and the number of single cells need to be known. The number of single cells can be derived from the settling curve (Figure 3) by correction for the settling of the cells, according equation 5. The total cell number can be determined off line by a particle counter.

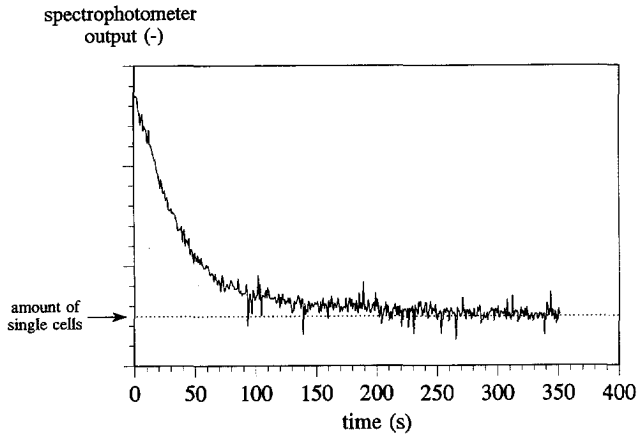

Figure 3. OD_{660} decline during a settling experiment.

If the total cell number ($N_{c,tot}$) and the number of single cells is known the number of flocs (N_f) can be calculated according the theory of fractals (Davis and Hunt, 1986; Fontana et al., 1991; Logan and Wilkinson, 1991),

$$N_f = \frac{N_{c,tot} - N_s}{\left(\frac{d_f}{d_c}\right)^D} \qquad (6)$$

where D is the fractal dimension of the yeast flocs.

Settling rate

Finally the settling rate of the particles is needed to complete the model. The settling rate (v_{sed}) of particles can be predicted by Stokes Law corrected for hindered settling,

$$v_{sed} = \frac{\Delta\rho \; g \; d^2}{18 \; \eta} \; (1 - \phi)^{6.5} \tag{7}$$

where $\Delta\rho$ is the density difference between a floc and the medium, the so-called effective density, η is the viscosity of the medium, and ϕ is the volume fraction of particles within the medium.

The hindered settling function is derived from the Richardson-Zaki equation (Richardson and Zaki, 1954). The exponent 6.5 is taken from Al-Naafa and Selim (1992) and can be applied in case of polydisperse settling at a low effective density (Davis and Gecol, 1994).

The effective density is defined as,

$$\Delta\rho = \left(\frac{d_f}{d_c}\right)^{D-3} (\rho_y - \rho_l) \tag{8}$$

where ρ_y is the density of the yeast and ρ_l is the density of the medium.

To calculate the hindered settling factor, the volume fraction of particles in the suspension has to be known. The volume fraction of the particles is defined as,

$$\phi = N_s \frac{\pi}{6} d_c^3 + N_f \frac{\pi}{6} d_{f,m}^3 \tag{9}$$

where $d_{f,m}$ is the average floc diameter.

By choosing a value for the average floc size and the standard deviation of the size distribution the optical density decline can now be calculated (Figure 1).

MATERIALS AND METHODS

Fermentation

Fermentations were done in a 2 l stirred vessel with standard geometry as described by Beek and Muttzall (1986). The cells were grown in standard brewery wort (12 °P) at a temperature of 9 °C. To provide a fixed amount of oxygen at the beginning of the fermentation the medium was saturated with air and the headspace was filled with nitrogen gas before inoculation. The inoculum of the flocculent strain of *Saccharomyces cerevisiae* (MPY3) was pregrown on wort at 15 °C. After pitching of the wort the initial cell number was 4×10^6 cells per ml. The fermentations were carried out without aeration.

During fermentation pH, temperature, extract, cell number, cell size, flocculence and flocculability were followed as described earlier (Van Hamersveld et al., 1993) and showed a normal course.

Fractal dimension

The fractal dimension of the flocs was measured by taking pictures of the flocs in a Couette device (Couette, 1890). For this, the fermentation broth was recycled via a Couette vessel. The vessel had a volume of 60 ml. The diameter of the inner cylinder was 100 mm and the annular gap was 1.5 mm. The vessel was of the 'Searle type' which means that the inner cylinder is rotating. By means of the rotation velocity the shear rate in the vessel could be varied between 30 s^{-1} and 100 s^{-1}. The pictures of the flocs were taken with a Nikon FM-2 camera equipped with a Nikon 105 'macrolens'. During an experiment the total cell number was constant. The pictures were analysed by an Image Analyser (Cue Series, Cue-2 image analyser versions 4.0, Olympus/Galai Productions Ltd., Israel). The image analysis gave the number of particles and the size of these

particles in a known volume. Since the total cell number was known, the fractal dimension could be calculated according to equation 5. The number of single cells was assumed to be 10% of the total number of yeast cells. The fractal dimension was determined at different shear rates (30 - 100 s^{-1}), different cell concentrations (25 - 35 10^6 cells per ml) and different floc strength. The floc strength was varied by adding calcium to the medium. The floc strength was varied such that the floc size increased by approximately 40% from the lowest to the largest floc strength.

Density/viscosity

The densities of the yeast and the medium were measured with a density meter (Paar DMA-48). The density of the medium was measured every 24 h. The density of the cells was detected by measuring the yeast suspension with different volume fractions of yeast. Extrapolation of the density of the suspension against the volume fraction of yeast gave the density of the cells. The viscosity of the medium was measured with an Ostwald viscosimeter. The viscosity measurements were carried out in a water bath to provide a constant temperature during the measurements.

Floc size

The size of the yeast flocs was determined from their settling curve. The measurements were done 'in situ' every 24 h from the moment the cells became flocculent. For the measurements a modified fermenter (Figure 2) was used. On the fermenter a pipe was fixed with a plunger in it. The plunger could be moved to take a sample from the yeast culture. A spectrophotometer was placed around the pipe to monitor the yeast suspension. Considering the position where the light beam crosses the pipe, the effect of light refraction is negligible. The pipe was placed at a height of 2 cm from the bottom of the vessel. If the sample is moved in front of the spectrophotometer the pipe is closed to prevent mixing or exchange with the vessel content and to guarantee a quiet settling of the flocs. The data from the spectrophotometer were collected by a computer. In Figure 3 an example of a settling curve is presented. Every data set of a settling experiment contained at least a hundred data points. All experiments were carried out in duplicate.

Because the floc strength increases from the moment the cells become flocculent the effect of floc strength on flocculation could be determined by carrying out experiments every 24 h after the moment the cells became flocculent.
The power input was changed during a set of experiments by varying the stirrer speed. The power input range was chosen based on the average power input during large scale brewery fermentation.
Next to power input, also the amount of yeast was varied. This was done at the end of the fermentation. At the end of the fermentation settling experiments at four different cell concentrations were carried out. For this, the cells were separated from the medium by centrifuge. After this, the cells were resuspended in the medium to reach the desired concentration in the vessel. This was done at a constant temperature (9 °C) under anaerobic conditions.

Number of single cells

During the settling of the yeast flocs the single yeast cells will remain in suspension due to their low settling rate compared to the flocs. When all flocs are settled, or at least have passed the light beam, the optical density decline will stop and reach a constant level (Figure 3). This level corresponds to the number of single cells. To determine this number, the apparatus was calibrated. The calibration was done with single cells suspended in an EDTA solution (50 mol/m^3) to prevent flocculation.

Model calculations

To calculate the optical density decline (equation 1) the size distribution of the flocs was divided into '*n*' compartments. The value of '*n*' was chosen based on a 99% accuracy level (n = 350). Regarding the standard deviation of the size distribution of the yeast cells (see below), the size distribution of yeast cells consists of one size class with a size equal to the average cell diameter.

From the plateau in the optical density decline curve (Figure 3) the number of single cells was determined and corrected for the settling rate of the flocs according equation 5. With a combination of equation 1 to equation 9, the decline of the optical density as a function of time can now be calculated and compared to the measured curves. The input

parameters are the average floc size and the standard deviation of the floc size distribution (Figure 1). From the calculated and measured optical density the total sum of squared errors was calculated. During the fit procedure this sum was minimized and new estimates for both parameters ($d_{f,m}$ and s_f) were generated by the Simplex like algorithm of Nelder Mead (Himmelblau, 1970). The calculations were carried out on a 486-DX processor based personal computer. At least 200 iteration steps were carried out during the fit procedure. The total fit procedure took approximately 1 hour.

RESULTS AND DISCUSSION

Yeast flocculation is an important phenomenon for the removal of the yeast from the green beer. The formation of yeast flocs enhances the sedimentation of the yeast at the end of the fermentation. To predict and control flocculation, quantification of the effect of flocculability, shear rate and cell concentration on flocculation is needed. The quantification of flocculation was carried out by determination of the floc size distribution and the number of single cells. These parameters were chosen because both play a role in the removal of the yeast. The floc size will determine the settling rate, whereas the number of single cells will determine the amount of yeast that remains in suspension after settling of the yeast flocs in case of quit settling. The effects of flocculability, turbulence and amount of yeast in suspension on flocculation were studied and the results are presented. Next to this, we will discuss the consequences for full scale fermentation in a cylindroconical tank.

Parameters

The set of parameters needed to calculate the floc size distribution from the settling curves is given in Table 1. A part of the parameters is determined in this study.

Fractal dimension

The fractal dimension was determined at different conditions in the Couette vessel to verify the independency of the fractal dimension. The fractal dimension was found to be 2.45 ± 0.03 and did not vary with floc strength, cell concentration and shear rate, in the range measured.

Table 1 Physical constants and parameters which were used for the model calculations and calculation of power input.

description	symbol	value	reference
power number	Po	5	Janssen and Warmoeskerken, 1987
diameter of Rushton turbine	D_{Rush}	0.045 m	this study
fermenter volume	V_f	1.5×10^{-3} m^3	this study
fractal dimension of the yeast flocs	D	2.45	this study
yeast cell diameter	d_c	7.2 µm	this study
total cell number	$N_{c,tot}$	variable	this study
number of single cells	N_s	variable	this study
density of the medium	ρ_l	variable	this study (Figure 4)
density of the yeast	ρ_y	1140 kg/m^3	this study
viscosity of the medium	η	2.3×10^{-3} Pa s	this study (Figure 4)
gravitational constant	g	9.81	Perry et al., 1984
radius of measuring tube	r	7.4 mm	this study
height of light beam in measuring tube	h_L	2.7 mm	this study
length of light path	L	13.7 mm	this study
calibration factor	K	2.3×10^{-3}	this study
incident optical density	$OD_{660,0}$	0.024	this study

The fractal dimension as determined in this study is within the range of values as found in literature. Davis and Hunt (1986) determined the fractal dimension of two types of strains of *Saccharomyces cerevisiae* (ATCC 58230 and ATCC 46758) and found values between 1.75 and 2.25. The fractal dimension was obtained by measurement of the floc size and counting the number of cells in each floc. Logan and Wilkinson (1991) determined the fractal dimension of flocs of *Saccharomyces cerevisiae* (ATCC 19623). Using the same approach as Davis and Hunt they found a fractal dimension of 2.66. Fontana et al. (1991) also determined the fractal dimension of two strains of *Saccharomyces cerevisiae* (27C and 38A) and found a value of 2.55 and 2.76 respectively.

The fractal dimension as found in this study was measured using beer as a medium. As can be concluded from the results in literature the fractal dimension is strain dependent.

In two cases measurement of different strains under equivalent conditions gave different values of fractal dimension. Next to this also the medium could play a role. However, in this study the medium was changed by adding calcium to it which showed no effect on the fractal dimension.

Cell diameter, yeast density and medium viscosity and density

Cell size measurements were carried out with a Coulter Counter. An average value of 7 μm was found. The diameter was the equivalent sphere diameter based on a volume basis. The size distribution could be described with a lognormal size distribution with a standard deviation of 0.14. Because of the low standard deviation the size distribution was assumed to consist of one class with a size equal to the average cell diameter.

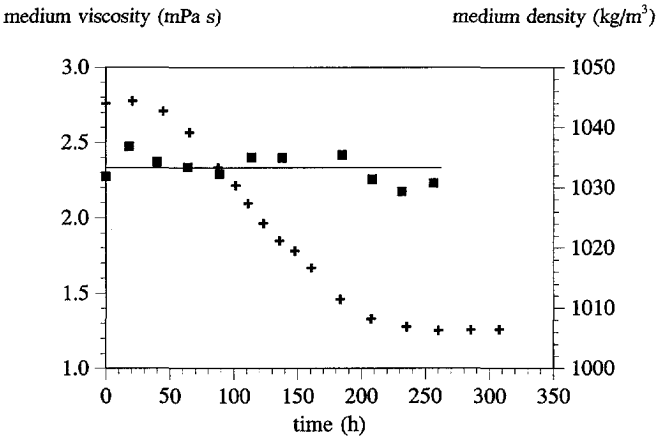

Figure 4. Viscosity (■) and density (+) of the medium during a beer fermentation.

The density of the yeast was found to be 1140 kg/m^3 ± 6 kg/m^3 and was constant during the fermentation. Fontana et al. (1991) derived the yeast density assuming a porosity of 30% of a floc with a diameter of 500 μm and found a value of 1129 kg/m^3. Davis and Hunt (1986) found a wet density of the yeast of 1110 kg/m^3. The range of densities is acceptable and the differences might be explained from the difference of strain and growth conditions.

Density and viscosity of the medium were measured during the whole fermentation. The results are shown in Figure 4. Due to sugar consumption and ethanol production the density of the medium decreased. The viscosity of the medium remained constant during the fermentation at a value of $2.3 \pm 0.1 \times 10^{-3}$ Pa s.

Calibration

The spectrophotometer was calibrated with yeast cells suspended in an EDTA solution to prevent floc formation. The calibration of the spectrophotometer gave the next results: $OD_{660,0} = 0.024$ and $K = 2.3 \times 10^{-3}$, both parameters are needed in equation 1. The optical density showed to be linearly dependent of the number of cells within the range measured (up to 50×10^{6} cells per ml).

Determination of the floc size distribution

The particle size distribution of a flocculating yeast suspension can be characterized by the number of single cells, the average cell size, the standard deviation of the cell size distribution, the number of flocs, the average floc size and the standard deviation of the floc size distribution. The cell diameter and standard deviation of the cell size distribution were found to be constant. The same result is found for the standard deviation of the floc size distribution. During all experiments, by which flocculability, shear rate and amount of yeast were varied, the standard deviation of the floc size distribution turned out to be 0.45 ± 0.1.

In this case the floc size distribution was assumed to be lognormal and the average floc size and standard deviation were determined iteratively by computer. This method of data handling can be improved by application of a square tube instead of a circular tube. In that case the size distribution can be derived directly from the settling curve without assumptions of the shape of the distribution. In that case the computational load will be reduced to one calculation step, which means an enormous reduction of the calculation time.

Effect of physical parameters on flocculation

Flocculability/floc strength

During fermentation of beer the flocculability of the cells increases due to among other things decreasing pH and decreasing sugar concentration (Smit et al., 1992). The effect of this on flocculation, which is expressed as floc size and number of single cells, is shown in Figure 5 and Figure 6. The floc size increases with approximately 60% as the flocculability increases. In the same range the number of single cells decreases. The single cell number is expressed in terms of the percentage of the total number of yeast cells in suspension. The number of single cells decreases with a factor four during fermentation as a result of the increasing flocculability.

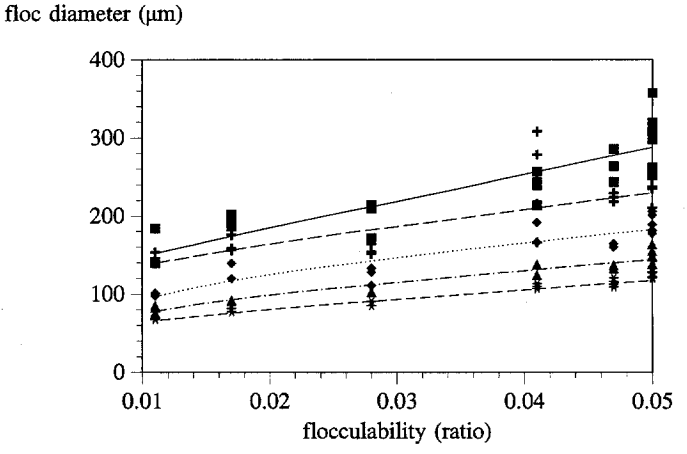

Figure 5. The relation between flocculability expressed as ratio and the diameter of yeast flocs at various power input.
ε = 0.14 W/m^3 (■), 0.5 W/m^3 (+), 1.2 W/m^3 (♦), 2.5 W/m^3 (▲) and 4.3 W/m^3 (*).

Because of this unambiguous effect on flocculation, flocculability could be an important factor in the control of flocculation. However, it is rather complicated to vary flocculability of the cells during fermentation without changing the composition of the medium, which is undesirable in most cases.

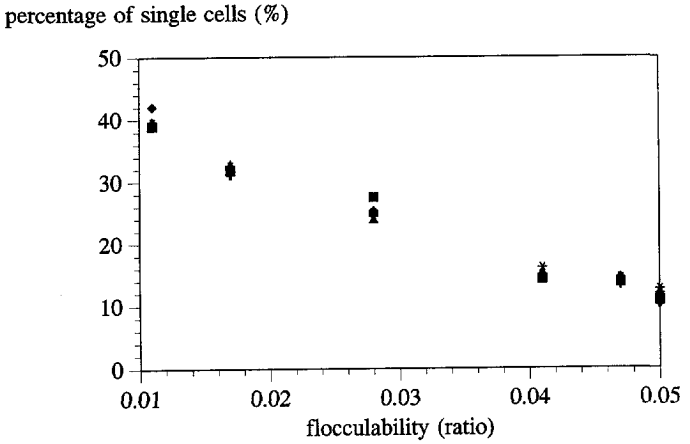

Figure 6. The percentage of single cells of the total number of yeast cells in suspension as function of the flocculability of the cells, the power input was varied during the experiments. ε = 0.14 W/m^3 (■), 0.5 W/m^3 (+), 1.2 W/m^3 (♦), 2.5 W/m^3 (▲) and 4.3 W/m^3 (*).

Little is known about the course of flocculability during full scale fermentation. However, the flocculability mainly depends on the composition of the medium, which can be expected to be equal on both lab scale and full scale. In this study a pure yeast strain was used resulting in a reproducible course of flocculability during fermentation. Some brewers, however, make use of heterogeneous strains. This will have consequences for the overall flocculability of the yeast that is in suspension in the tank. If the strains have different flocculation behaviour the settling behaviour will also vary. The most extreme case is the one in which a flocculent and a nonflocculent strain are present. The settling velocity of an individual cell is low, for this the cells of the nonflocculent strain will remain in suspension while the flocs of the flocculent strain will settle. In that case complete removal of the yeast cannot be achieved. The appearance of a heterogeneous strain can easily be detected by quantification of the flocculence or flocculability of the yeast during settling. If a representative sample is taken from the medium the ratio between the amount of flocculent cells and nonflocculent cells will decrease, because the number of flocculent cells decreases due

to settling. Consequently, the overall flocculence or flocculability of the sample will decrease.

Shear rate

During the experiments as described in this study the shear rate was varied by means of the revolution-speed of the stirrer in the vessel. The shear rate was derived from the power input in the vessel (Appendix). In Figure 7 the relation between power input and floc size is shown. The curve exhibits an exponential form with a sharp decrease in floc size between 0.1 W/m^3 and 2 W/m^3. In Figure 8 the effect of power input on the number of single cells is shown. As can be seen the number of single cells is constant at varying power input. In the range between 0.1 W/m^3 and 10 W/m^3 the percentage of single cells is approximately 10% of the total amount of yeast in suspension.

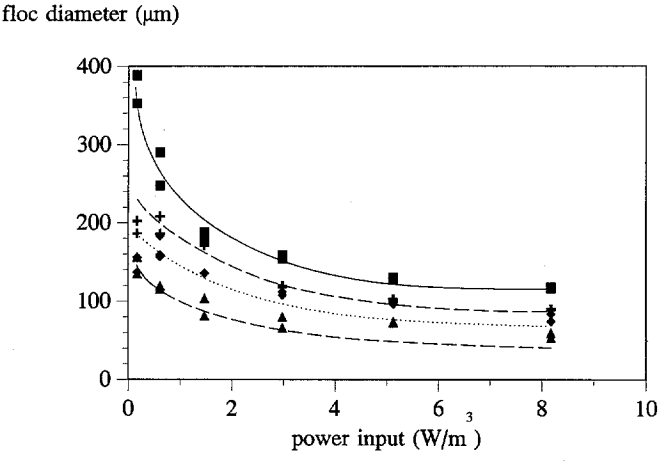

Figure 7. The relation between the diameter of yeast flocs and the power input in a stirred vessel. $N_{c,tot}$ = 49 × 10^6 cells/ml (■), 26 × 10^6 cells/ml (+), 14 × 10^6 cells/ml (♦) and 5 × 10^6 cells/ml (▲).

Full scale beer fermentations are mostly carried out in a cylindroconical tank. In a full scale tank the gas bubble formation as a consequence of the carbon dioxide production provides the power input from which the shear rate can be calculated (Appendix). The power input range during the experiments is chosen based on full scale conditions.

Renger (1988) reported a maximum carbon dioxide production rate per kg medium of 2.63×10^{-3} mol/kg s. The described cylindroconical vessel has got a height of 18.5 m, the volume is 400 m^3. From this the maximum power input can be calculated giving 3.7 W/m^3 (Appendix).

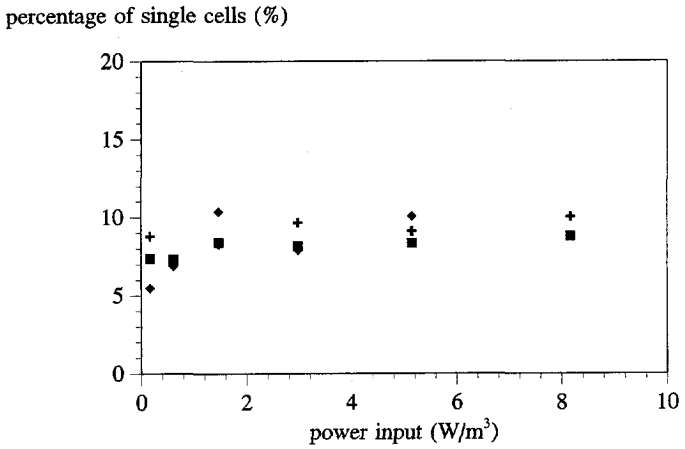

Figure 8. The percentage of single cells of the total number of yeast cells in suspension as function of the power input in a stirred vessel. $N_{c,tot}$ = 49 × 10^6 cells/ml (■), 26 × 10^6 cells/ml (+) and 14 × 10^6 cells/ml (♦).

To be able to translate the results from the power input variation in this study to full scale, the shear distribution and mixing time on lab scale and full scale have to be compared to the floc formation time.

In the range of power input applied in this study the mixing time varies between 10 s and 40 s (Voncken, 1960). The same range on full scale (V_f = 400 m^3; h_T = 11 m) will give mixing times between 100 s and 300 s (Joshi, 1980). The shear distribution in a stirred vessel is rather broad with a factor of 10 between the maximal and average power input (de Boer, 1987), resulting in a factor of 3 between the maximal and average shear rate. The floc formation time is about 10 s (Van Hamersveld, unpublished results). This means that the floc size distribution will be determined rather by the maximal than the average shear rate. On full scale the same power input will, regarding the large

mixing times, cause a floc size distribution following the shear distribution with an average value determined by the average shear rate.

As stated above the shear rate in a full scale tank depends on the carbon dioxide production rate. This production rate is not constant during fermentation. At the beginning of the fermentation the carbon dioxide production increases due to growth of the yeast. Partway through the fermentation, at the cell division stop, the production of carbon dioxide reaches its maximum. From then the carbon dioxide production starts to decrease due to the decreasing sugar concentration. This will lead to an increase in floc size, while the number of single cells remains constant.

Total amount of yeast present

The last factor studied, that will influence flocculation is the amount of yeast present. The effect of yeast amount on flocculation is shown in Figure 7 and Figure 8. The size of the flocs increases due to an increasing amount of yeast. The number of single cells is again constant at 10% of the total amount of yeast in suspension.

In this study the amount of yeast was varied by removing the yeast from the medium and resuspended it to the desired concentration. In a full scale tank, removal occurs by sedimentation of the yeast. At the end of the fermentation the conditions are favourable for sedimentation:
- the carbon dioxide production is low (giving a low shear rate),
- the flocculability is large (giving a large floc strength) and
- the yeast concentration is maximal.

These conditions are favourable for the formation of large flocs. Next to this, because of the low carbon dioxide production rate the fluid velocities will be low enough to allow settling of the flocs. As a result of this, the amount of yeast will decrease. The settling time on full scale has an order of magnitude of hours, while the floc formation takes a few seconds. Because of this, sedimentation and flocculation of the yeast will be in quasi steady state. During settling the yeast concentration will decrease resulting in decreasing floc size. This decrease in floc size will result in a decrease in settling velocity etc.

Estimation of flocculation on full scale

In Figure 9 the development of flocculation as can be expected during a full scale fermentation is shown. From the results of this study, the floc size and number of single cells were estimated. The flocculability course was taken from this study. From the sugar conversion the carbon dioxide production rate could be calculated. Assuming a cylindroconical tank (V_f = 400 m^3; h_T = 11 m) the power input could be derived (Appendix). Further, it was assumed that the settling of the flocs started at the moment the carbon dioxide production stops. In practice this will occur somewhat earlier, depending of the floc strength and fluid velocities in the tank.

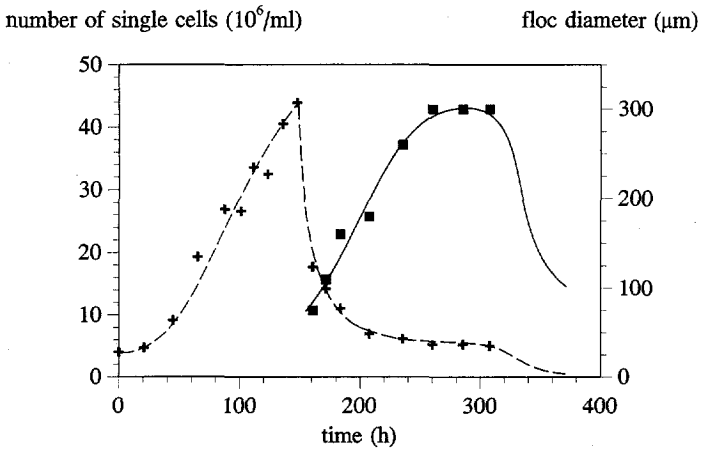

Figure 9. Development of the floc size (■) and the number of single cells (+) during the fermentation of beer.

The flocculation of the yeast suspension, expressed in terms of floc size and number of single cells, improves in the course of the fermentation. The development of flocculation is influenced by flocculability, shear rate and amount of yeast in suspension. The increase of flocculability and decrease of shear rate both cause an increase of the floc size. At the end of the fermentation, when the amount of yeast decreases due to settling, the floc size will decrease, which on its turn causes a decrease of the settling rate.

The number of single cells shows an almost similar course, due to increasing flocculability the number of single cells decreases between 150 and 200 h. When the settling of the flocs starts the number of single cells decreases proportionally to the amount of yeast in suspension.

CONCLUSIONS

Using a light extinction technique to measure the settling properties of yeast flocs is a powerful method to quantify flocculation. With this method the two parameters (floc size and number of single cells) that characterize flocculation can be measured simultaneously. The benefits of the measuring method as applied in this study are:
- flocculation can be quantified 'in situ' without disturbing flocculation.
- the results from the study can be translated to full scale conditions.
- within one step floc size and number of single cells can be measured.

The method can be used to quantify flocculation in a wide range of different yeast strains and growth conditions, such as temperature and medium composition. Next to this, the physical conditions can be varied as is shown in this study.

In view of the effect of floc strength, fluid shear and yeast concentration, the consequences for control of yeast flocculation are clear. Flocculation can be influenced directly by the floc strength, both floc size and number of single cells can be influenced. The shear rate has a strong effect on floc size. Via setting the power input the flocculation can be influenced.

Acknowledgements

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APPENDIX

Calculation of shear rate/power input in stirred tank and bubble column

The fluid flow within a vessel or tank strongly influences flocculation or aggregation processes. Shear rate is often used as a parameter in models that describe floc sizes (Dharmappa et al., 1993; Potanin, 1992; Tambo and Hozumi, 1979) or flocculation rates (Cleasby, 1984; Parker et al., 1972; Speers et al, 1992b). In case of laminar flow conditions (for example pipe flow and Couette flow) the shear rate can easily be calculated from respectively the flow rate (pipe) or the angular velocity (Couette device). However, usually flocculation occurs under turbulent flow conditions. For this situation the shear rate distribution is more complex. Camp and Stein (1943) derived an equation for the average shear rate ($\dot{\gamma}$) in turbulent flow,

$$\dot{\gamma} = \sqrt{\frac{\varepsilon \rho}{\eta}} \qquad (10)$$

where ε is the power input per mass, ρ is the density of the medium and η is the dynamic viscosity of the medium. Camp and Stein stated that this general equation 'is of equal validity in both viscous and turbulent flow'.

For calculation of the shear rate in turbulent flow the power input into the system must be known. In case of tube flow the power input can be calculated from the pressure drop in the pipe. For a Couette vessel the power input is related to the torque of the device. In stirred vessels the power input is provided by the stirrer and in case of bubble columns the gas input at the bottom of the vessel provides the power input.

Large scale brewery fermentations are nowadays mostly carried out in cylindroconical vessels. The power input for these vessels is provided by the yeast itself. Carbon dioxide is produced and gas bubbles rise in the fluid. The power input can be calculated from the total gas flow at the top of the fermenter (Delente et al., 1968), which in case of

saturation of carbon dioxide in the fluid is equal to the carbon dioxide production rate of the yeast.

$$\varepsilon = \frac{\varphi_m}{V_f} R\ T\left(\frac{a+1}{a}\ln(a+1) - 1\right)$$
$$a = \frac{\rho_l\ g\ h_T}{p_0} \tag{11}$$

where φ_m is the molar gasflow, V_f is the tank volume, R is the gas constant, T is the absolute temperature, p_0 is the atmospheric pressure, ρ_l is the density of the fluid, g is the gravitational constant and h_T is the height of the tank.

In this study we have made use of a stirred vessel to create a certain shear rate. The power input in a stirred vessel depends on the stirrer speed (N) and is defined as (Beek and Muttzall, 1986),

$$\varepsilon = \frac{Po\ N^3\ D_{Rush}^5}{V_f} \tag{12}$$

where Po is the power number, D_{Rush} is the diameter of the Rushton turbine and V_f is the volume of the vessel. The power number depends on the Reynolds number. For this study the power number is constant in the range of stirrer speeds that were applied (Po = 5, (Janssen and Warmoeskerken, 1987)).

NOMENCLATURE

ε	specific power input	W/kg
$\dot{\gamma}$	shear rate	s^{-1}
$\Delta\rho$	effective density	kg/m^3
Δd	range within a size class	m
η	viscosity	Pa s

ρ_l	density of the medium	kg/m^3
ρ_y	density of the yeast	kg/m^3
τ	turbidity	m^{-1}
φ_m	molar gas flow	mol/s
ϕ	volume fraction of particles in the suspension	-
a	dimensionless pressure	-
C	scattering cross section	m^2
D	fractal dimension of a floc	-
D_{Rush}	Rushton turbine diameter	m
d_c	cell diameter	m
d_f	floc diameter	m
$d_{f,m}$	average floc diameter	m
d_i	average particle diameter in size class 'i'	m
d_p	particle diameter	m
f	size distribution	-
g	gravitational constant	m/s^2
h_T	height of the tank	m
h_L	height at which the light beam passes the tube	m
K	calibration factor	-
L	length of the light path	m
N	stirrer speed	s^{-1}
N_0	particle number per unit of volume at t = 0 s	m^{-3}
$N_{c,tot}$	total cell number per unit of volume	m^{-3}
N_f	floc number per unit of volume	m^{-3}
N_s	single cell number per unit of volume	m^{-3}
N_t	particle number per unit of volume at t = t s	m^{-3}
OD_{660}	optical density of the suspension at a wavelength of 660 nm	-
$OD_{660,0}$	optical density of the medium at a wavelength of 660 nm	-
Po	power number	-
p_0	atmospheric pressure	Pa
Q_{sca}	scattering coefficient	-
R	gas constant	J/mol K
r	tube radius	m

S	fraction of the particle number	-
s_f	standard deviation of (ln d_f) of a lognormal floc size distribution	-
T	temperature	K
t	time	s
V_f	fermenter volume	m^3
v_{sed}	settling velocity	m/s

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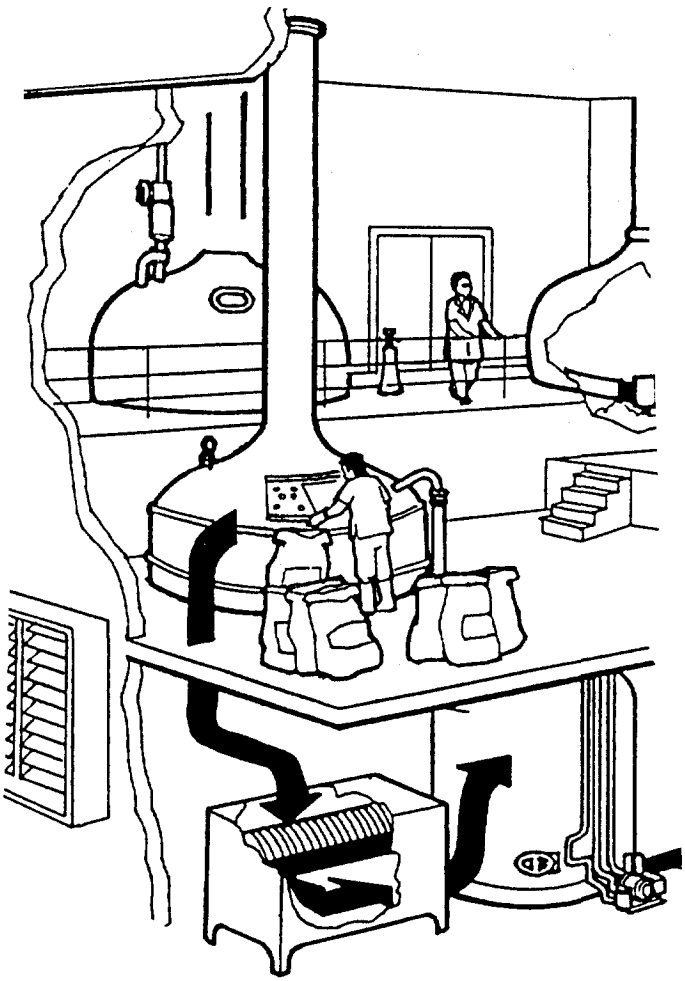

... the malting and brewing process ...

wort boiling	- After the separation of the wort from the spent solids the wort is boiled in the wort kettle. The process takes about 60-90 min. Some brewers nowadays use pressure boiling at 140 °C, this reduces the process time to approximately 4 min. After boiling the wort is clarified, cooled and aerated. - The effect of wort boiling is an arrest of the enzyme activity and sterilization of the wort. During wort boiling the wort is bittered by hop resins. Since oxygen is needed to allow yeast growth the wort is aerated after boiling, clarifying and cooling.

... to be continued on page 156 ...

CHAPTER VI

Modelling brewers' yeast flocculation

Abstract

Flocculation of yeast cells occurs during the fermentation of beer. Partway through the fermentation the cells become flocculent and start to form flocs. If the environmental conditions, such as medium composition and fluid velocities in the tank, are optimal the flocs will grow in size large enough to settle. After settling of the main part of the yeast the green beer is left, containing only a small amount of yeast necessary for rest conversions during the next process step, the lagering. The physical process of flocculation is a dynamic equilibrium of floc formation and floc breakup resulting in a bimodal size distribution containing single cells and flocs.

The floc size distribution and the single cell amount were measured under different conditions such as occurring during full scale fermentation. Influences on flocculation of floc strength, specific power input and total number of yeast cells in suspension were studied. A flocculation model was developed and the measured data used for validation. Yeast floc formation can be described with the collision theory assuming a constant collision efficiency. The breakup of flocs appears to occur mainly via two mechanisms, the splitting of flocs and the erosion of yeast cells from the floc surface. The splitting rate determines the average floc size and the erosion rate determines the number of single cells. Regarding the size of the flocs with respect to the scale of turbulence, only the viscous subrange needs to be considered. With the model the floc size distribution and the number of single cells can be predicted if the bond strength between the cells, the specific power input in the tank and the number of yeast cells that are in suspension in the tank are known.

INTRODUCTION

The phenomenon of particle aggregation is used in various types of processes, for example crystallization, polymerization, protein production, productions of ceramics, catalysts, removal of sludge from treated wastewater and the removal of yeast from beer. In the chemical industry, in water treatment, in the biochemical industry and the brewing industry, flocculation and/or aggregation processes play an important role for production and downstream processing. Here we focus on the flocculation of yeast cells, occurring during the fermentation of beer.

In the brewing industry, yeast flocculation occurs during the fermentation as one of the last steps of the brewing process. The yeast cells flocculate spontaneously, without addition of destabilizing chemicals, the so-called coagulants or flocculants. The flocculation of the yeast facilitates its removal at the end of the fermentation. However, due to its variability flocculation can also lead to complications in the fermentation. Weak flocculation will lead to problems during lagering and subsequent filtration of the beer. On the other hand strongly flocculating yeast will lead to early sedimentation of the yeast while complete attenuation has not yet been reached. To overcome these problems, control of yeast flocculation during the fermentation is required.

Beer fermentations are mostly carried out in cylindroconical vessels, with a height up to 20 m and a diameter up to 10 m (Hough, 1985). These vessels have a cone with an angle of 60° - 75°. The yeast suspension is mixed because of carbon dioxide production by the yeast and the subsequent bubble formation. The bubbles formed rise to the top of the tank and by that cause a fluid motion. The induced fluid velocities in the vessel can reach values up to 50 cm/s (Renger, 1991). Fluid flow within the vessel is therefore turbulent ($Re > 10^6$). Under these hydrodynamic conditions flocculation of the yeast cells can occur.

A beer fermentation carried out at 9 °C usually takes 10 to 12 days. The fermentation starts with inoculation of the wort with a certain amount of yeast. Partway through the fermentation the yeast cells become flocculent. The initiation of flocculence coincides with the stop of cell division (Straver et al., 1993). After initiation of flocculence, the flocculability (= the ability of yeast cells to flocculate in a given medium if the collision

rate is sufficient and the breakup forces are not too high and sufficient cells are present; Van Hamersveld et al., 1995a) starts to increase due to amongst other things, decreasing pH and sugar concentration (Smit et al., 1992).

Next to flocculability, flocculation depends on the collision rate of the flocs and the shear forces acting on the flocs. Both phenomena occur as a result of velocity gradients in the liquid. In a full scale tank these velocity gradients are directly correlated to the carbon dioxide production rate, which is maximal when the yeast cells become flocculent. Due to decreasing sugar concentration the carbon dioxide production will decrease from the moment the cells become flocculent. Flocculation will lead to sedimentation if the turbulence intensity or fluid velocities in the tank are low enough. So, from the moment the cells become flocculent the conditions for sedimentation to occur become more favourable. The flocculability increases whereas the shear forces decrease, resulting in bigger flocs and finally the turbulence intensity and fluid velocities will decrease leading to settling of the flocs.

Yeast removal by sedimentation can be characterized by two variables, the size of the flocs and the number of single cells (= freely suspended cells not forming part of a floc). The floc size determines the settling rate of the flocs and therefore the removal rate. The number of single cells determines the yeast amount that remains in suspension after settling of the flocs. In order to control flocculation and subsequent sedimentation, better understanding of the hydrodynamical/physical mechanism of the actual flocculation is needed. In this study we will present a model that describes yeast flocculation based on the theory of particle movement in turbulent flow. The input parameters will be evaluated on their suitability to serve as a control parameter.

MODEL DEVELOPMENT

Floc formation is a result of effective collisions between cells, flocs and cells and flocs. With the collision theory of Smoluchowski (1917) the number of collisions per time between solid particles in liquid or gas can be calculated. The particle collision frequency depends on the particle concentration, the size of the particles and the scale of turbulence. An effective collision between two particles will lead to a new particle. The fraction of effective collisions is defined by the collision efficiency.

Next to floc formation there is also floc breakup in the flocculation yeast suspension. The bond strength between particles within a floc or aggregate will, depending on the external forces acting on the floc, determine whether the aggregate will break up. Yeast flocs are very sensitive for breakup phenomena. Already small forces acting on the flocs cause breakup.

Several mechanisms may account for the breakup of flocs. Hydrodynamic forces, collisions between particles, collisions between particles and the wall of the tank or between particle and stirrer device can all cause the breakup of flocs. If the density difference between particle and fluid is low, particle disruption via collisions is relatively insignificant (Ayazi Shamlou et al., 1994a). In case of yeast flocculation the effective density is very low, so only hydrodynamic forces will need to be considered.

Floc breakup by hydrodynamic forces can occur in two ways, the flocs can be splitted into two or more parts or they can erode via the release of individual cells from the floc surface. Here we will study via which mechanisms yeast flocs are formed and broken up. The relations for floc formation, floc splitting and erosion as presented in literature will be evaluated on applicability to yeast flocculation in turbulent flow. Next to this, parameters influencing these processes will be studied.

Turbulence

Flocculation rate and the underlying mechanism depend strongly on the hydrodynamic regime, which is mainly the turbulent regime. Turbulent flow consists of a continuum of eddies ranging in size from the Eulerian macroscale to the Kolmogorov microscale. Turbulent fluid motion has a random character. Therefore, a useful approach to deal with turbulence is a statistical one. The instantaneous fluid velocity at a point is represented by a mean and a fluctuating part.

The fluctuating part, the so called root mean square (rms) velocity fluctuation, represents the spread of the velocity distribution. The average relative velocity between two points is given by the rms velocity difference of the fluid flow over the corresponding eddy scale. This velocity is used to determine the particle encounters in the collision theory.

The eddy scale can be divided into three regimes: Eulerian macroscale of turbulence, the inertial subrange and the viscous subrange.

Eddies with a length scale L, the Eulerian macroscale of turbulent flow, are the energy containing eddies. The size of these eddies is roughly of the same order as the size of the impeller blades in case of turbulent flow in a stirred tank (Kusters, 1991). In general in mechanically or pneumatically agitated tanks, the energy containing eddies have a size similar to the dimensions of the main flow stream (Kawase and Moo-Young, 1990). The average relative velocity of this turbulence scale can be calculated from the specific power input (ε; W/kg) (Taylor, 1935),

$$\Delta u(r) \sim \sqrt{2}\ (\varepsilon\ L)^{0.33} \tag{1}$$

where r is the radial distance between two points within the eddy.

Large eddies are instable and disintegrate into smaller eddies for which the Reynolds number rapidly decreases with the diameter until it is approximately unity for the smallest eddies.

The eddies of intermediate size are within the inertial subrange. These eddies transfer energy from the largest eddies to the smallest eddies. The average relative velocity within these eddies is equal to (Kolmogorov, 1941),

$$\Delta u(r) = 1.37\ (\varepsilon\ r)^{0.33} \tag{2}$$

Because of the low Reynolds' number, the small scale motion will be strongly affected by viscosity. The average relative velocity between two points within the smallest eddies is therefore equal to (Taylor, 1935),

$$\Delta u(r) = \left(\frac{1}{15}\right)^{1/2} \left(\frac{\varepsilon}{\nu}\right)^{1/2} r \tag{3}$$

The length of these small eddies is called the Kolmogorov length (λ_{Kol}). This length scale is defined as (Kolmogorov, 1941),

$$\lambda_{Kol} = \left(\frac{\nu^3}{\varepsilon}\right)^{0.25} \tag{4}$$

where ν is the kinematic viscosity.

The Kolmogorov length scale is normally used as a boundary between the viscous and the inertial subrange. Eddies of a size smaller than the Kolmogorov scale are within the viscous subrange whereas eddies with a larger size are within the inertial subrange.

Floc formation

Floc formation is caused by collisions between the particles in the medium. A collision between two yeast cells, two yeast flocs or a yeast floc and a yeast cell results in a newly formed particle, depending on the efficiency of the collision. The collisions between the particles are caused by movement of the particles. In general, three mechanisms are responsible for particle movement within a fluid: Brownian motion, entrainment by fluid shear and gravity resulting in settling of the particles.

- The first mechanism, Brownian motion, only applies to small particles. Particle collisions due to Brownian motion (perikinetic collision) occur between particles with a diameter smaller than 1 μm (Friedlander, 1977).
- The second mechanism via which particles collide is relative motion caused by velocity gradients within the fluid. For this mechanism no particle size restrictions apply.
- Finally particles will also move by gravity provided the turbulent forces are low enough (Davies, 1972). Different floc sizes will result in different settling velocities which may lead to collision.

Yeast cells have a diameter of approximately 7 μm. Due to the fractal buildup of yeast flocs, the density difference between flocs of different sizes is low. So, within in the

range of turbulence intensities as applied in this study, only particle collision caused by liquid velocity gradients is significant.

Particles in a shear field will collide due to their relative motion, see Figure 1. A particle with a higher velocity will collide with a particle with a lower velocity moving in the same plane. While moving with a given velocity, the particle will sweep out a certain volume per time. This volume is represented by the particle velocity times the cross-sectional area of the particle. The moving particle will collide with the particles contained in this volume. The collision rate of a particle is proportional to the volume swept out per time times the particle concentration. The magnitude of the collision rate depends on the scale of turbulence, since this determines the average relative fluid velocity.

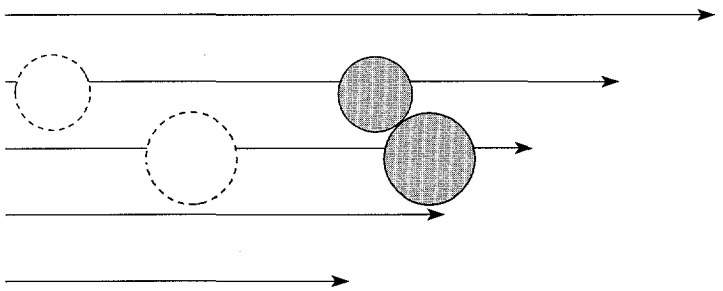

Figure 1. Schematic representation of particle collision due to velocity gradients in the fluid.

In case of collisions between particles with a size smaller than the Kolmogorov length the relation as defined for the viscous subrange should be applied (equation 2). At this turbulence scale the flow can assumed to be laminar. If the colliding particles are larger than the Kolmogorov length, the relation as defined for the inertial subrange should be applied (equation 3). The floc formation rate can now be written as (Appendix 1),

$$K_{ij} = \frac{dN_k}{dt} = \alpha\, k_{K,v}\, N_i\, N_j \left(\frac{\varepsilon}{\nu}\right)^{1/2} (d_i + d_j)^3 \qquad for\ \ d_i + d_j < \lambda_{Kol}$$

$$K_{ij} = \frac{dN_k}{dt} = \alpha\, k_{K,i}\, N_i\, N_j\, \varepsilon^{1/3}\, (d_i + d_j)^{7/3} \qquad for\ \ d_i + d_j > \lambda_{Kol} \tag{5}$$

where K_{ij} is the floc formation rate, α is the collision efficiency, $k_{K,v}$ and $k_{K,i}$ are the collision rate constants of the viscous subrange and the inertial subrange respectively and N is the particle number per volume.

Not all collisions will lead to a newly formed floc, therefore a collision efficiency is defined. According to Bell (1981), the collision efficiency in case of bioadhesion depends on: contact time, contact area, rate of bond formation per area and number of bonds required to bind two particles together. The first three parameters determine the number of bonds that will be formed during a collision between two particles. If the number of bonds that will be formed during a collision is higher than the number of bonds to hold two particles together, the collision will be effective.

Adler (1981) developed a more theoretical approach of collision efficiency based on particle trajectories in uniform shear flow. This resulted in a collision efficiency depending on particle size and shear rate. However, usually the collision efficiency is defined as a constant.

Floc breakup

Floc splitting

Floc splitting results from pressure differences caused by fluctuating fluid velocities acting on opposite sites of a floc (Thomas, 1964). These pressure differences are related to the eddy velocities, assuming that the only eddies influencing the floc are equal in size to the floc. Therefore, the rate of floc splitting depends on the eddy frequency. The eddy frequency can be calculated through division of the relative velocity (equation 2 and equation 3) by the diameter of the eddy. Next to this the splitting rate depends on particle concentration and splitting efficiency.

In Appendix 2 the different equations for the floc splitting rate are presented. The equations as presented by Kusters (1991) and Ayazi Shamlou et al. (1994b) are almost identical. Both defined a probability function for the splitting efficiency. Kusters (1991) used the ratio of the critical energy dissipation rate at which the flocs rupture (ε_c) and the minimum dissipation rate in the system (ε_{min}). Ayazi Shamlou et al. (1994b) used the ratio of the mechanical strength of a floc and the shear stress of the fluid. The shear stress (τ_s) can be calculated from the specific power input (Camp and Stein, 1943),

$$\tau_s = \rho_l \sqrt{\varepsilon \nu} \tag{6}$$

where ρ_l is the density of the medium.

The mechanical strength (σ) is defined as,

$$\sigma = \frac{9}{8} k_c \phi_{cf} F \frac{1}{\pi d_c^2}$$
$$\phi_{cf} = \left(\frac{d_f}{d_c}\right)^{D-3} \tag{7}$$

where k_c is the coordination number of the particles in the floc ($k_c = 15\ \phi_{cf}^{1/2}$; Kusters, 1991), ϕ_{cf} is the volume fraction of particles in a floc, F is the bond strength, d_c is the yeast cell diameter, d_f is the yeast floc diameter and D is the fractal dimension of the yeast flocs. This leads to the following equations for floc breakup via splitting,

$$S_k = \frac{dN_k}{dt} = \left(\frac{1}{15}\right)^{1/2} \exp(-\sigma/\tau_s)\ N_k \left(\frac{\varepsilon}{\nu}\right)^{1/2} \qquad \text{for } d_k < \lambda_{Kol}$$
$$S_k = \frac{dN_k}{dt} = \left(\frac{1}{15}\right)^{1/2} \exp(-\sigma/\tau_s)\ d_k^{-2/3}\ N_k\ \varepsilon^{1/3} \qquad \text{for } d_k > \lambda_{Kol} \tag{8}$$

where S_k is the floc splitting rate.

Surface erosion

The second breakup mechanism, which was proposed by Argaman and Kaufman (1970), is surface erosion. Primary particles are released from the floc surface by fluid shear. In Appendix 2 the relations for surface erosion are also presented.

The rate of particle erosion also depends on the eddy frequency. Eddies of a scale equal to the size of the floc will impart maximum surface shear (Parker et al., 1972). The number of cells released depends on floc surface area and particle volume fraction within the floc. A larger floc will have a larger surface area and will therefore loose more primary particles. On the other hand larger flocs will have a lower volume fraction of primary particles. Due to the relation between the aggregation number and the yeast floc size that can be described according to the theory for fractals, the volume fraction decreases with increasing floc size (Fontana et al., 1992; Logan and Wilkinson, 1991; Davis and Hunt, 1986). As for the floc formation rate and the floc splitting rate, the surface erosion rate also depends on particle concentration. This leads to the following relations for the surface erosion rate,

$$E_k = \left(\frac{\mathrm{d}N_1}{\mathrm{d}t}\right)_k = k_{E,v}\, N_k \left(\frac{\varepsilon}{\nu}\right)^{1/2} d_k^2 \qquad for \quad d_k < \lambda_{Kol}$$

$$E_k = \left(\frac{\mathrm{d}N_1}{\mathrm{d}t}\right)_k = k_{E,i}\, N_k\, \varepsilon^{1/3}\, d_k^{5/3} \qquad for \quad d_k > \lambda_{Kol} \tag{9}$$

where E_k is the erosion rate, $k_{E,v}$ and $k_{E,i}$ are the erosion rate constants of the viscous and subrange and the inertial subrange respectively.

Model in- and output

The evolution of the floc size distribution depends on the following parameters: bond strength between the cells, total number of yeast cells and the shear rate (specific power input).

- The bond strength will determine the erosion rate constant and the splitting efficiency. In case of erosion Parker et al. (1972) defined a floc surface shear strength

that was part of the erosion rate constant. In case of floc splitting a floc splitting efficiency was defined (Ayazi Shamlou et al., 1994b) containing the bond strength between two cells as one of the parameters.

- The total number of primary particles will influence the average floc size. This can be understood from the influence of particle number in both formation and breakup rate. Collisions occur between two particles which makes the formation rate proportional to the particle number squared. The breakup rate is first order with respect to the particle number. Because of this, the floc size will increase with increasing particle number.
- The specific power input influences both phenomena, floc formation rate and floc breakup rate. Increasing specific power input will result in an increasing collision rate, because the relative velocity between the particles will increase. Further, increasing the specific power input will result in higher forces acting on the flocs leading to a decreasing average floc size.

MODEL VALIDATION

Experimental data

The model was validated by a series of experiments as described earlier (Van Hamersveld et al. 1995b). Then the influence of floc strength, number of yeast cells and specific power input on yeast flocculation was described.

In this study it is confirmed that the equilibrium floc size distribution in case of yeast flocculation is bimodal as was found by several authors (Davis and Hunt, 1986; Stratford et al., 1988; Miki et al., 1982a and 1982b). The total size distribution consists of a lognormal distribution of flocs with an average standard deviation of 0.45 and a narrow exponential distribution of single cells and small flocs. The single cell amount in the experiment turned out to be constant at approximately 10%, the average floc size varied between 50 μm and 350 μm. The experiments as described by Van Hamersveld et al. (1995b) were carried out at a power input between 0.1 W/m^3 and 10 W/m^3, which is comparable to the power input at a full scale brewery fermentation. The medium used

was green beer (pH 4.2; E_s = 3.8 °P; $[Ca^{2+}]$ = 1.2 mol/m^3). The total cell number was varied between 5 × 10^6 and 50 × 10^6 cells per ml.

Model calculations

The model calculations were carried out on a 486-DX processor based personal computer. The differential equations as presented in the Appendices 1 and 2 were solved iteratively. To reduce the calculation time the model was discretisized (Appendix 3). In this way the calculation of one equilibrium size distribution took about 15 minutes.

The model was first tested on application of the breakup mechanisms and the scale of turbulence. From this the model structure was optimized, by choosing the most appropriate mechanism and turbulence scale. With the optimized model the experimental data were compared to the model calculations in the range of specific power input and cell number as described above. Hereby the collision efficiency, bond strength and erosion rate constant were varied until the modelled data fitted the measured ones. Because a computational fit procedure would at least need 100 iterations (= 24 h), the evaluation of the best fit was done manually.

RESULTS AND DISCUSSION

Application of breakup phenomena

The model was first tested by applying only one of the breakup mechanisms. The use of collision theory and floc breakup via surface erosion as presented by Argaman and Kaufman (1970) resulted in a bimodal size distribution. However, the percentage of primary particles (yeast cells) as predicted by the model was much higher than the 10% measured. This high single cell amount was caused by the fact that the erosion rate mainly depends on the size of the flocs formed. Relatively large flocs will release numerous cells causing the high number of small flocs and single cells.

In the same way the model was tested using floc formation by particle collision and floc breakup by floc splitting. In this way a floc size distribution was obtained with an

acceptable average floc size. However, the particle size distribution was not bimodal. The flocs are splitted only, while a significant amount of primary particles will only arise if individual cells are sheared from the flocs surface or if very small flocs are splitted. However, the mechanical strength is very high for small flocs (equation 7), resulting in a relative low splitting rate for small flocs. These flocs have a higher cell density, which explains their relative high mechanical strength. Therefore floc splitting only will not lead to a bimodal size distribution.

From the results presented above, it was decided to take both breakup mechanisms into account, floc splitting as well as erosion of cells from the floc surface. The two-way approach of floc breakup resulted in a bimodal floc size distribution with a number of single cells and average floc size comparable to the experimental results. Hereby the erosion rate determines the number of single cells and the splitting rate determines the average floc size. The relations for the floc formation rate as presented in Appendix 1 were used. For the collision rate constant in the viscous subrange the value as calculated by Camp and Stein (1943) was chosen. This collision rate constant was derived for undisturbed laminar flow. Regarding the density of yeast cells this seems to be the best approach in case of yeast flocculation.

Application of turbulence scale

Next to the breakup phenomena the turbulence scale was evaluated. Most authors use the Kolmogorov length (equation 4) as a boundary between the viscous subrange and the inertial subrange. However, some authors use a value of six times the Kolmogorov length to be the upper limit of the viscous subrange (Table 1).

Table 1 The scale of turbulence as presented by Kusters (1991) derived from Liepe (1980) and Obuchov and Jaglom (1958), r is the eddy size, λ_{Kol} is the Kolmogorov length and L is the Eulerian macroscale.

subrange	eddy size
viscous	$r < 6\lambda_{Kol}$
transition	$6\lambda_{Kol} < r < 25\lambda_{Kol}$
inertial	$25\lambda_{Kol} < r < \frac{1}{2}L$
macro	$\frac{1}{2}L < r \approx L$

The model was first tested by applying the Kolmogorov length as the boundary between the viscous and inertial subrange. The results were unacceptable. Because the floc formation rate as well as the floc breakup rate changes at the Kolmogorov length the floc size distribution resulting from the model shows unregularities around this point. Application of the turbulence scale as proposed by Kusters gave much better results. The size of the yeast flocs is lower than the Kolmogorov length (Figure 2), so in this case only the viscous subrange has to be considered. This makes the model far more simple, because the equations applying for the inertial subrange can be left out.

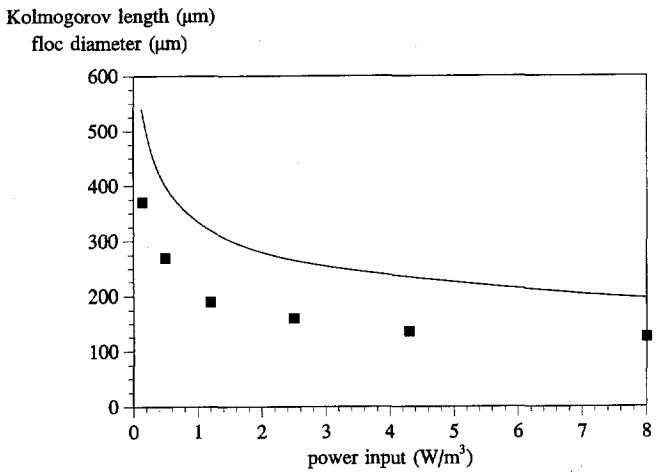

Figure 2. Floc size (■) and Kolmogorov length (solid line) against power input. The floc size was determined at a cell concentration of 50×10^6 cells per ml.

Simulation results

In Table 2 the parameters used in the model for flocculation of yeast are given. The fractal dimension of the flocs, the kinematic viscosity of the medium, the yeast cell diameter and the medium density are assumed to be constant. During beer fermentation the fractal dimension and the viscosity are constant, the cell diameter and the density of the medium only change slightly, less than 5%.

Power input, bond strength between the cells and total number of yeast cells change during fermentation. The power input which is proportional to the carbon dioxide production rate changes between 0 and 4 W/m^3 during full scale beer fermentation. The bond strength between two yeast cells depends on flocculability, values from 5×10^{-9} N (Van Hamersveld et al., 1994) to 0.02×10^{-9} N (Speers et al., 1993) are reported in literature. The flocculability of the cells increases during fermentation, but, at the end of the fermentation it is normally constant. Finally, the total number of yeast cells that is in suspension also changes during fermentation. Due to sedimentation this number will decrease at the end of the fermentation.

The collision efficiency and the erosion rate constant were estimated using the measured data. Because the bond strength was not measured, it was also estimated by the model. The collision efficiency is a constant in case of yeast flocculation and had a value of 1.0×10^{-3}, the erosion rate constant showed to be 2.0×10^{4} m^{-2}, and the bond strength was 0.25×10^{-9} N. The latter value is of the same order of magnitude as measured earlier with the same yeast strain (Van Hamersveld et al., 1994).

Table 2 The parameters used for modelling yeast flocculation.

parameter	symbol	value
constant parameters		
fractal dimension	D	2.45
kinematic viscosity	ν	2.3×10^{-6} m^2/s
yeast cell diameter	d_c	7 μm
medium density	ρ_l	1005 kg/m^3
variable parameters		
power input	ε	0.15 - 10 W/m^{-3}
total cell number	$N_{c,tot}$	$5 - 50 \times 10^{12}$ m^{-3}
fit parameters		
collision efficiency	α	1.0×10^{-3}
bond strength	F	0.25×10^{-9} N
erosion rate constant	k_E	2.0×10^{4} m^{-2}

In Figure 3 the results of the simulated floc size distribution compared to the measured ones are shown. In this Figure the markers represent the simulated floc number in a size class, the solid lines are calculated from the measured average floc size and standard

deviation of the lognormal size distribution. The size distributions were obtained at a total cell number of 50 × 10^6 cells per ml and power inputs of 0.5 W/m^3 and 1.5 W/m^3. In case of changing the involved parameters similar results were obtained. The size distribution of small flocs is an exponential one with a large slope. For this, data were fitted on cross-sectional area instead of number of single cells according to the way the data were obtained (Van Hamersveld et al., 1995b).

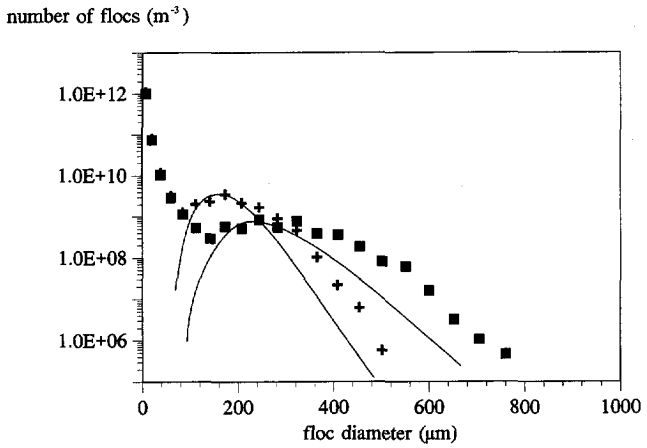

Figure 3. Example of simulated floc size distribution (markers) compared to the floc size distribution as was measured (solid lines). The cell number was 50 × 10^6 cells per ml and the power input had a value of 0.5 W/m^3 (■) and 1.5 W/m^3 (+).

In Figure 4, Figure 5 and Table 3 results of the model simulations are shown. In Figure 4 the average floc size is plotted as a function of the power input. The symbols are the measured data while the lines represent the simulation results. The power input and the total cell number were varied as indicated in Table 2. The bond strength was constant in all cases. With the model it was possible to simulate the measured data, whereby the best results were obtained in case of high cell numbers (50 × 10^6 cells per ml). At lower cell numbers the floc size is overestimated by the model, especially in case of a low power input. The introduction of a size dependent collision efficiency as proposed by Adler (1981) will reduce this effect. The collision efficiency for small flocs

is lower than that for big flocs because of the size density relation, which can be described by the theory of fractals.

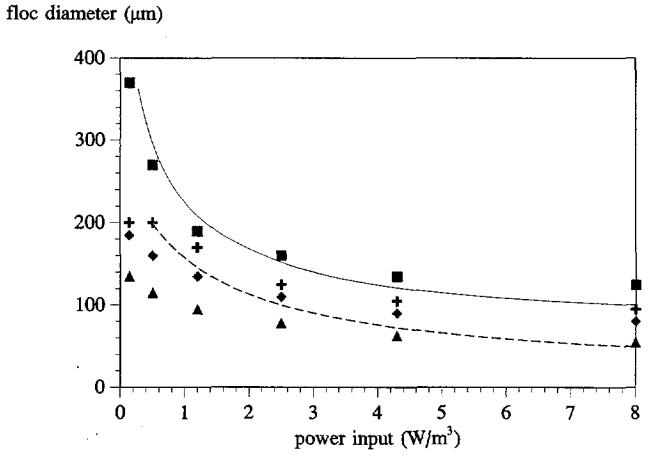

Figure 4. Influence of the power input on the average floc size in case the total cell number is 50 (■), 26 (+), 14 (♦), 5×10^6 (▲) cells per ml. The plotted lines are the simulated relations according the model presented for a cell number of 5×10^6 (dotted line) and 50×10^6 cells per ml (solid line).

In Table 3 the number of single cells that was measured and simulated are presented. Variation of power input between the values as reported in Table 2 has no effect on the number of single cells at a constant total cell number and a constant floc strength and varying power input. The same result is found from the model simulations. On the other hand the total cell number influences the percentage of single cells. A decrease of total cell number leads to relatively more single cells according to the model simulations. The same effect was seen during experimental verification, however, these results are less convincing due to the spread in the measurements. This effect can be understood from the influence of the particle number on the collision rate and erosion rate. The particle number is second order with respect to the collision rate, while it is first order with respect to the erosion rate. This leads to a relatively high single cell number at a decreasing total cell number.

Table 3 Comparison between experimental and simulated data for the number of single cells expressed in terms of the percentage of the total cell number. The values are obtained by varying the power input between 0.1 W/m^3 and 8.0 W/m^3 and showed to be constant within this range.

total cell number (10^6 ml^{-1})	number of single cells (%)		
	simulated	*experimental*	*standard deviation*
50	5.7	8.1	0.7
26	7.0	8.7	1.6
14	8.9	7.8	2.6
5	15.5	14.3	9

In Figure 5 the simulated effect of bond strength on average floc size and number of single cells are shown. The simulation is carried out at a total cell number of 50 $\times$ 10^6 cells per ml and a power input of 0.5 W/m^3 and 8.0 W/m^3. The simulations could not be validated because experimental data on bond strength were not available. However floc size and single cell number were measured at varying flocculability (Van Hamersveld et al., 1995b). The floc size thereby varied between 150 μm and 250 μm at a total cell number of 50 $\times$ 10^6 cells per ml and a power input of 0.5 W/m^3. In the same range the number of single cells decreased from 40% to 10% of the total cell number. In Figure 5 it is shown that this is approximately the range where the bond strength increases from 0.04 $\times$ 10^{-9} N to 0.4 $\times$ 10^{-9} N. At the same total cell number and at a power input of 8.0 W/m^3, the number of single cells is identical, while the average floc size is lower, what is also shown in Figure 4.

Sensitivity analysis and controllability

The parameter sensitivity of the model was determined by changing the parameter concerned by 10%. The power input and total cell number were 0.5 W/m^3 and 50 $\times$ 10^6 cells per ml respectively. The collision efficiency, the erosion rate constant, the bond strength of the cells and the fractal dimension of the flocs were tested on their sensitivity to the average floc size and the percentage of single cells. Figure 6 shows that the fractal dimension is the most sensitive parameter. It is therefore important to determine this parameter accurately. An increasing fractal dimension leads to compacter and thus smaller flocs. In case of the number of single cells the collision efficiency and

erosion rate constant are most sensitive. This can be understood from the erosion rate and collision theory. A higher erosion rate will automatically lead to more single cells. On the other hand a higher collision efficiency will lead to fewer single cells, because the cells will then rapidly form flocs.

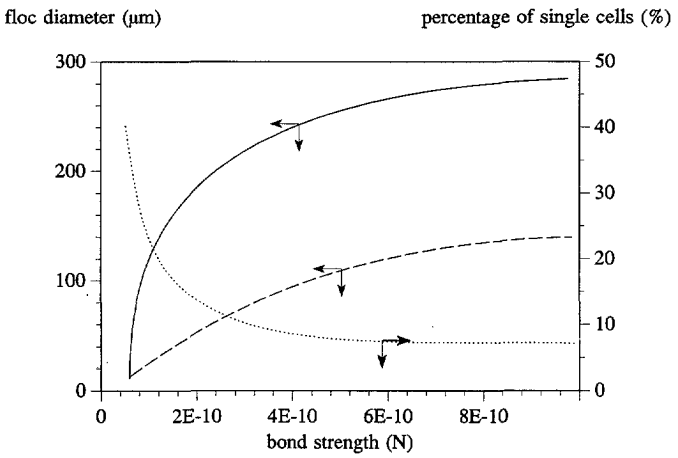

Figure 5. The influence of bond strength between the yeast cells on average floc size and the number of single cells expressed as the percentage of the total cell number. The relation between average floc size, number of single cells and bond strength is calculated for a total cell number of 50×10^6 cells per ml and a power input of 0.5 W/m^3 (solid line) and 8.0 W/m^3 (dashed line) percentage of single cells (dotted line).

Control of yeast sedimentation can be achieved by regulation of flocculation. In the flocculation model four parameters can be used to influence the floc size distribution: i) fractal dimension, ii) power input, iii) total cell number and iv) the bond strength. The nature of floc buildup as described by the fractal dimension is a difficult subject and not much is known about this yet. However, in literature different values for the fractal dimension of yeast flocs are presented, indicating that it is probably a strain dependent value. Earlier it was shown that the fractal dimension is independent of power input and floc strength, which fortifies the conclusions on strain dependence. Therefore, this fractal dimension can only serve as a control parameter by choosing the appropriate yeast strain.

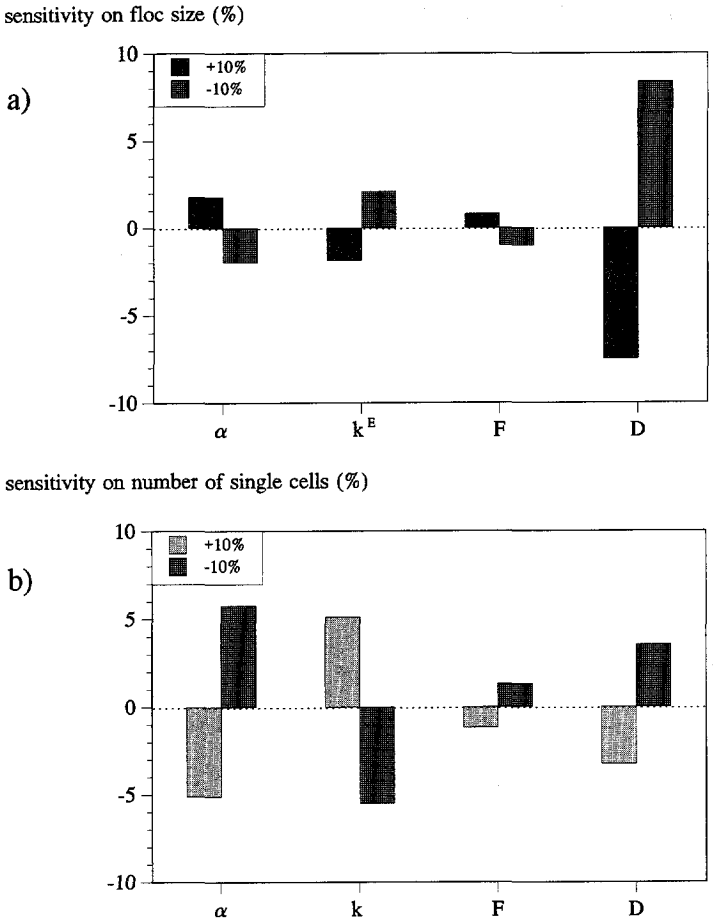

Figure 6. Sensitivity analysis of collision efficiency, erosion rate constant, bond strength and fractal dimension to the average floc size (a) and the number of single cells (b). The involved parameter is changed by 10% and the percentual change of floc diameter and single cell number are calculated.

The effect of power input is clear: increasing or decreasing power input leads to decreasing or increasing floc size respectively. However, the number of single cells is

independent of power input. This parameter can therefore be used to control the sedimentation via regulation of the floc size during fermentation.
The total number of yeast cells influences both single cell number and average floc size. Control via this parameter can be achieved by regulation of the amount of biomass formed during the growth phase of the fermentation.
Finally, the bond strength via which also both floc size and single cell number can be influenced can be used to control yeast sedimentation. The bond strength depends on yeast strain, growth conditions and the composition of the medium. This makes control via bond strength during fermentation complex. However, it is possible to influence flocculation by choosing the appropriate yeast strain, growth conditions and medium composition.

CONCLUSIONS

The floc size distribution of yeast as occurring in 'turbulent' flow is determined by an equilibrium of three phenomena: floc formation by particle collision and floc breakup resulting from erosion of primary particles from the floc surface and from the splitting of the flocs. In case of yeast flocculation occurring at a power input between 0.1 W/m^3 and 10 W/m^3 the eddy subranges according to Kusters (1991) as presented in Table 1 can be applied. Regarding the average size of the flocs this means that in case of yeast flocculation only the viscous subrange has to be considered.

The collision efficiency turned out to be constant at varying power input and for all involved floc sizes (up to 1 mm). The model is simple and only a few parameters must be known to be able to calculate average floc size and the number of single cells. Once the parameters that are to the system, such as fractal dimension of the flocs and viscosity, are known, only bond strength, number of single cells in suspension and the carbon dioxide flow, from which the power input can be calculated, should be determined in order to predict the floc size distribution.

From the parameter sensitivity analysis it can be concluded that the fractal dimension is the most sensitive parameter regarding the average floc size.

Control of yeast sedimentation during the fermentation can be achieved by varying the power input. Influencing flocculation before the fermentation can be accomplished by choosing the appropriate yeast strain, growth conditions and medium composition.

Acknowledgements

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APPENDIX 1

Overview of collision rate constants in literature

The definition of the collision rate in literature is rather consequent except for the definition of the inertial and viscous subrange and the value of the collision rate constant. In Table 4 the collision rate constants as found in literature are given.

The differences between the collision rate constants can be explained from the assumptions made at the derivation of the equations. The most simple derivation is made by Smoluchowski (1917) and Camp and Stein (1943) for undisturbed laminar shear flow giving a collision rate constant of 1/6.

The boundary between the inertial and viscous subrange is mostly made at the Kolmogorov length. An exception is made by Kusters (1991), who defined the boundary at six times the Kolmogorov length.

Table 4 Relations to describe the collision rate of particle '*k*' according to the collision theory of particles in a fluid shear field. Particle '*k*' is formed by collision between the particles '*i*' and '*j*'. α is the collision efficiency, K_{ij} is the collision rate between the particles '*i*' and '*j*'. k_K is the collision rate constant, ε is the specific power input, ν is the kinematic viscosity, d is the particle diameter and N is the particle number per volume.

viscous subrange

$$K_{ij} = \frac{dN_k}{dt} = \alpha\ k_K\ N_i\ N_j \left(\frac{\varepsilon}{\nu}\right)^{1/2} (d_i + d_j)^3 \qquad for\ \ d_i + d_j < \lambda_{Kol} \tag{10}$$

$k_{K,\nu}$	reference
$(\pi/120)^{1/2} \approx 0.16$	Saffman and Turner (1956), Spielman (1978), Kusters (1991)
$\pi\ 60^{-1/2} \approx 0.41$	Delichatsios and Probstein (1975)
$1/6 \approx 0.17$	Camp and Stein (1943), Friedlander (1977), Ives (1978)

inertial subrange

$$K_{ij} = \frac{dN_k}{dt} = \alpha\ k_{K,i}\ N_i\ N_j\ \varepsilon^{1/3}\ (d_i + d_j)^{7/3} \qquad for\ \ d_i + d_j > \lambda_{Kol} \tag{11}$$

$k_{K,i}$	reference
$(\pi/6)^{1/2}\ (1/2)^{1/3} \approx 0.57$	Baldyga and Pohorecki (1993)
$(8\ \pi/3)^{1/2} \approx 2.89$	Kuboi et al. (1972)
$\pi/2\ 1.37 \approx 2.15$	Delichatsios and Probstein (1975)
$1.37\ \pi\ (1/2)^{7/3} \approx 0.54$	Spielman (1978)
$1.37\ (\pi/2)^{1/2}\ (1/2)^{1/3} \approx 1.36$	Kusters (1991)

APPENDIX 2

Floc breakup mechanisms

In literature two floc breakup mechanisms are reported, floc splitting and surface erosion of primary particles. The first mechanism was proposed by Thomas (1964), who stated that floc splitting was due to pressure differences on opposite sides of the floc. Under turbulent flow conditions these pressure differences cause bulgy deformation and rupture. Later the theory was further refined (Table 5).

Table 5 Floc breakup by floc splitting. S is the splitting rate, $k_{S,v}$ and $k_{S,i}$ are the splitting rate constants for the viscous and inertial subrange respectively, σ is the mechanical strength of a floc, τ is the shear stress ε_{min} is the minimal specific power input in the tank and ε_c is the critical specific power input for floc splitting.

viscous subrange		
equation		reference
$S_k = \frac{dN_k}{dt} = k_{S,v} N_k \left(\frac{\varepsilon}{\nu}\right)^{1/2}$	(12)	Thomas (1964)
$S_k = \frac{dN_k}{dt} = \left(\frac{1}{15}\right)^{1/2} \exp(-\sigma/\tau_s) N_k \left(\frac{\varepsilon}{\nu}\right)^{1/2}$	(13)	Ayazi Shamlou et al. (1994b)
$S_k = \frac{dN_k}{dt} = \left(\frac{4}{15\pi}\right)^{1/2} \exp(-\varepsilon_c/2\varepsilon_{min}) N_k \left(\frac{\varepsilon}{\nu}\right)^{1/2}$	(14)	Kusters (1991)
inertial subrange		
equation		reference
$S_k = \frac{dN_k}{dt} = k_{S,i} d_k^{2/3} N_k \varepsilon^{1/3}$	(15)	Thomas (1964)
$S_k = \frac{dN_k}{dt} = \left(\frac{1}{15}\right)^{1/2} \exp(-\sigma/\tau_s) d_k^{2/3} N_k \varepsilon^{1/3}$	(16)	Ayazi Shamlou et al. (1994b)
$S_k = \frac{dN_k}{dt} = \left(\frac{2}{\pi}\right)^{1/2} 1.37 \exp(-\varepsilon_c/2\varepsilon_{min}) N_k \varepsilon^{1/3} d_k^{2/3}$	(17)	Kusters (1991)

The second floc breakup mechanism as proposed by Argaman and Kaufman (1970) is the erosion of primary particles from the floc surface. The rate at which primary particles are released depends on the floc surface and the eddy velocity (Table 6).

Table 6 Floc breakup by surface erosion in the viscous and the inertial subrange. E is the erosion rate and $k_{E,v}$ and $k_{E,i}$ are the erosion rate constants of the viscous subrange and the inertial subrange respectively.

viscous subrange		
equation		reference
$E_k = \left(\frac{dN_1}{dt}\right)_k = k_{E,v} N_k \left(\frac{\varepsilon}{\nu}\right)^{1/2} d_k^2$	(18)	Argaman and Kaufman (1970), Cleasby (1984)
$E_k = \left(\frac{dN_1}{dt}\right)_k = k_{E,v} \phi_{cf}^{2/3} N_k \left(\frac{\varepsilon}{\nu}\right)^{1/2} d_k^2$	(19)	Parker et al. (1972)
inertial subrange		
equation		reference
$E_k = \left(\frac{dN_1}{dt}\right)_k = k_{E,i} \phi_{cf}^{2/3} N_k \varepsilon^{1/3} d_k^{5/3}$	(20)	Parker et al. (1972)

APPENDIX 3

Computer model

Discretization of the model

Yeast flocs can be described according to the theory of fractals (Fontana et al., 1992; Davis and Hunt, 1986; Logan and Wilkinson, 1991). This means that the floc size can be derived from the aggregation number, which is the number of cells per floc. A floc with a size of 1.5 mm contains over 500.000 cells. If this is assumed to be the largest floc formed, 500.000 population balances should be solved for modelling the evolution of the floc size distribution. This would lead to a computational load far too big to handle. To limit this number the size distribution was divided in a confined number of classes. The size distribution, containing flocs with a diameter between 0 and 3 mm, was divided into 55 classes. The average aggregation number of a class is defined as follows,

$$agnu[k] = k^{3.75} \tag{21}$$

where *agnu*[*k*] is the average aggregation number of size class '*k*', which is the number of cells per floc of size class '*k*' and *k* is the index of the size class. The exponent 3.75 follows from the range of floc diameters and the number of classes.

The upper boundary of size class '*k*' is defined as,

$$bound[k] = 2\ agnu[k] - bound[k\text{-}1] \tag{22}$$

where *bound*[*k*] is the upper boundary of a class. So particles with an aggregation number between *bound*[*k*] and *bound*[*k*-1] belong to size class '*k*'.

The average floc size can be derived from the average aggregation number, according the theory of fractals,

$$d_f[k] = d_c\ agnu[k]^{\frac{1}{D}} \tag{23}$$

where *df*[*k*] is the average floc size of class '*k*' and *D* is the fractal dimension.

Mathematically only collisions between flocs with the average size of a class occurs. Because of this, the number of flocs of the average size of a class were multiplied by the total number of sizes in a class to yield the collisions of the total size class. The number of sizes per class can be calculated by the following equation,

$$NumbSize[k] = bound[k] - bound[k\text{-}1] \tag{24}$$

where *NumbSize*[*k*] is the number of sizes in a class.

The total number of flocs in a class (*m*[*k*]) can now be defined,

$$m[k] = n[k]\ NumbSize[k] \tag{25}$$

Collision between two flocs yield a floc with an aggregation number that is the summation of the two colliding flocs. From this number the new floc size can be calculated. Based on the boundaries of a size class it is decided to which class the floc will be added. The increase of the floc number of this class is calculated through dividing the aggregation number of the newly formed floc by the aggregation number of the involved size class. Collision between a floc from class '*i*' and a floc from class '*j*' will yield a floc to be added to class '*k*'. The increase of floc number of class '*k*' is now defined as,

$$\mathrm{d}m[k] = \frac{agnu[i] + agnu[j]}{agnu[k]} \tag{26}$$

In case of floc splitting the same procedure is followed.

For erosion the procedure is somewhat different because the loss of a cell from a floc will never lead to a shift to a lower size class. In this way only the number of flocs in a class will decrease due to erosion. To overcome this problem, in case of erosion a floc will shift to a lower class. The floc number is then proportionally adapted as stated above.

Solution of population balances

To calculate the equilibrium floc size distribution at a given cell number, specific power input and floc strength, the differential equations for floc formation, floc splitting and surface erosion should be solved. This is done numerically by computer.

Due to collision particles from size class '*k*' will be lost and gained. The rate of loss of particles *k* is determined by collision of particle *k* with every other particle. The rate of gain of particle is determined by collisions between flocs from class '*i*' and flocs from class '*j*' yielding a floc within the boundaries of size class '*k*'. The net rate of generation of particles *k* is given by the following equation

$$\frac{dN_k}{dt} = \frac{1}{2} \sum_{i+j=k}^{\infty} K_{ij} - \sum_{i=1}^{\infty} K_{ik} \tag{27}$$

where K_{ij} is the floc formation rate. The factor ½ in the first term is to prevent double counting of collisions between flocs from size class 'i' and 'j'.

For floc splitting we assumed that half of the flocs where splitted into two equal sized flocs. The remaining half was splitted into a part of 0.25 and a part of 0.75. In case of splitting there will also be a simultaneous gain and loss to the floc number in the size class.

The particle number of size class 'k' will decrease due to splitting. On the other hand there will be an increase of the particle number in class 'k' due to splitting of flocs resulting in a floc within size class 'k'.

$$\frac{dN_k}{dt} = -\sum_{k=2}^{\infty} S_k + \sum_{i=2k}^{\infty} S_i + \frac{1}{2} \sum_{i=4k}^{\infty} S_i + \frac{1}{2} \sum_{i=1.33k}^{\infty} S_i \tag{28}$$

In case of erosion of primary particles from the floc surface the same approach is used, in the sense that there is a gain and loss of particles within the same class, yielding the next balance,

$$\frac{dN_k}{dt} = \sum_{k=2}^{\infty} - E_k + E_{k+1}$$

$$\frac{dN_1}{dt} = \sum_{k=2}^{\infty} E_k \tag{29}$$

NOMENCLATURE

α	collision efficiency	-
$\dot{\gamma}$	shear rate	s^{-1}
$\Delta u(r)$	relative fluid velocity	m/s
ε	specific power input	W/kg
ε_c	critical specific power input for floc splitting	W/kg
ε_{min}	minimal specific power input in the system	W/kg
λ_{Kol}	Kolmogorov microscale of turbulence	m
ν	kinematic viscosity	m^2/s
ρ_l	density of the medium	kg/m^3
σ	mechanical strength of a floc	N/m^2
τ_s	shear stress	N/m^2
ϕ_{cf}	volume fraction of primary particles in a floc	-
D	fractal dimension of yeast flocs	-
d_c	diameter of a yeast cell	m
d_f	floc diameter	m
d_i	diameter of particles in size class '*i*'	m
d_Q	fluid flow diameter	m
E_k	erosion rate of particles in size class '*k*'	$m^{-3}\ s^{-1}$
F	bond strength between two yeast cells	N
K_{ij}	floc formation rate from the particles '*i*' and '*j*'	$m^{-3}\ s^{-1}$
k_c	coordination number of primary particles in a floc	-
$k_{E,i}$	erosion rate constant (inertial subrange)	m^{-2}
$k_{E,v}$	erosion rate constant (viscous subrange)	m^{-2}
$k_{K,i}$	collision rate constant (inertial subrange)	-
$k_{K,v}$	collision rate constant (viscous subrange)	-
$k_{S,i}$	splitting rate constant (inertial subrange)	-
$k_{S,v}$	splitting rate constant (viscous subrange)	-
L	Eulerian macroscale of turbulence	m
$N_{c,tot}$	total number of yeast cells	m^{-3}
N_k	number of particles of size class '*k*'	m^{-3}

r	separation distance between the centres of two particles approaching	m
S_k	splitting rate of flocs in size class 'k'	m^{-3} s^{-1}
t	time	s
u	fluid velocity	m/s
Re	dimensionless number of Reynolds; Re = $u\ d_Q/\nu$	-

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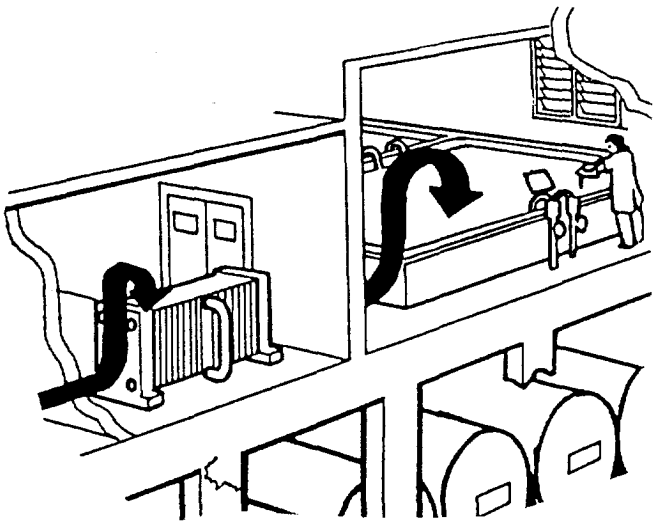

... the malting and brewing process ...

fermentation	- The fermentation is mostly carried out in a cylindroconical fermentation vessel. The aerated wort is inoculated with a certain amount of yeast. At the end of the fermentation the main part of the yeast is removed and used for inoculation of fresh wort. Lager fermentation is carried out at circa 10 °C and takes about 200 h. - During fermentation sugars in the wort are being converted into ethanol and carbon dioxide resulting in green beer as product. Next to this also flavour components and yeast are formed.

... to be continued on page 194 ...

CHAPTER VII

Modelling full scale brewery fermentation

Abstract

At the end of a beer fermentation the main part of the yeast has to be removed. This removal is facilitated by the ability of brewers' yeast to form flocs. Yeast sedimentation depends on many factors of which the most important are, the flocculence of the cells and the carbon dioxide production rate. If the conditions are favourable the yeast flocs will settle. In this study a model is presented that describes the fermentation of beer. The model can be used to calculate the amount of yeast remaining in suspension during the fermentation. The model is divided into three parts namely: i) the part where yeast growth and carbon dioxide production are described, ii) the part that describes the formation of the flocs and iii) the part describing the sedimentation of the flocs. With the model it was possible to simulate the growth of biomass and the behaviour of cells in suspension in a full scale tank. The simulations match the experimental data on yeast in suspension as well as on sugar consumption and carbon dioxide production. The settling of the flocs starts at a critical value of the carbon dioxide flow. In view of this the carbon dioxide flow is the most suitable parameter to control sedimentation. By recycling an amount of carbon dioxide, the total carbon dioxide flow can be kept above the critical value.

INTRODUCTION

One of the last steps in the beer brewing process is the fermentation. In the course of this process step sugars are converted into ethanol and carbon dioxide . Next to this, flavour compounds are formed by the yeast cells. At the end of the fermentation the bulk of yeast has to be removed, which is achieved by flocculation. The yeast flocs will either settle to the bottom of tank (bottom fermenting yeast) or rise in the tank and form a layer at the top (top fermenting yeast). In this study only bottom fermenting yeast is used. After settling of the yeast flocs a part of the yeast remains in suspension and is needed for rest conversions to take place in the next step, the lagering. After the lagering the beer is filtered to remove the remaining yeast (Figure 1).

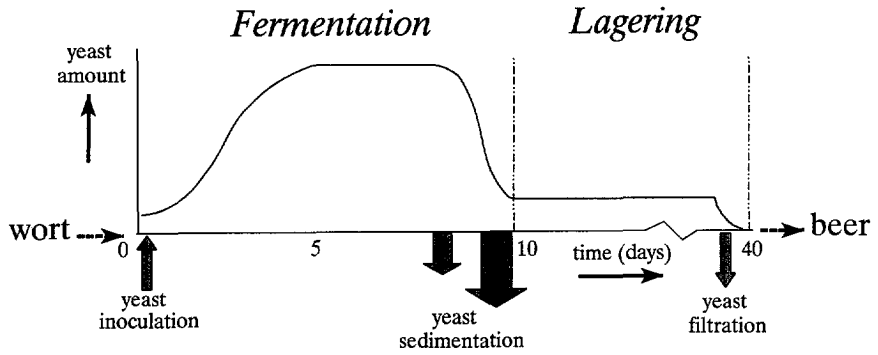

Figure 1. Desired course of the amount of yeast in suspension during fermentation and lagering.

Many brewers nowadays make use of cylindroconical vessels to carry out the fermentation of wort. These vessels have a lot of advantages relative to the former used open rectangular vessels (Hough, 1985). Some advantages are:

- cleaning in place (CIP),
- temperature control with cooling jackets,
- self generating mixing currents and
- efficient yeast removal from the cone.

However, in spite of these advantages, problems may arise at the end of the fermentation when the main part of the yeast has to be removed.

Yeast removal at the end of the fermentation is carried out by settling of the yeast, which is facilitated by the ability of brewers' yeast to flocculate under certain conditions. However, due to strain variability the flocculation of the yeast may be too strong or too weak. Weakly flocculating yeast will lead to a yeast concentration during lagering that is too high. In that case problems may arise after the lagering when these high concentrations of yeast have to be removed by filtration. On the other hand a strongly flocculating yeast strain will lead to early sedimentation of the yeast while complete attenuation has not yet been reached. By trial and error most brewers are capable of controlling yeast sedimentation within certain boundaries. However, for a more structural solution to overcome these problems more insight in the flocculation and sedimentation mechanisms is required.

In this study we make an inventory of the factors involved in the sedimentation of yeast. With this a model to describe the sedimentation of the yeast during fermentation will be developed. The model must be able to predict the sedimentation of the yeast based on the properties of the yeast and the process conditions. The model will be validated by experimental results obtained from full scale fermentations. Finally a proposal to control yeast sedimentation is given.

MODEL DEVELOPMENT

Lager beer fermentations are normally carried out at a temperature of approximately 9 °C. At this temperature a fermentation takes about ten days. At the start of the fermentation the wort is inoculated with yeast to reach an initial yeast concentration of approximately 5×10^6 cells per ml. The cells start to divide and ethanol and carbon dioxide is produced. The carbon dioxide produced will dissolve in the medium until the saturation concentration is reached. From then on gas bubbles will be formed, which rise to the top of the tank.

Partway through the fermentation the yeast cells become flocculent. The initiation of flocculence coincides with the stop of cell division (Smit et al., 1992). At that point the

cell number is 30 - 35 $\times$ 10^6 cells per ml. From the moment the cells become flocculent the flocculability also increases. Flocculability is the ability of yeast cells to flocculate in a given medium if the hydrodynamical conditions are favourable (sufficient collision rate and not too high breakup forces) and sufficient cells are present (Van Hamersveld et al., 1995a). The increase of flocculability is due to two factors: i) the increasing flocculence of the yeast cells and ii) the changing properties of the medium caused by sugar conversion, production of ethanol, etc.

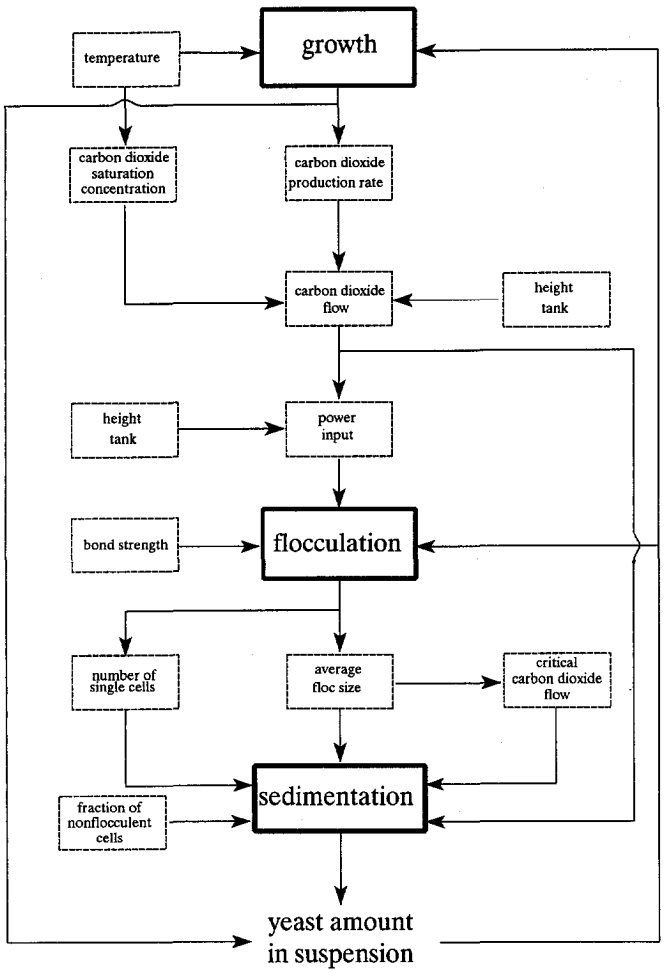

Figure 2. Schematic representation of the fermentation model.

At the end of the fermentation the conditions are favourable for sedimentation to occur. The flocculability is high and the fluid shear is low, this results in the formation of large flocs with a diameter up to 400 μm, depending on the exact conditions. Next to this the fluid velocities in the tank will be low enough to enable settling of the flocs.

Here, a fermentation model is presented that describes the total process whereby the amount of yeast in suspension will be the main model output. The fermentation model consists of three parts, a growth model, a flocculation model and a sedimentation model (Figure 2).

Growth

The growth model describes the production of yeast and carbon dioxide resulting from nitrogen uptake and sugar conversion.

Table 1 The input parameters and output variables of the growth model. These parameters can be obtained experimentally (exp.) or from literature (lit.) as indicated. The temperature trajectory during the fermentation has to be known to be able to calculate both biomass concentration and carbon dioxide production rate.

output variables		
- biomass concentration		
- carbon dioxide production rate		
input parameters		**source**
yeast metabolism	- maximum specific growth rate	exp./lit.
	- Monod constant for biomass production	exp./lit.
	- maximum specific substrate uptake	exp./lit.
	- Monod constant for substrate conversion	exp./lit.
	- yield on nitrogen uptake	exp.lit.
medium	- nitrogen concentration at t = 0 s	exp.
	- substrate concentration at t = 0 s	exp.
	- biomass concentration at t = 0 s	exp.
	- temperature	-

The input parameters and output variables of the model are listed in Table 1. From the amount of yeast the total cell concentration can be calculated, which is an important

factor in the formation rate of yeast flocs. The carbon dioxide production rate determines the flocculation rate (i) and the critical carbon dioxide flow (ii).

ad i. From the carbon dioxide production rate the carbon dioxide flow can be calculated (Appendix). With this, the power input in the tank can be calculated, which determines the flocculation rate.

ad ii. The critical carbon dioxide flow is the carbon dioxide flow at which settling of the flocs starts. If the carbon dioxide flow is above this critical value the flocs will not settle and remain in suspension.

Flocculation

The flocculation model describes the process of floc formation and floc breakup. The processes of floc formation and breakup are in a dynamic equilibrium with each other. This dynamic equilibrium determines the size of the yeast flocs and the number of single cells, which form the output of this part of the model (Table 2). Single cells are flocculent cells that do not form part of a floc, but are freely suspended in the medium. The rate of floc formation depends on local shear rate in the tank and cell concentration. The breakup rate mainly depends on the actual bond strength between the cells and the shear rate.

Table 2 The input parameters and output variables of the flocculation model. These parameters can be obtained experimentally (exp.), from literature (lit.) or from the growth model (grow.). The power input in the tank can be calculated from the carbon dioxide flow (Appendix).

output variables	
- average floc size	
- number of single cells	
input parameters	**source**
- erosion rate constant	exp./lit.
- collision efficiency	exp./lit.
- fractal dimension of yeast flocs	exp.
- cell concentration	exp./grow.
- power input in the tank/shear rate	calc. (Appendix)
- bond strength between the cells	exp.

Sedimentation

Finally, the sedimentation of the yeast is described in the sedimentation model. Yeast sedimentation is the main factor determining the amount of yeast in suspension. The settling rate of yeast flocs depends mainly on their size. Whether or not the flocs will settle depends on the fluid velocity in the tank, which is caused by the carbon dioxide production. The process of sedimentation and flocculation is in quasi steady state. Because of settling the cell concentration decreases, by this the flocculation will be influenced resulting in smaller flocs, which in its turn will cause a decrease in the settling rate of the flocs. The input and output of the sedimentation model is presented in Table 3.

Table 3 The input parameters and output variables of the sedimentation model are listed. The parameters can be obtained experimentally (exp.), from literature (lit.), from the growth model (grow.) or from the flocculation model (flocc.).

output variables	
- amount of yeast in suspension	
input parameters	source
- particle concentration	exp./grow.
- floc size	exp./flocc.
- number of single cells	exp./flocc.
- carbon dioxide flow	exp./grow.
- yeast floc density	calc. (eq. 17)
- fractal dimension of the flocs	exp./lit.
- tank diameter	exp.

In Figure 2 a schematic representation of the fermentation model and its main parameters is given.

THE FERMENTATION MODEL

Main model equations

For the fermentation model a two compartment flow system was assumed. The tank was divided into two parts, the upper part (u) where the fluid is moving and the bottom part

(*b*) where the fluid is quiescent. To describe the amount of yeast in the upper and bottom part of the tank respectively, the next balance equations were used,

$$\frac{\mathrm{d}(\text{yeast amount})_u}{\mathrm{d}t} = \text{yeast production} - \text{yeast sedimentation}$$
$$\frac{\mathrm{d}(\text{yeast amount})_b}{\mathrm{d}t} = \text{yeast sedimentation} \qquad (1)$$

where t is the time.

Because the cell division stop coincides with the initiation of flocculence, the yeast production was assumed to be zero in the bottom compartment of the tank. Next to this, it was assumed that the yeast in the bottom compartment in inactive. So the carbon dioxide production of yeast in the bottom part is neglected.

The sedimentation of the yeast is assumed to take place at the interface between the upper part of the tank and the bottom. The cone of the cylindroconical tank is defined as the bottom. The amount of yeast in the upper part of the tank is depending on production and sedimentation of the yeast. The yeast production depends on the biomass production rate (r_x). The sedimentation of the yeast depends on the number of flocculent cells, the settling velocity of the flocs (v_{sed}), the settling area (A_{sed}) and the tank volume (V_T),

$$\frac{\mathrm{d}N_{c,u}}{\mathrm{d}t} = r_x\, CN_{conv} - \frac{v_{sed}\, A_{sed}\, N_{c,u}}{V_T} (1 - \Psi_{nfl}) (1 - \Psi_s) \qquad (2)$$

yeast production yeast sedimentation

where $N_{c,u}$ is the number of cells in the upper part of the tank, CN_{conv} is a conversion factor to calculate the cell number from the biomass amount, Ψ_{nfl} is the fraction of nonflocculent cells and Ψ_s is the fraction of single cells.

Yeast production

The biomass production rate can be derived from the growth model, which will be described below. The conversion factor to calculate the cell number form the biomass concentration can be obtained by dividing the biomass concentration by the cell number. This conversion factor was assumed to be constant during the fermentation. This assumption could be made because glycogen production was not taken into account during fermentation.

Yeast sedimentation

The sedimentation of the yeast depends on five parameters, the settling rate of the flocs (i), the critical carbon dioxide flow (ii), the settling area (iii), the amount of flocculent cells (iv) and the fraction of single cells (v).

i) The settling rate of the flocs is determined by the size of the flocs resulting from the flocculation model. The settling rate will result from the sedimentation model.
ii) The critical carbon dioxide flow determines whether or not the flocs will settle. This critical value depends on the size of flocs and their density. It is assumed that no settling will take place if the carbon dioxide flow is higher than the critical value for particle suspension.
iii) The settling area is defined as the area in the cone were settling takes place. Because of the sedimentation of the yeast the cone will be filled partly, and hence the settling area will decrease in time. However, if all yeast cells settle into the cone and the volume fraction of the cells in the cone is assumed to be 0.5, this decrease is only 5%. Therefore the settling area is assumed to be constant and equal to the surface area of the cone.
iv) A part of the cells will not become flocculent during the fermentation, depending on whether or not a pure strain is used. Nonflocculent cells are assumed not to settle since the settling velocity of individual cells is very low (1 cm/h). Because of the settling of the flocculent cells the fraction of nonflocculent cells in the upper part of the tank increases during the fermentation. This increase is linearly dependent on the concentration of cells in the upper part of the tank and will reach unity if all flocculent cells are settled.
v) Next to the nonflocculent cells, single cells will settle neither. On the contrary, the fraction of single cells is a fixed fraction of the concentration of cells in suspension.

Consequently, the number of single cells in suspension will decrease during settling of the yeast.

The number of single cells is calculated in the flocculation model.

Growth model

From the growth model the biomass production rate and the carbon dioxide flow can be derived. Both are needed to describe the amount of yeast in suspension during fermentation. The first parameter will determine the total amount of yeast that will be formed, while the second parameter is involved in the formation and settling of the flocs (Figure 2).

Biomass production rate

During the first part of the fermentation the biomass concentration increases. Nitrogen is indirectly the limiting compound for the formation of biomass. Hence the biomass production rate depends on the nitrogen concentration in the medium. The production of biomass can be described according to Monod kinetics (Roels, 1983),

$$\frac{dC_x}{dt} = r_x = \mu_{max}\,\kappa\,\frac{C_n - C_{n,e}}{K_n + (C_n - C_{n,e})}\,C_x \qquad (3)$$

where C_x is the concentration of biomass, μ_{max} is the maximum specific growth rate at 9 °C, C_n is the nitrogen concentration, $C_{n,e}$ is the nitrogen concentration at the end of the fermentation and represents the amount of nitrogen that is not available to biomass production, K_n is the Monod constant for biomass production and κ is the Arrhenius number, which is defined as,

$$\kappa = \exp\left(\frac{T_a}{282} - \frac{T_a}{T}\right) \qquad (4)$$

where T_a is the Arrhenius temperature and T is the actual temperature.

The Arrhenius number is introduced to correct the maximum specific growth rate and the maximum specific substrate uptake rate for temperature during deepcooling or temperature increase during fermentation.

The rate of nitrogen uptake by the yeast cells is assumed to be proportional to the biomass production rate,

$$\frac{dC_n}{dt} = r_n = -\beta\, r_x \tag{5}$$

where r_n is the nitrogen consumption rate, β molar fraction of nitrogen in dry biomass. From the equations 3 to 5 the biomass production rate (biomass concentration) can be calculated.

Carbon dioxide production rate

Under anaerobic conditions yeast cells mainly produce ethanol and carbon dioxide according to the carbon balance as presented in Table 4 (Renger, 1991).

Table 4 Carbon balance of a brewery fermentation (Renger, 1991). The amount of consumed and formed components are expressed in Cmol/l.

	component	concentration [Cmol/l]	
consumed	sugars	2.7	
	amino acids	0.05	
produced	ethanol		1.5
	carbon dioxide		0.84
	glycerol		0.06
	biomass		0.1
	glycogen		0.05
total		2.75	2.55

As can be derived from this balance, approximately 10% of the amount of sugars is converted to glycerol, biomass and glycogen. For this it is assumed that sugars are only converted to ethanol and carbon dioxide.

From the biomass concentration the substrate uptake can be calculated according to Monod kinetics,

$$\frac{dC_s}{dt} = -r_s = -q_{s,max} \kappa \frac{C_s - C_{s,e}}{K_s + (C_s - C_{s,e})} C_x \qquad (6)$$

where r_s is the rate of substrate consumption, C_s is the substrate concentration, $C_{s,e}$ is the substrate concentration at the end of the fermentation, which is equal to the concentration of nonfermentable sugars, $q_{s,max}$ is the maximum specific substrate uptake rate and K_s is the Monod constant for substrate consumption.

Finally from the substrate uptake the carbon dioxide production rate and carbon dioxide concentration can be calculated,

$$\begin{aligned} \frac{dC_c}{dt} &= r_c = \frac{1}{3} r_s \qquad \text{if} \quad C_c < C_c^* \\ \frac{dC_c}{dt} &= 0 \qquad\qquad\quad \text{if} \quad C_c > C_c^* \end{aligned} \qquad (7)$$

where C_c is the carbon dioxide concentration in the medium, r_c is the carbon dioxide production rate and C_c^* is the saturation concentration of carbon dioxide in the medium.

If the medium is completely saturated with carbon dioxide the carbon dioxide production rate is equal to the carbon dioxide flow. In the Appendix of this paper the carbon dioxide flow is modelled. Next to this, the relation between the power input and the carbon dioxide flow is presented.

Flocculation model

Yeast flocs are formed by collisions between cells, flocs and cells and flocs. These collisions are caused by velocity gradients within the fluid which originate from the

carbon dioxide production (Appendix). By these collisions the flocs will increase in size. However, next to be formed, flocs can also break up. So flocculation of yeast consists of two mechanisms: floc formation and floc breakup. Simultaneous floc formation and breakup lead to a dynamic equilibrium of the floc size distribution containing flocs and single cells. To predict the floc size distribution, the formation rate and breakup rate of the flocs must be modelled.

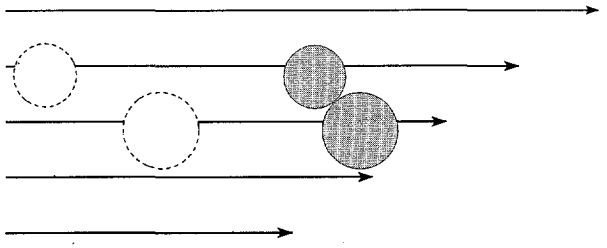

Figure 3. Particle collision caused by a velocity gradient in the fluid.

Floc formation

The floc formation rate can be derived from the collision theory (Smoluchowski, 1917). Particles collide due to their relative motion (Figure 3). While moving a particle sweeps out a certain volume depending on the size of the particle. The moving particle collides with the particles contained in this volume. Therefore, the rate of collisions between the particles depends on their relative velocity, the particle concentration and the size of the particles. The velocity gradients can be derived from the power input in the tank (Appendix). Whether a collision between two particles will lead to a new floc depends on the collision efficiency. The collision efficiency is usually defined as a constant. The floc formation rate can be described with (Camp and Stein, 1943; Kusters, 1991),

$$\frac{\mathrm{d}N_k}{\mathrm{d}t} = \alpha \frac{1}{6} N_i N_j \left(\frac{\varepsilon}{\nu}\right)^{1/2} (d_i + d_j)^3 \qquad \text{for} \quad d_i + d_j < 6\lambda_{Kol} \tag{8}$$

where N_k is the number of particles in size class 'k', α is the collision efficiency, ε is the specific power input, ν is the kinematic viscosity, d is the particle size of a certain size class and λ_{Kol} is the Kolmogorov microscale of turbulence. The Kolmogorov length is used as the upper limit of the size range in which equation 8 can be applied and is defined as,

$$\lambda_{Kol} = \left(\frac{\nu^3}{\varepsilon}\right)^{0.25} \tag{9}$$

In case of yeast flocculation the size of the yeast flocs is smaller than the Kolmogorov length (Van Hamersveld et al., 1995c).

Floc breakup

Floc breakup mainly depends on floc strength and shear rate in the fluid. A high floc strength will result in a lower floc breakup rate whereas higher velocity gradients (shear rate) will increase the floc breakup rate. Floc breakup consists of two mechanisms, the splitting of the floc into two parts and the erosion of individual cells from the floc surface.

Erosion of yeast cells from the floc surface is caused by fluid velocity gradients near the floc surface. The number of cells released depends on the floc surface area and the number of flocs. The velocity gradients can be calculated from the power input in the tank (Appendix). The erosion rate can be described by (Argaman and Kaufman, 1970),

$$\left(\frac{\mathrm{d}N_1}{\mathrm{d}t}\right)_k = k_E N_k \left(\frac{\varepsilon}{\nu}\right)^{1/2} d_k^2 \qquad \text{for} \quad d_k < 6\lambda_{Kol} \tag{10}$$

where N_1 is the number of single cells (= particles in size class '1') and k_E is the erosion rate constant.

The second mechanism of floc breakup, floc splitting, is a result of velocity fluctuations in the fluidum causing pressure differences on opposite sites of a floc (Thomas, 1964).

The velocity fluctuations are proportional to the shear rate (Ayazi Shamlou et al., 1994), which can be calculated from the power input (Appendix). Next to this, the splitting rate depends on the splitting efficiency of the floc. This efficiency is defined as a probability function of the mechanical strength (σ) and the shear stress (τ_s) (Ayazi Shamlou et al., 1994). The floc splitting rate can be described by the next formula (Kusters, 1991),

$$\frac{dN_k}{dt} = \left(\frac{1}{15}\right)^{1/2} \exp(-\sigma/\tau_s)\ N_k \left(\frac{\varepsilon}{\nu}\right)^{1/2} \qquad \text{for} \quad d_k < 6\lambda_{Kol} \tag{11}$$

The shear stress can be calculated from the specific power input (Camp and Stein, 1943),

$$\tau_s = \rho_l \sqrt{\varepsilon\ \nu} \tag{12}$$

where ρ_l is the density of the fluidum.

The mechanical strength mainly depends on the floc diameter (d_f) and the bond strength (F) between two yeast cells. In this the fractal buildup of a floc is an important factor. Because of this, the mechanical strength decreases proportionally to the size of the floc. The mechanical strength (σ) is defined as (Ayazi Shamlou et al., 1994)

$$\sigma = \frac{9}{8}\ k_c\ \phi_{cf}\ F\ \frac{1}{\pi\ d_c^2}$$

$$\phi_{cf} = \left(\frac{d_f}{d_c}\right)^{D-3} \tag{13}$$

where k_c is the coordination number of the particles in the floc ($k_c = 15\ \phi_{cf}^{1,2}$; Kusters, 1991), ϕ_{cf} is the volume fraction of cells in a floc, d_c is the cell diameter and D is the fractal dimension of the yeast flocs.

With the flocculation model the floc size distribution and number of single cells can be calculated. To reduce calculation time, 55 classes were used in the population balances (Van Hamersveld et al., 1995c). From the floc size distribution the average floc size is derived. The average floc size and the number of single cells are both input parameters for the sedimentation model.

Sedimentation model

If yeast flocs are formed and reach a certain size the flocs can settle. The critical value of the carbon dioxide flow for floc settling depends on floc size and density and carbon dioxide production rate in the tank. If the critical carbon dioxide flow is reached, the flocs start to settle and the amount of yeast in suspension will decrease.

The critical carbon dioxide flow, ($\varphi_{m,c}$) can be calculated from the critical superficial gas velocity for particle suspension (U_c),

$$\varphi_{m,c} = \frac{U_c \frac{\pi}{4} d_T^2}{V_m} \tag{14}$$

where V_m is the gas molar volume and d_T is the tank diameter.

The critical superficial gas velocity is derived from Koide et al. (1983, 1986), whereby the last term of the equation is added to correct for the fact that the gas is not injected at the bottom of the tank but produced over the whole height,

$$U_c = K \frac{\Delta\rho^{1.4}}{\rho_l^{0.6}} \left(\frac{\phi_f}{\rho_f}\right)^{0.15} \frac{g^{0.9}}{\nu^{0.8}} d_T^{0.1} d_f^{1.6} \frac{a \ln(a+1)}{(a+1)\ln(a+1) - a} \tag{15}$$

where K the suspension constant, $\Delta\rho$ is the effective floc density, ϕ_f is the floc volume fraction in the medium, ρ_f is the floc density, g is the gravitational constant and a is the dimensionless pressure, which is defined as,

$$a = \frac{\rho_l \, g \, h_l}{p_0} \tag{16}$$

where g is the gravitational constant, h_l is the liquid height in the tank and p_0 is the atmospheric pressure.

The floc density can be calculated by taking the sum of the mass of yeast and the mass of medium within a floc and divide this by the floc volume,

$$\begin{aligned} \rho_f &= \frac{N_{cf} \frac{\pi}{6} d_c^3 \rho_y + \left(\frac{\pi}{6} d_f^3 - N_{cf} \frac{\pi}{6} d_c^3 \right) \rho_l}{\frac{\pi}{6} d_f^3} \\ &= N_{cf} \left(\frac{d_c}{d_f} \right)^3 (\rho_y - \rho_l) + \rho_l \end{aligned} \tag{17}$$

where N_{cf} is the number of cells per floc and ρ_y is the density of the yeast.
The number of cells per floc (N_{cf}) can be described by the theory of fractals. This theory has proven to be applicable to yeast flocs (Fontana et al., 1992; Davis and Hunt, 1986; Logan and Wilkinson, 1991),

$$N_{cf} = \left(\frac{d_f}{d_c} \right)^D \tag{18}$$

Combination of equation 17 with equation 18 gives the relation for the effective floc density,

$$\Delta\rho = \rho_f - \rho_l = \left(\frac{d_f}{d_c}\right)^{D-3} (\rho_y - \rho_l) \tag{19}$$

Finally the floc volume fraction is needed to calculate the critical superficial gas velocity,

$$\phi_f = N_f \frac{\pi}{6} d_f^3 \tag{20}$$

where N_f is the number of flocs, which can be calculated from the total cell number ($N_{c,tot}$) the number of cells per flocs and the number of single cells (N_s),

$$N_f = \frac{N_{c,tot} - N_s}{N_{cf}} \tag{21}$$

The suspension criterion determines whether or not the flocs will settle. If the carbon dioxide flow is higher than the critical value, the settling rate will be zero. In case the carbon dioxide flow is lower the Stokes equation corrected for hindered settling was used to calculate the sedimentation velocity,

$$\begin{aligned} v_{sed} &= \frac{\Delta\rho\, g\, d^2}{18\,\eta} (1 - \phi_f)^{6.5} \qquad \text{for} \quad \varphi_m < \varphi_{m,c} \\ v_{sed} &= 0 \qquad\qquad\qquad\qquad\quad \text{for} \quad \varphi_m > \varphi_{m,c} \end{aligned} \tag{22}$$

where η is the dynamic viscosity of the medium and φ_m is the molar gas (carbon dioxide) flow. The hindered settling function is derived from the Richardson-Zaki equation (Richardson and Zaki, 1954). The exponent 6.5 is taken from Al-Naafa and Selim (1992) and can be applied in case of polydisperse settling at a low effective density (Davis and Gecol, 1994).

Together with the fraction of single cells and the fraction of nonflocculent cells the settling velocity determines the yeast sedimentation. The fraction of nonflocculent cells was calculated according,

$$\Psi_{nfl} = \frac{N_{nfl}}{N_{c,u}} \tag{23}$$

where N_{nfl} is the number of nonflocculent cells.

MATERIALS AND METHODS

The presented model was evaluated by comparing the results with full scale brewery fermentations. The flocculation part of the model was validated previously (Van Hamersveld et al., 1995c). The experimental data used here come from three full scale fermentations. The first fermentation (I) was used to validate the carbon dioxide flow, the extract and the carbon dioxide concentration in the liquid as predicted by the growth model. The second (II) and third (III) fermentation were used to validate the fermentation model. For these fermentations (II and III) it was not possible to measure the carbon dioxide flow. To determine the power input in the tank for these fermentations, the carbon dioxide production rate was derived from the growth model.

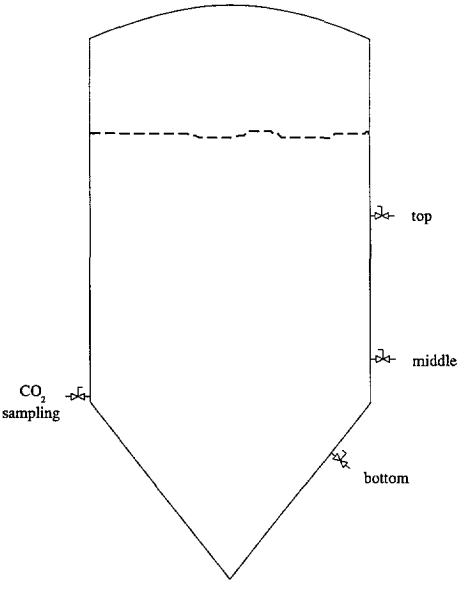

Figure 4. Position of sample points at the fermentation tank.

Table 5 Listing of parameters as used for the model calculations.

description	symbol	value	reference
biomass concentration at t = 0 h	$C_{x,0}$	0.7 g/l	this study
nitrogen concentration at t = 0 h	$C_{n,0}$	0.075 Cmol/l	Renger, 1991
concentration unavailable nitrogen	$C_{n,e}$	0.025 Cmol/l	Renger, 1991
sugar concentration at t = 0 h	$C_{s,0}$	12 - 15 °P	this study
concentration nonfermentable sugars	$C_{s,e}$	3 - 4 °P	this study
molar mass of water	C_{H20}	55.5 mol/l	Perry et al., 1984
conversion factor	CN_{conv}	3.37×10^{14} Cmol^{-1}	this study
yeast cell diameter	d_c	7.2 µm	Chapter II
tank diameter	d_T	7.3 m	this study
fractal dimension of yeast flocs	D	2.45	Chapter V
bond strength of yeast cells	F	0.1×10^{-9} N	this study
gravitational constant	g	9.81 m/s^2	Perry et al., 1984
height of the cone	h_{con}	4.76 m	this study
liquid height in the tank	h_L	8 - 12 m	this study
erosion constant	k_E	20000	Chapter VI
suspension constant	K	5×10^{-3}	this study
Monod constant	K_n	0.001 Cmol/l	Renger, 1991
Monod constant	K_s	0.1 Cmol/l	Renger, 1991
molar gas constant	R	8.31 J mol/K	Perry et al., 1984
tank volume	V_T	260 - 400 m^3	this study
maximum specific substrate uptake	$q_{s,max}$	2.18×10^{13} h^{-1}	Renger, 1991
collision efficiency	α	0.001	Chapter VI
oxygen molar fraction in dry biomass	ß	0.17	Renger, 1991
nitrogen molar fraction in dry biomass	γ	0.28	Renger, 1991
dynamic viscosity	η	2.3×10^{-3} Pa s	Chapter 5
maximum specific growth rate	μ_{max}	1.45×10^{12} h^{-1}	Renger, 1991
medium density	ρ_l	1005 kg/m^3	Chapter V
yeast density	ρ_y	1140 kg/m^3	Chapter V
kinematic viscosity	ν	2.3×10^{-6} m^2/s	Chapter 5

The fermentations were carried out in a cylindroconical vessel with a volume of 430 m^3, the diameter of the tank is 7.3 m. The vessel was filled with aerated wort containing yeast (0.7 g/l). The starting point of fermentation was defined as the moment that the tank was completely filled. The initial extract was 12 °P (1 °P equals 10 g sugars per

kg medium), the temperature was between 4 °C and 12 °C. The yeast strain used was an industrial lager strain (*Saccharomyces cerevisiae*).

During fermentation I the following parameters were measured: extract, carbon dioxide flow and carbon dioxide concentration within the medium. During fermentation II and III the following parameters were measured: extract, number of yeast cells in suspension and flocculence of the yeast. The extract, the number of yeast cells and flocculence of the yeast were measured off-line. The samples were taken at three different heights in the tank (Figure 4). The samples were analysed as described previously (Van Hamersveld et al., 1993). The carbon dioxide flow was measured with a Yew vortex meter at the top of the tank. The carbon dioxide concentration was measured with a probe (Procal) just above the cone (Figure 4).

The model calculations were based on parameter values determined in an earlier study (Table 5). Only the bond strength and the suspension constant were estimated by minimizing the total sum of squared errors between the simulated and measured data of the fermentations II and III. The actual temperature during fermentation was used as model input.

RESULTS

Growth model

The growth model was validated by comparison of the modelled and measured carbon dioxide flow, extract and carbon dioxide concentration in the medium. In Figure 5 the course of carbon dioxide flow and the total amount of carbon dioxide produced during fermentation I are shown. In the same Figure the results of the model calculations are presented. The parameters used for the calculations are shown in Table 5.

The carbon dioxide flow depends on the active biomass concentration and the sugar concentration in the medium. During the first 40 h the carbon dioxide flow is zero due to undersaturation of the medium. In the course of the fermentation the biomass concentration is increasing and consequently the carbon dioxide flow. Partway through

the fermentation the amount of biomass is constant while the sugar concentration is decreasing, causing a decrease in carbon dioxide flow until zero at the end of the fermentation.

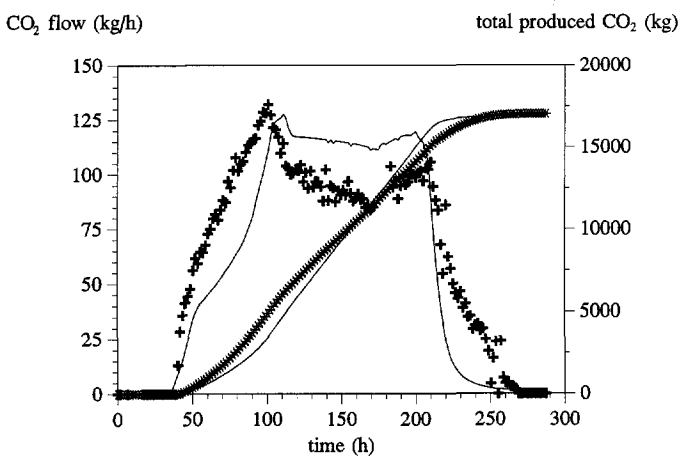

Figure 5. Measured (+) and simulated (solid line) carbon dioxide flow, cumulative carbon dioxide flow (*) and simulated total carbon dioxide production (solid line) during fermentation I.

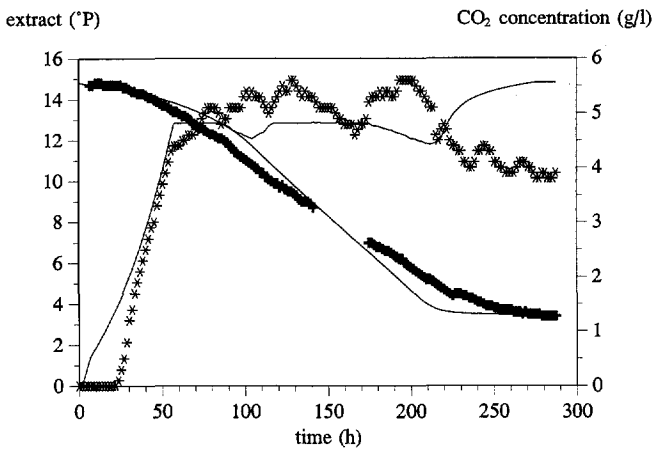

Figure 6. Measured (+) and simulated extract (solid line) and carbon dioxide concentration as measured (*) and simulated (solid line) for fermentation I.

Figure 6 shows the measured sugar concentration together with the carbon dioxide concentration in the medium in the bottom part of the tank during fermentation I. Next to this, the simulated extract and carbon dioxide concentration in the medium are shown. The irregularities of the simulated carbon dioxide concentration are caused by failure of cooling, which was also accounted for in the simulation.

Fermentation model

The flocculence of the cells determines their bond strength, which is one of the parameters in the flocculation model. In Figure 7 the flocculence as measured during fermentation III is shown. The samples were taken at three heights in the tank. After approximately 50 h the cells become flocculent, from then the flocculence increases reaching a maximum at 125 h. At 250 h all cells remaining in suspension are nonflocculent.

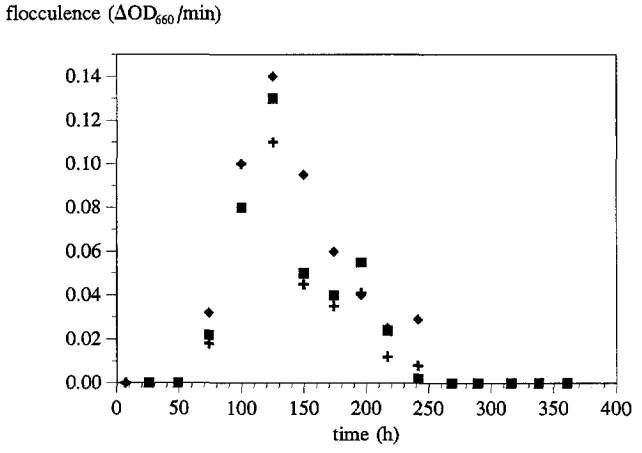

Figure 7. The flocculence of the yeast cells during fermentation at three heights in the tank; top (■), middle (+) and bottom (♦) (fermentation III).

Figure 8 shows the amount of yeast at three heights in the tank for fermentation III. During the first part of the fermentation the cell number increases due to cell division. At 100 h after the start of the fermentation the yeast starts to settle and the cell number

reaches a constant value at 175 h after the fermentation start. The cell number is approximately constant over the height of the tank. At the moment the flocculence reaches zero (250 h) the cell number is about 15×10^6 cells per ml, for fermentation II this is 22×10^6 cells per ml. So these numbers represent the number of nonflocculent cells during both fermentations.

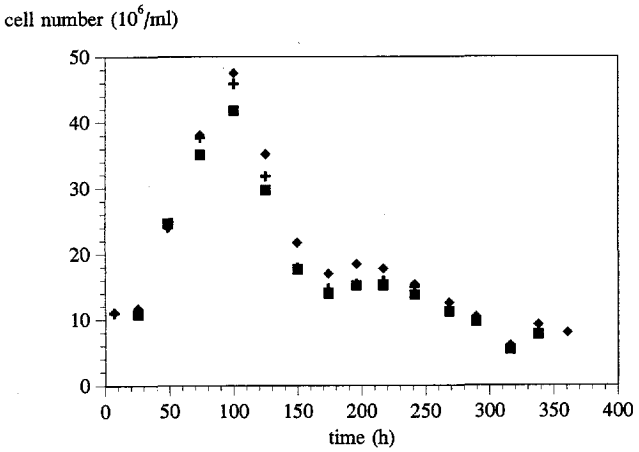

Figure 8. Yeast cell number at three heights in the tank during the fermentation; top (■), middle (+) and bottom (♦) (fermentation III).

Also the extract values were measured at three heights in the tank. The average values of the measurements are shown in Figure 9a and 9b for fermentation II and III respectively. The extract was constant over the height of the tank with an absolute error smaller than 0.05 °P. Next to this the modelled extract and carbon dioxide flow during the fermentation are shown.

In Figure 10a and 10b the measured and simulated yeast cell numbers for respectively fermentation II and III are given. The cell number is the average of the samples taken at the three heights in the tank. The parameters bond strength and suspension constant were estimated using the model. This resulted in the following values, bond strength: 0.1×10^{-9} N and suspension constant: 5×10^{-3}. The input parameters for the simulations of fermentation II and III were identical apart from the number of nonflocculent cells.

The critical carbon dioxide flow for suspension was 120 kg/h for fermentation II and 110 kg/h for fermentation III. The average floc size at that point was 115 μm and 105 μm for fermentation II and III respectively.

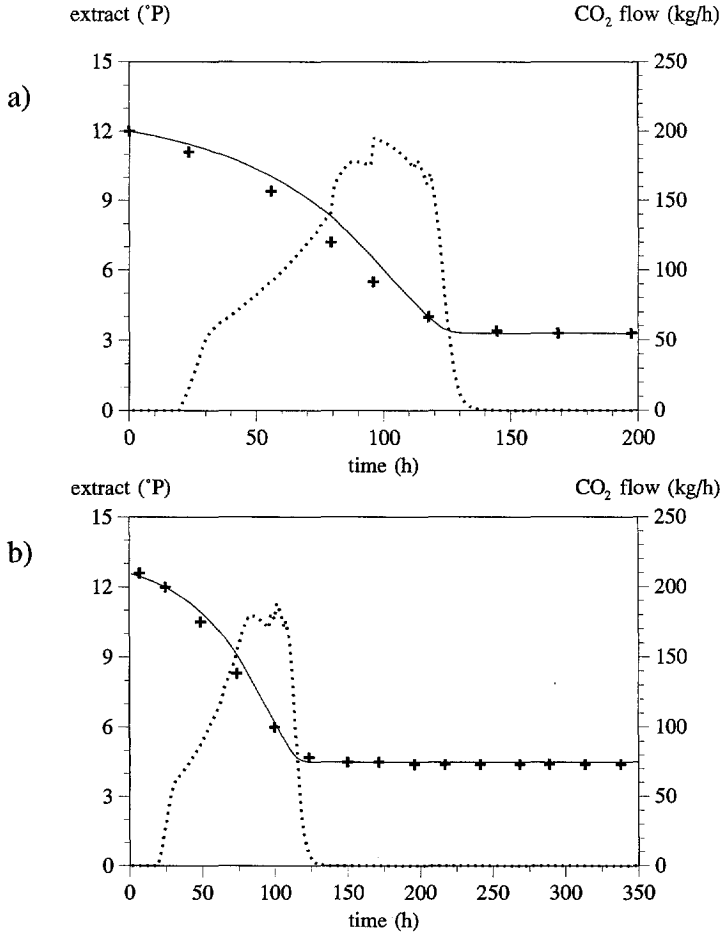

Figure 9. a) Extract (+) as measured during the fermentation. The lines are the simulations of the extract (solid line) and the carbon dioxide flow (dotted line) (fermentation II).
b) idem (fermentation III).

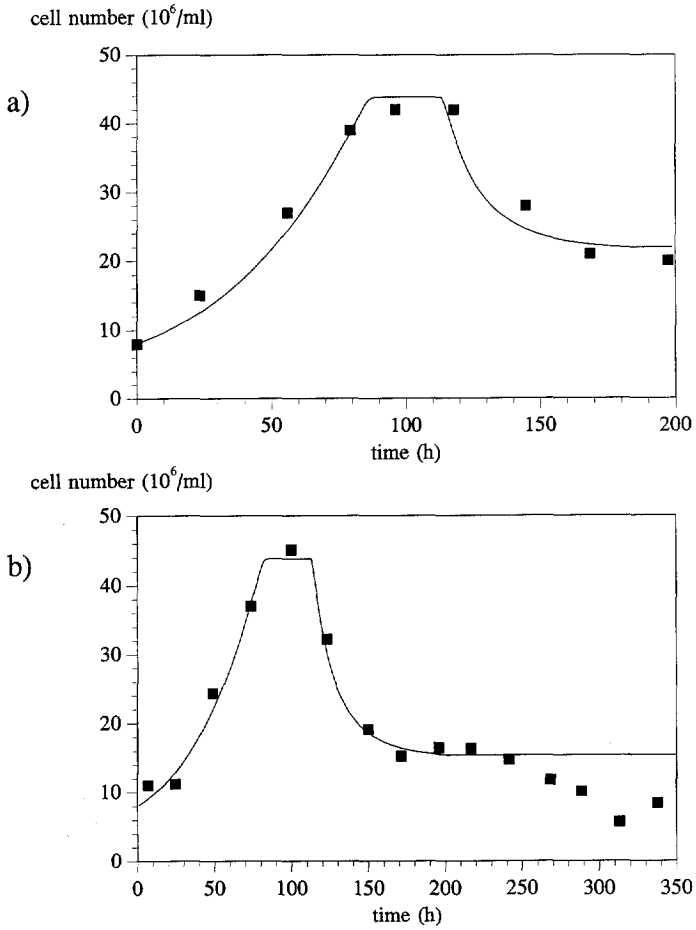

Figure 10. a) The number cell in suspension during fermentation, measured (■) and simulated (solid line) (fermentation II).
b) idem (fermentation III).

DISCUSSION

Flocculence

The occurrence of flocculence during beer fermentation is the most important factor in the removal of the yeast. The degree of flocculence determines the floc size and thereby the settling rate of the flocs. From lab scale experiments it is known that yeast cells do not loose their flocculence during fermentation. The decrease in flocculence shown in Figure 7 can be explained by the fact that a part of the cells are flocculent and a part is nonflocculent. Individual cells hardly settle, so at the moment the sedimentation starts (Figure 8) the amount of flocculent yeast starts to decrease. The nonflocculent cells remain in suspension causing a decrease of the flocculence of the yeast in suspension. Consequently the fraction of nonflocculent cells increases until it gets near unity at the end of the fermentation (250 h). This demonstrates that the fraction of nonflocculent cells is a determining factor in the removal of yeast. The fraction of nonflocculent cells determines the amount of yeast remaining in suspension at the end of the fermentation. In this nonflocculent cells must be distinguished from single cells, which are flocculent but do not form part of a floc. Under the conditions as occurring at full scale fermentation the number of single cells is approximately 10% of the total number of flocculent cells that are in suspension (Van Hamersveld et al., 1995b). Consequently, the number of single cells will decrease during settling of the yeast.

Two compartment approach

In this study it was assumed that the tank could be divided into two compartments, the upper part of the tank and the bottom part. This choice was made because of the low settling velocity of the yeast flocs with respect to the high fluid velocities in the tank, which prevents the formation of a yeast gradient over the height of the tank. The experimental results (Figure 8) confirm the fact that a tow compartment approach is sufficient in this case. The cell number appeared to be constant over the height of the tank (Figure 8). If a more strongly flocculating yeast strain is used, big flocs and small flocs might not be equally dispersed over the height of the column causing a yeast

gradient. In that case the model should be enlarged by one or more compartments describing the amount of yeast in those parts.

Carbon dioxide

Next to ethanol, carbon dioxide is produced during the fermentation of beer. A part of the carbon dioxide produced, dissolves in the medium. If the medium is saturated with carbon dioxide, bubbles will arise causing a carbon dioxide flow from the top of the tank. The saturation concentration of carbon dioxide in water at atmospheric pressure and a temperature of 9 °C and pH 7 is 2.3 g/l (Appendix). However, at the bottom of the tank the solubility of the carbon dioxide will be about 4.5 g/l. The average value will be approximately 3.4 g/l. Figure 6 compares the measured concentration of carbon dioxide in beer at the bottom of the tank, with the calculated value. During the fermentation the pH drops from 5 to 4, regarding this it was not accounted for in the model. The results show that the relations as presented in the Appendix of this paper form a good approach of the solubility of carbon dioxide in beer.

According the model simulations the carbon dioxide flow is nihil during the first part of the fermentation, before the saturation point at atmospheric pressure is reached. This is confirmed by the experiments (Figure 5). The carbon dioxide flow will start after approximately 35 h. Saturation of the whole tank contents is reached after 55 h, whereafter the carbon dioxide flow equals the carbon dioxide production rate. Because of this, the mixing will be poor during the first part of the fermentation. Mixing might be improved by carbonating the wort before inoculation or by recycling the carbon dioxide flow from neighbouring tanks.

Carbon dioxide solubility also plays a role during fermentation if the temperature is changed. By this the solubility of the carbon dioxide will change, which will influence the carbon dioxide flow. This is demonstrated in Figure 5. At about 75 h and 175 h the temperature changes due to a failure of cooling causing an increase of the carbon dioxide flow. This effect is enhanced by increasing substrate consumption by the yeast, which is also caused by the increase in temperature.

Glycogen formation is neglected in modelling the carbon dioxide production from sugar consumption. However, glycogen is formed during the last 50 h of the fermentation. Because of the assumption that glycogen will not be formed, the carbon dioxide production will be overestimated. For example in case of fermentation II the overestimation of the total carbon dioxide production is 850 kg or 17 kg/h overestimation in carbon dioxide flow. The consequence is that in the model settling starts a few hours later. In view of the total time of sedimentation this can be neglected.

Fermentation model

With the model as presented above it is possible to calculate the amount of yeast in suspension during fermentation. From the growth model the increase in yeast cell number during the first part of the fermentation can be simulated and the sedimentation of the yeast can be simulated by the sedimentation model (Figure 10a and 10b). Next to this, the course of the extract (Figure 6, 9a and 9b), of the carbon dioxide flow and total produced amount of carbon dioxide (Figure 5) as well as the carbon dioxide concentration in the medium (Figure 6) as simulated by the model shows good agreement with the measured data.

With only two fitparameters it was possible to simulate the fermentations II and III with acceptable results. The number of nonflocculent cells were different for the fermentations II and III, despite this it was possible to simulate both fermentation with the same parameter set, which gives confidence in the model. Figure 9a and 9b show that the carbon dioxide flow decreases sharply at 105 h and 120 h respectively. The sharp decrease coincides with the onset of sedimentation of the yeast. Because the critical carbon dioxide flow is reached the sedimentation starts.
Sedimentation of the yeast depends on cell number in two ways. Firstly, decreasing cell number causes a decrease in floc size as predicted by the flocculation model. In the second place a lower cell concentration will decrease the overall removal rate as defined in the second term of equation 1. These findings are confirmed by the shape of the sedimentation curve, which is exponential and not linear as could be expected in the absence of a concentration effect.

Control of sedimentation

The mechanism of yeast sedimentation has strong consequences for the control of yeast sedimentation. The experiments show that the cells need to be flocculent to be able to settle. Nonflocculent cells will not settle. The most important parameter is the carbon dioxide flow. The carbon dioxide flow determines the settling rate of the flocs in two ways:

- Firstly, the carbon dioxide flow determines the power input in the tank, which in its turn will determine the size of the yeast flocs and thereby their settling rate.
- Secondly, the carbon dioxide flow determines the settling rate via the suspension criterion. If this criterion is met the flocs start to settle. If the carbon dioxide flow is higher than this critical value the settling velocity is zero.

In this case the critical carbon dioxide flow is about 100 kg/h. At that point the cell number was 45×10^6 cells per ml, the power input calculated from the suspension criterion is circa 2 W/m^3. From the flocculation model the floc size (100 μm) and the fraction of single cells (0.20) could be calculated. Because the critical value is below the maximum carbon dioxide flow reached during the fermentation, the carbon dioxide flow can be used as a control parameter. The carbon dioxide flow can be influenced in two ways: via recycling of the gas flow from the top of the tank, or by changing the temperature. Temperature adjustment will only have an effect if enough sugar is left in the medium to cause a significant increase of the carbon dioxide production rate.
Recycling of carbon dioxide is preferable because it will not influence the fermentation. Temperature adjustment, on the other hand, might influence the formation of secondary metabolites, which is not wanted. Most brewers already change the temperature at the end of the fermentation, in that case a more flexible temperature trajectory based on model calculations will increase fermentation controllability.

CONCLUSIONS

Sedimentation of yeast only occurs when the cells are flocculent. Individual cells have a settling velocity far too low to contribute to the removal of the yeast. In view of their terminal velocity the same applies for small flocs up to 50 μm.

Yeast sedimentation only occurs at the bottom of the tank in the cone. In the tank the yeast is homogeneously dispersed. Because of this, a two compartment model is sufficient to describe the sedimentation of the yeast.

The removal of the yeast is mainly determined by the carbon dioxide flow, the fraction of nonflocculent cells and the degree of flocculence of the flocculent cells. Control of yeast sedimentation can be achieved by manipulation of one or more of these parameters.

Via a simple model presented in this study it is possible to predict the carbon dioxide flow and the sugar consumption of the yeast. The carbon dioxide flow is used to calculate the power input, necessary for predicting the floc size and thereby the critical carbon dioxide flow.

The model describes the experimental data reasonably well. The increase of cell number during the first part of the fermentation, the moment at which sedimentation starts as well as the decrease of cell number during settling can be predicted, whereby only two parameters need to be estimated.

Acknowledgements

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APPENDIX

Calculation of carbon dioxide concentration in beer and carbon dioxide flow, power input and local shear rate in the tank

From the carbon dioxide production rate the carbon dioxide flow at the top of the tank can be calculated. For this, the saturation concentration of carbon dioxide in beer is needed, because carbon dioxide bubble formation will only occur if the fluid is saturated with carbon dioxide. The saturation concentration of carbon dioxide in water (C_c^*) (atmospheric pressure, pH 7) can be calculated from Henry's Law (Janssen and Warmoeskerken, 1987),

$$p = H\,x$$
$$x = \frac{C_c^*}{C_{H_2O}} \tag{24}$$

where p is the local static pressure, H is the Henry constant, x is the molar fraction and C_{H2O} is the molar density of water.

The static pressure is the summation of the atmospheric pressure (p_0) and the pressure in the tank by the liquid (p_{liq}).

Between 0 °C and 30 °C the Henry constant for carbon dioxide in water is almost linear and can be estimated by the next relation, derived from data from Janssen and Warmoeskerken (1987),

$$H = 3.96 \times 10^6\ T - 1.01 \times 10^9 \tag{25}$$

The liquid pressure depends on the position in the tank, for this a saturation depth (d_{sat}) is defined,

$$d_{sat} = \frac{H\dfrac{C_l}{C_{H_2O}} - p_0}{\rho_l\, g} \qquad \text{for} \quad C_l > C_c^*\ (p = p_0) \tag{26}$$

where C_l is the apparent concentration of carbon dioxide in the liquid. If the apparent concentration of carbon dioxide in the liquid is lower than the saturation concentration at atmospheric pressure the saturation depth is zero and so is the carbon dioxide flow. If the apparent concentration of carbon dioxide in the liquid exceeds the saturation concentration at atmospheric pressure the saturation depth starts to increase until the tank is completely saturated with carbon dioxide. At that point the apparent carbon dioxide concentration reaches its maximum. If the saturation depth equals the height of

the column the carbon dioxide flow is equal to the carbon dioxide production rate. If the tank volume is partly saturated with carbon dioxide the carbon dioxide flow is calculated proportionally according the saturation depth,

$$\varphi_m = \frac{d_{sat}}{h_l} r_c \tag{27}$$

where φ_m is the molar gas flow, h_l is the liquid height in the tank and r_c is the carbon dioxide production rate.
Using the equations presented above the carbon dioxide flow can be calculated. From the carbon dioxide flow the specific power input in the tank can be calculated (Delente, 1968),

$$\varepsilon = \frac{\varphi_m}{V_T \rho_l} R\, T \left(\frac{a+1}{a} \ln(a+1) - 1 \right)$$
$$a = \frac{\rho_l g h_l}{p_0} \tag{28}$$

where V_T is the fermenter volume, R is the molar gas constant, a is the dimensionless pressure, ρ_l is the density of the fluid, g is the gravitational constant.
from the specific power input the local shear rate ($\dot{\gamma}$) can be derived,

$$\dot{\gamma} = \sqrt{\frac{\varepsilon}{\nu}} \tag{29}$$

NOMENCLATURE

α	collision efficiency	-
β	oxygen molar fraction in dry biomass	mol/Cmol
$\dot{\gamma}$	shear rate	s^{-1}

$\Delta\rho$	effective floc density	kg/m^3
ε	specific power input	W/kg
η	dynamic viscosity	Pa s
κ	Arrhenius number	-
λ_{Kol}	Kolmogorov microscale of turbulence	m
μ_{max}	maximum specific growth rate	s^{-1}
ν	kinematic viscosity	m^2/s
ρ_f	density of a floc	kg/m^3
ρ_l	density of the medium	kg/m^3
ρ_y	density of the yeast	kg/m^3
σ	mechanical strength of a floc	N/m^2
τ_s	shear stress	N/m^2
ϕ_{cf}	volume fraction of cells in a floc	-
ϕ_f	floc volume fraction	-
φ_m	molar gas flow	mol/s
$\varphi_{m,c}$	critical molar gas flow	mol/s
Ψ_{nfl}	fraction of nonflocculent cells	-
Ψ_s	fraction of single cells	-
A_{sed}	sedimentation area	m^2
a	dimensionless pressure	-
C_c^*	carbon dioxide saturation concentration	Cmol/l
C_c	carbon dioxide concentration	Cmol/l
C_{H2O}	molar concentration of water	mol/l
C_n	nitrogen concentration	Cmol/l
$C_{n,e}$	nitrogen concentration at the end of the fermentation	Cmol/l
CN_{conv}	conversion factor (biomass → cell number)	Cmol
C_s	sugar concentration	Cmol/l
$C_{s,e}$	sugar concentration at the end of the fermentation	Cmol/l
C_x	biomass concentration	Cmol/l
D	fractal dimension of yeast flocs	-
d_c	cell diameter	m
d_f	floc diameter	m
d_k	particle diameter in size class *'k'*	m

d_{sat}	saturation depth of carbon dioxide in the tank	m
d_T	tank diameter	m
F	bond strength between two yeast cells	N
g	gravitational constant	m/s^2
H	Henry constant	Pa
h_l	liquid height in the tank	m
K	suspension constant	-
K_n	Monod constant for biomass production	Cmol/l
K_s	Monod constant for substrate consumption	Cmol/l
k_c	coordination number of primary particles in a floc	-
k_E	erosion rate constant	m^{-2}
N_{cf}	number of cells per floc	-
$N_{c,tot}$	total cell number per unit of volume	m^{-3}
$N_{c,u}$	total cell number per unit of volume in upper part of the tank	m^{-3}
N_f	floc number per unit of volume	m^{-3}
N_k	number of particles of size class 'k'	m^{-3}
N_{nfl}	number of nonflocculent cells	m^{-3}
N_s	single cell number per unit of volume	m^{-3}
p	local pressure	Pa
p_0	atmospheric pressure	Pa
$q_{s,max}$	maximum specific substrate uptake rate	s^{-1}
R	molar gas constant	J/mol K
r_c	carbon dioxide production rate	Cmol/l s
r_n	nitrogen consumption rate	Cmol/l s
r_s	sugar consumption rate	Cmol/l s
r_x	biomass production rate	Cmol/l s
T	temperature	K
T_a	Arrhenius temperature	K
t	time	s
U_c	critical superficial gas velocity	m/s
V_m	molar gas volume	m^3/mol
V_T	tank volume	m^3
v_{sed}	settling velocity	m/s
x	molar fraction	-

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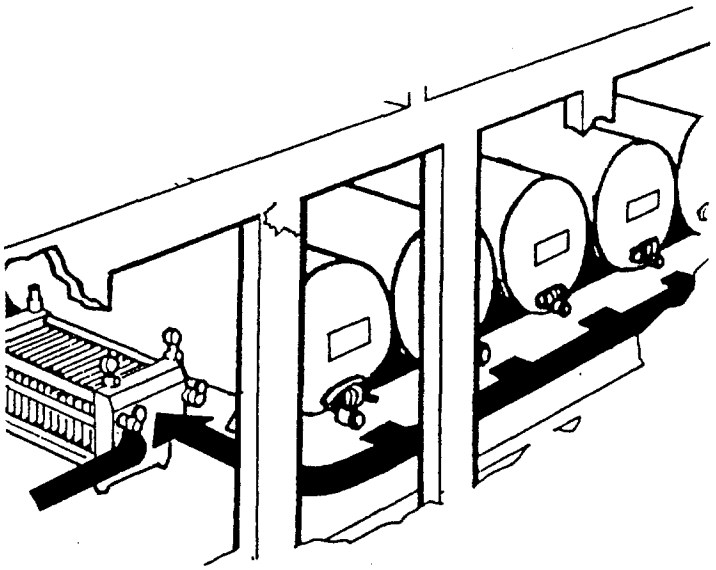

... the malting and brewing process ...

lagering	- After fermentation the maturation of the green beer starts. This process in carried out in the lager tanks. The green beer is left standing for circa 30 days. - During lagering the remaining part of the yeast accomplishes the rest conversions of sugars and reduction of off-flavours.

... to be continued on page 206 ...

CHAPTER VIII

Reflections

INTRODUCTION

To maintain a high product quality is of prime concern to a brewer. A major factor in this is the flocculation characteristic of the yeast strain. Weak flocculation leads to a high yeast concentration in the beer (Figure 1a; dotted line). The removal of the yeast will then be difficult and expensive. On the other hand, strong flocculation of the yeast leads to early sedimentation, which will slow down the fermentation (Figure 1; dashed line). Therefore, control of yeast flocculation and subsequent sedimentation is important.

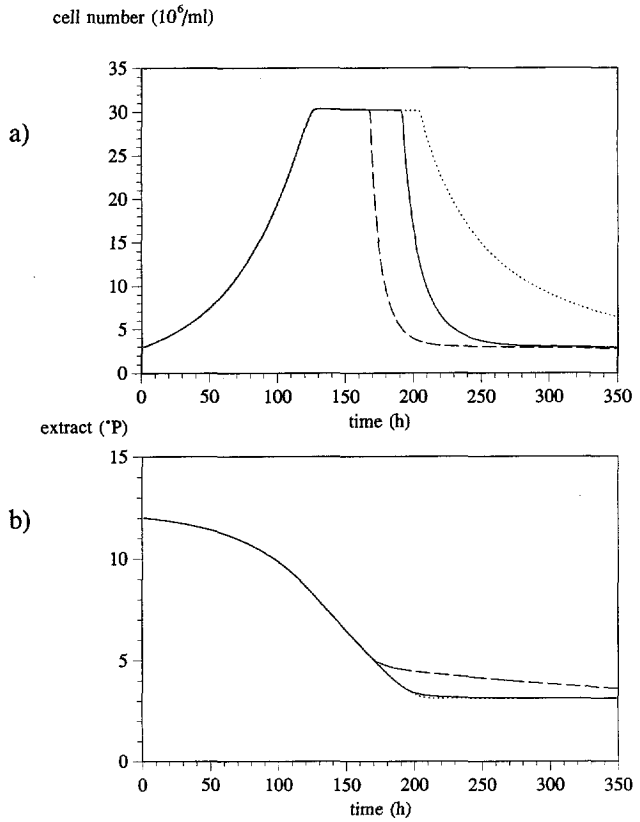

Figure 1. The course of the yeast cell number (a) and the extract (b) are shown during a fermentation (carried out at 9 °C). The solid line represents the course in case of optimal flocculation characteristics of the yeast, the dashed line and the dotted line represent the course in case of strong and weak flocculation respectively.

In Figure 2 the factors and parameters involved sequentially in the sedimentation of yeast from the green beer are shown. In principle all items mentioned can be used to control yeast sedimentation. However, some may be undesirable or too complex to use.

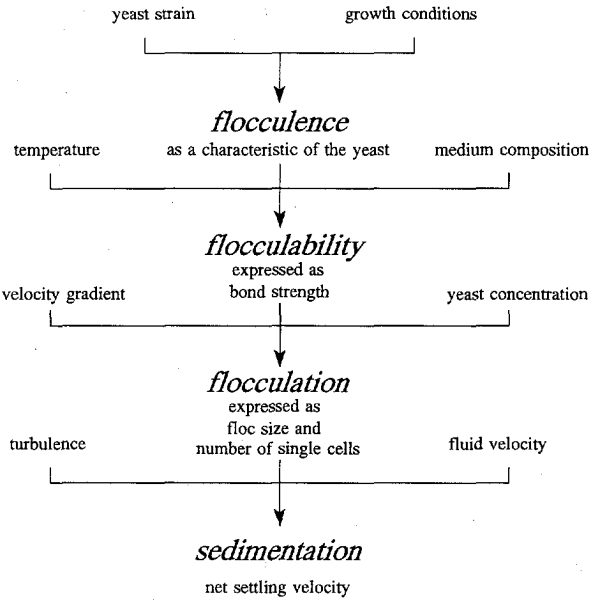

Figure 2. Factors and parameters involved in the settling of the yeast.

The flocculence of the yeast depends on the strain used and the growth conditions. The presence of oxygen and the temperature of the medium are the main factors determining flocculence during the growth phase. The cells become flocculent at the moment the cell division stops. After that the flocculence is constant, provided that the process conditions remain anaerobic. Therefore, control during the fermentation via flocculence is less suitable.

- Flocculability of yeast depends on physiological conditions such as temperature and composition of the medium. Under brewing conditions, temperature and pH of the medium and the presence of calcium, ethanol and sugars are the main factors

determining flocculability. Normally, at full scale, these conditions are favourable for flocculability.

- The actual flocculation depends on physical factors such as the fluid shear, bond strength between the cells and the concentration of yeast cells. At the end of the fermentation these factors are favourable for flocculation.
- Sedimentation depends on floc size, number of single cells, turbulence and fluid velocity in the tank. At the end of the fermentation when the carbon dioxide production rate is low, the turbulence and the fluid velocities will be low enough for the flocs to settle.

The aim of this study was to improve controllability of yeast flocculation and sedimentation. To achieve this the mechanism of yeast flocculation should be examined. Another important aspect in this is the quantification of yeast flocculation.

In this study most attention has been payed to the measurement (Chapter III, IV and V) and the physical aspects (Chapter V, VI and VII) of yeast flocculation. Next to this some attention has been given to the physicochemical (Chapter II) and physiological aspects (Chapter IV). Table 1 gives a summary of the state of the art before this study and of the main results from this study. Here we will discuss the relevance of the results from this study for the brewing industry and give suggestions for further research. Finally, the feasibility of controlling yeast flocculation and sedimentation on full scale will be evaluated.

IMPLICATIONS TO THE BREWING INDUSTRY

Despite all research, yeast flocculation is still an important issue in the brewing industry. The aim of this study was to improve the understanding of the physical mechanism of flocculation, in order to increase controllability. An important aspect in this is the measurement of flocculation. Without a suitable method to monitor flocculation during the fermentation, control will be impossible.

Table 1 State of art before and after the research as described in this thesis.

(1876 - 1989)	(1990 - 1995)
monitoring Several methods to monitor flocculation are available, however most of them are based on the settling behaviour of the flocs formed, leading to a qualitative result. For translation of the results to the industry and for a reliable comparison between different strains, a quantitative test is needed.	In this study three methods to determine flocculation are presented. The first method is the determination of bond strength between the cells, via dispersion of the flocs in a laminar shear field. The second method measures floc size and number of single cells 'in situ' at actual hydrodynamic conditions, without influencing flocculation during the measurement. The last method is a qualitative one. With a so-called Photometric Dispersion Analyser flocculation can be determined on-line during fermentation. For the last two methods the hydrodynamic conditions turned out to be one of the determining factors.
physiological aspects Flocculation is shown to be dependent on pH and temperature. Specific sugars like maltose and mannose inhibit flocculation, whereas calcium is specifically required. However, the precise role of calcium is still unknown and a mechanistic explanation of the effects is lacking.	Regarding the physiology the following conclusions were drawn: the calcium concentration in wort and beer as used in this study is sufficient for flocculation to occur, increasing this concentration only results in a small increase in flocculation characteristics of the yeast. Yeast flocculation in beer is optimal at a temperature of 15 °C. Above 30 °C the flocculability disappears. The flocculation in beer is optimal at pH 4.5, below pH 4 flocculation decreases sharply. Flocculation decreases slowly between pH 4.5 and pH 8.
physicochemical aspects Regarding the physicochemical interactions, zeta-potential and hydrophobicity of yeast cells have been measured. From the results it was suggested that physicochemical interactions are involved in yeast flocculation.	The zeta-potential and hydrophobicity of nonflocculent and flocculent yeast cells were measured. Using these, the physicochemical interaction between the cells, according the DLVO theory, was calculated. From the results it was concluded that flocculation cannot be described by physicochemical interaction between the cells only.
physical aspects Some physical mechanisms were discovered by chance (Stratford and Keenan, 1987). It was found that shaking a yeast suspension leads to floc formation. Flocculation was related to collision frequency by which the force of collision was considered to be rate limiting.	Yeast flocculation can be described with classical collision theory. Collisions between cells and flocs lead to new flocs. Next to being formed, the flocs also break up via two mechanisms, splitting of a floc and the erosion of single cells from the floc surface. The floc splitting determines the average floc size whereas the erosion of cells determines the number of single cells. The process of floc formation and floc breakup leads to an equilibrium floc size distribution in the medium.

Monitoring

Monitoring of flocculation is necessary to predict the sedimentation of the yeast. A brewer has two opportunities to determine flocculation, qualitative and quantitative. In the first case application is simple and cheap. The on-line method presented in Chapter III, which is easy to apply, is suitable for that. Operators should be trained to recognize the proper flocculation course and the maximum deviation from this course that is acceptable, to prevent sedimentation problems.

The other approach is a quantitative determination of flocculation. The advantage of this approach is that the flocculation course can be predicted using a fermentation model. By quantifying a few parameters the sedimentation of the yeast can be predicted (Chapter VII). Sedimentation problems can be detected at an early stage giving the opportunity to prepare the necessary process adjustments. This method will be more expensive because of monitoring the model input parameters, and because more research on the fermentation model is needed to improve flexibility. However, the method will be more accurate leading to optimization of the total process time.

To enable a comparison between the results obtained by different research groups the quantification of yeast flocculation should be unambiguous. In this study it is shown that especially temperature and hydrodynamics are important quantities which are unfortunately underexposed in literature. During a flocculation assay both need to be defined. The temperature and fluid shear should be kept constant during the assay. A temperature between 15 °C and 20 °C is preferable. As for hydrodynamics a laminar flow regime is favoured, because shear forces and velocity gradients are accurately known in that case. Proper setting of these parameters will lead to an unambiguous flocculation assay that is reproducible and reliable.

Physiological aspects

Flocculation of brewers' yeast depends strongly on the medium. Much research has been carried out on this subject. So far several types of medium effects have been investigated, which leads to a better understanding of the type of molecules involved in

flocculation. For the brewing industry, however, the medium composition is more or less fixed. Only the temperature is used for control.

Physicochemical aspects

Regarding the physicochemical aspects of yeast flocculation it was shown that the interaction between yeast cells cannot be described by the DLVO theory. However, colloid science in general can be of help to understand flocculation of brewers' yeast. Measurement of zeta-potential, determination of the iso-electric point and hydrophobicity can be helpful to determine which types of molecules on the cell wall are involved in flocculation.

An aspect of colloid science which is not studied here is the viscosity of yeast suspensions. Speers et al. (1992) stressed the importance of this parameter as for the flow behaviour of suspensions. An aspect not mentioned by these authors is the effect of flocculation on viscosity. This effect is twofold.

- In the first place flocculation will lead to a higher volume fraction of particles. Because of the spacial buildup of yeast flocs which can be described by the theory of fractals, flocs formed out of cells take up more space than individual cells. According to the relation of Einstein (1906) the viscosity of a suspension is linear to its volume fraction of particles. At volume fractions above 0.10 this relation is exponential (Krieger, 1972). At a cell concentration of 50×10^6 cells per ml and a floc size of 1 mm the viscosity increases with 30%. In this study the most extreme situation (cell concentration: 50×10^6 cells per ml; floc size: 350 μm) corresponds to a viscosity increase of 15%.
- The second effect occurs at high volume fractions, i.e. volume fractions of individual cells of more that 0.10, in brewers' terms a consistency of 10%. In this case flocculation will lead to a situation whereby almost all particles will be in contact with each other. This leads to a so-called 'giant floc' (Van de Ven, 1985) resulting in a large viscosity increase, up to a factor of 10. In the brewing industry this phenomenon will arise at the transport of dense flocculating suspensions (up to 50% consistency) that occurs during inoculation of the medium and while harvesting the yeast from the cone after settling of the flocs at the end of the fermentation. Because of the high viscosity a high pumping capacity may be needed. More research on this subject is necessary to obtain more insight in this problem.

Physical aspects

The physical mechanism of yeast flocculation is hardly mentioned in literature. In this study floc formation is described by the classical collision theory combined with two mechanisms to account for floc breakup. With the model developed in this study, it is possible to calculate the size of the yeast flocs and the amount of single cells if the bond strength between the cells, the cell concentration and the velocity gradients in the vessel are known. Future research should focus on the applicability of the model for flocculation of different strains in different reactor configurations.

CONTROL OF YEAST FLOCCULATION AND SEDIMENTATION

Control can be achieved via the four phenomena, flocculence, flocculability, flocculation and sedimentation as depicted in Figure 1. Here the possibilities of control via these phenomena will be discussed.

Flocculence

If a brewer has structural flocculation problems a way to solve this is to adapt the growth conditions or to choose another yeast strain with the appropriate flocculation characteristic. However, this will most likely lead to another taste of the beer, which will be undesirable for commercial and, since most brewers have a long tradition in brewing, sentimental reasons. Next to this, proper fermentation conditions will not guarantee that flocculation problems will never arise. Therefore, control via adapting flocculence is possible, but in practice hard to realise.

Flocculability

Control via flocculability gives two possibilities, adjustment of the medium composition or the medium temperature.

The medium composition can be changed by addition of calcium to enhance flocculability or fermenting at a higher sugar concentration. In both cases the taste of the product might be influenced. Another way to increase flocculability is to add polymers (flocculants) to the medium. A necessity is that they can be removed from the beer easily after settling of the yeast. A possibility is the addition of latex beads coated with a cationic polymer, for example Perol-63.

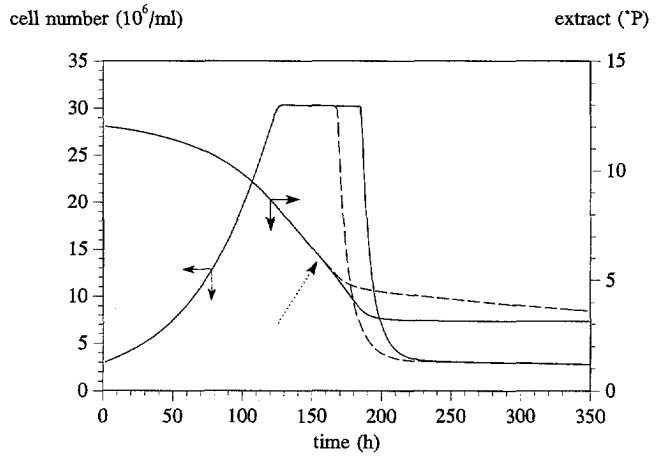

Figure 3. The course of a fermentation (carried out at 9 °C) with a strong flocculating yeast strain (dashed line). If the flocculation is controlled by temperature the problem is solved (solid line). The dotted arrow shows the point from which the temperature is raised to 13 °C with a rate of 0.085 °C/h.

Another way to control yeast sedimentation is adaption of the temperature during the fermentation. The effect is twofold: the flocculability changes and secondly the power input changes due to changing of the carbon dioxide production rate and carbon dioxide saturation concentration. In Figure 3 the effect of a temperature increase is illustrated for a strong flocculating yeast strain, as predicted by model calculations (Chapter VII). The situation in Figure 1 was used and the temperature was increased from 9 °C to 13 °C. As a result of this the flocculability (bond strength) increases. Next to this, the carbon dioxide flow increases due to an increase of the sugar conversion rate and a decrease of the carbon dioxide saturation concentration. The net effect is an increase of the floc size but also an increase of the fluid velocity in the tank. In this case the

increase of flocculation resulted in floc sizes that are 1.5 times higher than before the temperature adjustment. This, however, was not high enough to withstand the increase in fluid velocity so the settling of the flocs was suppressed. In case this last factor is not dominant, temperature control will not be successful. The increase in carbon dioxide flow will not be sufficient to keep the flocs from settling.

In fact temperature control has an effect in two directions, if the temperature is decreased the opposite effect occurs. In that case the relation between flocculability and temperature determines the extent of the effect.

Flocculation/sedimentation

Flocculation can be influenced by the forces acting on the flocs by means of the velocity gradients in the medium. This will always be in combination with control via the suspension criterion which is the power input at which settling of the flocs starts. At this critical power input the fluid velocity is slightly lower than the settling velocity of the flocs.

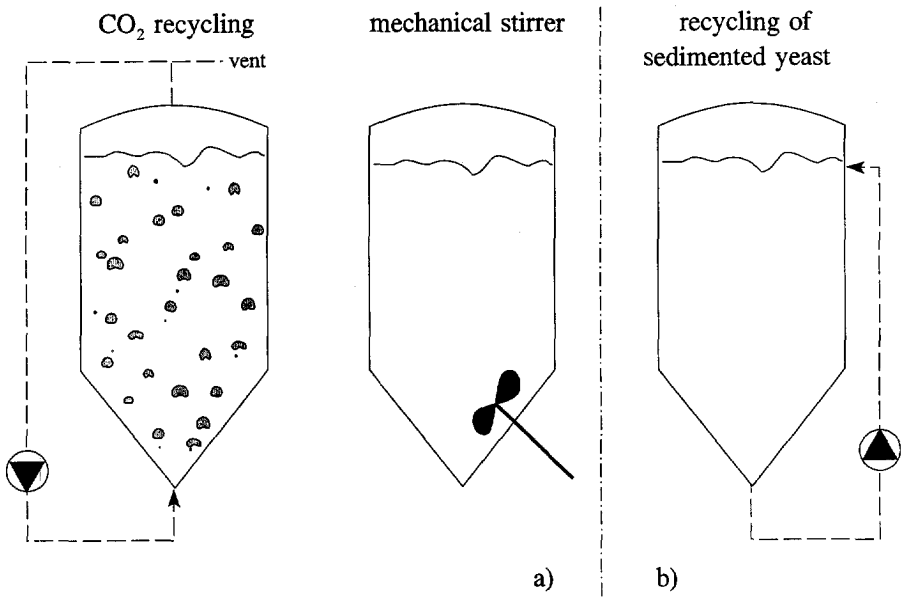

Figure 4. Ways of controlling yeast sedimentation/suspension. a) via power input b) via yeast resuspension.

In Figure 4a two ways of controlling sedimentation via power input are presented. The power input in the tank can be influenced by recycling the carbon dioxide formed. In this way the power input can be regulated to prevent the sedimentation of the yeast. This method is easy to conduct, and even if the produced carbon dioxide flow is insufficient, the carbon dioxide of neighbouring tanks can be transported to the 'problem tank'. In that case, the tanks must be connected via a system of tubes.

Installation of a mechanical stirrer device in the tank is another approach. Despite the costs it is an easy method of power input control not only for regulating yeast sedimentation but also for use as a mixing device, if desired.

A last method of sedimentation control is resuspending the yeast by recycling the sedimented yeast (Figure 4b). This method is cheap and easy to perform, but only limited control can be achieved by this method.

Control of yeast sedimentation is not an easy task, but not impossible. Flocculation problems are easiest to solve when using strongly flocculating yeast. In case of weak flocculation the possibilities of control are very limited, improving flocculability via addition of flocculants seems to be the only way in that case. Therefore, the best way to operate a fermentation is to use a strongly flocculating yeast of which the sedimentation is controlled via power input as shown in Figure 4a. Carbon dioxide recycling is in view of the costs and the feasibility the best solution. In this way product quality will not be influenced and yeast sedimentation is under control.

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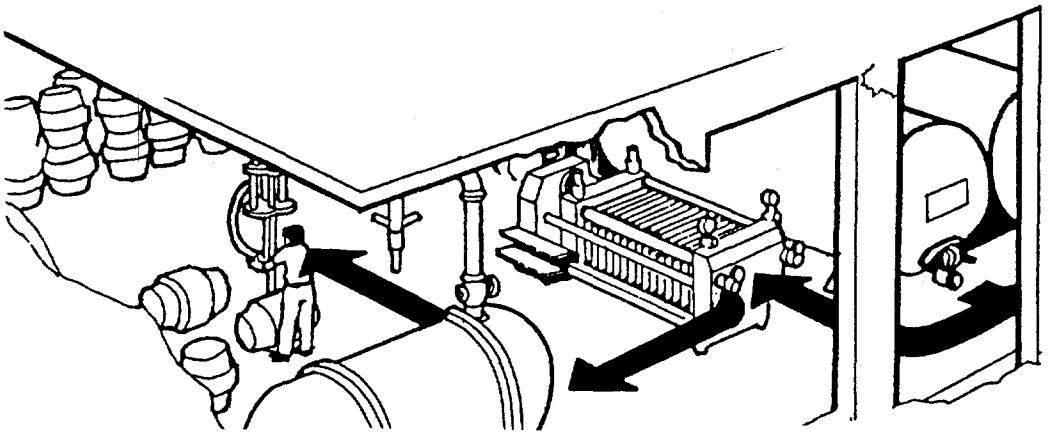

... the malting and brewing process ...

bottling	- After the lagering the beer is clarified, pasteurised (by most brewers) and bottled.

... the end.

SUMMARY

Flocculation of brewers' yeast

- quantification, modelling and control -

Introduction

Beer has been brewed and drunk for many ages. Its production is therefore one of the oldest biotechnological processes. However, it was not until 1857 when Pasteur discovered the relation between beer fermentation and living matter namely yeast. From then, a lot of research has been carried out, which was mainly focused on the fermentation step. For living organisms being a 'bag full of enzymes' (Tubb[1], 1987), another bag of research is necessary to unravel all aspects involved. Considering yeast as a black box is simple: sugars (mainly maltose) go in and ethanol and carbon dioxide go out. Of course it is not as simple as that, next to the two mentioned products a wide range of secondary metabolites is formed which are responsible for the taste of the product. The formation of secondary metabolites also depends on environmental conditions such as temperature, pH and the local static pressure. Therefore the main concern of a brewer, after selecting a yeast strain that results in a tasteful beer, is to maintain a constant product quality and to be able to control the process if things tend to go wrong.

Lager fermentations are mostly carried out at a temperature between 9 °C and 15 °C. At this temperature the process takes about 200 hours. The fermentation is carried out in cylindroconical tanks equipped with cooling jackets to regulate the temperature in the vessel. The mixing in the tank is provided by the yeast itself via carbon dioxide production. Before the tank is filled with hopped wort and yeast the wort is aerated. The oxygen is needed to reach the desired amount of yeast. Yeast cells only divide if oxygen is available. However, this amount of oxygen should be limited because growing yeast hardly produces ethanol. Normally cell division stops partway through the fermentation

[1] Tubb, R.S., 'Introduction', In: Symposium on Brewers' Yeast (1986, Helsinki), Verlag Hans Carl (Brauwelt Verlag), Nürnberg, 1987.

whereafter only ethanol, carbon dioxide and secondary metabolites are formed. At the cell division stop the yeast cells become flocculent.

Fermentation problems are often due to variable flocculation behaviour of the yeast. Early flocculation followed by sedimentation of the flocs results in incomplete attenuation of the wort (a so called 'hung' or 'stuck' fermentation). On the other hand flocculation may be hampered, leaving a green beer containing too much yeast. In that case problems may arise in the next process step, the lagering. Thus, control of flocculation and sedimentation of the yeast is wanted.

In a larger project combining the efforts of different disciplines, flocculation was studied. The major aim of the total yeast flocculation project was to improve the controllability of yeast flocculation and sedimentation in any fermentation configuration. This should lead to a more constant product quality. In this thesis the physical mechanism of yeast flocculation is described to contribute to the overall aim.

Definition of flocculation characteristics

Yeast flocculation is a complex phenomenon consisting of several consecutive mechanisms. To be able to quantify yeast flocculation, flocculation has to be defined first. In this study we have used the following definitions:

- *Flocculence* is the ability of cells to flocculate if all environmental conditions are favourable. Environmental conditions are: presence of the right inducer, temperature of the medium, sufficient cells to allow floc formation etc.

- *Flocculability* is the ability of yeast cells to flocculate in a given medium if the hydrodynamical conditions are favourable (sufficient collision rate and not too high breakup forces). To have a certain degree of flocculability, flocculence is a necessary condition.

- *Flocculation* is the actual floc formation under the actual environmental conditions. For flocculation to occur flocculability is an essential condition.

- *Sedimentation* is the actual settling of flocs. Sedimentation is only possible under favourable hydrodynamical conditions (low turbulence intensity or fluid velocities and not too large breakup forces). Flocculation is a necessary condition for sedimentation.

Measurement of flocculation characteristics

An important factor in examining yeast flocculation is the measurement of flocculation characteristics. Next to this, measurement of flocculation characteristics is relevant to the brewing industry with respect to the control of flocculation.

The physicochemical aspects of yeast flocculation are presented in Chapter II. By measurement of zeta potential and hydrophobicity the net attraction forces between two flocculent cells could be calculated according to the DLVO theory. However, from measurement of the bond strength between the cells it was concluded that the DLVO theory was not applicable to yeast flocculation.

To detect the degree of flocculability of the yeast an on-line method is used. With a Photometric Dispersion Analyser (PDA) it is possible to measure under defined hydrodynamic conditions. The apparatus measures turbidity and turbidity fluctuations of a suspension flowing through a transparent tube. With a constant number of yeast cells the signal is proportional to the size of the flocs formed. This method is optimized to determine yeast flocculability on-line during the fermentation (Chapter III). With the PDA it is possible to determine yeast flocculability quantitatively as well as qualitatively. In the last case the bond strength of the cells can be derived from the measurements (Chapter II and IV).

Flocculation characteristics are determined by measurement of floc size and number of single cells (single cells are cells not forming part of a floc). The method used is a combined light extinction and sedimentation technique. A sample of the flocculating well-mixed suspension is moved to a quiet zone where the flocs settle, while the single cells remain in suspension. This process is monitored by a spectrophotometer. The decline of the optical density caused by the settling of the flocs is registered by a computer. From the settling curve, both floc size and number of single cells can be derived (Chapter V).

The effect of environmental conditions on flocculation

Yeast flocculation is determined by the state of the cells, the composition and temperature of the medium and the hydrodynamic interactions. In this study the effect of medium and hydrodynamic conditions have been examined. For this, a production strain (*Saccharomyces cerevisiae*) is used. Apart from the scale, the fermentations are all carried out under the conditions as occurring in a brewery.

Medium effects

The effect of pH, calcium concentration and temperature on yeast flocculation in beer is determined (Chapter IV).

- The effect of temperature on flocculation turned out to be strongest. Yeast flocculation is optimal at a temperature of 15 °C. At a temperature of 30 °C the flocs are completely dispersed in the medium.
- The effect of pH is less strong. Beer has an acidity between pH 5 and pH 3.5. The optimum acidity of the medium for flocculation is pH 4.5. Below pH 4 the flocculation decreases sharply, whereas above pH 5 the decrease is less pronounced.
- Calcium is absolutely necessary for flocculability. In wort and beer the calcium concentration is approximately 1 mol/m^3 which is sufficient for flocculation to occur. Higher calcium concentrations only have a slightly positive effect on flocculability of yeast in beer.

The effect of hydrodynamic interactions

Next to the effect of the medium also the effect of hydrodynamic interactions was investigated (Chapter V). Flocculation of yeast is influenced by velocity gradients in the fluid and by the concentration of the yeast. Variation of shear rate in the medium strongly influences the size of the flocs. High shear rate leads to small flocs and vice versa. The number of single cells is hardly influenced by fluid shear and is 10% of the total yeast amount. The yeast concentration also influences flocculation. Decreasing cell number leads to a decrease in the floc size. The number of single cells decreases linearly proportional to the yeast cell concentration.

Modelling of flocculation and sedimentation

To get more insight in the physical mechanisms of flocculation, the process of flocculation was modelled (Chapter VI). The flocculation of yeast is a dynamic

equilibrium between the formation and breakup of flocs. Flocs are formed by collisions between particles in the medium (cells and flocs). The number of these collisions per time depends on the particle size, the particle number and the relative particle velocity (shear rate). In case of yeast flocculation, floc breakup occurs via two mechanisms, the splitting of flocs into smaller flocs and the erosion of single cells from the floc surface. Both mechanisms depend on shear rate and floc strength.

It was found that flocculation depends on three parameters, the bond strength between the cells, the velocity gradients in the medium and the concentration of yeast cells. With the developed model it is possible to calculate the size of the flocs and the number of single cells in a flocculating yeast suspension. The results from the model calculations are in agreement with the results from experiments carried out at hydrodynamic and medium conditions as occurs during full scale brewery fermentations.

To predict the amount of yeast in suspension, the total process of yeast growth, flocculation and sedimentation during fermentation is modelled (Chapter VII). The sedimentation of the yeast is determined by the size of the yeast flocs, the number of single cells and the turbulence intensity in the tank. The floc size and number of single cells can be calculated from the flocculation model, which forms part of the overall model. Turbulence intensity can be derived from the carbon dioxide production, which is described by the growth model. It was found that the settling of the flocs starts at a critical value of the carbon dioxide production.
By means of the model it was possible to calculate the yeast amount that is in suspension during a full scale fermentation. The results from the model calculations matched the data obtained from a full scale fermentation.

Control of yeast flocculation and sedimentation

For control of flocculation and sedimentation two parameters are suitable, the bond strength of cells and the power input in the tank.

- Bond strength can be influence in two ways: by adjusting flocculence or by regulation of the flocculability of the yeast. Flocculence of the yeast can be controlled via genetic modification or by changing the growth conditions during fermentation. Flocculability can be adapted via regulation of the medium composition (for example temperature). The bond strength directly influences the size of the flocs and thereby their settling velocity.

- The second way of control, via power input adjustment, affects both flocculation and sedimentation. By means of the velocity gradients the flocculation is influenced, and by the turbulent forces the sedimentation will be affected. Increasing power will result in smaller flocs and higher turbulent forces acting on them. Both effects enhance the suspension of the yeast. Decreasing power input will have an opposite effect by enhancing sedimentation. Regarding this, bond strength is a suitable parameter for structural control of flocculation, whereas power input is more appropriate for control on the spot if flocculation variability comes up.

In this study it is shown that a physical approach of yeast flocculation is a way to understand the mechanisms of yeast flocculation. Via modelling the process, the flocculation and sedimentation mechanisms were found. Flocculation can be described by three parameters (bond strength, shear rate and cell concentration). The sedimentation is mainly determined by the flocculation and the turbulence intensity induced by carbon dioxide production. Control of the process can be achieved via one of these parameters. An important aspect related to this is the measurement of yeast flocculation. In this study three methods to quantify or qualify yeast flocculation are developed.

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SAMENVATTING

Flocculatie van brouwersgist

- kwantificeren, modelleren and beheersbaarheid -

Inleiding

Het brouwen van bier is een van de oudste biotechnologische processen. Reeds in de oudheid werd bier geproduceerd en geconsumeerd. Het duurde echter tot 1876 voordat het besef doordrong dat er levende organismen betrokken waren bij het brouwproces. In dat jaar ontdekte Louis Pasteur dat gist verantwoordelijk was voor de omzetting van suikers naar alcohol in het bier. Sindsdien is er veel onderzoek verricht naar de vergisting van bier.

Het brouwproces bestaat uit een flink aantal stappen waarbij gerst, water en hop de grondstoffen vormen. Een van de laatste processtappen is de vergisting waarbij alcohol en kooldioxide worden gevormd alsmede een reeks smaakstoffen. De vergisting wordt meestal uitgevoerd bij een temperatuur tussen 9 °C en 15 °C en duurt ongeveer 200 uur. De vergistingstank wordt gevuld met beluchte wort waarna de gist wordt toegevoegd. De wort bevat voldoende zuurstof om gistgroei door celdeling te laten optreden. Tijdens de vergisting wordt geen zuurstof meer toegevoegd om te voorkomen dat alleen gist en geen alcohol wordt geproduceerd. Ongeveer halverwege de vergisting stopt de celdeling en worden de gistcellen flocculent. Hierdoor zijn de cellen in staat vlokken te vormen die aan het eind van de vergisting kunnen bezinken. Een deel van de gist blijft achter in het 'jongbier' en is nodig voor de restomzettingen die plaatsvinden tijdens de lagering van het bier. Na de lagering wordt het bier gefiltreerd en opgeslagen in de helderbiertank waarna het gebotteld kan worden.

Als er tijdens de vergisting problemen optreden is dat meestal het gevolg van onvoldoende of juist te sterke flocculatie van de gist. In het eerste geval zal de gist niet bezinken waardoor er te veel gist aanwezig is tijdens de lagering die dan niet goed zal verlopen. Een bijkomend probleem is dat al die gist verwijderd zal moeten worden door filtratie, wat een extra inspanning vraagt. In het tweede geval waarbij de gist te sterk

flocculeert zal deze vroegtijdig bezinken. Het gevolg is dat de vergisting zeer traag zal verlopen. Een brouwer is daarom ook gebaat bij een beheersbare flocculatie van de gist.

Het onderzoek zoals dat in dit proefschrift staat beschreven is een onderdeel van een groter project, waarbij gistflocculatie vanuit verschillende disciplines werd bestudeerd. Het doel van het project was om de stuurbaarheid van gistflocculatie te verbeteren in elke gewenste vergistingsconfiguratie. Het hier beschreven onderzoek is voornamelijk gericht op het bestuderen van de fysische aspecten van gistflocculatie om zodoende de beheersbaarheid van flocculatie te kunnen vergroten. Een deel van het onderzoek is gewijd aan het meten van gistflocculatie.

Definiëring van flocculatie kenmerken

Er worden verschillende begrippen gebruikt om flocculatie aan te duiden. Voor de meting van flocculatie en het vertalen van het meetresultaat is het belangrijk onderscheid aan te brengen. De volgende begrippen worden in dit proefschrift gebruikt: flocculentie, flocculerend vermogen, flocculatie en sedimentatie.

- *Flocculentie* is een gisteigenschap. Gistcellen zijn flocculent als ze in staat zijn vlokken te vormen als alle omstandigheden gunstig zijn.

- *Flocculerend vermogen* is de eigenschap die gist heeft in een bepaald medium. Gistcellen hebben flocculerend vermogen als ze in een gegeven medium (bijvoorbeeld bier) in staat zijn vlokken te vormen als de hydrodynamische condities gunstig zijn (voldoende botsingen tussen de cellen, niet te grote vloeistofkrachten op de vlok). De cellen moeten hiertoe flocculent zijn.

- *Flocculatie* is een proces. Flocculatie is het verschijnsel waarbij onder gegeven omstandigheden vlokvorming optreedt. Voorwaarde is dat de gist flocculerend vermogen bezit.

- *Sedimentatie* is de uiteindelijke bezinking van de gevormde vlokken. Sedimentatie treedt op als de vloeistofsnelheden in de tank voldoende laag zijn om de gevormde vlokken te laten bezinken.

Meting van flocculatie kenmerken

Een belangrijke factor bij de bestudering van flocculatie kenmerken is het meten ervan. Daarnaast is een ondubbelzinnige meting van flocculatie een voorwaarde om het flocculatiegedrag van de gist tijdens de vergisting te volgen.

Het onderzoek is gestart met een colloïd chemische studie naar gistflocculatie (Hoofdstuk II). Door het meten van de zeta-potentiaal en de hydrofobiciteit van flocculente gistcellen kan met behulp van de DLVO theorie de aantrekkingskracht tussen twee gistcellen worden berekend. De resultaten van de berekeningen werden vergeleken met de resultaten van de meting bij die zelfde aantrekkingskracht. De resultaten bleken niet overeen te komen. Gistflocculatie kan niet worden beschreven met de DLVO theorie, de binding tussen de flocculente cellen vindt op een andere manier plaats waarbij hoogstwaarschijnlijk moleculen op de celwand de hoofdtoon voeren.

In dit onderzoek zijn een aantal methoden ontwikkeld om gistflocculatie te meten (Hoofdstuk III, IV en V). Hierbij werd gebruik gemaakt van een 'Photometric Dispersion Analyser' (PDA) en een turbidostaat.
Met de PDA kan men de turbiditeit en de fluctuaties in de turbiditeit van een gistsuspensie die door een buis stroomt meten. Het signaal is evenredig met de grootte van de vlokken in de suspensie. Door deze manier van meten is de methode geschikt voor on-line meting. De vloeistof wordt hierbij continu rondgepompt tussen de PDA en de vergistingstank. Afhankelijk van het gekozen medium kan zowel flocculentie als flocculerend vermogen worden gemeten.
Met hetzelfde apparaat kan ook de bindingssterkte tussen de cellen worden gemeten. Op deze manier is het mogelijk een kwantitatieve waarde toe te kennen aan flocculentie of flocculerend vermogen.

De turbidostaat werd in dit onderzoek gebruikt om flocculatie te kwantificeren. Met dit apparaat is het mogelijk de grootte van de vlokken en het aantal 'vrije cellen' te bepalen onder verschillende hydrodynamische omstandigheden. Tijdens de meting wordt een gedeelte van de suspensie in een pijp gebracht die aan een geroerde tank is bevestigd. In deze pijp is geen vloeistofstroming zodat de vlokken bezinken terwijl de 'vrije cellen' in suspensie blijven. De sedimentatie van de gist wordt gevolgd met behulp van een spectrofotometer die rond de pijp is geplaatst. Door sedimentatie van de vlokken neemt de optische dichtheid van de suspensie in de pijp af. Door nu het gemeten signaal te

vergelijken met het signaal dat uit modelberekeningen volgt kan de vlokgrootteverdeling alsmede het aantal 'vrije cellen' worden bepaald.

Het effect van mediumsamenstelling en de kwantificatie van fysische interacties

Met de ontwikkelde meetmethoden is de flocculatie van gist onder verschillende omstandigheden gemeten.

Medium

De samenstelling en de temperatuur van het medium bepalen het flocculerend vermogen van de gist. De calciumconcentratie, de pH en de aanwezigheid van bepaalde suikers zijn hierbij belangrijke factoren. In Hoofdstuk IV staat beschreven wat het effect van calciumconcentratie, pH en temperatuur is op het flocculerend vermogen.

- De aanwezigheid van Ca^{2+}-ionen is een absolute voorwaarde voor flocculerend vermogen. De wort en het bier zoals gebruikt tijdens dit onderzoek bevatten ongeveer 1 mol/m^3 calcium, wat voldoende is. Het toevoegen van nog meer calcium leidt slecht tot een kleine verbetering van het flocculerend vermogen van de gist.
- De zuurgraad van bier ligt tussen pH 3.5 and pH 5. Het flocculerend vermogen van gist is optimaal bij pH 4.5. Bij een pH waarde lager dan pH 4 neemt het flocculerend vermogen sterk af. Boven een pH waarde van pH 5 is deze afname minder sterk.
- Het effect van temperatuur op flocculerend vermogen is vrij sterk. De onderzochte giststam vertoonde geen flocculerend vermogen boven een temperatuur van 30 °C. Het optimum ligt bij 15 °C.

Fysische interacties

Flocculatie wordt sterk bepaald door de stromingscondities in de tank en door de concentratie gistcellen. De snelheidsgradiënten in de vloeistof zorgen ervoor dat de gistcellen met elkaar botsen met als gevolg dat er vlokken ontstaan. Aan de andere kant zorgen de snelheidsgradiënten er ook voor dat de vlokken weer breken. Het gevolg is een dynamisch evenwicht tussen vorming en afbraak van gistvlokken.

De snelheidsgradiënten in de vloeistof worden bepaald door de vermogensinbreng in de tank. Bij een geroerde tank wordt het vermogen ingebracht door de roerder. Bij vergisting op industriële schaal wordt de vermogensinbreng bepaald door opstijgende kooldioxidebellen, als gevolg van de kooldioxideproductie door de gist zelf. Met behulp van de turbidostaat is bij verschillende waarden van flocculerend vermogen,

gistconcentratie en vermogensinbreng in de tank, flocculatie gemeten. De omstandigheden kwamen overeen met die bij vergisting op industriële schaal. Het aantal vrije cellen bleek onafhankelijk van de vermogensinbreng en bedraagt aan het eind van de vergisting 10% van de gistconcentratie. Bij een lager flocculerend vermogen loopt dit percentage op tot 40% van de gistconcentratie.

Modellering van flocculatie en sedimentatie

Om het inzicht in de fysische mechanismen van gistflocculatie te vergroten werd het proces gemodelleerd. Flocculatie is het geheel van vormings- en afbraakprocessen van gistvlokken. De vormingssnelheid van een vlok hangt af van het aantal cellen, de botsings-efficiency van de deeltjes en de snelheidsgradiënten in de vloeistof. Vlokafbraak vindt plaats langs twee routes, door splitsing van vlokken en door de erosie van losse cellen van het vlokoppervlak. De bindingssterkte tussen de cellen en de snelheidsgradiënten in de vloeistof zijn hier de bepalende factoren.
Met het ontwikkelde model is het mogelijk om het aantal 'vrije cellen' en de vlokgrootte te voorspellen aan de hand van de concentratie gistcellen, de bindingssterkte tussen de cellen en de snelheidsgradiënten in de vloeistof.

Het hierboven beschreven flocculatiemodel werd gebruikt om de hoeveelheid gist die in suspensie is gedurende de vergisting te modelleren. Ongeveer halverwege de vergisting worden de gistcellen flocculent waarna het flocculerend vermogen toeneemt als gevolg van de veranderende mediumsamenstelling. De kooldioxideproductiesnelheid die uiteindelijk de snelheidsgradiënten in de vloeistof bepaalt, bereikt een maximale waarde rond het flocculent worden van de gist. Daarna daalt de productiesnelheid van kooldioxide met als gevolg dat de snelheidsgradiënten in de vloeistof ook afnemen. De omstandigheden voor flocculatie zijn daarom aan het eind van de fermentatie zeer gunstig. Doordat de vloeistofsnelheden in de tank aan het eind van de fermentatie afnemen zullen de gevormde vlokken uiteindelijk bezinken. Hierdoor zal de gistconcentratie afnemen, wat weer zijn invloed heeft op de flocculatie van de gist, met als gevolg dat de sedimentatie ook verandert. Dit leidt ertoe dat aan het eind van de fermentatie flocculatie en sedimentatie in 'quasi steady state' verkeren. Het model werd gevalideerd met de resultaten van experimenten op industriële schaal. Met het ontwikkelde fermentatiemodel bleek het mogelijk de gistgroei, de kooldioxideproductie alsmede de sedimentatie van de gist aan het eind van de vergisting te berekenen op industriële schaal.

Beheersbaarheid van gistsedimentatie

Gistsedimentatie hangt af van een aantal factoren. Diezelfde factoren kunnen ook worden gebruikt om het totale proces te sturen.
Sedimentatie van gist kan worden beïnvloed via flocculentie, flocculerend vermogen en flocculatie van de gist.
De flocculentie van de gist hangt af van het soort gist dat wordt gebruikt en de groeiomstandigheden. Na het moment van flocculent worden kan de flocculentie van de gist nauwelijks meer worden beïnvloed, waarmee het minder geschikt wordt om de sedimentatie te sturen.
Het flocculerend vermogen van de gist wordt bepaald door de mediumsamenstelling. Door deze te variëren kan het flocculerend vermogen worden veranderd, echter de smaak en kwaliteit van het product kunnen hierdoor worden beïnvloed.
Flocculatie is sterk afhankelijk van de vermogensinbreng in de tank. Door deze te sturen kan de flocculatie van de gist worden verandert. Op deze manier zal ook de sedimentatie worden beïnvloed. Door de vermogensinbreng te veranderen zullen namelijk ook de vloeistofsnelheden in de tank veranderen. Gezien de invloed op het product lijken de fysische factoren het meest geschikt om sedimentatie te sturen.

Door het modelleren van gistflocculatie en sedimentatie zijn de fysische mechanismen van deze processen duidelijker geworden. Flocculatie kan worden beschreven met slechts drie parameters (bindingsterkte, snelheidsgradiënten en celconcentratie). De sedimentatie van gist wordt naast flocculatie voornamelijk bepaald door de turbulentie in de tank. Deze turbulentie wordt veroorzaakt door de kooldioxideproductie van de gist. Flocculatie en sedimentatie kunnen worden gestuurd via één van deze parameters. Een belangrijk onderdeel in relatie tot dit is het meten van flocculatie. Tijdens het hier beschreven onderzoek zijn drie meetmethoden ontwikkeld om gistflocculatie te meten, zowel kwalitatief als kwantitatief.

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OP EEN DAG ...

ben je geen student meer maar AIO. Je krijgt een onderzoeksvoorstel en dan begint het. Gelukkig zijn er dan diverse mensen die bereid zijn de helpende hand te bieden.
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Een belangrijk onderdeel van promoveren (ik kan het niet vaak genoeg zeggen) is het uitvoeren van experimenten. Nu ben ik niet zo'n enthousiaste experimentator, maar voor een 'bierproefje' wilde ik nog wel naar het lab. Gelukkig werd ik in de loop van het onderzoek bijgestaan door Stef en de studenten Jeroen, Edward en Maarten. Stef heeft hierbij alle mogelijkheden van de PDA onderzocht. Jeroen heeft mij echt versteld doen staan door het zeer grote aantal experimenten dat hij heeft uitgevoerd. Een deel van de resultaten staat beschreven in hoofdstuk IV. Edward heeft tijdens z'n afstuderen iets gedaan wat absoluut onmogelijk leek, namelijk het fotograferen van Fast Moving Coalescerende Gist in een Couette vat, zodat we er eindelijk eens achter kwamen hoe groot die vlokken nu werkelijk zijn. Als laatste kwam Maarten opdagen. Maarten heeft als informaticus helpen sleutelen aan het model zoals beschreven in hoofdstuk VI. Waarbij hij een flinke winst in het terugdringen van de rekentijd wist te behalen. Allen bedankt.
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Dit onderzoek maakte onderdeel uit van een multidisciplinair project waarbij naast de TU-Delft twee onderzoeksgroepen van de Rijksuniversiteit te Leiden en de onderzoeksafdeling van een brouwerij betrokken waren. Het hieruit voortvloeiende fameuze flocculatieoverleg met Jan Kijne, Gerrit Smit, Marika Straver, Yde Steensma en Aloys Teunissen uit Leiden en de mensen van de brouwerij in wisselende bezetting was zeer leerzaam en vooral ook gezellig.
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Tot slot, Netje, 'ondanks' jou is dit proefschrift er toch gekomen. Maar dankzij jou was het overgrote deel van mijn tijd in Delft onvergetelijk!

Zo en nu eerst ...

Eddy van Hamersveld

CURRICULUM VITAE

Everardus Hendrikus van Hamersveld werd op zaterdag 19 juni 1965 geboren te Baarn. Na de kleuterschool en de lagere school (Aloysiusschool Baarn) werd in 1977 aangevangen met het ongedeeld VWO aan het Alberdingk Thijm College te Hilversum. In 1984 werd het VWO diploma behaald. In datzelfde jaar begon de auteur van dit proefschrift de studie scheikunde aan de Universiteit van Amsterdam. In het kader van de bedrijfsstage werd onderzoek verricht naar bodemverontreinigingen in het Ketelmeer. Dit onderzoek werd uitgevoerd bij de Dienst Binnenwateren van het RIZA te Lelystad. Het afstudeeronderzoek werd uitgevoerd bij de vakgroep Chemische Technologie, sectie bioproceskunde. Tijdens het afstuderen werd onderzoek verricht aan stoftransport naar methanogeen korrelslib. Voor de scriptie werd een rekenmodel onwikkeld dat de grootteverdeling van methanogeen korrelslib in afvalwaterzuiveringsinstallaties voorspelt. In 1990 werd het doctoraal examen afgelegd en in datzelfde jaar werd begonnen met het in dit proefschrift beschreven onderzoek. In 1992 werd het University College London bezocht en wel het Department of Civil Engineering. Bij deze vakgroep werd onderzoek gedaan naar de toepassingmogelijkheden van de 'Photometric Disperion Analyser' voor het promotieonderzoek.