Study of bioadhesion on a flat plate with a yeast/glass model system

M. Mercier-Bonin, a K. Ouazzani, b P. Schmitz, a, * and S. Lorthois c

a Laboratoire de Biotechnologie et Bioprocédés, UMR CNRS 5504 UMR INRA 792, INSA, 135, avenue de Rangueil, 31077 Toulouse Cedex 4, France
b Laboratoire de Génie des Procédés et de l’Environnement, EST de Fès, Route d’Imouzzer, Fez, Morocco
c Institut de Mécanique des Fluides, UMR CNRS/INP-UPS 5502, Allée du Professeur Camille Soula, 31400 Toulouse, France

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Abstract

The attachment of microorganisms to a surface is a critical first step of biofilm fouling in membrane processes. The shear-induced detachment of baker’s yeast in adhesive contact with a plane glass surface was thus experimentally studied, using a specially designed shear stress flow chamber. The yeast was marketed either as rod-shaped pellets (type I yeast) or as spherical pellets (type II yeast). A complete series of experiments for measuring the shear stress necessary to detach a given proportion of individual yeast cells of type I or II was performed under different environmental conditions (ionic strength, contact time). In parallel, the surface physicochemical properties of the cells (surface charge, hydrophobicity, and electron donor and electron acceptor components) were determined. For the first type of yeast cells, which were rather hydrophilic, adhesion to the glass plate was weak. This was due to both electrostatic effects and hydrophilic repulsion. Furthermore, adhesion was not sensitive to any variation of the ionic strength. For yeast of the second type, adhesion was drastically increased. This could be explained by their physicochemical surface properties and especially their hydrophobic and electron acceptor components, which caused strong attractive van der Waals and Lewis acid–base interactions, counterbalancing the electrostatic repulsion. For increasing ionic strengths, adhesion was greater, due to lower electrostatic repulsion. The results were quantified through the definition of a critical wall shear stress (τw 50%) required to detach 50% of the yeast cells initially deposited on the glass surface. The influence of the contact time was also evaluated and it was shown that, whatever the type of yeast, macromolecules such as proteins were released into the extracellular medium due to cell lysis and could contribute to the formation of a conditioning film. As a result, the cells were more strongly stuck to the glass plate.

Keywords: Adhesion; Removal; Yeast; Glass plate; Hydrodynamics; Surface physicochemical properties

1. Introduction

Microorganisms, both prokaryotic and eukaryotic, have a strong ability to bind to surfaces (Bos et al. [1]). Once they are attached, a multistep process starts, leading to the formation of a complex adherent microbial community, which is termed a “biofilm.” Biofilms can be beneficial, for instance, to degrade environmental substances in the soil (MacDonald and Rittmann [2]) or in a bioreactor (Diks and Otten graph [3]), but also detrimental, as on heat exchangers for food processing (Lalande et al. [4]), ship hulls (Cooksey and Wigglesworth-Cooksey [5]), or medical implants (Gristina [6]). Another adverse application deals with membrane filtration systems in which biofilms up to 500 µm thick can build up (Flemming et al. [7]). So several effects on filtration performance are observed: membrane flux decline due to the formation of a low-permeability film on the membrane surface, which produces an increase in the pressure drop and the feed pressure, membrane biodegradation, and increased energy requirements. It is therefore important to focus attention on how to prevent and control biofilm formation in such applications. While previous studies of biofilm development have been largely devoted to bacterial species, relatively little is known about fungal biofilms because many of the organisms that form these structures are not amenable to genetic approaches (O’Toole et al. [8]). In search of a model system for fungal biofilms, Reynolds and Fink [9] recently investigated whether the well-characterized baker’s yeast Saccharomyces cerevisiae could form biofilms. According to O’Toole et al. [8], bacteria are said to be able to form biofilm if they adhere to plastic. Following this definition, Reynolds and Fink [9] concluded that S. cerevisiae could initiate biofilm formation since cells adhered to

* Corresponding author.

E-mail address: schmitz@insa-tlse.fr (P. Schmitz).
polystyrene and to polypropylene and, to a lesser degree, to polyvinylchloride. *S. cerevisiae* was thus chosen as the biological model for the present study and for convenience, suspensions were prepared by simply rehydrating cells in saline solutions of various ionic strengths.

The attachment of microorganisms to a surface is the critical first step of biofilm formation and subsequent fouling. In a previous work (Mercier-Bonin and Fonade [10]) relating to gas-sparged microfiltration of unwashed baker’s yeast suspended in physiological serum, the adhesion of cells to the membrane surface was quantified by soaking the membrane (of a mineral type) in the suspension for 30 min and recording the time variations of the pure water permeate flux before and after soaking. A 75% decrease in water flux was obtained, which was correlated by scanning electron microscopy with the formation of a surface deposit of randomly scattered cells, covering small patches. The role of the extracellular medium was also pointed out (Mercier-Bonin and Fonade [10]). These results demonstrated the importance of adhesion phenomena in the reduction of flux performance, especially when biological particles are involved.

The present study on a glass/yeast model system was thus devoted to the characterization of this initial phase of adhesion. The model surface was not a mineral membrane but a glass plate, due to transparency constraints. The effect of fluid velocity, type of yeast, and environmental conditions (ionic strength, contact time) on the detachment of yeast cells from a glass surface was investigated using a shear stress flow chamber. This setup, previously used for several applications such as particle/membrane interactions during drinking water production (Elzo et al. [11]), fibrin/fibrin specific molecular interactions in blood clot fragmentation (Lorthois et al. [12]), and removal of alumina particles suspended in various chemical solutions (Negri et al. [13]), was carefully designed to give a fully developed laminar two-dimensional Poiseuille flow, resulting in accurate knowledge of the hydrodynamic forces and torques acting on individual biological particles. Direct observation of the detachment process in situ was thus possible under a well-controlled hydrodynamic and physicochemical environment. Results of detachment experiments were then interpreted on the basis of the physicochemical characteristics of the yeast surface such as charge, hydrophobicity, electron donor, and electron acceptor components. These direct quantitative measurements of adhesion in combination with the determination of the physicochemical properties of cells provide mechanistic insight into fungal biofilm fouling, particularly the early stages of cell attachment to the membrane surface.

2. Materials and methods

2.1. Yeast cells

Experiments were performed with *Saccharomyces cerevisiae* as model biological particle. *S. cerevisiae* is a baker’s yeast, each cell being considered approximately spherical. The cells, provided by Lesaffre (Marcq-en-Baroeul, France), were initially packaged as dry aggregates of (i) small rod shape, named type I (Saf Instant), or (ii) sphere shape, named type II (Saf Levure). These differences were due to distinct drying procedures. The mean diameter measured by laser granulometry (Mastersizer, Malvern Instruments, Malvern, UK) was found to be 3.65 and 3.49 µm for type I and type II, respectively, with a weak dispersion in size, as seen in Fig. 1. A dilute suspension of washed cells was used. First, the primary suspension was prepared by dispersion and rehydration of 10 mg of aggregates in ultrafiltered distilled water at room temperature, with gentle agitation for 20 min. Second, it was filtered through a flat membrane of polyamide (0.2-µm pore diameter) and the cake resulting from the accumulation of cells at the membrane surface was resuspended in ultrafiltered distilled water. The same operation was executed twice to eliminate all submicrometric particles (broken cells) and soluble components (proteins). Third, the carefully washed cells were resuspended in 20 ml ultrafiltered distilled water containing a given quantity of NaCl with respect to the experiment to be performed. Three concentrations of saline solution were tested (0.2, 0.9, and 2.0% w/v), corresponding to ionic strengths of 33, 150, and 330 mM, respectively. The final concentration of cells in the suspension was about 2 × 10^7 cells/ml.

2.2. Glass plate

A glass plate (Planilux Saint Gobain, France) was carefully cleaned before use as substrate in the shear flow chamber. It was immersed at room temperature in a sulfochromic acid solution (K_2Cr_2O_7 (2.7 M)/H_2SO_4 (4% v/v)) for 1 h, rinsed three times in distilled water, and then air-dried. A new clean glass plate was used for each experiment.
2.3. Analytical methods

It should be noted here that each analytical method was tested on both types of yeast.

2.3.1. Electrophoretic mobility

Yeast cells, suspended in a 1.5 mM NaCl solution at a concentration of about $10^7$ cells/ml, were washed by two centrifugations at 7000g, 4°C for 5 min, discarding the supernatant and resuspending the pellet. The electrophoretic mobility was measured at a temperature of 17.5°C using a 100-V electric field and a laser Zetameter (Zetaphorimeter III, CAD Instruments, Les Essarts le Roi, France). The pH of the suspension was adjusted from 3 to 7 by adding either nitric acid (HNO₃) or potassium hydroxide (KOH). The results were based on automated video analysis of about 200 particles for each measurement. The typical standard deviation was $0.3 \times 10^{-8}$ m²/V/s.

2.3.2. MATS analysis

The “microbial adhesion to solvents” method is based on the comparison between microbial cell affinity to a polar solvent and to a nonpolar solvent by simply measuring the fraction of cell removal from the aqueous phase in the presence of these solvents (Bellon-Fontaine et al. [14]). The polar solvent can be an electron acceptor or an electron donor, but both solvents must have similar van der Waals surface tension components. The following pairs of solvents, as described by Bellon-Fontaine et al. [14], were used: on the one hand chloroform, an electron acceptor solvent, and hexadecane, a nonpolar solvent, and on the other hand ethyl acetate, a strong electron donor solvent, and decane, a nonpolar solvent. Due to the surface tension properties of these solvents, differences between the results obtained with chloroform and hexadecane and the results obtained with ethyl acetate and decane indicated that there were electron donor/electron acceptor interactions at the bacterial cell surface and revealed hydrophobic and hydrophilic properties. First, the yeast suspension was prepared in physiological serum (NaCl, 150 mM) at a concentration of about $4 \times 10^7$ cells/ml and washed twice using the same procedure, as detailed in Section 2.3.1. Then 2.4 ml of the washed yeast suspension (at an optical density of about 0.8 at 400 nm) was vortexed for 60 s with 0.4 ml of solvent. A high ionic strength electrolyte was used to avoid charge interference by a masking cell charge, because some solvent droplets, especially hexadecane, become negatively charged in aqueous suspensions (Geertsema-Doornbush et al. [15]). The mixture was allowed to stand for 15 min to ensure that the two phases were completely separated before a sample of 1 ml was carefully removed from the aqueous phase and the optical density at 400 nm was measured. The percentage of cell affinity for each solvent was subsequently calculated using the equation

$$\text{% affinity} = 100 \times (1 - A/A_0),$$

where $A_0$ is the optical density at 400 nm of the yeast suspension before mixing and $A$ the absorbance after mixing. Each assay was performed in triplicate.

2.3.3. Detection of proteins

The yeast suspension, prepared in physiological serum (150 mM NaCl) at a concentration of $2 \times 10^7$ cells/ml, was washed twice following the procedure presented in Section 2.1 and left under stirring at room temperature for 40 h. Samples were regularly taken and the supernatant was analyzed using a UV detector (Shimadzu, Croissy Beaubourg, France) at a wavelength of 280 nm.

2.3.4. Determination of chitin, β-glucan, and mannan contents in the yeast wall

The proportions of chitin, β-glucan, and mannan in the yeast wall were determined using a procedure adapted from Dallies et al. [16]. Cells were rehydrated, washed three times, and suspended in 0.5 ml of cold deionized water in the presence of 0.5 g of glass beads. Disruption was carried out by vortexing the yeast suspension at full speed for 6–7 periods of 20 s, with 20-s intervals on ice. Microscopic observation indicated that breakage was complete. The cell suspension was collected and the glass beads were extensively washed with cold deionized water. The supernatant and washings were pooled and centrifuged again at 3800g for 5 min. The pellet, containing the cell walls, was washed several times with cold deionized water until the supernatant became clear. Cell walls (100 µl) were wetted with 50 µl of 72% (w/w) H₂SO₄ and left at room temperature for 3 h, after which 650 µl of deionized water were added to obtain a final H₂SO₄ concentration of 1 M. The slurry was heated to 100°C for 4 h and then cooled on ice. The hydrolysate was diluted by adding 6 ml of Milli-Q water. Sulfate ions were precipitated by dropwise addition of saturated Ba(OH)₂ until neutral pH was reached. The volume was adjusted to 25 ml and the BaSO₄ precipitate was pelleted at 3800g for 10 min. Any remaining sulfate ions were removed by a second centrifugation. The supernatant was ready to be used for monosaccharide analysis with HPAEC-PAD using a Dionex Bio-LC system (Sunnyvale, CA, USA). Detection of sugars was performed with a pulsed amperometric detector (PAD) equipped with a gold electrode. Quantification of sugars was based on the response factors calculated from the peak areas of reference monosaccharides pretreated with the corresponding acid solution.

2.4. Shear stress flow chamber

The shear stress flow chamber and the associated experimental device used in the present study were derived from three other chambers used in previous studies concerning the adhesion of particles on surfaces (Elzo et al. [11]; Lorthois et al. [12]; Negri et al. [13]).

A schematic representation of the flow chamber is presented in Fig. 2. The chamber consisted of a bottom glass
plate \((210 \times 90 \times 4 \text{ mm})\) used as a substrate onto which yeast cells were deposited, of an upper Plexiglas plate \((210 \times 90 \times 10 \text{ mm})\), and of a hollowed stainless steel shim \((210 \times 90 \times 0.2 \pm 0.0025 \text{ mm})\) for channelling the fluid flow. The three plates were held together with aluminum clamps. The fluid entered the chamber through a 1-mm-width slit pierced perpendicularly in the upper plate and exited from the chamber through a 2-mm-diameter hole. A third orifice \((0.26\text{-mm diameter})\) topped by a syringe valve was used to inject the yeast suspension. The rectangular flow channel in which the adhesion and removal of yeast cells were studied followed a diverging–converging channel, in order to ensure uniform flow at the entrance of the channel rectangular part (ESDU [17]).

In such a channel, the flow is theoretically a laminar two-dimensional Poiseuille flow and the wall shear stress \(\tau_w\) is uniform except in the boundary layers confined near the channel side walls and over a short entrance region whose length \(Le\) is about

\[
Le = 1.092 \frac{Qh^3}{v},
\]

where \(Q\) (\(\text{m}^3/\text{s}\)) is the flow rate, \(v\) is the fluid kinematic viscosity (\(\text{m}^2/\text{s}\)), and \(l\) and \(h\) are respectively the channel half width and half thickness (m). For the maximum flow rate used in the experiments, the entrance region was about 0.22 mm long, which is much smaller than the total length (50 mm) of the rectangular channel. In particular, in the observation area where the yeast cells were deposited (Fig. 3), i.e., downstream from the entrance region and in the central region of the flow channel, \(\tau_w\) can be written

\[
\tau_w = \frac{3\mu Q}{4h^2l},
\]

where \(\mu\) is the fluid dynamic viscosity (\(\text{Pa}\cdot\text{s}\)).

More details concerning the flow in the chamber are reported by Lorthois et al. [12].

In order to check for the existence of a laminar two-dimensional Poiseuille flow downstream the entrance region in the rectangular flow channel, a test chamber with a fourth orifice \((0.26\text{-mm diameter})\) topped by a syringe valve, located 10 mm from the rectangular channel entry, was built. Experimental measurement of the flow rate and of the pressure drop (\(\Delta P\)) between the fourth and third orifices (distance \(\Delta x\)) showed that

\[
\frac{\Delta P}{Q} = \frac{3\mu \Delta x}{4h^2l},
\]

which is the theoretical relationship for a laminar two-dimensional Poiseuille flow (Lorthois et al. [18]). Thus, Eq. (3) is valid in the whole observation area. In parallel, the nondeformation of the flow chamber, even at higher applied pressures, was demonstrated.

In order to evaluate the order of magnitude of the forces applied by such a flow upon the yeast cells, let first consider the ideal case of an individual spherical particle in contact with a plate under a linear shear flow. The particle experiences hydrodynamic drag \(D\), torque \(C\), at the center of the particle and lift \(L\). These forces, given by O’Neil [19] and Krishnan and Leighton [20], can be expressed by

\[
\begin{cases}
D = \frac{32\tau_w a^2}{\alpha}, \\
C = 0.38 a D, \\
L = 9.257\tau_w a^2 Re_p,
\end{cases}
\]

where \(a\) is the particle radius (m). In this expression, \(Re_p\) denotes the particulate Reynolds number,

\[
Re_p = \frac{a^2 \tau_w}{v\mu}.
\]

As seen in Table 1, \(Re_p\) is always lower than 1 in the range of laminar shear stress considered in the paper. Lift may thus be ignored compared to drag in the detachment mechanism (Table 1). Earlier workers already assumed that lift might be

<table>
<thead>
<tr>
<th>Shear stress (\tau_w) (Pa)</th>
<th>1</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particulate Reynolds number (Re_p)</td>
<td>0.00306</td>
<td>0.0306</td>
</tr>
<tr>
<td>Drag (D) (pN)</td>
<td>98</td>
<td>980</td>
</tr>
<tr>
<td>Lift (L) (pN)</td>
<td>0.0867</td>
<td>8.67</td>
</tr>
</tbody>
</table>
ignored in the theory of detachment of particles from flat surfaces by laminar shear flow (Hubbe [21]; Sharma et al. [22]).

Let us now consider the real case of a three-dimensional laminar flow past an array of spherical particles in contact with the bottom side of a rectangular channel. If the particles are sufficiently far apart (typically a distance between particles greater than 5a) and the ratio of the particle radius a to the channel half height h is less than 1/15, Eq. (5) still holds (Brooks and Tozeren [23]). Both conditions are fulfilled in the experiments reported in the present paper. To interpret the experimental results, which describe the particle detachment as a function of the wall shear stress applied, the relationship between the adhesion force and the wall shear stress at detachment must be elucidated. Whatever the mechanism of detachment of particles from the surface, one of the most important and sensitive parameters is the radius of the contact area between particles and surface. This contact area may be related either to the microroughness, to the elastic and adhesive deformation of the surfaces, or to the presence of specific bonds between the particles and the surface. Moreover, the use of Eq. (5) in Newton’s second law to determine the adhesion force needs to assume a particular detachment mechanism for particle release such as rolling, sliding, or bond rupture (Lorthois et al. [12]). Due to the complexity of the particle–flat surface system considered in the paper, it was not possible to estimate the contact area and to choose a pertinent detachment mechanism. Therefore the adhesion force has not been determined.

2.5. Detachment experiments

The chamber was assembled and fixed on the stage of an inverted phase-contrast microscope (Nikon Diaphot TDM) coupled to a 5.9 M pixel digital camera (Nikon D1X) with a video image-processing system for the visualization and counting of particles. With this setup, a direct observation of the detachment process in situ was thus possible. The observation area was located far downstream of the rectangular channel inlet, in order to avoid entrance effects. The flow chamber was filled with distilled ultrafiltered water mixed in each case with the desired quantity of NaCl. The pH of the yeast suspension that was brought in contact with the support was roughly adjusted (between 5 and 6). Yeast cells were slowly injected into the flow chamber through a syringe valve. They were allowed to settle under gravity for at least 20 min, resulting in a cell separation of about 5 cell radii, and allowing them to adhere. This separation distance was chosen to minimize the artefacts caused by hydrodynamic interactions between particles such as shielding of the shear field as previously mentioned. It can be noticed that below 20 min, cells did not adhere to the glass surface. So 20 min was the minimal time for cell sedimentation. Two contact times were tested, 1 and 15 h, the contact time being defined as the time during which cells are kept in contact with the glass plate without any mechanical stress. It corresponds in fact to the lapse of time between the end of the settling phase and the beginning of the stepwise procedure for the variation of \( \tau_w \).

After the contact time, the flow rate in the channel \( (Q) \) was increased step by step (a typical time step was 3 min). Flow rates ranging from 1 to 4.5 ml/min were generated by gravity, controlling the height of a constant head vessel located upstream of the chamber. Flow rates greater than 4.5 ml/min were obtained using a gear pump (Ismatec, Fisher Bioblock Scientific, Illkirch, France). At the end of each flow rate step, the number of cells remaining at the glass surface was counted using the video image-processing system. Homemade software was used to select the cells of the observation area to be counted according to the following criteria: (i) each entity was not an aggregate of several cells, (ii) the cell was deposited in the observation area at the beginning of the experiment (after the prescribed contact time), (iii) the cell had not moved from its initial location at the beginning of the experiment, (iv) budding cells were not considered, and (v) the separation distance between two neighboring cells was higher than 5 cell radii. The shear stress applied was calculated using Eq. (3) from the value of the flow rate measured by weighing the fluid output on an electronic balance. \( \tau_w \) varied in the range 0–16 Pa.

At least three experiments were performed for a given set of parameters. Each result presented in the paper is the mean of three independent experiments.

3. Results

3.1. Characterization of surface physicochemical properties of Saccharomyces cerevisiae cells: influence of the yeast type

Fig. 4 presents the electrophoretic mobility of the two types of \( S. \) cerevisiae as a function of pH in 1.5 mM NaCl.

![Electrophoretic mobility of S. cerevisiae cells as a function of pH and yeast type](image)

Fig. 4. Electrophoretic mobilities of \( S. \) cerevisiae cells suspended in 1.5 mM NaCl as a function of pH and yeast type: (●) type I and (○) type II.
suspension. Electrophoretic mobility became more negative as pH increased from 3 to 5 and then stabilized at pH 5 to 7. The isoelectric point was around 3, which is in accordance with previously published results on other yeast strains (Mozes et al. [24]; Vernhet et al. [25]). No difference in electrophoretic mobility was observed between the two types of yeast.

The MATS results for the two kinds of yeast, suspended in a 150 mM NaCl solution, are displayed in Fig. 5. Despite the relatively low affinities, that of the yeast of type I was higher with chloroform (an electron acceptor solvent) than with hexadecane (a nonpolar solvent). The differences in affinity between these two solvents were due to Lewis acid–base interactions, i.e., electron donor/electron acceptor interactions resulting from the electron donor nature of the yeast. Likewise, yeast affinity was lower with ethyl acetate (a strong electron donor) than with decane, indicating that the electron acceptor nature of the yeast was weak. On the basis of these results, it was concluded that yeast cells of type I were rather electron donor and thus showed quite hydrophilic properties (van Oss [26]). Yeast of type II presented totally different surface characteristics. Cells which exhibited a high affinity with nonpolar solvents coupled with a more pronounced electron acceptor nature were strongly hydrophobic.

3.2. Detachment experiments: influence of the environmental conditions

3.2.1. Comparison of detachment data between the two types of yeast

The number of cells initially present in the observation area was typically between 50 and 100. In order to check for the statistical validity of each experiment, the results obtained in both half observation areas were analyzed as follows: the percentage of particles remaining attached in each half observation area at each flow rate step was plotted as a function of the percentage of particles remaining attached in the entire observation area. In the ideal case (no variability of the particle radius, perfect contact area, etc.), the curve obtained should be the first bisecting line (see Lorthois et al. [18]). Therefore, results were systematically rejected when deviation from the bisecting line was greater than 15%.

The results of all detachment experiments were plotted as a function of the wall shear stress and the yeast type (type I and type II). Ionic strength of 150 mM and contact time of 1 h.

As expected, the percentage of detached cells increased with the wall shear stress applied, for any type of yeast. However, the two kinds of yeast exhibited a totally different behavior. For the first type, adhesion to the glass plate was weak whereas, for the second type, it was significantly stronger. In fact, the percentage of detached cells under a given wall shear stress was drastically decreased. As biological cell size distribution was found to be almost symmetric (see Fig. 1), results can also be interpreted in terms of the wall shear stress that causes 50% biological cell detachment, which is also the wall shear stress necessary to detach cells with a radius equal to the mean radius of the distribution (Lorthois et al. [12]). This threshold wall shear stress was denoted \( \tau_{w, 50\%} \). As expected, \( \tau_{w, 50\%} \) for type I was low (0.19 ± 0.01 Pa) and \( \tau_{w, 50\%} \) for type II was enhanced by a factor of 12.

3.2.2. Influence of the contact time and the ionic strength

The influence of the contact time was first studied. Results are presented in Table 2 for the two kinds of yeast and an ionic strength of 150 mM. It can be seen that \( \tau_{w, 50\%} \) drastically increased when the contact time increased for both
type I and type II yeast. This means that cells were more strongly stuck to the glass plate after 15 h of contact time than after 1 h.

The influence of the ionic strength was then evaluated for the two kinds of yeast and results are summarized in Table 3 for a contact time of 15 h. As previously found with the same experimental device for inert particles in contact with the glass plate, such as glass beads (Elzo et al. [11]) or latex particles (Lorthois et al. [12]), the results for the type II yeast were in good agreement with the DLVO (Derjaguin, Landau, Verwey, Overbeek) theory, which describes the energy of interaction between two charged surfaces in a polar medium and its decay with separation distance. According to this classical approach, the net energy of interaction is the sum of the attractive van der Waals energy and the repulsive double-layer energy (the interested reader can refer for instance to Israelachvili [27] for the details of the theory). Increasing the ionic strength decreases the double-layer thickness, which results in lower electrostatic repulsion and significantly enhanced adhesion. This is in accordance with the aforementioned experimental results. The drastic increase in the wall shear stress necessary to remove 50% of yeast cells from the glass surface as ionic strength increased from 33 to 150 mM can be clearly seen in Table 3 (enhancement of τw,50% by a factor of 3).

However, as can also be seen in Table 3, results for the type I yeast were not sensitive to any ionic strength effect, since no significant variation of τw,50% was observed.

3.2.3. Determination of the wall composition for the two types of yeast

Another point to deal with concerned the differences in the physicochemical properties of the two kinds of yeast, which were probably related to different cell wall compositions. The yeast cell wall accounts for about 20% of the dry weight and therefore represents an important part of the cell (Fleet [28]). It has an intricate structure based on an internal skeletal layer and an outer protein layer. The skeletal layer consists mostly of β1,3- and β1,6-glucans and chitin and is responsible for the shape and the strength of the wall (Zlotnik et al. [29]). The outer layer is formed of manno-proteins which are generally heavily glycosylated and determine most of the surface properties of the cell (Zlotnik et al. [29]). Glucan, manno-proteins, and chitin constitute about 60, 40, and 1% of the total cell wall, respectively.

The concentrations of chitin, β-glucan, and mannan in the yeast wall were measured for types I and II cells and the results are summarized in Table 4. The chitin and mannan contents were close for both types of yeast but the glucan content was significantly higher for yeast of type II.

4. Discussion

The weak adhesion of the yeast of type I could be explained by the nature of the glass, which is a polar, extremely hydrophilic (Vernhet et al. [25]), and negatively charged surface (Mozes et al. [24]). A strong repulsion occurred, due to electrostatic effects (same sign for the surface charge of cells and glass) and hydrophilic repulsion (electron donor surface properties of cells and glass). It was also found that yeast cells of type II were more strongly stuck to the glass plate than cells of type I yeast under the same environmental conditions (ionic strength and contact time). This could be explained by the physicochemical surface properties of the yeast cells of type II and especially their hydrophobic and electron-acceptor components, which caused strong attractive van der Waals and Lewis acid–base interactions, counterbalancing the electrostatic repulsion.

Furthermore, for long contact times of about 15 h, adhesion was stronger whatever the yeast type. This was related to the increase in the OD_{280 nm} of the suspension supernatant over the first 20 h (Fig. 7), which undoubtedly corresponded to the release of some compounds such as proteins into the extracellular medium, due to cell lysis. These macromolecules could contribute to the formation of a conditioning film which influences the interaction of the cells with the bare glass plate. Unlike Bos et al. [1], who concluded that adhering microorganisms were more easily stimulated to detach when adhering to a conditioning film than when adhering directly to the substratum, the adhesion was here stronger in the presence of the conditioning film, thus demonstrat-
of hydrophilic yeast strains to champagne bottles. Hett and Bellon-Fontaine [31] in their study of the adhesion phenomena observed, confirming the conclusions of Verh- ...

was not controlled by electrostatic interactions. Our data preliminary observations, it can be concluded that adhesion different under the same environmental conditions. From these results, strong repulsion still persisted.

interfacial water, the reorientation of dipoles or particles as yield stronger bonds, due to the progressive removal of bond aging yielded stronger bonds, due to the electron donor nature of both the cells and the support, was not sensitive to the increase in ionic strength, unlike the electrostatic interactions and, as a whole, or an increase in the number of hydrogen bonds.

Concerning the influence of the ionic strength, results for the type II yeast were in good agreement with the DLVO theory. However, yeast cells of type I were not sensitive to any ionic strength increase. Therefore, as previously reported (Bos et al. [11]), DLVO theory does not systematically apply, particularly when microorganisms are involved. In fact, it can be necessary to include short-range Lewis acid–base interactions (hydrophobic attractive and hydrophilic repulsive forces). In the case of yeast of type I, the hydrophilic repulsive force, due to the electron donor nature of both the cells and the support, was not sensitive to the increase in ionic strength, unlike the electrostatic interactions and, as a result, strong repulsion still persisted.

Despite the same surface charge, the behavior of the two types of yeast toward the glass plate was completely different under the same environmental conditions. From these preliminary observations, it can be concluded that adhesion was not controlled by electrostatic interactions. Our data suggested that Lewis acid–base interactions, together with van der Waals attractive forces, were rather involved in the phenomena observed, confirming the conclusions of Vernhet and Bellon-Fontaine [31] in their study of the adhesion of hydrophilic yeast strains to champagne bottles.

The modified glucan content between the two types of yeast could contribute to the differences in their surface properties and thus in their adhesive behavior. In particular, a larger deformability of the wall of yeast of type II could increase the contact area between the cells and the glass plate, thus increasing the net adhesion force. In future work, it would be useful to quantify the effect of the glucan content on the shape and the strength of the yeast wall. Therefore, the balance of forces and torques on the yeast cells, assuming a given detachment mechanism, would give an estimate of the net adhesion force, as done by Lorthois et al. [12]. Nevertheless, further work should now be focused on the role of mannoproteins, which are mainly responsible for the surface properties of the cells. For example, studies on the cell wall of the pathogenic yeast Candida albicans suggested a relationship between the mannoproteins and the cell surface hydrophobicity (Masuoka and Hazen [32]). In our case, even though mannan content is very similar for both types of baker’s yeast, it is possible that, for the type II, some specific mannoproteins are present on the cell surface, explaining its hydrophobicity. In particular, Reynolds and Fink [9] showed that attachment of baker’s yeast to plastic required Flo11p, a member of a large family of fungal surface glycoproteins, which allowed hydrophobic properties of cells to be enhanced.

However, with these two types of industrial yeast cells, which are simply rehydrated in saline solutions, it is difficult to control their physiological behavior and to maintain strictly identical biological parameters. The relationship between the type of yeast and its surface physicochemistry also remains unclear, due to the lack of knowledge about culture conditions. Further work will thus have to be devoted to adhesion tests on biologically active cells, issued from fresh cultures. The influence of the physiological state and the growth conditions will be evaluated. Experiments on mutant baker’s yeast strains, with modified cell wall properties, will be carried out to investigate the role of the composition and the structure of the wall on the cell physicochemical characteristics and to determine the consequences on adhesion. From a more technological point of view, the flow chamber will be adapted to allow non-transparent surfaces like membranes to be observed. For that purpose, a metallurgical microscope, based on incident reflected light, will be setup.

5. Conclusion

In order to characterize the attachment of microorganisms to a surface, which is the critical first step of biofilm fouling in membrane processes, the in situ shear-induced detachment of baker’s yeast in adhesive contact with a plane glass surface was experimentally studied. The work, carried out with two types of yeast marketed in different forms, focused on the effect of ionic strength and contact time. For any type of yeast, the percentage of detached cells increased with the wall shear stress applied. For the first type of yeast...
cells, which were rather hydrophilic, adhesion to the glass plate was weak. This was due to both electrostatic effects and hydrophilic repulsion. When yeast cells of the second type were initially deposited, the percentage of detached cells under a given shear flow was drastically decreased. This higher adhesion could be explained by the physicochemical surface properties of the cells and especially their hydrophobic and electron acceptor components, which caused strong attractive van der Waals and Lewis acid–base interactions, counterbalancing the electrostatic repulsion. For increasing ionic strengths, adhesion was greater, due to lower electrostatic repulsion. However, with the first type of yeast, adhesion was not sensitive to variations of the ionic strength. This showed that DLVO theory did not systematically apply and in order to predict the cell behavior, it was necessary to include short-range Lewis acid–base interactions such as hydrophobic attractive and hydrophilic repulsive forces. For long contact times, cells stuck more strongly to the glass plate which was related to the release of macromolecules such as proteins into the extracellular medium.

This work, achieved on a simplified yeast/glass model system, needs now to be extended to more realistic conditions in terms of microorganisms and support media.

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