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Modeling of orthokinetic flocculation of *Saccharomyces cerevisiae*

Jean W.C. Hsu, R. Alex Speers*, Allan T. Paulson

Department of Food Science and Technology, Dalhousie University, P.O. Box 1000, Halifax, Nova Scotia, Canada, B3J 2X4

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Abstract

This study examined the flocculation behavior of two *Saccharomyces cerevisiae* strains expressing either Flo1 (LCC1209) genotype or NewFlo (LCC125) phenotype in a laminar flow field by measurement of the fundamental flocculation parameter, the orthokinetic capture coefficient. This orthokinetic capture coefficient was measured as a function of shear rate (5.95–223 s^{-1}) and temperature (5–45 °C). The capture coefficients of these suspensions were directly proportional to the inverse of shear rate, and exhibited an increase as the temperature was increased to 45 °C. The capture coefficient of pronase-treated cells was also measured over similar shear rate and temperature range. A theory, which predicts capture coefficient values due to zymolectin interactions, was simplified from that developed by Long et al. [Biophys. J. 76: (1999) 1112]. This new modified theory uses estimates of: (1) cell wall densities of zymolectins and carbohydrate ligands; (2) cell wall collision contact area; and (3) the forward rate coefficient of binding to predict theoretical capture coefficients. A second model that involves both zymolectin interactions and DLVO forces was used to describe the phenomenon of yeast flocculation at intermediate shear ranges, to explain yeast flocculation in laminar flow.

Keywords: Brewing yeast; Flocculation; Capture coefficient; Shear flow

1. Introduction

The mechanisms of cell-to-cell adhesion have drawn the attention of researchers in various fields, including medical, pharmacological, biophysical, immunological, and microbiological areas. Brewing yeast flocculation is one of the most studied types of microbial aggregations. Yeast flocculation is often examined from a biochemical/microbiological viewpoint and less often using colloidal/biophysical approaches developed by medical researchers [1]. From a biochemical/microbiological standpoint, yeast flocculation can be simply defined as the non-sexual aggregation of yeast cells into clumps, dispersible by ethylenediaminetetraacetic acid (EDTA) or specific sugar(s) [2]. The mechanisms of zymolectin (or lectin-like protein–carbohydrate interactions) have been hypothesized to explain the bonding between yeast cells [3–8]. However, the exact details of yeast flocculation remain unclear.

Viewing the fermentation process from a colloidal/biophysical standpoint has, unfortunately, not been discussed by many researchers. The 'DLVO' theory (Derjaguin, Landau, Verwey and Overbeek) and related kinetic theories have been employed to explain flocculation from a colloidal

* Corresponding author. Tel.: +1-902-494-3146; fax: +1-902-420-0219.

E-mail address: alex.speers@dal.ca (R.A. Speers).

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viewpoint [9]. Orthokinetic flocculation is accepted to be the major mechanism for yeast cell-to-cell interactions [10]. As yeast cells are relatively large, perikinetic flocculation is not important in this brewing process [8]. Determining the orthokinetic capture efficiency can help to understand the flocculation behavior of yeast cells in shear flow. Orthokinetic capture coefficients of a flocculent ale strain, experimentally estimated by Speers et al. [11], were much higher than those predicted by Duszyk and Doroszewski [12] based on the DLVO theory. Thus, the DLVO theory alone could not be used to explain brewing yeast flocculation [11].

When two particles collide under a shear flow, formation of doublets depends on the collision frequency. The total two-body collision frequency per unit volume (H_c) can be described as follows [1,13,14]:

$$H_c = \frac{16}{3}\dot{\gamma}\, N_s^2 r^3 \tag{1}$$

where $\dot{\gamma}$ is the shear rate, N_s is the density of singlets in particle suspension, and r is the radius of the particle. A kinetic theory for the rate of particle flocculation in dispersions was first described by von Smoluchowski and more recently modified by van de Ven and Mason [13]:

$$\frac{N_t}{N_0} = e^{-(4\alpha_0\dot{\gamma}\varphi_0/\pi)t} \tag{2}$$

where N_t is the concentration of particles at time t, N_0 is the initial concentration of particles, and φ_0 is the initial volume fraction of particles. The orthokinetic capture coefficient, α_0, is an important parameter in the equation for it reflects the sum of all forces and interactions acting on colloidal particles as they approach each other in the laminar shear field [8].

Cell adhesion during shearing flow has been intensely studied by biophysicists. Bell [15] developed a model for cell-to-cell interactions mediated by reversible receptor–ligand bonds, which describes the kinetics of bond formation and breakage. A few years later, Bell [16] created a probabilistic formulation for cell aggregation in Couette flow by considering: the duration of a cell collision; rate of bond formation (k_f); the contact area between cells (A_c); shear rate ($\dot{\gamma}$); and cell diameter. According to Bell [16], the orthokinetic capture coefficient (α_0), or sticking probability, depended on shear rates: at low $\dot{\gamma}$, the α_0 value is proportional to $\dot{\gamma}^{-1}$; and at high $\dot{\gamma}$, α_0 is proportional to $\dot{\gamma}^{-2}$.

Receptor–ligand bonding is an interaction often observed in cell-to-cell associations. These receptor–ligand interactions include those between antigens and antibodies, lectins and carbohydrates, or enzymes and substrates. Lectins have been defined as glycoproteins of non-immune origin that contain at least two sugar binding sites [17]. A zymolectin is defined as an anchored yeast cell-wall protein that contains one or more mannose or gluco-mannose binding sites [7,8]. The general reversible reaction between particles governed by receptor–ligand bonding can be written as follows [1,18,19]:

$$\nu_r M_r + \nu_l M_l \underset{k_r}{\overset{k_f}{\rightleftarrows}} \nu_b M_b \tag{3}$$

where M_r, M_l, and M_b are the molecular species of receptor, ligand, and bond, ν_r, ν_l, ν_b denote the corresponding stoichiometric coefficients, and k_f and k_r are the forward and reverse rate coefficients. When two cells collide, a doublet will form if sufficient intercellular bonds form during the collision. The collision time has been reported to be approximately equal to $5\pi/6\dot{\gamma}$ at low shear rates [1].

A simplified master equation [18], which describes the binding kinetics of a small number of receptors and ligands, can be expressed as follows [1]:

$$\begin{aligned}\frac{dp_n}{dt} = {} & A_c m_r m_1 k_f^{(n)} p_{n-1} \\ & - (A_c m_r m_1 k_f^{(n+1)} + n k_r^{(n)}) p_n \\ & + (n+1) k_r^{(n+1)} p_{n+1}\end{aligned} \tag{4}$$

where p_n is the probability of having n bonds at time t, A_c is the contact area (μm^2), m_r is the number densities of receptor (μm^{-2}), m_l is the number densities of ligand (μm^{-2}), $k_f^{(n)}$ is the forward rate coefficient per unit density for the formation of the nth bond ($\mu m^2\ s^{-1}$), and $k_r^{(n)}$ is

the reverse rate coefficient for the dissociation of the nth bond (s^{-1}). This modified McQuarrie master equation [1] governs the rate of change of bond number between the cells.

In 1999, Long et al. [1] reported on the construction of a model to predict the flocculation of sheared cells attached by antigen–antibody bonds. If one considers initial two cell collisions at low shear rates (where the rate of bond break-up is low), the orthokinetic capture coefficient (α_0) can be defined as:

$$\alpha_0 = \frac{\pi}{\dot{\gamma}} A_c m_r m_l k_f \quad (5)$$

where A_c is the contact area between the cells during a collision (μm^2), m_r and m_l are number densities of receptors and ligands (μm^{-2}), and k_f is forward coefficient per unit density ($\mu m^2\ s^{-1}$).

The objective of this study was to examine the behavior of yeast cells using colloidal and biophysical cell theories to assist our understanding of the mechanism of yeast flocculation. The prime focus of this work was to test the application of the orthokinetic capture coefficient theory proposed by Long et al. [1] (for particles flocculated by antigen–antibody interactions) to zymolectin-mediated yeast flocculation.

2. Materials and methods

2.1. Yeast strains

The two ale yeast strains used in this study were flocculent *S. cerevisiae* strains 125 and 1209 obtained from the Labatt Breweries culture collection (LCC; London, ON). Strain LCC125 has been defined as a NewFlo phenotype strain, whereas Strain LCC1209 is a haploid strain of Flo1 genotype [20].

2.2. Polystyrene microspheres

Polystyrene ($[–CH_2–CH(C_6H_5)–]_n$) microspheres (Duke Scientific Corporation, Palo Alto, CA, USA) with 4.988 ± 0.035 μm diameter were used. These spheres were synthesized from the monomeric precursor, styrene.

2.3. Yeast preparation

The yeast strains were cultured on yeast extract peptone dextrose (YEPD) agar. Isolated colonies were inoculated into 100 ml of YEPD broth in 250 ml Erlenmeyer flasks. They were incubated aerobically at 30 °C in an orbital shaker (Eberbath Corporation, Ann Arbor, MI, USA) at 100 rev. min^{-1}. Yeast samples were inoculated at a pitching rate of 15×10^6 ml^{-1} as determined by a hemocytometer (Hausser Scientific Partnership, Horsham, PA, USA). Yeast samples were harvested by centrifugation ($450 \times g$ for 2 min). The standard incubation time for the yeast culture was 24 h.

A yeast washing procedure described by Speers et al. [21] was used. The yeast washing steps were as follows: twice with double distilled water; once with 10 mM EDTA; twice with double distilled water; and washed with 0.1 M sodium acetate standard buffer [pH 4.5 containing 1.0 mM Ca (as $CaCl_2$) and 5% ethanol]. Yeasts were then resuspended in the standard sodium acetate buffer.

A hemocytometer was used for counting cells under a light microscope. The method employed was based on the ASBC standard method [22]. Washed cells were stored at −30 °C if the measurement could not be performed immediately. The flocculation ability of frozen cells (−30 °C) has been found to be unchanged after 6 months storage [23].

The volume of a yeast cell (V_y) was calculated from the formula for a prolate spheroid:

$$V_y = 4/3\pi ab^2 \quad (6)$$

where *a*, *b* are the lengths of the major and minor semi-axes of a prolate spheroid.

2.4. Treatment with pronase

The method of pronase treatment was based on Hodgson et al. [24]. Yeast cells were treated with 200 μg ml^{-1} of pronase (protease type XIV, from Streptomyces griseus, Sigma Chemical Co., St. Louis, MO, USA) dissolved in 50 mM sodium phosphate, 50 mM EDTA at pH 7.50. The suspension was incubated at 30 °C for 1 h. The cells were washed as previously described.

2.5. Determination of orthokinetic flocculation

The original orthokinetic flocculation experimental procedure described by Speers et al. [11] was employed with minor modifications. Yeast cells were suspended in a standard sodium acetate buffer (pH 4.5, 0.1 M with 1.0 mM $CaCl_2$ and 5% ethanol). Flocculation rates were measured using cell suspensions of 10^5–10^7 cells ml^{-1}. The deflocculated suspensions were loaded onto the flat plate of a Bohlin VOR rheometer (Bohlin Instruments, Cranbury, NJ, USA) with a 5°, 3.0 cm diameter cone. All samples were sheared at 9.21 s^{-1} and 15 °C for up to 30 min in duplicate. Temperature and shear rates were varied from 5 to 45 °C and 5.95 to 223 s^{-1}. At set periods, a sample was carefully taken and the number concentration of flocs was counted (N_t) using a hemocytometer. With these data, the orthokinetic capture coefficients (α_0) were calculated from Eq. (2), the modified von Smoluchowski equation [13].

2.6. Zymolectin determination assay

The original zymolectin sites determination method using FITC-avidin fluorescent probe (Molecular Probes, Inc., Eugene, OR, USA) was described by Patelakis et al. [6]. This method was performed with minor modifications. Stock solutions of 0 to 200 μg ml^{-1} of the probe were prepared in the standard sodium acetate buffer (pH 4.5, 0.1 M with 1.0 mM $CaCl_2$ and 5% ethanol). A 100-μl aliquot of the stock solution (instead of 20 μl) was added into 4-sided clear cuvettes (Fisher Scientific, Whitby, ON), then 2900 μl of the yeast suspension (1×10^6 ml^{-1}) in the standard buffer was added. The suspension was mixed well, and centrifuged (Centra-MP4R, IEC. Needham Heights, MA, USA) at $1240\times g$ for 3 min. Fluorescence intensity measurements of supernatant fluid were performed at an excitation wavelength of 495 nm and an emission wavelength of 520 nm with 10 nm slit widths with a spectrophotofluorimeter (LS 50, Perkin–Elmer Ltd., Beaconsfield, Buckinghamshire, UK). The non-specific binding of probe (background) was determined under addition of 10 mM EDTA in the standard buffer. Langmuir analysis was used to calculate zymolectin density. All experiments were carried out at 20 °C in triplicate.

2.7. Ligand sites determination assay

Ligand density was determined by using Alexa Flour™ 488 conjugate concanavalin-A probe (Molecular Probe, Inc., Eugene, OR, USA). Stock solutions of 0–20 μg ml^{-1} of the probe were prepared in the standard sodium acetate buffer (pH 4.5, 0.1 M with 1.0 mM $CaCl_2$ and 5% ethanol). An amount of 2900 μl of yeast suspension (1×10^6 ml^{-1}) in the desired buffer was added into 4-sided clear cuvettes (Fisher Scientific, Whitby, ON), then 100 μl of the probe was added. The suspension was mixed well and incubated at 20 °C for 30 min, covered by aluminum foil to avoid light. The cuvette was centrifuged (Centra-MP4R, IEC Needham Heights, MA, USA) at $1240\times g$ for 3 min. Fluorescence intensity measurement of the supernatant was performed at 20 °C and an excitation wavelength of 495 nm and emission wavelength of 520 with 10-nm slit widths with a spectrophotofluorimeter (described previously). Langmuir analysis was used to calculate ligand density.

3. Results and discussion

3.1. Orthokinetic flocculation

The orthokinetic flocculation of yeast cells was examined at an initial stage during shearing flow. A semi-logarithmic decline in the number of flocs was observed during the shearing. The modified von Smoluchowski equation, Eq. (2), was fit to the data and orthokinetic capture coefficients calculated.

3.2. Effect of shear rates on capture coefficients

As one might expect, elevating the shear rate lowered the capture coefficients of Flo1 LCC1209 and NewFlo LCC125 strains. Fig. 1 shows the linear relationship between the capture efficiency of Flo1 cells, NewFlo cells, and polystyrene

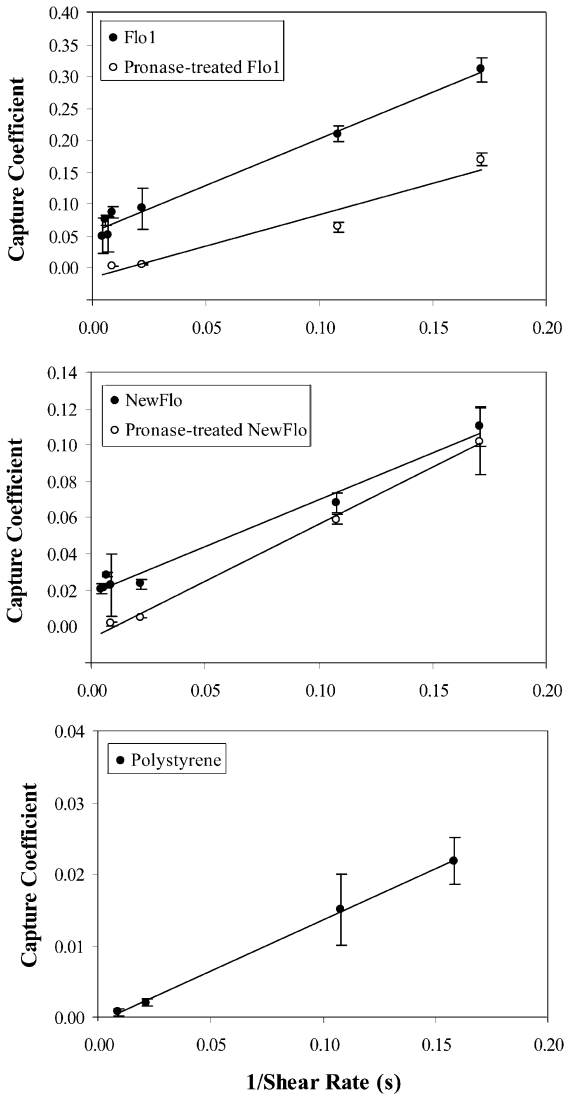

Fig. 1. Effect of shear rate on capture coefficients of Flo1 LCC1209, NewFlo LCC125 and polystyrene microspheres at a temperature of 15 °C. Values are given as means ± S.D.

microspheres, and the reciprocal of shear rate. The capture efficiency of pronase-treated cells of both strains was also reduced when the shear rate was increased. These results indicate that the capture efficiency is directly proportional to the inverse of shear rate at low shear rates as predicted by Eq. (5).

3.3. Effect of temperature

The capture coefficients of Flo1 and NewFlo cells were significantly influenced by temperature (5–45 °C) as illustrated in Fig. 2 ($P<0.01$ and $P<0.001$, respectively). The capture efficiency of both Flo1 and NewFlo strains increased at higher temperature, and the same trend was found after treating both strains with pronase. The reason for the increase in α_0 may be due to conformational changes in the zymolectin–carbohydrate binding sites and/or increased hydrophobic interactions. Elevating temperatures did not significantly change capture coefficient values of polystyrene latex ($P>0.05$).

3.4. Effect of pronase treatment

From examination of the flocculation behavior of the Flo1 and NewFlo cells in Fig. 1, it is clearly that pronase substantially reduced flocculation ($P<0.001$). This finding agrees with previous studies [25]. Presumably, the proteins on the cell surface were degraded by the protease, preventing receptor–ligand binding.

In the absence of zymolectins one might expect the α_0 values of pronase treated cells to approximate polystyrene values. However, the capture coefficient of polystyrene microspheres was much lower than that of both yeast strains, whether treated with pronase or not. While non-specific forces involved in the interactions between polystyrene microspheres and pronase treated cells (hydrophobic interactions, van der Waals attraction, or electrostatic forces) might be expected to be similar this is clearly not the case. One possible reason might be that the protease did not completely break down proteins involved in zymolectin binding.

3.5. Orthokinetic capture efficiency: theory vs. experimental results

The experimental orthokinetic capture efficiency of Flo1 LCC1209 and NewFlo LCC125 was calculated from the modified von Smoluchowski equation, Eq. (2) as discussed previously. The

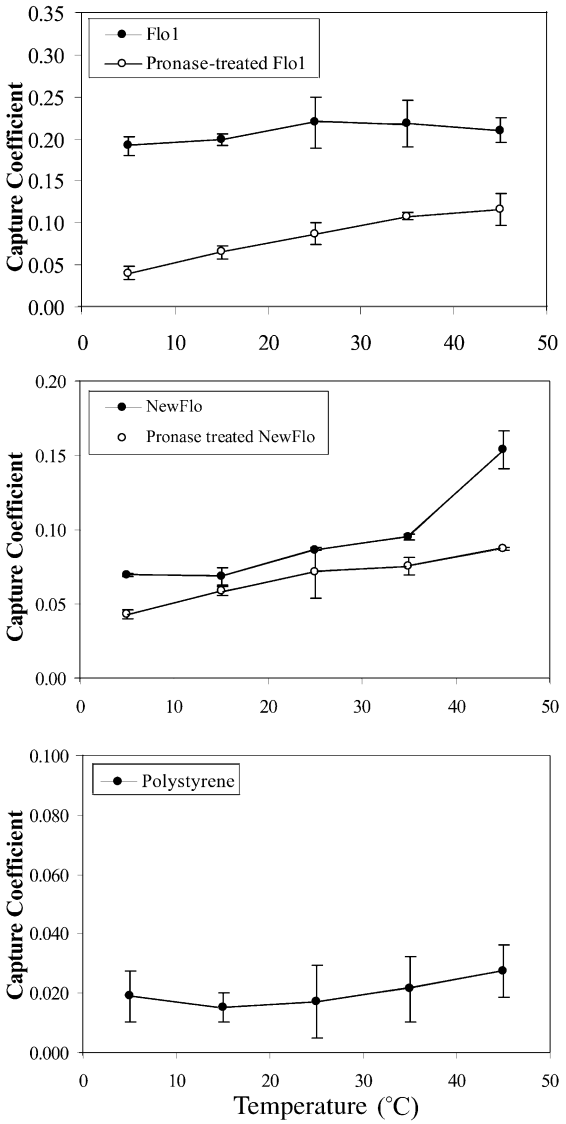

Fig. 2. Effect of temperature on capture coefficients of Flo1 LCC1209, NewFlo LCC125 and polystyrene microspheres at 9.27 s^{-1}. Values are given as means $\pm$ S.D.

theoretical capture efficiency of both strains was estimated from the theory of Long et al. [1].

To test Long's theory zymolectin and ligand densities of both yeast strains were determined experimentally. Estimation of the contact area between two particles (i.e. cells) and the forward kinetic rate constant was then undertaken to calculate theoretical capture coefficients.

3.6. Receptor and ligand densities of yeast cells

In the literature, the distinction between receptors and ligands is ambiguous, especially when classifying lectins and carbohydrates. Bell [15] mentioned that a carbohydrate is a receptor and a lectin is a ligand. Goldstein and Poretz [26] stated that a lectin, such as Con A, binds to carbohydrate ligands. Stratford and Assinder [27] stated that receptors of flocculation lectins are sugars. Before further examination of the zymolectin–carbohydrate interactions, the usage of 'receptor' and 'ligand' needs to be clarified. In general, a ligand is a low molecular weight molecule that exhibits specific binding to a high molecular weight molecule site (e.g. proteins) [28]. With lectin and sugar interactions, the lectin is the receptor while the sugar is the ligand. In yeast, zymolectins are defined as 'cell-wall anchored, proteinaceous, mannose or gluco-mannose receptors' [6,7]. Clearly zymolectins are the receptors and carbohydrate residues serve as the ligands, even though they are attached to the mannan backbone on yeast cell surfaces.

The zymolectin hypothesis has become an important tenet of yeast flocculation since the 1980s [8]. Non-reducing termini of α-1,3-linked mannoside branches, two or three mannopyranosyl residues in length are expected to act as ligands for zymolectin binding of both Flo1 and NewFlo strains [27].

The density of zymolectin receptors and carbohydrate ligands affect the probability of cell adherence after a collision. According to Long et al. [1], two-body capture efficiency increases when there are more receptors or ligands on the cell surfaces. The zymolectin densities of Flo1 LCC1209 and NewFlo LCC125 cells were $(2.63 \pm 0.79) \times 10^5$ and $(1.00 \pm 0.49) \times 10^5$ μm^{-2}; whereas, the apparent carbohydrate densities of Flo1 and NewFlo cells were $(8.83 \pm 1.99) \times 10^4$ and $(8.92 \pm 5.91) \times 10^4$ μm^{-2}. These data are used later in prediction of theoretical α_0 values. After applying the pronase treatment, the zymolectin density of Flo1 cells was significantly reduced

$[(0.53\pm0.16)\times10^5\ \mu m^{-2}; P<0.05]$, but not zymolectin density of NewFlo cells $[(0.65\pm0.25)\times10^5\ \mu m^{-2}; P>0.05]$. The apparent carbohydrate densities of both strains did not significantly change after pronase treatment was applied ($P>0.05$).

3.7. Estimation of contact area

The contact area size (A_c) cannot be controlled by an experimenter, since it varies in the cell system. Contact area estimation is traditionally difficult [19]. Long et al. [1] and Goldsmith et al. [29] hypothesized that the contact area of a spherical particle is equal to $2\pi rh$ where h is the 'contact zone' of the particle. Using this theory, the length of a zymolectin was assumed equal to the length of Flo1p (1537 amino acids long [30]) minus the cell wall thickness (25–200 nm [31,32]). Thus, feasible h values of Flo1 and NewFlo cells were approximately equal to 5×10^{-6} to 1×10^{-5} μm, respectively. Therefore, the contact areas (maximum) of Flo1 and NewFlo cells were estimated to be 7.8×10^{-5} and $2.3\times10^{-4}\ \mu m^2$. This method of contact area estimation assumes that the ligands and receptors are flexible enough to form bonds within the contact area.

The problem of estimating the contact area between two yeast cells is that both receptors and ligands are present on each cell. This is a different situation from an antigen–antibody bond where antibodies and antigens are present on different cells. Jin [23] assumed that the zymolectin is monovalent. If this assumption is true, it may be that the 'true' contact area is much smaller than calculated above and is closer to the area of a zymolectin binding site. Unfortunately, the exact dimension and shape of zymolectin are still unknown. Since Con A is a well-studied lectin which also binds to mannose and glucose residues, the Con A binding site area was used as an approximate value of the contact area of flocculating yeast cells. The dimensions of a subunit of Con A is approximately 4.2×10^{-3} μm high, 4.0×10^{-3} μm wide, and 3.0×10^{-3} μm thick [33]. Hence, the carbohydrate binding site could be assumed as $2.4\times10^{-5}\ \mu m^2$.

3.8. Estimation of forward kinetic rate coefficient

The forward rate coefficient (k_f) is also very difficult to determine [19]. The forward rate coefficient decreases as applied force increases, due to higher rates of shear increasing the average force acting on the bonds [1,34]. This coefficient is dependent on the attachment time of receptor–ligand bonds and the ligand densities [35,36]. Cozens-Roberts et al. [35] have indicated that the forward rate coefficient of cells ranged from 10^{-6} to 1 $\mu m^2\ s^{-1}$ [37–39]. In experimental modeling of a receptor–ligand system, Cozens-Roberts et al. [36] assumed that the forward rate coefficient was $2.33\times10^{-7}\ \mu m^2\ s^{-1}$. The mean forward rate coefficient determined by Long et al. [1] from Kwong et al. [40] data was equal to $8.38\times10^{-7}\ \mu m^2\ s^{-1}$ (between $1.5\times10^{-6}\ \mu m^2\ s^{-1}$ at low shear rates, and $2.27\times10^{-7}\ \mu m^2\ s^{-1}$ at high shear rates). In our estimations a mean forward rate coefficient of $8.38\times10^{-7}\ \mu m^2\ s^{-1}$ was used to calculate the theoretical yeast cell capture coefficient.

3.9. Estimation of orthokinetic capture coefficient

Theoretical orthokinetic capture coefficients of Flo1 LCC1209 and NewFlo LCC125 can be estimated by Eq. (5) using four main assumptions:

1. yeast cells are spheres;
2. the minimum contact area is equal to the area of two sugar binding sites of Con A;
3. no doublets are split during the contact time (i.e. the reverse rate coefficient is negligible); and
4. forward rate coefficient is equal to the mean forward rate coefficient determined by Long et al. [1] and Kwong et al. [40].

Using these assumptions, Fig. 3 illustrates both the theoretical (from receptor–ligand theory) and experimentally determined orthokinetic capture coefficients of both Flo1 and NewFlo strains.

Table 1 shows both the experimental and predicted capture coefficients calculated from *both* minimum and maximum contact areas discussed earlier. From Eq. (5) one would expect the capture efficiency should approach nearly zero when shear

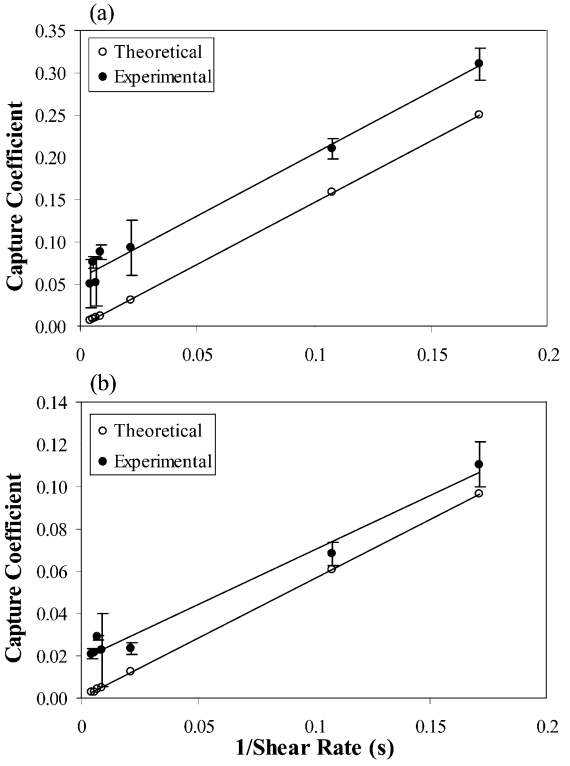

Fig. 3. Comparison of the theoretical and experimental orthokinetic capture coefficients of (a) Flo1 LCC1209 and (b) NewFlo LCC125 cells.

rate is infinite, but in Fig. 3, when shear rate reached infinity, there was a minimum value for capture efficiency (i.e. at the intercept). This value could be caused by other forces involving in colloidal interactions, such as non-specific electrostatic forces and hydrophobic interactions. The receptor–ligand theory was modified to fit yeast cells:

$$\alpha_0 = \frac{\pi}{\dot{\gamma}} A_c m_r m_1 k_f + \alpha_{min} \quad (7)$$

where α_{min} is the minimum of capture coefficient as the shear rate approaches infinity. The α_{min} values may be dependent on non-specific attractive and repulsive forces between cells. Non-specific attractive and repulsive forces of the cells decrease when elevating shear rate, however their ratio (i.e. the attractive and repulsive forces) may be shear rate independent. The α_{min} values could also depend on other forces, such as hydrophobic interactions, whose hydrodynamic behavior is poorly understood.

Minor DLVO forces might be involved in yeast flocculation along with the zymolectin–carbohydrate interactions. The Flo1 cells were found to have more negatively charged groups on the cell surface than the NewFlo cells [41]. The theoretical values of the DLVO capture coefficient [12] can be estimated using the cell surface charge information. Speers et al. [25] reported that a flocculent ale strain at pH 4.0 has zeta potential ranging from -2 to -5 mV; whereas Smart et al. [42] reported a zeta potential of -42 mV for a brewing strain (KS1, formerly BB5). Most zeta potentials of yeast cells range from -4 to -40 mV [41–44]. Dimensionless attractive (C_A) and repulsive (C_R) forces [13] could be determined assuming 'typical' parameters ($A = 2.5 \times 10^{-21}$ J, $\eta = 1.0$ mPa·s, $\psi = \zeta =$ 4 or 40 mV, $\varepsilon = 81$ and $\varepsilon_0 = 8.85 \times 10^{-12}$ J^{-1} C^2 m^{-1} [10]):

$$C_A = \frac{A}{36\pi\eta\dot{\gamma}r^3} \quad (8)$$

$$C_R = \frac{2\varepsilon\varepsilon_0\psi^2}{3\eta\dot{\gamma}r^2} \quad (9)$$

where A is the Hamaker factor, η is viscosity of the medium, ε is the dielectric constant of the medium, ε_0 is permittivity of free space and ψ is the surface potential (taken to be ζ the zeta potential). Using Duszyk's method [12], approximate DLVO capture coefficients ($\alpha_{0,DLVO}$) of Flo1 and NewFlo cells at various shear rates were calculated as summarized in Table 1. The $\alpha_{0,DLVO}$ values of Flo1 and NewFlo cells at $\psi = 4$ mV ranged from 0.06 to 0.14; whereas the $\alpha_{0,DLVO}$ values of both cells at $\psi = 40$ mV were negligible due to the high values of C_R (strong repulsive forces).

By use of Eq. (7), the theoretical and experimental data of the Flo1 strain agreed. The theoretical values of NewFlo also seemed to agree with the experimental data. The α_{min} values of Flo1 and NewFlo (0.0578 and 0.0184, respectively) were close to $\alpha_{0,DLVO}$ values; hence, the DLVO forces might also play a minor role in yeast flocculation.

Table 1
Experimental, predicted and DLVO capture coefficients of Flo1 LCC1209 and NewFlo LCC125 cells at various shear rates

Strain	Shear rate (s^{-1})	Experimental α_0 ($N=2$)	Measured $A_c k_f$[a] ($\mu m^4 \cdot s^{-1}$)	α_0 from receptor–ligand [1] theory[b]		$\alpha_{0,DLVO}$[c]	
				A_c (min)	A_c (max)	$\psi=4$ mV	$\psi=40$ mV
Flo1	5.85	$3.11\pm0.19\times10^{-1}$	2.49×10^{-11}	2.51×10^{-1}	8.15×10^{-1}	0.140	<0.000
	9.27	$1.99\pm0.12\times10^{-1}$	2.53×10^{-11}	1.58×10^{-1}	5.14×10^{-1}	0.130	<0.000
	46.3	$9.32\pm3.30\times10^{-2}$	5.91×10^{-11}	3.17×10^{-2}	1.03×10^{-1}	0.100	<0.000
	117	$8.78\pm0.85\times10^{-2}$	1.41×10^{-10}	1.25×10^{-2}	4.08×10^{-1}	0.085	<0.000
	147	$5.24\pm2.78\times10^{-2}$	1.06×10^{-10}	9.98×10^{-3}	3.24×10^{-2}	0.084	<0.000
	185	$7.61\pm0.75\times10^{-2}$	1.93×10^{-10}	7.93×10^{-3}	2.58×10^{-2}	0.082	<0.000
	233	$5.06\pm2.82\times10^{-2}$	1.62×10^{-10}	6.30×10^{-3}	2.05×10^{-2}	0.080	<0.000
NewFlo	5.85	$3.11\pm0.11\times10^{-1}$	2.31×10^{-11}	9.63×10^{-2}	9.23×10^{-1}	0.112	<0.000
	9.27	$2.10\pm0.05\times10^{-1}$	2.27×10^{-11}	6.08×10^{-2}	5.83×10^{-1}	0.105	<0.000
	46.3	$9.32\pm0.28\times10^{-2}$	3.90×10^{-11}	1.22×10^{-2}	1.17×10^{-1}	0.085	<0.000
	117	$8.78\pm1.73\times10^{-2}$	9.60×10^{-11}	4.82×10^{-3}	4.62×10^{-2}	0.070	<0.000
	147	$5.24\pm0.12\times10^{-2}$	1.50×10^{-10}	3.83×10^{-3}	3.67×10^{-2}	0.068	<0.000
	185	$7.61\pm0.01\times10^{-2}$	1.43×10^{-10}	3.05×10^{-3}	2.92×10^{-2}	0.063	<0.000
	233	$5.06\pm0.27\times10^{-2}$	1.74×10^{-10}	2.42×10^{-3}	2.32×10^{-3}	0.061	<0.000

Note. Experimental α_0 values were measured at 15 °C; Flo1: $C_R/C_A=2.13\times10^3$ at $\psi=4$ mV and $=2.13\times10^5$ at $\psi=40$ mV; NewFlo: $C_R/C_A=4.45\times10^3$ at $\psi=4$ mV and $=4.45\times10^5$ at $\psi=40$ mV.

[a] Measured $A_c k_f$ was calculated from Eq. (5).

[b] Receptor–ligand capture coefficients were calculated assuming $k_f=8.38\times10^{-7}$ $\mu m^2 \cdot s^{-1}$, A_c (min) $=2.4\times10^{-5}$ μm^2, A_c (max) for Flo1 $=7.8\times10^{-5}$ μm^2 and A_c (max) for NewFlo $=2.3\times10^{-4}$ μm^2. A_c (min) was estimated from Con A binding site area, while A_c (max) was estimated from the 'contact zone' of the particle.

[c] $\alpha_{0,DLVO}$ is values are approximate values from Fig. 3 in Duszyk and Doroszewski [12].

If the modified receptor–ligand interaction equation, Eq. (7), is valid and experimental capture coefficients determined from von Smoluchowski theory are also correct, $A_c k_f$ values can be determined. The Experimental value of $A_c k_f$ of Flo1 is equal to 1.98×10^{-11} $\mu m^4\ s^{-1}$, and that of NewFlo is equal to 5.57×10^{-12} $\mu m^4\ s^{-1}$; whereas assuming A_c of 2.4×10^{-5} μm^2 and k_f of 8.38×10^{-7} $\mu m^2\ s^{-1}$ leads to $A_c k_f$ values of 2.01×10^{-11} $\mu m^4\ s^{-1}$ for both strains.

Chesla et al. [19] found that $A_c k_f$ is equal to $(2.62\pm0.32)\times10^{-7}$ $\mu m^4\ s^{-1}$ for the CD16A–higG interaction and $(5.7\pm0.31)\times10^{-7}$ $\mu m^4\ s^{-1}$ for the CD16A-rigG interaction. The CD16A-higG and CD16A-rigG interactions are protein–protein interactions with antigen–antibody bonding. The reasons for the large difference in the $A_c k_f$ values are not clear. It is possible that electrostatic repulsion, surface roughness or a very short zymolectin length may result in very small effective contact areas. Since zymolectin–sugar interactions in yeast flocculation are protein–carbohydrate interactions, one can expect that $A_c k_f$ values may be different from those reports based on antigen–antibody bonding. Finally, the forward reaction rate may also be lower in this system than the antigen–antibody interactions studied by Long et al. [1].

Fig. 4 illustrates the maximum and minimum predicted capture efficiency range of both Flo1 and NewFlo strains with experimental and predicted DLVO capture coefficients. The shaded area represents the possible values from the receptor–ligand binding theory [1], using values predicted from the maximum and minimum contact areas discussed and reported earlier in Table 1. The experimental capture coefficients were closer to the minimum predicted values. Hence, the contact area between the cells could be equal to the size of the zymolectin binding area. The experimental α_0 values of Flo1 at lower shear rates (<46.3 s^{-1}) fall in this range. However, agreement between theory and experiment fails at high shear rates where zymolectin binding is not expected to occur [11]. The receptor–ligand binding theory

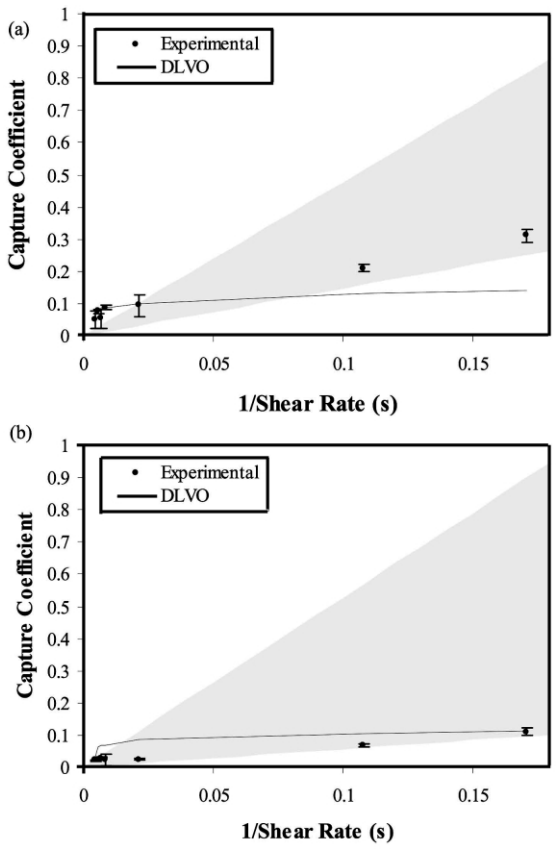

Fig. 4. Maximum and minimum predicted, experimental and DLVO capture coefficients of (a) Flo1 LCC1209 and (b) NewFlo LCC125 cells. Shaded area ■‘ ’ the possible values for the theory of Long et al. [1].

might govern the main interactions of Flo1 cells under shearing flow at low shear rate. The predicted DLVO α_0 values of Flo1 were close to the experimental values at high shear rates. Since the predicted DLVO capture coefficients of NewFlo strain were greater than the experimental values, DLVO forces might not be major forces involved in flocculation of NewFlo cells under shearing flow. On the other hand, DLVO forces might play a role in flocculation of Flo1 strain in laminar flow.

4. Conclusions

The colloidal interactions of brewing yeast cells are governed by complex mechanism(s). The phenomenon of yeast flocculation involves several different forces: zymolectin–carbohydrate interactions; hydrophobic interactions; and electrostatic forces. Examining the behavior of yeast cells from a colloidal and a biophysical point of view can help us to understand the mechanism of yeast flocculation.

The rate of yeast flocculation is governed by von Smoluchowski theory. The antigen–antibody binding theory of Long et al. [1] is useful to help understand yeast flocculation, but cannot be directly applied to the complex process of yeast flocculation. It is not unreasonable to assume that more than one type of force may be involved in yeast flocculation. A new semi-empirical model was proposed to explain yeast flocculation under shearing flow.

Determination of the forward rate coefficient of yeast cells would be a key in assisting future investigations of brewing yeast flocculation. Knowing the length of zymolectins and the surface architecture of zymolectin–carbohydrate interactions is also required to estimate a more accurate contact area between cells. Determination of quantitative surface charge values (or zeta potentials) will also help to develop a model describing yeast flocculation in the future.

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