Quantification of Brewers' Yeast Flocculation in a Stirred Tank: Effect of Physical Parameters on Flocculation

E. H. van Hamersveld,[1] R. G. J. M. van der Lans,[2] K. C. A. M. Luyben[2]

[1]NIZO, Department of Process Engineering, P.O. Box 20, 6710 BA Ede, The Netherlands; telephone: 31-318-659 563; fax: 31-318-650 400; e-mail: hamersve@nizo.nl
[2]Delft University of Technology, Department of Biochemical Engineering, Julianalaan 67, 2628 BC Delft, The Netherlands

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Abstract: Quantification of yeast flocculation under defined conditions will help to understand the physical mechanisms of the flocculation process used in beer fermentation. Flocculation was quantified by measuring the size of yeast flocs and the number of single cells. For this purpose, 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 fermentation, without influencing flocculation. The effects of three physical parameters, floc strength, fluid shear, and yeast cell concentration, on flocculation during beer fermentation, were examined. Increasing floc strength results in larger flocs and lower numbers of single cells. If the fluid shear is increased, the size of the flocs decreases, and the number of single cells remains constant at approximately 10% of the total cells 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 in linear proportion to the cell concentration. This means that, during yeast settling at full scale, the number of single cells decreases. The results of this study are 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. *Biotechnol Bioeng* **56:** 190–200, 1997.
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INTRODUCTION

One of the steps in the beer brewing process is the fermentation of wort. In this step, ethanol, carbon dioxide, and flavor 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 rest conversions to take place during the lagering. One of the advantages of brewers' yeast is the ability of the cells to form flocs under certain environmental conditions. This property facilitates yeast 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 yeast sedimentation takes place must be known. Knowledge of the factors involved in both flocculation and sedimentation is necessary for this. Here we will focus on the factors influencing flocculation. The conditions for sedimentation will be the 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 favorable) (Amory, 1988).
2. The physiological conditions must be favorable (presence of sufficient calcium, favorable temperature and pH of the medium, etc.) to enable a certain bond strength between the cells.
3. The hydrodynamical conditions must be favorable (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) vary 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 favorable environmental conditions, also increases (van Hamersveld et al., 1996). The pH and sugar concentration of the medium, which both change during fermentation, are factors influencing the flocculability (floc strength) of the cells (Smit et al., 1992).

Correspondence to: E. H. van Hamersveld
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- 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 thereby cause a turbulent fluid motion in the vessel. 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 that point 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 flocculability (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 have already been determined by Kihn et al. (1988) and Stratford et al. (1988). However, their experiments were carried out under undefined hydrodynamic conditions and Kihn's group obtained only qualitative results. Here the effects of the parameters mentioned will be quantified under conditions such as those occurring during full scale fermentation.

Determination of floc size is complicated in turbulent flow. Because of the high velocities within the vessel, simply taking pictures of the particles is impossible. Separation of the particles from the medium also seems to be 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 modeling this settling process a floc size distribution can be obtained 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 modeling the optical density decline caused by settling of the flocs and comparing the modeling results with the experimental optical density decline, the floc size distribution can be determined. In what follows we will explain how the optical density decline is modeled.

Optical Density

The optical density is linearly proportional to the turbidity of the suspension, which is proportional to the particle concentration and the scattering cross-section. Because of settling of the flocs the particle concentration in the light beam depends on the settling rate of the flocs, which on its turn depends on the floc size (Fig. 1).

The optical density as a function of time, at a wavelength of 660 nm [$OD_{660}(t)$], can be described by:

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

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.

Turbidity

A flocculating yeast suspension contains flocs and single cells (Miki et al., 1982a, 1982b), which makes the total size distribution of a flocculating yeast suspension bimodal. Therefore, the turbidity of the suspension can be derived from the turbidity caused by cells and the turbidity caused by the flocs. In the 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

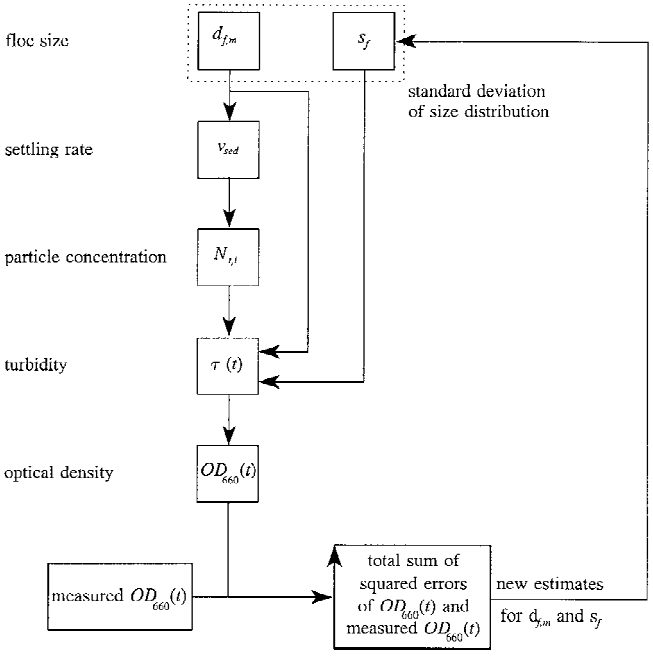

Figure 1. Schematic representation of the calculation procedure for the determination of the floc size distribution.

within a size class, and C is the scattering cross-section of a particle, which is defined as:

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

where d_p is the particle (cell or floc) diameter and Q_{sca} is the scattering coefficient, which equals 2 in the case of a particle diameter >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 [$f(d)$] and, in this case, a lognormal distribution is assumed:

$$S(d_i) = \int_{d-\frac{1}{2}\Delta d}^{d+\frac{1}{2}\Delta d} f(d_{f,i})\mathrm{d}d$$

$$\int f(d_{f,i})\mathrm{d}d = \tfrac{1}{2}\,\mathrm{erf}\,\frac{\left(\frac{\ln d_{f,i} - \ln d_{f,m}}{s_f}\right)}{\sqrt{2}} \tag{4}$$

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 settling measurements were carried out in a curved tube (Fig. 2); for this purpose, the particle number as a function of time must be corrected for the curvature of the tube leading to the following equation, describing the number of particles at the height of the light beam (h_L) in the tube:

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

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

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 the 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).

The number of particles at $t = 0$ s can be calculated from the total cell number and the number of single cells. The number of single cells can be derived from the settling curve (Fig. 3) by correction for the settling of the cells, according to Eq. (5). The total cell number can be determined off-line by a particle counter.

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 to the theory of fractals (Davis and Hunt, 1986; Fontana et al., 1991; Logan and Wilkinson, 1991),

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

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. This settling rate (v_{sed}) can be predicted by the Stokes law corrected for hindered settling:

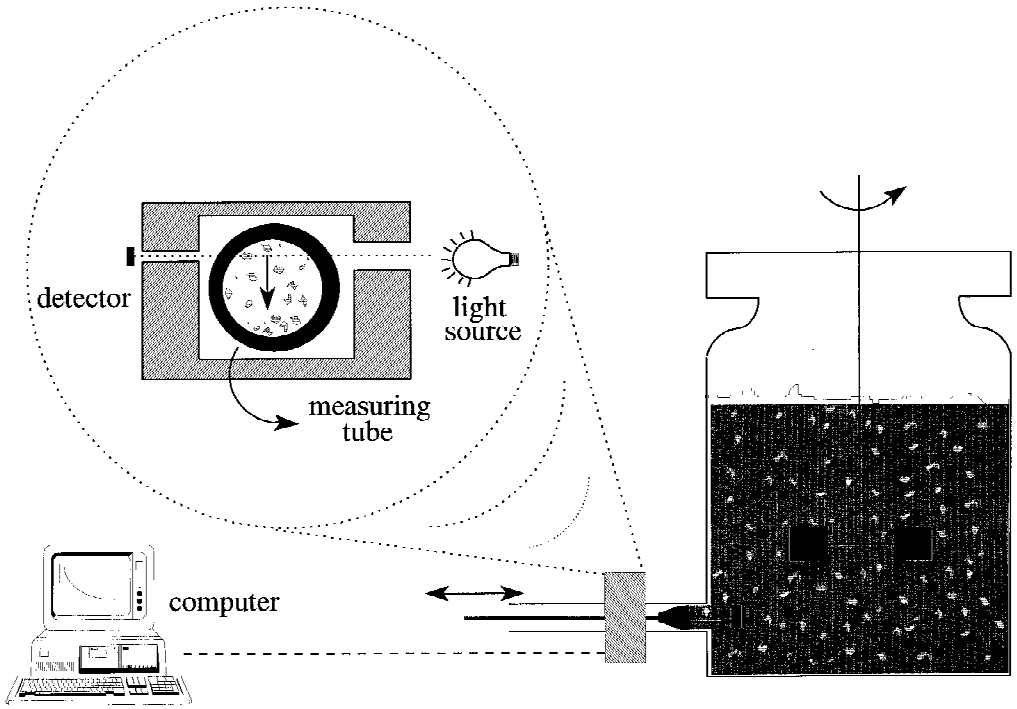

Figure 2. Experimental setup for in situ measurement of the size of yeast flocs formed in turbulent flow.

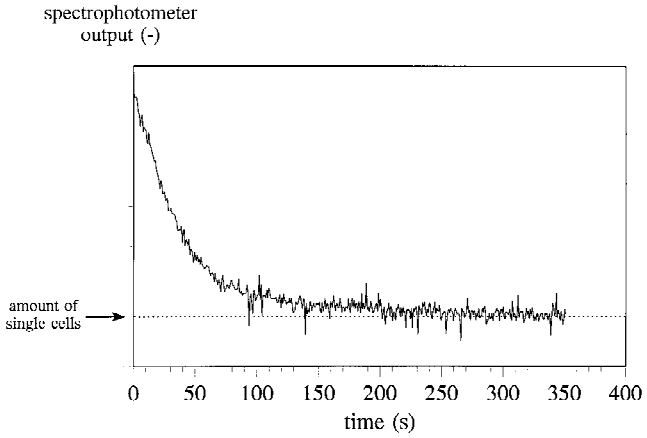

Figure 3. OD_{660} decline during a settling experiment.

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

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 Sami 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 an initial value for the average floc size and the standard deviation of the size distribution the optical density decline can now be calculated iteratively (Fig. 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 in wort at 15°C. After pitching of the wort the initial cell number was 4×10^6 cells/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.

The results presented in this study are based on measurements carried out during one fermentation. Each measurement was repeated several times; all individual data are presented in the figures.

Flocculability

Floculability was measured with a Photometric dispersion analyzer (PDA). With the PDA it is possible to determine the turbidity of a flowing suspension. The output of the apparatus is a ratio between two signals. This ratio is proportional to the size of the particles in the suspension (van Hamersveld et al., 1996).

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 rotates. 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 ''macro-lens.'' During the experiment, the total cell number was constant. The pictures were analyzed 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. Because the total cell number was known, the fractal dimension could be calculated according to Eq. (6). 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 to 100 s^{-1}), different cell concentrations (25 to 35 $\times$ 10^6 cells/mL), and different floc strength. The floc strength was varied by adding calcium to the medium so that floc size increased by approximately 40% from the lowest to the highest 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 density of 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.

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 fermentor (Fig. 2) was used. A pipe containing a plunger was fixed on the fermentor at a height of 2 cm from the bottom of the vessel. The plunger could be moved to take a sample from the yeast culture. To monitor the yeast suspension a spectrophotometer was placed around the pipe. 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. Considering the position where the light beam crosses the pipe, the effect of light refraction is negligible. 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 100 data points. All experiments were carried out in duplicate.

Because floc strength increased from the moment the cells became flocculent the effect of floc strength on flocculation was determined by carrying out experiments every 24 h after the moment the cells became flocculent.

The power input was changed during each set of experiments by varying the stirrer speed. The range was chosen based on the average power input during large-scale brewery 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 and 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 remained in suspension due to their low settling rate compared with the flocs. When all flocs were settled, or at least passed the light beam, the optical density decline stopped and reached a constant level (Fig. 3). This level corresponded to the number of single cells. To determine this number, the apparatus was calibrated. 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 [Eq. (1)] the size distribution of the flocs was divided into ''n'' compartments. The value of ''n'' was chosen based on a 99% accuracy level ($n = 350$). The first size class represents the single cells.

From the plateau in the optical density decline curve (Fig. 3) the number of single cells was determined and corrected for the settling rate of the flocs according to Eq. (5). With a combination of Eq. (1) to Eq. (9), the decline of the optical density as a function of time can be calculated and compared with the measured curves. The calculations are started with an initial value for the average floc size and the standard deviation of the size distribution (Fig. 1). From the floc size the settling velocity can be derived with which the particle concentration as a function of time can be calculated. From the particle concentration and the floc size distribution (average floc size and standard deviation) the turbidity can be calculated, from which the optical density follows. The optical density as a function of time is compared with the measured optical density and the total sum of squared errors is calculated. After this, new estimates for the average floc size and the standard deviation are generated by the simplex-like algorithm of Nelder–Mead (Himmelblau, 1970). During the fitting procedure, the total sum of squared errors was minimized. At least 200 iteration steps were carried out during the fitting procedure. The calculations were carried out on a 486-DX processor-based personal computer. The total fit procedure took approximately 1 h.

RESULTS AND DISCUSSION

Yeast flocculation is an important phenomenon to enhance the removal of the yeast from the green beer. 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 quiet settling. The effects of flocculability, turbulence, and amount of yeast in suspension on flocculation were studied and the results are presented. We then discuss the consequences of 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 I.

Fractal Dimension

The fractal dimension was determined at different conditions in the Couette vessel. The fractal dimension was found

Table I. Physical constants and parameters used for the model calculations and calculation of power input.

Description	Symbol	Value	Reference
Power number	P_O	5	Janssen and Warmoeskerken (1987)
Diameter of Rushton turbine	D_{Rush}	0.045 m	This study
Fermentor 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
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
Gravitational constant	g	9.81 m/s^2	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

to be 2.45 ± 0.03 and did not vary with floc strength, cell concentration and shear rate, in the range measured.

The fractal dimension as determined in this study is within the range of values found in the 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. They derived the fractal dimension from the floc size and 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 and they found a fractal dimension of 2.66. Fontana et al. (1991) determined the fractal dimension of two strains of *Saccharomyces cerevisiae* (27C and 38A) and found values 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 the literature the fractal dimension was strain-dependent. In two cases, measurement of different strains under equivalent conditions gave different values of fractal dimension. Also, the medium could play a role; however, in this study, the medium, which was changed by adding calcium, 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 volume. The size distribution could be described as log-normal, 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.

The density of the yeast was found to be 1140 ± 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 yeast wet density of 1110 kg/m^3. The range of densities was acceptable and the differences might be explained by the differences in strain and growth conditions.

Density and viscosity of the medium were measured during the whole fermentation. Due to sugar consumption and ethanol production the density of the medium decreased from 1045 to 1006 kg/m^3. The viscosity of the medium remained constant during the fermentation at a value of 2.3 ± 0.1 × 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 following results: $OD_{660,0} = 0.024$ and $K = 2.3 \times 10^{-3}$, both parameters are needed in Eq. (1). The optical density was shown to be linearly dependent on the number of cells within the range measured (up to 50×10^6 cells/mL).

Determination of Floc Size Distribution

The particle size distribution of a flocculating yeast suspension can be characterized by both the shape of the cell and

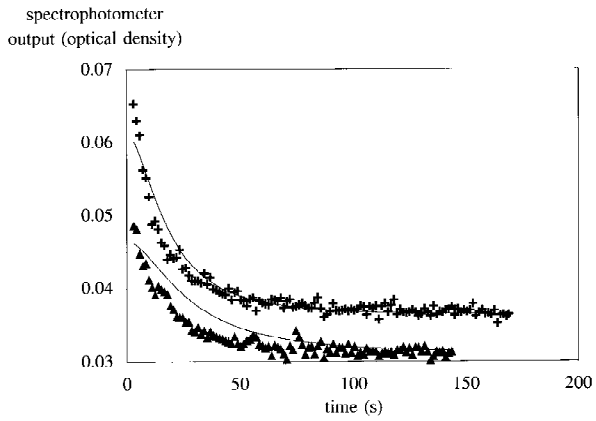

Figure 4. Example of optical density as a function of time as predicted by the model compared to the measured values of the optical density. $N_{c,tot} = 49 \times 10^6$ cells/mL; $d_f = 240$ μm (+); $N_{c,tot} = 26 \times 10^6$ cells/mL, $d_f = 175$ μm (▲). $\epsilon = 0.5$ W/m^3.

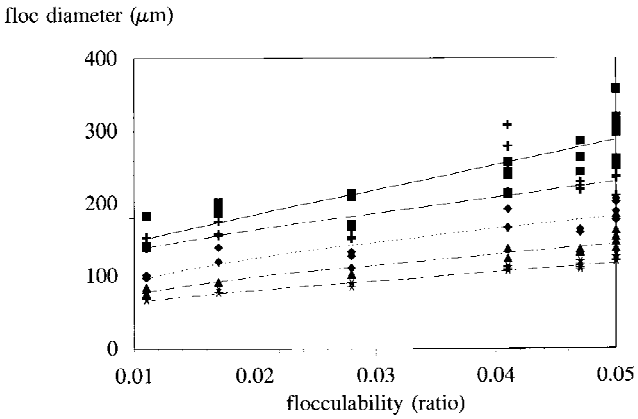

Figure 5. The relation between flocculability expressed as a ratio proportional to the floc size (van Hamersveld et al., 1996) and the diameter of yeast flocs at various power input levels. $\epsilon = 0.14$ W/m^3 (■), 0.5 W/m^3 (+), 1.2 W/m^3 (♦), 2.5 W/m^3 (▲), and 4.3 W/m^3 (∗).

the floc size distribution. The cell diameter and standard deviation of the cell size distribution were found to be constant. The same result was found for the standard deviation of the floc size distribution. During all experiments the standard deviation of the floc size distribution turned out to be 0.45 ± 0.1. In Figure 4 an example of the measured and modeled optical density is shown as a function of time.

The floc size distribution was assumed to be log-normal 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 this case the size distribution can be derived directly from the settling curve without assumptions of the shape of the distribution. Also, the computational load will be reduced to one calculation step, which means an enormous reduction in 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 increase on flocculation, which is expressed as floc size and number of single cells, is shown in Figures 5 and 6. The floc size increased by approximately 60% as the flocculability increased. In the same range the number of single cells decreased. 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 decreased by a factor of four during fermentation as a result of the increasing flocculability.

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.

Little is known about the course of flocculability during full scale fermentation. However, flocculability depends mainly on the composition of the medium, which can be expected to be equal at 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 make use of heterogeneous strains. This will have consequences for the overall flocculability of the yeast in suspension in the tank. If the strains have different flocculation behavior the settling behavior 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, and for this the cells of the nonflocculent strain will remain in suspension while the flocs of the flocculent strain will settle. In such a case, complete re-

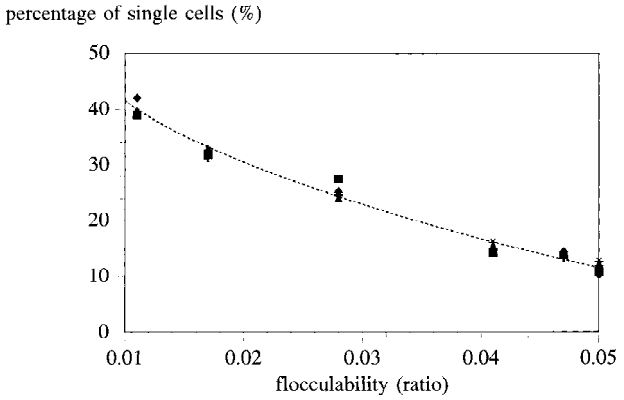

Figure 6. The percentage of single cells of the total number of yeast cells in suspension as a function of the flocculability of the cells. The power input was varied during the experiments. The flocculability is expressed as ratio proportional to the floc size (van Hamersveld et al., 1996). $\epsilon = 0.14$ W/m^3 (■), 0.5 W/m^3 (+), 1.2 W/m^3 (♦), 2.5 W/m^3 (▲), and 4.3 W/m^3 (∗).

moval 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 of the amount of flocculent cells and nonflocculent cells will decrease in time, 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 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 and 2 W/m^3. The power input had no effect on the number of single cells. In the range 0.1 to 10 W/m^3 the percentage of single cells was approximately 10% of the total amount of yeast in suspension.

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 (see Appendix). The power input range during the experiments is chosen based on full scale conditions. Renger and Luyben (1988) reported a maximum carbon dioxide production rate per kilogram of medium of 2.63×10^{-3} mol/kg s. The described cylindroconical vessel has a height of 18.5 m and a volume of 400 m^3. From this the maximum power input can be calculated giving 3.7 W/m^3 (see Appendix).

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

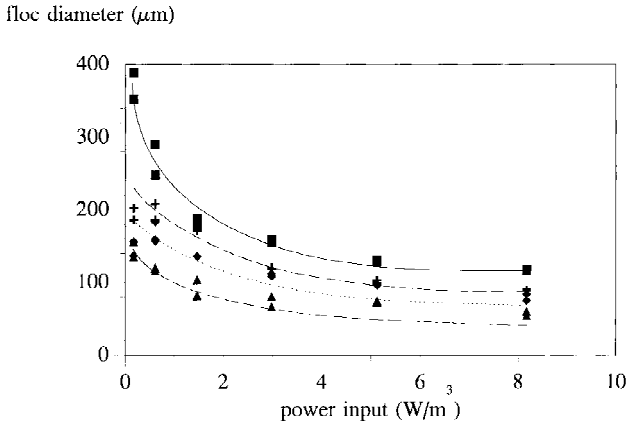

Figure 7. The relation between the diameter of yeast flocs and the power input in a stirred vessel. $N_{c,tot} = 49 \times 10^6$ cells/mL (■), 26×10^6 cells/mL (+), 14×10^6 cells/mL (♦), and 5×10^6 cells/mL (▲).

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 a full scale ($V_f = 400$ m^3; $h_T = 11$ m) will give mixing times of 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 by the maximal rather than the average shear rate. At full scale, the same power input will, in regard to the long mixing times, cause a floc size distribution that follows a shear distribution with average value determined by average shear rate.

The shear rate in a full scale tank depends on the carbon dioxide production rate, which varies 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 that point 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. The size of the flocs increases due to an increasing amount of yeast. The number of single cells is constant at 10% of the total amount of yeast in suspension.

Estimation of Flocculation on Full Scale

In this study, the amount of yeast was varied by removing the yeast from the medium and resuspending 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 favorable for sedimentation:

- The carbon dioxide production is low (giving a low shear rate).
- The flocculability is high (giving a large floc strength).
- The yeast concentration is maximal.

These conditions are favorable for the formation of large flocs. Thus, because of the low carbon dioxide production rate, the fluid velocities will be low enough to allow settling of the flocs. The settling time at full scale has an order of magnitude of hours, whereas the floc formation takes a few seconds. Because of this, sedimentation and flocculation of the yeast will be in quasi steady state.

In Figure 8 the development of flocculation expected during full scale fermentation is shown. The flocculability

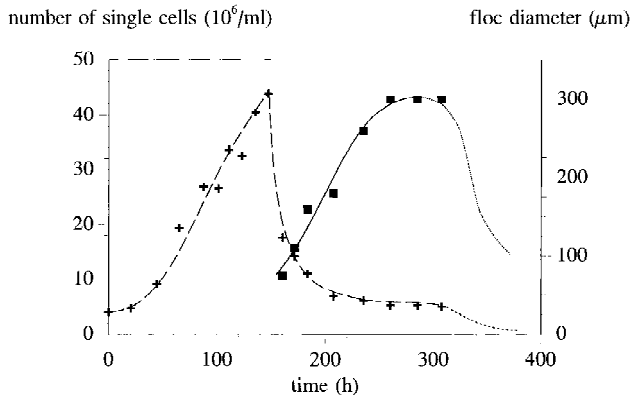

Figure 8. Development of the floc size (■) and the number of single cells (+) during the fermentation of beer.

course was taken from this study. From the sugar conversion the carbon dioxide production rate could be calculated. Assuming a cylindrical tank ($V_f = 400$ m^3, $h_T = 11$ m) the power input was derived (see Appendix). With this, the floc size and number of single cells were estimated. Further, it was assumed that the settling of the flocs started at the moment the carbon dioxide production stopped. In practice, this will occur somewhat earlier, depending on the floc strength and fluid velocities in the tank. The extrapolated lines in Figure 8 are based on the settling rate of the flocs and the height of the tank.

The development of flocculation is influenced by flocculability, shear rate, and amount of yeast in suspension. The increase in flocculability and decrease in shear rate both cause an increase in floc size and a decrease of single cell number. At the end of the fermentation, when the amount of yeast decreases due to settling, the floc size decreases, which in turn causes a decrease in the settling rate. The number of single cells decreases proportionally with 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 over a wide range of yeast strains and growth conditions, such as temperature and medium composition. In addition, the physical conditions can be varied, as 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, and both floc size and number of single cells can also be influenced. Shear rate has a strong effect on floc size. By setting the power input flocculation can be influenced.

Edward van Wezel is acknowledged for his contribution to the Couette experiments. We thank the members of the ''flocculation group'' for stimulating discussions.

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_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)
D_{Rush}	Rushton turbine 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 (−)
p_0	atmospheric pressure (Pa)
Po	power number (−)
Q_{sca}	scattering coefficient (−)
r	tube radius (m)
R	gas constant (J/mol K)
s_f	standard deviation of (ln d_f) of a log-normal floc size distribution (−)
S	fraction of the particle number (−)
T	temperature (K)
t	time (s)
V_f	fermentor volume (m^3)
v_{sed}	settling velocity (m/s)

APPENDIX

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 size (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 (e.g., pipe flow and Couette flow) the shear rate can be easily calculated from the flow rate (pipe) or the angular velocity (Couette device), respectively. However, flocculation usually occurs under turbulent flow conditions. In 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:

$$\dot{\gamma} = \sqrt{\frac{\epsilon\rho}{\eta}} \tag{10}$$

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 the 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 the 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 fermentor (Delente et al., 1968), which, in the case of saturation of carbon dioxide in the fluid, is equal to the carbon dioxide production rate of the yeast.

$$\begin{aligned} \epsilon &= \frac{\varphi_m}{V_f} RT \left(\frac{a+1}{a} \ln(a+1) - 1\right) \\ a &= \frac{\rho_l g h_T}{p_0} \end{aligned} \tag{11}$$

where φ_m is the molar gas flow, 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):

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

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).

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