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Characterization of the physical properties of yeast flocs

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Summary. Physical characteristics, namely floc density function, floc size distribution, and relative floc strength, of a number of flocculent yeast types were measured. A straight-line relationship was found to exist between log values of size and density for the yeasts examined. Each yeast type had coefficients from this relationship which could be used to interpret settling behaviour. Indices of relative floc strength were also obtained and together with the floc density function allowed fuller interpretation of yeast settling than with simpler theories.

Introduction

Tower fermentation systems have been used for the production of microbial protein (Paca and Gregr 1977), power ethanol (Prince and Barford 1982) antibiotics (Konig et al. 1982) and other products (Smith and Greenshields 1974). One such system, the tower fermentation of beer was based on the principle of fluidized particles, in this case yeast flocs, and is well documented by several authors (Royston 1966; Smith and Greenshields 1973; den Blanken 1974). However, Greenshields and Smith (1971) point out that before such systems gain popularity they must first be fully characterized in the physical sense and the operating parameters determined. This study correlates (a) yeast floc size and density and (b) floc size and agitation intensity, for several yeast types which allows coefficients to be developed. These coefficients indicate the ability of the yeast flocs to settle and also their strength which will determine their size under different conditions of shear. They reflect some of the physical aspects of yeast flocculence.

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Symbols: a = constant ($g \cdot cm^{-3}$); B_2/B_1 = floc binding strength of $floc_2$ relative to $floc_1$; d_f = floc diameter (cm); d_i = image diameter on print (cm); d_{max} = maximum floc diameter (cm); f_d = floc effective density ($g \cdot cm^{-3}$); g = gravitational constant (981 $cm \cdot s^{-1}$); K_p = constant (−); R_1 = rate of enlargement on film; R_2 = rate of enlargement on print; S_s = density of suspending liquid phase ($g \cdot cm^{-3}$); S_f = density of solid (floc) phase ($g \cdot cm^{-3}$); U_t = terminal settling velocity ($cm \cdot s^{-1}$); u = liquid viscosity ($g \cdot cm^{-3} \cdot s^{-1}$)

Methods

Yeasts and growth conditions. The six strains of *Saccharomyces cerevisiae* and two strains of *S. carlsbergensis* used in these experiments were supplied courtesy of Arthur Guinness and Son Ltd., James's Street, Dublin, Ireland. The strains of *S. cerevisiae* were divided into three classes, CII, CIII, and CIV after the classification of Gilliland (1951). Each class was sub-divided into coarse (C) or fine (F) type. The two strains of *S. carlsbergensis* were classed simply as flocculent and very flocculent and are hereafter referred to as type F and type VF.

All strains were maintained and grown as described previously (Brohan and McLoughlin 1984).

Preparation of cells. Stationary phase cells were harvested and prepared as described previously (Brohan and McLoughlin 1984).

Estimation of Burns number. Harvested yeast (1 g wet weight) was transferred to a 10-ml graduated tapered centrifuge tube. The total volume in the tube was made up to 10 ml with acetate buffer. The yeast was resuspended by repeated inversion for 3 min after which time the tube was tapped sharply to dislodge any yeast trapped in foam in the top of the tube. The yeast was allowed to settle in the tube and the sediment volume after 10 min was taken as the Burns number.

Measurement of optical density. Optical density measurements were carried out on the upper 5 ml of liquor in the Burns number tubes after settling. The upper 5 ml was carefully removed with a pasteur pipette and transferred to a test tube containing an equal volume of fresh buffer. The percentage transmission at 660 nm was determined using a spectrophotometer (Coleman, model 295 E).

Measurement of floc size and settling velocity. Approximately 1 g · l^{-1} of yeast (wet weight) was suspended in buffer. An aliquot of this suspension was transferred by means of a wide-mouth pipette to a specially constructed perspex settling chamber (170 mm high × 9 mm deep × 35 mm wide). Flocs in the pipette were allowed to settle in to the buffer solution in the settling chamber without disturbance. Individual flocs were allowed to reach terminal settling velocity and then timed over a 5-cm fall. After 5 cm of timed descent the floc was photographed with a single lens reflex camera fitted with extension tubes. Illumination was by electronic flash. The diameter of a photographed floc was given by Eq. (1).

$$d_f = \frac{d_i}{R_1 R_2} \tag{1}$$

where d_f = floc diameter (cm), d_i = image diameter on print (cm), R_1 = rate of enlargement on film (−), R_2 = rate of enlargement on print (−).

Calculation of floc density. When the size and terminal settling velocity of the flocs were known the density could be calculated using a settling equation, once the properties of the suspending liquid were known. For this work the Stokes settling Eq. (2), used by Greenshields and Smith (1971) for calculating yeast floc terminal velocity was used:

$$U_t = \frac{g(S_f - S_s)\, d_f^2}{18\,\mu} \tag{2}$$

where U_t = terminal settling velocity (cm · s^{-1}), g = gravitational constant (981 cm · s^{-1}), S_f = density of solid phase (g · cm^{-3}), S_s = density of liquid phase (g · cm^{-3}), d_f = floc diameter (cm), μ = liquid viscosity (g · cm^{-3} · s^{-1}).

Measurement of effect of agitation intensity on floc size distribution. Harvested yeast (1 g wet weight) was resuspended in 1 l acetate buffer in a specially constructed perspex vessel (90 mm deep × 90 mm wide × 200 mm high). Agitation was provided by means of a centrally located shaft-driven propellor (3 blade of diameter 32 mm and pitch 80 mm, Baird and Tatlock, London), which was positioned above the base of the vessel at a distance which gave optimal vertical lift to the flocs at low agitation rates.

The agitation rate was high initially and was decreased step-wise as the experiment progressed. Each agitation rate was maintained for at least 10 min before readings were taken to allow the system to equilibrate. Equilibration was confirmed when consecutive estimations of floc size were similar. Floc size was measured photographically in a similar manner to that used for measuring floc size above. Electronic flash was used to "freeze" the images of the rapidly moving flocs.

Results

Evaluation of yeast flocculence by the Burns number

The Burns number is an index of flocculation mainly used in the brewing industry. The numerical value of the Burns number is the volume of sediment of yeast flocs (or biomass volume index) in a tapered centrifuge tube after a set time and under standard conditions of initial biomass levels, temperature, buffer etc. It is generally accepted that the greater the Burns number the more flocculent the yeast but this would appear to be an over simplification because it does not relate to information on (a) floc structure, (b) how flocs pack within the sediment, and (c) the ratio of flocculated to unflocculated cells.

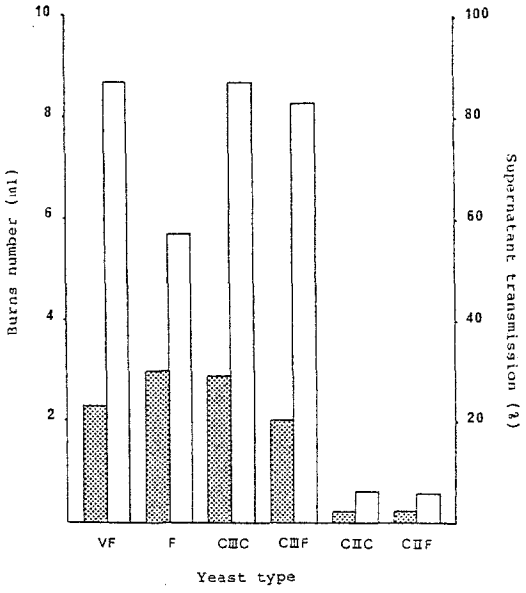

Fig. 1. Comparison of methods of flocculence measurement of six yeast types in acetate buffer after 5 min settling. ▨ Burns number (yeast sediment volume); □ percentage transmission of upper 5 ml of supernatant

Burns numbers for six yeast types were measured and the optical density (as percent transmission) of the upper 5 ml of liquor in the Burns number tubes after 10 min was estimated. Results are reported in Fig. 1.

In this study four of the six yeast types behaved as flocculent; the two strains of *Saccharomyces carlsbergensis* and the *S. cerevisiae* strains CIIIC and CIIIF. Strains CIIF and CIIC behaved as non-flocculent. This was probably due to the fact that the conditions of growth used in these experiments were not necessarily those at which flocculence in each yeast was optimally expressed nor indeed were they the conditions at which the yeasts were originally classified.

Figure 1 shows that types CIIF and CIIC were both found to be non-flocculent. This was reflected in a low Burns number and low transmission reading for the upper 5 ml. Types CIIIC and CIIIF were both found to be flocculent. Type CIIIC was found to have more cells remaining in suspension after 10 min settling than type CIIIF and the former also had a higher Burns number (2.9) as opposed to the latters (2.0). The higher Burns number of type CIIIC

suggested it was more flocculent than type CIIIF and this was supported by the optical density of the liquor in the tubes after settling.

Saccharomyces carlsbergensis type VF was found to have a lower Burns number than type F, the former with a Burns number of 2.3 and the latter 3.0, suggesting that type F was more flocculent than type VF. However, it was found that the percentage transmission of type F was significantly lower than that of type VF (57% as opposed to 87%). Therefore, more cells remained in the supernatant of type F than that of type VF. This result suggested that the Burns number is a function not only of the amount of yeast removed from suspension but also of the packing density of these flocs within the sediment once removed from suspension. Consequently, this reflects several interdependent variables which may affect the measurement of flocculation and is one of the limitations of methods based on the biomass volume index of a standard amount of yeast. It was, therefore, decided to investigate the variables which may be used to characterize the physical behaviour of yeast flocs.

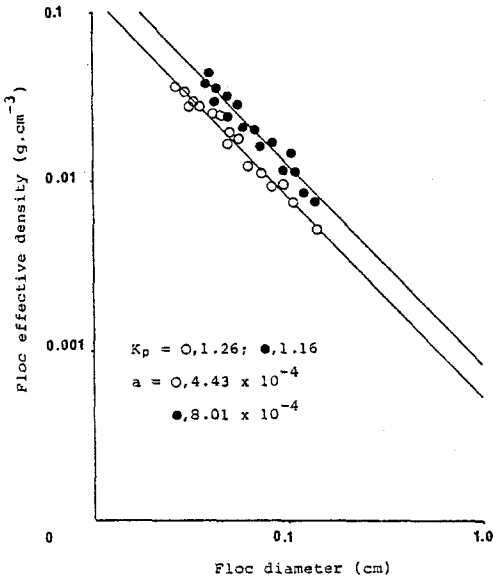

Fig. 2. Log-log plot of variation of floc effective density with floc diameter for two strains of *S. cerevisiae*. O, type CIIIC; ●, type CIIIF. Correlation coefficient = O, −0.99; ●, −0.98. Degrees of freedom = 14. Level of significance = ($P > 99.9\%$)

Yeast floc size-density relationship

It has been shown that changes in floc size, strength, and density can alter the biomass volume index [or sludge volume index (SVI)] of an activated sludge (Magara et al. 1976). These authors suggest that floc density exerts the greatest influence on the SVI of an activated sludge. Tambo and Watanabe (1967) have reported a floc size-density relationship for aluminium flocs while Lagvanker and Gemmel (1968) have reported the same relationship for iron (III) flocs using the Vold model floc (Vold 1963). More recently Tambo and Watanabe (1979) have studied the size density relationship for aluminium, iron, magnesium, and activated sludge flocs and have found a floc effective density (effective density = floc density − suspending liquid density) when plotted on logarithmic paper. The relationship has been termed the floc density function and its existence has been verified by model floc simulation. The floc density function can be expressed as Eq. (3)

$$S_f - S_s = f_d = \frac{a}{(d_f/1.0)^{K_p}} \tag{3}$$

where S_f and S_s = density of the floc and suspending liquid respectively, f_d = floc effective density (g · cm^3), d_f = floc diameter (cm), $d_f/1.0$ = dimensionless floc diameter (cm/cm), "a" = constant (g · cm^{-3}) and K_p = constant (−). The constant "a" is the effective density of a hypothetical 1-cm floc while K_p is the slope of the log-log plot and consequently reflects the rate of change of density with floc diameter. Equation (3) was applied to data obtained for yeast flocs. Figures 2 and 3 show plots of the floc density function for the four flocculent yeasts under study. Significant correlation coefficients indicated a straight-line relationship in each case. From Fig. 2 it is seen that type CIIIF had a significantly lower K_p value and a higher "a" value than the corresponding values for type CIIIC, suggesting that flocs of similar diameter had different effective densities. The rate of change of density with diameter also varied for the two yeast types.

With types F and VF a similar trend was found but the difference between the K_p and "a" values was much greater in this case suggesting that flocs of type F were significantly denser, for a given diameter, than those of type VF.

The floc density function curves show that for a particular yeast type, floc density decreases as floc size increases which is in agreement with the results of Tambo and Watanabe (1979). Each floc type has its own floc density function which would appear to be strain specific. However, density is not the major influence on floc settling velocity but floc diameter as can be seen from the Stokes settling Eq. (2). From Figs. 2 and 3 floc size and density correlate but floc size may be made a dependent variable by varying the rate of shear imposed on the system. Thus the effect of shear on floc size was investigated, a parameter

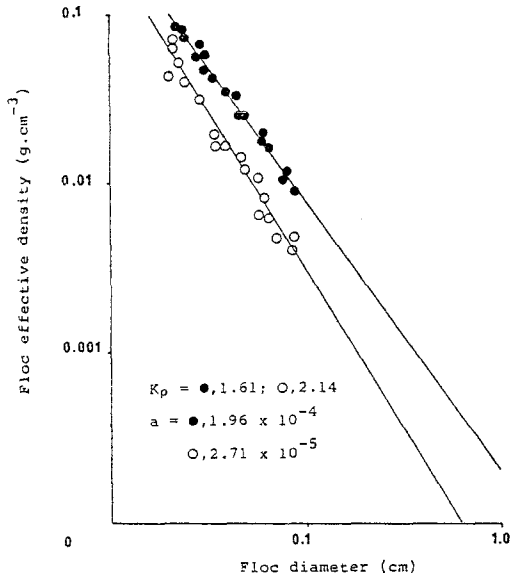

Fig. 3. Log-log plot of variation of floc effective density with floc diameter for two strains of *S. carlsbergensis*. ●, type F; ○, type VF. Correlation coefficient = ○, −0.99; ●, −0.96. Degrees of freedom = 16. Level of significance = ($P > 99.9\%$)

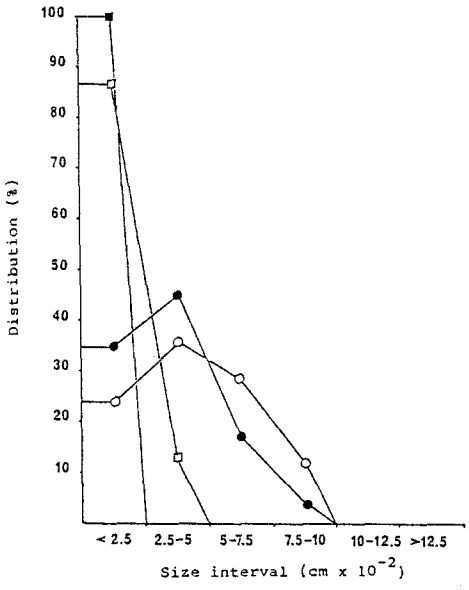

Fig. 5. Effect of agitation intensity on floc size (diameter) distribution of *S. cerevisiae* type CIIIF in acetate buffer. ○, 250 rpm; ●, 500 rpm; □, 750 rpm; ■, 1,000 rpm

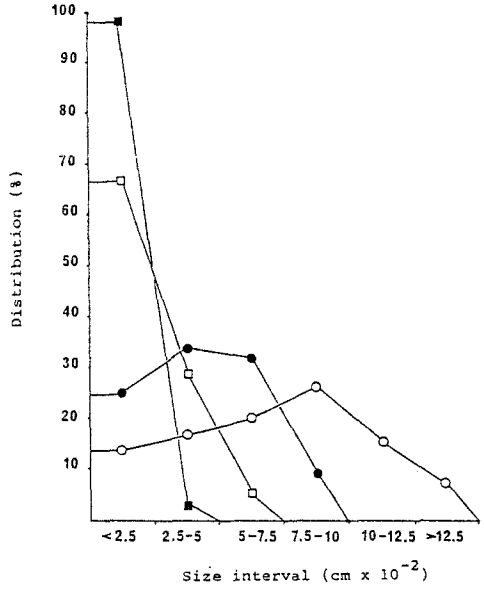

Fig. 4. Effect of agitation intensity on floc size (diameter) distribution of *S. cerevisiae* type CIIIC in acetate buffer. ○, 250 rpm; ●, 500 rpm; □, 750 rpm; ■, 1,000 rpm

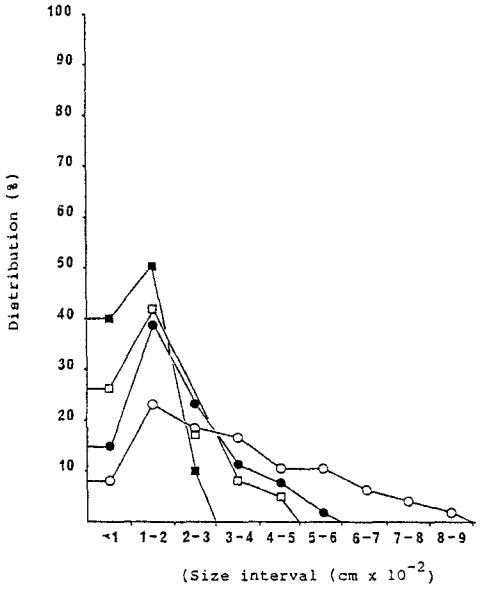

Fig. 6. Effect of agitation intensity on floc size (diameter) distribution of *S. carlsbergensis* type VF in acetate buffer. ○, 250 rpm; ●, 500 rpm; □, 750 rpm; ■, 1,000 rpm

which obviously reflects the strength of individual flocs.

Effect of agitation on floc-size distribution

Parker et al. (1971) proposed maximum floc size to be limited by the rate of floc breakup. Bradley and Krone (1971) proposed that the settling characteristics of activated sludge flocs were a function of shearing intensity and time. Magara et al. (1976) employed the method used by Nambu (1971) to evaluate the strength of biological flocs under given conditions. More recently Tambo and Hozumi (1979) have derived a measure of the relative floc binding strength from the floc density function. The effect of shear on yeast floc size was determined for the four flocculent yeast types under study.

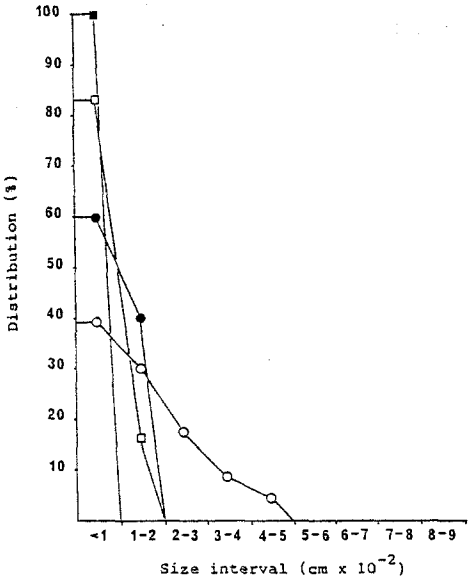

Fig. 7. Effect of agitation intensity on floc size (diameter) distribution of *S. carlsbergensis* type F in acetate buffer. ○, 250 rpm; ●, 500 rpm; □, 750 rpm; ■, 1,000 rpm

Figures 4–7 show the effect of agitation intensity on the floc size distributions for the four floc types under study. Figures 4 and 5 compare the effect of agitation intensity on floc size distributions of types CIIIC and CIIIF. For comparable agitation intensities the distribution of type CIIIC contained a greater percentage of large flocs than type CIIIF. For example, at the lowest agitation rate the largest flocs observed for type CIIIF were in the range 0.075–0.10 cm while for type CIIIC 7% of the floc population were greater than 0.125 cm. This trend was maintained as agitation intensity was increased. This suggested that type CIIIC flocs were stronger than type CIIIF flocs.

For the two strains of *S. carlsbergensis* it was found that type VF contained a greater proportion of large flocs than type F under similar levels of agitation (Figs. 6 and 7). At the lowest agitation rate of 250 rpm type F was observed to have no flocs greater than 0.05 cm while type VF had 22% of its floc population greater than this diameter at the same agitation rate. As with the two *S. cerevisiae* strains this trend was maintained at the higher agitation rates. Again, this suggested that type VF flocs were stronger than type F flocs.

Comparing the distributions between the two species of *Saccharomyces* it was found that at similar agitation rates the *S. cerevisiae* strains both formed larger flocs than the *S. carlsbergensis* strains, suggesting that the flocs of the former were stronger than those of the latter.

Tambo and Hozumi (1979) have formulated a relationship between maximum diameter of aluminium flocs and the effective energy dissipation rate of the system. From this relationship they have derived an expression for relative floc binding strength in terms of the floc density function and the maximum floc diameter. The equation is given in (4) below

$$\frac{B_2}{B_1} = \left(\frac{a_2}{a_1}\right)^{-\frac{2}{3}} \left[\frac{d_{2\,\max}^{(3+K_p)}}{d_{1\,\max}^{(3+K_p)}}\right]^{-\frac{2}{3}} \tag{4}$$

where B_2/B_1 = the floc binding strength of $floc_2$ relative to $floc_1$. The suffixes 1 and 2 denote the characteristic values of $floc_1$ and $floc_2$, respectively and d_{max} = maximum floc diameter (cm). A modification of this equation was used to calculate the strength of each floc type relative to *S. carlsbergensis* type-F flocs. The modified form employed the statistical mean diameter of the distributions rather than the maximum floc diameter.

Table 1. Relative floc binding strength (B_2/B_1) of the four yeast types calculated from Eq. 4 at different agitation rates

Yeast type	B_2/B_1 at			
	250 rpm	500 rpm	750 rpm	1,100 rpm
F	1	1	1	1
VF	12.7	13.2	14.3	16.4
CIIIF	39.1	34.5	30.7	33.9
CIIIC	73.2	55.8	48.6	49.7

The results are summarised in Table 1. Over the range of agitation rates studied the results predicted by Eq. (4) were consistent with the pattern observed in Figs. 4, 5, 6, and 7 and placed *S. cerevisiae* type CIIIC as the strongest floc type followed by type CIIIF. *S. carlsbergensis* type VF while weaker than the two *S. cerevisiae* strains had a higher relative floc binding strength than type F. These results agree well with the floc size distributions at the various agitation rates. The rather high value of relative floc binding strength obtained for yeast type CIIIC at 250 rpm may be an artefact due to the inability of the agitator to fully suspend these large flocs at this, the lowest, agitation rate.

Discussion

Of the many methods reported for measuring yeast flocculence (for a review see Greenshields et al. 1972) most depend on a function of yeast sedimentation rate to obtain their index of flocculation. These methods of flocculence measurement are analogous to the sludge volume index used to characterize biological sludge in waste water treatment. Dick (1972) has listed the factors which may influence the

Table 2. Summary of results

Yeast type	Burns no. (ml)	O. D. (−)	K_p (−)	a (g · cm^{-3})	d_f (mean at 250 rpm) (cm)
VF	2.3	87	2.14	2.71×10^{-5}	0.0335
F	3.0	57	1.61	1.96×10^{-4}	0.0158
CIIIC	2.9	87	1.26	4.43×10^{-4}	0.0706
CIIIF	2.0	76	1.16	8.01×10^{-4}	0.0477
CIIC	0.2	6	–	–	–
CIIF	0.2	5	–	–	–

results at the experimental level. In the present study it was found that in certain instances indices of flocculation conflicted depending on the method of measurement.

It has long been known (Richardson and Zaki 1954) that the nature of the discrete particle influences the hindered settling pattern of a collection of such particles in close proximity. It was therefore proposed that the discrepancies in the Burns numbers measured for the various yeast types should be reflected in the physical characteristics of the different floc types. Evidence for this was found in the fact that:

a) Each of the floc types was found to have a straight-line relationship when its floc density function was plotted on a log-log axis but the magnitude of the K_p and "a" values were different in each case (Figs. 2 and 3).

b) The floc size distributions at various agitation intensities yielded significantly different mean floc diameters for each of the floc types studied (Fig. 4–7).

c) The relative floc strength of each of the floc types, measured relative to the weakest floc type, were significantly different (Table 1).

These results are summarised in Table 2 and were interpreted as follows. In the Burns number tests yeast types VF and CIIIC contained similar levels of biomass in their sediments (as judged from the optical density of the supernatants). The larger and significantly stronger flocs of type CIIIC undergo interfloc cross-linking to a greater extent than the smaller weaker flocs of type VF. The degree of interfloc cross-linking would appear to be a function of floc strength and determines the extent to which the flocs remain discrete. Although flocs may be large with a high effective density, cross-linking may lead to a lowering of the settling velocity due to a resistive mechanical support within the tenuous floc matrix. Conversely, with yeast type F very little cross-linking between flocs was observed to occur. However, in this case the factor limiting the settling rate is the small diameter of the flocs which form.

Although intrinsically denser than flocs of the other yeast, Stokes Law predicts that settling velocity is proportional to the floc effective density. There would appear to be a number of interdependent parameters affecting the overall sedimentation pattern, namely the floc size-density relationship, floc strength, and interfloc cross-linking.

Indices of flocculation based on a function of floc hindered settling are widely used for the screening of flocculent strains of yeast and are carried out quickly once flocs have been obtained. Although such indices are very useful their value could be extended by coupling them to more specific information about the individual flocs. The Burns number alone tells us nothing about how flocs will behave in a process where shear forces and density gradients may be present. A floc which performs best in a quiescent system may not be the optimum choice for a turbulent system such as the tower fermentation of power alcohol. The screening of yeast strains for performing in such systems should include specific information on floc density, size distribution and strength in addition to its fermentative ability and sedimentation index.

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