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Removal forces and adhesion properties of *Saccharomyces cerevisiae* on glass substrates probed by optical tweezer

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In agroindustry, the hygiene of solid surfaces is of primary importance in order to ensure that products are safe for consumers. To improve safety, one of the major ways consists in identifying and understanding the mechanisms of microbial cell adhesion to nonporous solid surfaces or filtration membranes. In this paper we investigate the adhesion of the yeast cell *Saccharomyces cerevisiae* (about 5 μm in diameter) to a model solid surface, using well-defined hydrophilic glass substrates. An optical tweezer device developed by Piau [J. Non-Newtonian Fluid Mech. **144**, 1 (2007)] was applied to yeast cells in contact with well-characterized glass surfaces. Two planes of observation were used to obtain quantitative measurements of removal forces and to characterize the corresponding mechanisms at a micrometer length scale. The results highlight various adhesion mechanisms, depending on the ionic strength, contact time, and type of yeast. The study has allowed to show a considerable increase of adhering cells with the ionic strength and has provided a quantitative measurement of the detachment forces of cultured yeast cells. Force levels are found to grow with ionic strength and differences in mobility are highlighted. The results clearly underline that a microrheological approach is essential for analyzing the adhesion mechanisms of biological systems at the relevant local scales. © *2007 American Institute of Physics*.
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I. INTRODUCTION

Microorganisms are present in both natural and industrial environment and their adhesion onto nonporous solid surfaces or filtration membranes is unavoidable. The growth of these adhered microorganisms can lead to the formation of biofilms. These biofilms are either wished for biofiltration processes or hazardous for health such as in agroindustry or surgery. Understanding the initial adhesion and removal of microorganism is a first decisive step to control the formation of these biofilms. In this aim, micron-scale adhesion mechanisms of yeast cells on a model surface have been investigated by means of an optical tweezer.

Various studies have considered the adhesion of microorganisms on inert surfaces probed by optical tweezer or atomic force microscopy (AFM). For example, Simpson *et al.*[1] have characterized and quantified the detachment forces of a single *Staphylococcus aureus* cell from a surface coated with specific proteins that are generally present in the extracellular matrix. Bowen *et al.*[2] used AFM to study the adhesion of *Saccharomyces cerevisiae* yeast on hydrophilic mica surfaces coated with a hydrophobic material. A single yeast cell was grafted on the cantilever tip, which was brought into contact with the surface. These authors have demonstrated that the contact time was of primary importance in the adhesion phenomenon. Klein *et al.*[3] used a single-beam gradient optical trap to micromanipulate a spherical bacterium against a flat glass surface, working out forces ranging from 0.01 to 4 pN and obtaining Derjaguin, Landau, Verwey, and Overbeek (DLVO) profiles versus the separation distance. Furthermore, measurements of the adhesion properties of *S. cerevisiae* have already been carried out with a shear stress flow chamber on glass[4,5] as also on polystyrene, polypropylene,[4] and stainless steel[4,6] materials.

The yeast cell *S. cerevisiae* was selected for performing the present work. This system is widespread in agroindustry and is retained as a model for studying the eukaryotes owing to its entirely sequenced genome and its ability to initiate a biofilm.[7] In this paper, two types of yeast, rehydrated and cultured, will be studied. The cell wall of yeast is the only interface of the adhesion phenomenon and plays a significant role in the process. The cell wall is 100–200 nm thick,[8] and envelopes the whole cell. Its rigidity[9] and its unique macromolecular organization give the yeast a specific shape. It is an exoskeleton which accounts for 15%–25% of the total dry weight of the cell.[10] The outer layer consists of heavily glycosylated mannoproteins emanating from the cell surface.[11] Owing to its selective-permeability wall, yeast controls the concentration of solutes inside the cell as also intracellular osmotic pressure (potential values of 0.81 and 0.61 MPa in stationary and exponential phase cells, respectively[8,12]). Moreover, ionic transfer between the yeast and the extracel-

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lular medium, via channels, is now well understood.[8,13,14] When osmoregulation promotes the ejection of cations, it might be assumed that the electrical double layer (Gouy-Chapman and Stern layers) will be seen to be altered and thus that the ionic force imposed by the medium will disturb physicochemical interactions in the vicinity of the wall. Studies have assumed that yeast adapts to ambient conditions by secreting macromolecules such as proteins (flocculins and adhesins), which are the direct expression of several genes of various yeast strains.[15]

To probe the relevant scales affecting the mechanisms of yeast adhesion on surfaces, an optical tweezer device was set up. This is a carefully customized instrument for handling objects at micron scale[16,17] that provides a way of quantifying forces needed to remove microorganisms from surfaces. In order to compare the measurements presented here at local micron scale using the optical tweezer with those obtained previously at a larger scale,[5] the contact surfaces were chosen to be a microscope glass coverslip and a slice of glass. These surfaces are hydrophilic; thus the hydration forces help our device to detach microorganisms and specific interactions can be highlighted. They were both physically and chemically identified and were similar to those used for previous assays in the shear stress flow chamber.[4] The adhesion has been studied accordingly in three ways: (a) to quantify the forces needed by the optical trap to remove yeast from the surface, (b) to provide statistics concerning the number of yeast cells that the optical trap is able or unable to remove using maximum power, and (c) to perform real-time observations of the movements imposed on the adhered yeasts.

The paper is organized as follows. In Sec. II, we present the yeast *S. cerevisiae*, the various glass surfaces used in the experiments, and the experimental device. Section III provides results for two different methods of investigation that correspond to positioning the adhesion surface horizontally and vertically. Finally, in Sec. IV, the results are discussed, allowing us to state that adhesion is locally governed by binding sites. Additional information is given in Appendixes A and B, where are presented, respectively, a short review on photodamage and configuration required to avoid any potential damage to the yeast by the laser beam and details concerning the calibration force of the optical trap.

II. MATERIALS AND METHODS

A. Yeast cells

Dried baker yeast, *S. cerevisiae*, has been provided by Lesaffre (Marcq-en-Baroeul, France). The material was initially packaged as dry aggregates of small rod shape. The physicochemical properties of the surface have been given in a previous work.[4]

1. Rehydrated yeast cells

Yeast cells were rehydrated in saline solutions (NaCl) with 0.2 μm filtered and demineralized water (Milli-Q, Millipore, Billerica, MA). Three NaCl ionic strengths were tested: 1.5, 33, and 150 mM. The suspension has been prepared by dispersing and rehydrating 1 g/l aggregates in the saline solution at room temperature (23 °C), with gentle agitation for a time of 20 min. Then, yeast cells were collected by centrifugation for 2 min at 15 000 rpm (Biofuge Stratos, Heraeus Instruments, Osterode, Germany), washed twice, and suspended again in NaCl solution, leading to a *p*H of 5.7 at $T=(25.0\pm0.1)$ °C.

2. Cultured yeast cells

A preculture was prepared by placing a few aggregates of dry yeast in 100 ml of sterile yeast extract peptone dextrose (YEPD) broth [1% yeast extract (Fluka Chemika, Buchs, Switzerland), 2% anhydrous glucose (Fluka Chemika, Buchs, Switzerland), and 1% bactopeptone (Sigma-Aldrich and Co., Saint Louis, MO)] in a 500 mL Erlenmeyer flask and incubated overnight at 30 °C under a fixed agitation speed (about 600 rpm). Firstly, a volume of this preculture was adjusted in order to obtain the same starting optical density (OD) and secondly, was inoculated into another flask of 200 ml sterile YEPD broth, incubated under the same conditions for 48 h until the stationary growth phase was reached (with a final cell concentration of 7.5 g/l). Cells were harvested by centrifugation as described above, washed twice, suspended again in saline solution (NaCl 150, 180, 200, 250, and 330 mM), and diluted just before starting the experiment. The final *p*H was 5.9 at $T=(23.0\pm0.1)$ °C.

B. Glass substrates

1. Quantitative approach: A glass coverslip

The glass surface used was an 18×24 mm^2 microscope glass coverslip of thickness 0.15 mm (Marienfeld-Superior, Marienfeld Laboratory glassware, Lauda-Koenigshofen, Germany). This surface was carefully cleaned by the means of a sulfochromic mixture [$K_2Cr_2O_7$ (2.7*M*)/H_2SO_4(4% v/v), Chimie Plus Laboratoires, Denicé, France] for 1 h, rinsed three times with distilled water, and stored in Milli-Q water (filtered on 0.2 μm) at room temperature. A clean new plate was used for each test. They were dried just before the experiment. Such conditions are close to those of previous protocols adopted by Guillemot *et al.*[4] for detachment experiments performed in a stress flow chamber. The two sides of the coverslips were also distinguished: Side 1 denotes the upper face of the coverslip when laid out initially in its original packing and side 2 the lower face.

2. Qualitative approach for observation: A slice of glass

In this approach, we are interested in the mechanisms of yeast adhesion to the substratum. For the best observation of these mechanisms in the vertical plane, a slice of glass was placed between the microscope slide and the coverslip in order to position the adhesion surface vertically. This slice of glass has come from an ordinary calcosodic silicate glass plate (Planilux, Saint-Gobain, France), whose energy properties obtained by the contact angle measurement method (see below) have been given in previous works.[4]

A made-to-order fragment was shaped from this glass plate ($210\times90\times4$ mm^3) using the lithopreparation method (abrasion on each side) in order to obtain a slice of 10 mm

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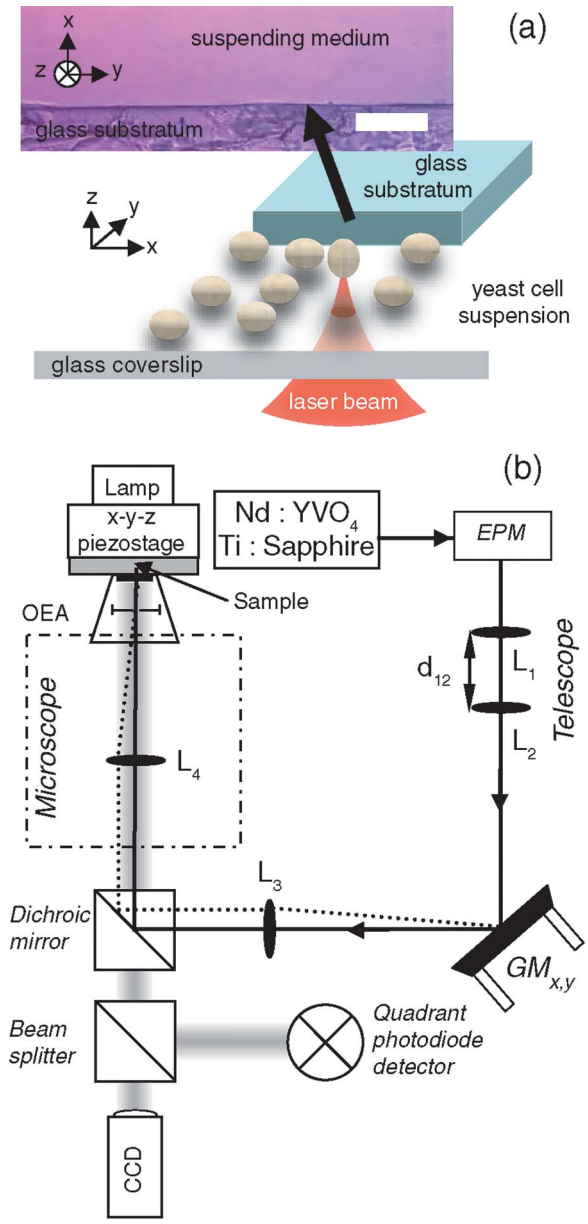

FIG. 1. (Color online) (a) Schematic layout of the sample slide, coverslip, yeast cell suspension, and glass fragment. The side where yeast cells adhere is shown in profile (photo). Note that the schematic is not to scale. The scale bar is 20 μm. (b) Schematic layout of the optical tweezer system. With externally positioned mirrors (EPM) objective entrance aperature (OEA) and galvanometric mirrors ($GM_{x,y}$)

long, 4 mm wide, and 250±3 μm thick. The area concerned by is perpendicular to the slide-coverslip system and of similar nature [energy properties and average roughness (0.25 nm)] to the top face of the original plate [see Fig. 1(a)]. Cleaning was carried out with the sulfochromic mixture for 1 h.

3. Surface properties

Surface properties of substrates were determined by contact angle measurements. Water (demineralized and filtered at 0.2 μm), glycerol (Selectipur, Merck, Darmstadt, Germany), and diiodomethane (Sigma, Sigma-Aldrich and Co., Saint Louis, MO) contact angles were determined by the sessile drop technique using a goniometer (Digidrop, GBX Scientific Instruments, Romans, France) coupled with a WINDROP++ software for capturing and analyzing the images.

The results have consisted of an average on ten measurements on both sides aimed at discovering possible differences due to the coverslip preparation method. Contact angles were then converted into free surface energy values using the modifications of the Young equation proposed by Van Oss,[18] which ignore spreading pressure and distinguish Lifshitz–van der Waals and Lewis acid/base free surface energy components according to the equation

$$\gamma_L(1+\cos\theta) = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^{+}\gamma_L^{-}} + \sqrt{\gamma_S^{-}\gamma_L^{+}}\right), \quad (1)$$

where γ is the total surface energy, γ^{LW}, γ^{+}, and γ^{-} the Lifshitz–van der Waals, electron-acceptor, and electron-donor components of the free surface energy, respectively, and θ the contact angle. The subscripts S and L denote the solid and liquid samples, respectively.

C. Experimental setup and methods

1. Optical tweezer

The experimental setup employed is capable of trapping micron-sized objects with index of refraction different from those of the suspending medium. However, two distinct techniques can be used to move the trap arbitrarily in relation to the suspending medium along the x, y, and z axes, either by manipulating the focal point of the laser beam or by manipulating the medium with a piezoelectric micropress. Such system, inspired by Fällman and Axner,[19] has been developed and described by Piau.[20] Couvin and Piau[21] used it in rheometric measurements. A short description will be given below according to the diagram shown in Fig. 1(b).

The laser (Millennia V, Spectraphysics, Mountain View, CA) emits a 532 nm beam of a maximum of 5 W. The wavelength of this beam is adjusted to 800 nm, which is considered as the minimum absorption level of the suspending medium by means of a Ti: Sapphire (3900S, Spectraphysics, Mountain View, CA). Literature data and our own experiments allow us to confirm that all the necessary precautions had been taken in order to minimize damaging laser effects on the microorganisms and their subsequent adhesion, i.e., to avoid an increase of the local temperature which caused the cell lysis (see Appendix A).

The beam is magnified by the telescopic system consisting of two lenses (L_1 and L_2) and then directed into the galvanometric system which consists of two perpendicular mobile mirrors. Then, the beam is sent into the microscope and focused on the sample through the objective. Changing the distance d_{12} between the two lenses L_1 and L_2 alters the vertical position (z) of the optical trap in the sample.

The micropress possesses a reinforcement built on a piezoelectric system (Tritor 3D 101SG, Piezosystem Jena GmbH, Jena, Germany), allowing displacements in the horizontal plane (x,y) and along the z axis. The sample was sealed between the slide and coverslip with an autoadhesive

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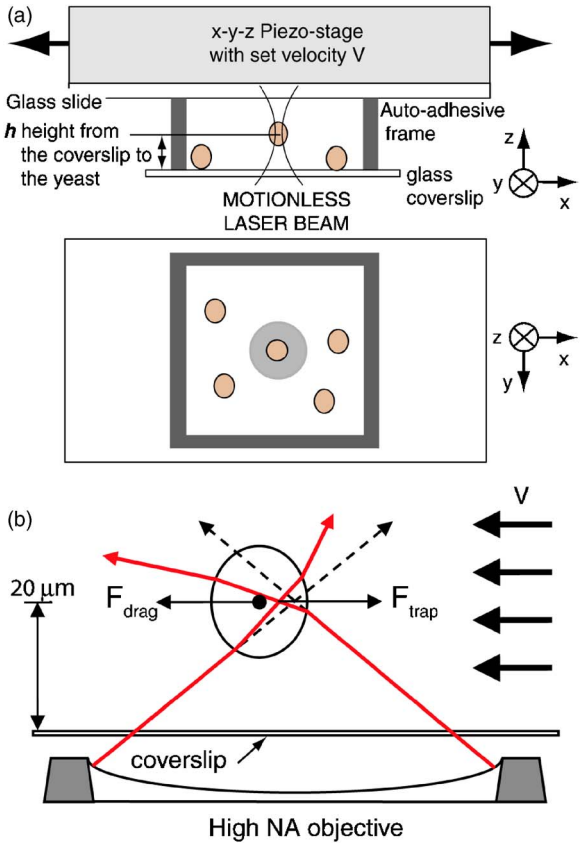

FIG. 2. (Color online) Creation of flow around yeast maintained by the optical trap by generating oscillatory displacement with the slide-coverslip-yeast suspension-piezostage system. (a) Here, the adhesion glass surface is the microscope coverslip. The tethered yeast is seen beneath. (b) Laser tweezer experimental geometry. A yeast cell is held 20 μm from the sample interface using an optical trap. The escape force is measured when the stage velocity increase makes the drag force stronger than the trap. The known drag force gives the corresponding trap force. Note that schematics are not to scale and the thermal probe is not displayed.

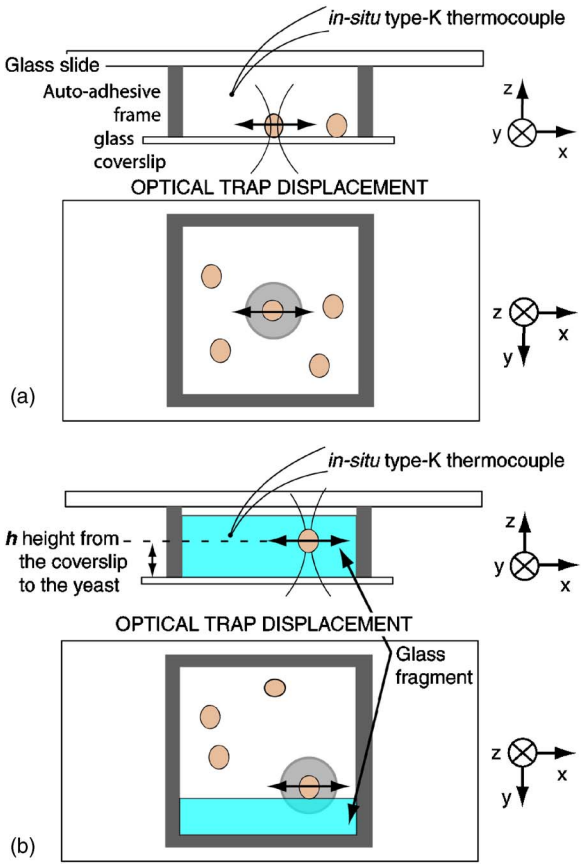

FIG. 3. (Color online) Schematic layout of the sample slide, coverslip, autoadhesive frame, and yeast cell suspension. The double black arrow represents the displacement of the laser beam, which exerts an optical force on the tethered yeast cell. The type-K thermocouple can measure the *in situ* temperature of the sample during the experiments. In the experiment, the sample is observed from beneath, through the coverslip (second part of the schematics). (a) The adhesion surface is horizontal. (b) Adhesion occurs at a height h from the coverslip. The adhesion surface is vertical and consequently the adhering yeast cell is seen in profile. Note that schematics are not to scale.

frame (Geneframe 25 μl, ABgene, Epsom, UK) and held on the micropress. Flow around the particle can be generated by moving either the micropress or the optical trap. In the present study, the optical trap was kept immobile and the induced motions of the particles were observed [see Fig. 2(a)].

The optical tweezer has been built around an inverted light microscope (Olympus IX70; Olympus, Melville, NY). Micron-sized particles require the use of a high magnification objective ($\times$100) and a large numerical aperture increases beam stability. Thus, an oil immersion objective is used with a numerical aperture of 1.4. The refractive index of the oil used is 1.52. The display system consists of a 25 image per second charge coupled device camera (JVC KYF55B; JVC UK Ltd., London, UK) and a quadrant photodiode detector (SPOT series, UDT Sensor Inc., Hawthorne, CA) assembled on two parallel optical benches and positioned after a double exit (beam splitter). The video camera has a resolution of 768$\times$494 pixels and the ocular unit and sensor have been assembled on an optical bench, thus producing a larger final image ($\times$7.8), like a slide displayed onto a screen.

2. First method: Surface positioned horizontally

Sample preparation and experimental protocol. Before performing the test, the coverslip and slide are carefully cleaned with the sulfochromic mixture and dried with clean-room paper. To assemble a sample, a quantity of 25 μl of yeast suspension (4×10^6 cells/ml) is introduced into the chamber formed by the slide and the adhesive frame stuck onto it. The coverslip is then set down on the frame, thus forming an airtight cavity as the measurement chamber, containing the yeast suspension [see Fig. 3(a)].

A type-K thermocouple probe of 0.25 mm thick and accurate to within 0.1 °C is positioned in the measurement chamber. The room temperature is regulated at 23 °C and, before each test, we use another type-K thermocouple (sheathed cable, accurate to within 0.1 °C). The two probes are plugged into a displayer thermometer (CHY 506 thermometer, Taiwan). The temperature, measured for each assay, was found to be in the range of (25.5±0.6) °C for all the results of this paper.

The assembled sample was turned over so that the coverslip was beneath the slide. The sample was placed on the piezoelectric stage with the coverslip downwards, facing the

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TABLE I. Surface tension components of the probe liquids used for contact angle measurement (water, glycerol, and diiodomethane).

Probe liquids	γ_L (mJ/m^2)	γ_L^{LW} (mJ/m^2)	γ_L^{AB} (mJ/m^3)	γ_L^+ (mJ/m^2)	γ_L^- (mJ/m^2)
Water	72.8	21.8	51.0	25.5	25.5
Glycerol	64.0	34.0	30.0	3.9	57.4
Diiodomethane	0.8	50.8	0	0	0

objective. The yeast suspension was left for 20 min to sink down to the bottom of the chamber and onto the coverslip so as to form a deposit. Afterwards, time 0 was defined as being the initial yeast/glass contact time. When the yeasts had formed a deposit and were in contact with the coverslip, the time was left to run until the desired contact time is reached.

At a given contact time (1,2,...,17 h), about 30 different yeast cells were examined by bringing the focal point of the laser beam into the center of the yeast cell. With a set laser power, an attempt was made to remove each yeast cell from the coverslip by moving the laser beam parallel to the glass wall. The laser was focused at a z position equal to an average yeast radius (about 3 μm) from the coverslip. A horizontal displacement of approximately 10 μm in amplitude was imposed along the x axis. The laser power at the objective end was initially set to its lowest level, i.e., 27 mW, for each yeast cell and then gradually increased up to 120 mW, its maximum value. It was noted whether the yeast cell could be removed or not, and when possible, the force exerted by the laser on the yeast cell was measured using the same power level by a force calibration using the Stokes drag equilibrium. To do this, a laminar flow is generated in the suspending medium around the yeast cell by the piezostage, which is kept immobile by the laser beam. As soon as the steady flow is secured, two forces are in opposition during displacement in a given direction [see Fig. 2(b)]: the springlike force F_{trap} of the optical trap and the drag force F_{drag} the fluid exerts on the yeast (see Appendix B for more details).

3. Second method: Surface positioned vertically

In this configuration, we were interested only in the adhesion mechanisms without force measurements. The stresses are created by the optical trap on the particle parallel to the adhesion surface.

Sample preparation and experimental protocol. In this part, only rehydrated yeast cells were used. Before each test, the slide, coverslip, and slice of glass were carefully cleaned with the sulfochromic mixture and dried with cleanroom paper. To assemble an analysis chamber, an autoadhesive frame was fixed on the microscope coverslip. Then the slice of glass was placed on the plate, inside the frame, so as to position the adhesion surface (smooth face of the fragment) in the vertical plane. 25 μl of yeast suspension was introduced into the chamber formed by the coverslip and the adhesive frame stuck to it. The analysis chamber was then sealed by laying the slide above, evenly stuck on the frame, while taking care to eject any air bubbles. Figure 3(a) shows the assembly, positioned such that the microorganisms form a deposit on the glass fragment (adhesion surface) for 20 min. The analysis chamber was then positioned in the microscope, coverslip downwards, facing the oil immersion objective. The time 0 was thus defined as previously described with the first method. After a contact time of 1 h, the yeast cells had reached the surface of the fragment and adhered to it. Since this was defined only as a qualitative approach, only a few isolated yeast cells were examined for a known contact time. The temperature was measured as described in the first method.

III. RESULTS

A. Glass substrates

Contact angle measurements were only made on the glass coverslip used in the first method [Fig. 3(a)]. The energy properties of the probe liquids the average angles achieved with the three probe liquids are given in Tables I and II, respectively. The resulting components in terms of the free surface energy of the glass coverslips, calculated with Eq. (1), are provided in Table III. Concerning the cleaning procedure, the nonpolar component of the free surface energy remains similar, such as a γ_S^{LW} value of 40±3 mJ/m^2 for the two sides of the coverslip. It may also be noted that the acid component value γ_S^+ remains low, with a maximum of 1 mJ/m^2. Conversely, the basic component γ_S^- of the free surface energy and consequently the Lewis acid/base component is found to vary considerably. The two sides do not display significant energy discrepancies; they are found to be in the range of the standard measurement error. Cleaning with sulfochromic mixture leads to increase the hydrophilicity of glass. This can be explained by the persistent presence of a contamination layer. Indeed, water angle decreases from (63.2±3.1)° to (14.7±0.7)° and from (68.6±3.4)° to (15.2±0.7)° for side 1 and side 2, respectively. The basic

TABLE II. Contact angles and standard deviation (deg) at room temperature ($T \sim 23$ °C). The contact angles were obtained by measurement of angle between probe liquid (water, glycerol, and diiodomethane) and glass coverslips.

	θ_{water}		$\theta_{diiodomethane}$		$\theta_{glycerol}$	
	Side 1	Side 2	Side 1	Side 2	Side 1	Side 2
No cleaning[a]	63.2±3.1	68.6±3.4	44.5±2.2	44.3±2.2	65.1±3.2	63.8±3.2
Cleaning[b]	14.7±0.7	15.2±0.7	38.3±1.9	37.9±1.9	18.4±0.9	16.8±0.8

[a]Coverslips were rinsed with distilled water and air dried.
[b]Coverslips were left in the sulfochromic mixture for 1 h, rinsed with distilled water, and dried with cleanroom paper.

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TABLE III. Free surface energy values (mJ/m^2) for the two sides of the coverslips. Contact angles obtained previously (see Table II) were then converted into surface free energy values using the van Oss' modification of the Young equation [see Ref. 18 and Eq. (1)], which ignored spreading pressure and distinguished Lifshitz–van der Waals and Lewis acid/base surface free energy components.

	γ_S^{LW}		γ_S^+		γ_S^-		γ_S^{AB}	
	Side 1	Side 2	Side 1	Side 2	Side 1	Side 2	Side 1	Side 2
No cleaning[a]	37.3±1.0	37.4±0.8	0.5±0.8	1.0±1.2	30.7±2.1	27.0±2.1	8.1±0.9	10.6±0.6
Cleaning[b]	40.5±1.5	40.7±1.5	1.0±0.9	0.9±0.9	53.6±2.3	53.4±2.3	14.4±0.5	14.0±0.5

[a]Coverslips were rinsed with distilled water and air dried.
[b]Coverslips were left in the sulfochromic mixture for 1 h, rinsed with distilled water, and dried with cleanroom paper.

component γ_S^- of the free surface energy thus increases from 30 to 53 mJ/m^2, making it more electron donor. Similar values have been previously obtained by Guillemot *et al.*[4] for wall shear stress experiments, when the glass was cleaned with sulfochromic acid.

B. Adhesion of yeast cells to glass and influence of ionic strength and contact time: A statistical study

The first method was used here, i.e., with the adhesion surface positioned horizontally [Fig. 3(a)]. The yeast cell was trapped by the optical tweezer, with a very weak laser power such as 27 mW. A beam x-axis displacement of 10 μm amplitude was imposed. If the yeast cell was easily caught by the trap at this power of 27 mW, it was regarded as "not adhering." If not, the yeast cell was considered as "adhering" and thus, we tried to detach it by increasing the laser power and consequently the trap force. Adhering yeast cells were counted for an entire scanned population, allowing us to plot a distribution of the yeast cell population that had adhered in percentage with a given contact time.

Rehydrated and cultured yeast cells issued from the same package adhered differently. The rehydrated yeast cells adhered more strongly than the cultured ones. Figure 4 shows the distribution of the population that stuck to the glass for a given entire population, according to the ionic strength (millimolar NaCl), with different contact times. In the case of rehydrated yeast cells, with a contact time of 1 h, almost the entire population remained tethered. For a longer contact time (2 h), all the yeast cell population examined was stuck to the glass, whatever the ionic strength.

The results obtained with yeasts resulting from culture have revealed a greater dependence on the contact time and ionic strength. Generally, with a fixed ionic strength, the kinetic parameter of adhesion should be considered since it was noted that the yeast population adhering to the glass had increased with contact time. Furthermore, the amount of adhering yeast increased considerably with the ionic strength and with a fixed contact time. Indeed, with 1.5 mM NaCl and ranging from 1 to 17 h contact time, no yeast had adhered to the surface. With a higher ionic strength, the amount of adhering yeast increased. For example, at 1 h contact time, the percentages of yeast cell remained stuck to glass were significantly enhanced: 41% at 150 mM, 64% at 180 mM, 75% at 200 mM, 95% at 250 mM, and 100% at 330 mM. In addition, the kinetic parameter was more noticeable in the 150–330 mM range. Indeed, at 150 mM, 41% (1 h), 55% (2 h), and 95% (17 h) of the yeast population were found to adhere. At 180 mM, the percentages were 64% (1 h), 72% (2 h), and 97% (17 h) and finally, at 200 mM, the same increase in the distribution of tethered yeasts was observed: 75% (1 h), 83% (2 h), and 100% (17 h). A range of ionic strengths from 150 to 330 mM was thus identified, where cultured yeast cells adhere to the glass coverslip.

C. Optical-trap-induced detachment of cultured yeast cells: Removal force measurement and detachment statistics

This part of the study concerns cultured yeast cells adhering to glass. After the yeast had adhered, we tried to detach it with the optical tweezer. The laser beam was displaced 10 μm along the x axis and its power was increased (27–120 mW) until the particle was once again put into suspension. When detachment occurred, the force was calibrated by the means of Eq. (3), as described previously. For

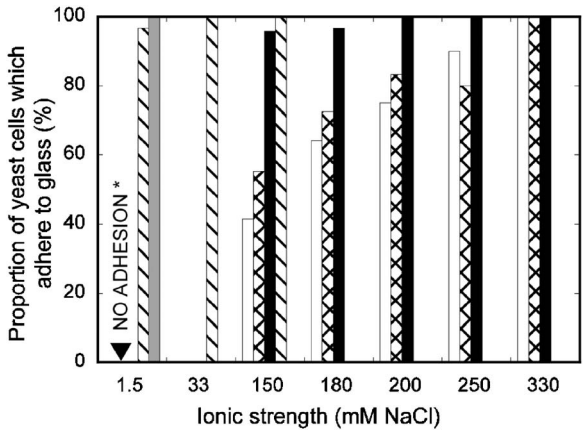

FIG. 4. Proportion of rehydrated (Rh) and cultured (C) yeast cells which adhere to glass for different contact times [rehydrated: 1 h, (▨), and 2 h (▩); cultured: 1 h (□), 2 h (▩), and 17 h (■)] at different ionic strengths (mM NaCl). Note that "no adhesion" of cultured yeast cells occurred for any contact time at 1.5 mM ionic strength. Temperatures measured *in situ* are the following. Rehydrated yeast cells: 25.1 °C (1.5 mM, 1 h); 25.4 °C (1.5 mM, 2 h); 25.6 °C (33 mM, 1 h); and 25.5 °C (150 mM, 1 h). Cultured yeast cells: 25.2 °C (1.5 mM, 1 h); 25.1 °C (150 mM, 1 h); 25.4 °C (150 mM, 2 h); 25.3 °C (150 mM, 17 h); 25.3 °C (180 mM, 1 h); 25.5 °C (180 mM, 2 h); 25.0 °C (180 mM, 17 h); 25.3 °C (200 mM, 1 h); 25.7 °C (200 mM, 2 h); 25.0 °C (200 mM, 17 h); 25.7 °C (250 mM, 1 h); 25.7 °C (250 mM, 2 h); 25.4 °C (250 mM, 17 h); 25.1 °C (330 mM, 1 h); 25.4 °C (330 mM, 2 h); and 25.3 °C (17 h).

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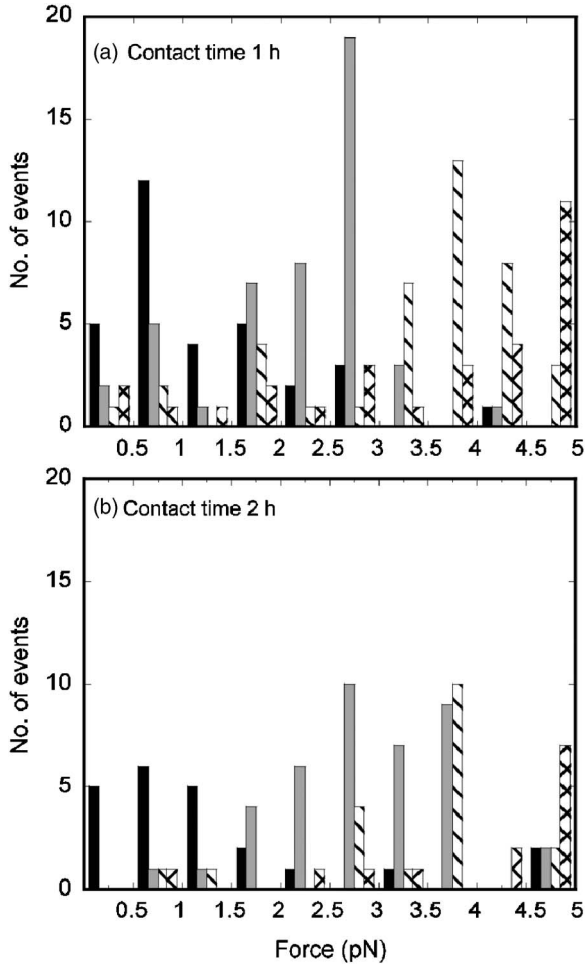

FIG. 5. Histogram showing the distribution of events vs detachment force in the case of cultured yeast cells with contact times of (a) 1 h and (b) 2 h at ionic strengths (NaCl) of 150 mM, (■), 180 mM, (▨), 200 mM, (▧), and 250 mM, (☒). $T_{in\ situ}=(25.5\pm0.5)$ °C.

each successful detachment, the applied force (piconewtons) was measured quantitatively, in the range of 0.2–5 pN, for a given contact time and ionic strength of the suspending medium.

Figures 5(a) and 5(b) present results on 249 detachments within 549 tests at 1 h and 485 tests at 2 h, respectively. We choose to present these results as a variation in the number of detachment events according to the applied force level (piconewtons).

With a 1 h contact time [Fig. 5(a)], four spreading zones are observed, each around a peak. Indeed, at 150 mM, we clearly observed a peak at 0.75 pN. Depending on the ionic strength, other peaks can be observed, such as 2.75 pN (180 mM), 3.75 pN (200 mM), and 4.75 pN (250 mM). In the last case, it seems that the real peak is not visible and may be located around 5.25 pN, by extrapolating the other three similar zones towards a higher force level. At 330 mM, only one detachment occurred with 0.86 ± 0.27 pN. Force levels usually increase with the ionic strength, in the range of 150–250 mM.

With a contact time of 2 h [Fig. 5(b)], the peaks remained at the same force level as previously observed, but occurred less frequently. Indeed, 28% of detachments occurred at 1 h contact time and 20% at 2 h (no detachment occurred at a contact time of 17 h, regardless of ionic strength). Consequently, the decrease in 8% for the number of yeast cells detached from 1 to 2 h contact time confirms the kinetic parameter involved in the adhesion process, in good agreement with the results of Fig. 6. It can also be noticed that these 8% of yeasts stuck to the glass are not detached by the optical tweezer. Thus, the force level has considerably increased.

D. Yeast adhesion on glass: The main mechanisms

In this study, we were also interested in the mechanisms brought into play during the adhesion process. With adhesion without detachment, two types of mechanisms were observed: Either involving highly mobile yeast or involving yeast with reduced mobility. Mobility is defined as the movement of a yeast cell produced by displacing the position of the optical trap in a given direction (translational or rotational displacement). Mobility is found to be influenced particularly by the ionic strength and the contact time. Thus, these mechanisms can be observed with the two aforementioned methods related to horizontal or vertical positioning of the adhesion surface.

1. First method: Surface positioned horizontally [Figure 3(a)]

The cultured and rehydrated yeasts have been found to be very mobile even though tethered to the coverslip, without detachment occurring. These observations suggest us that bonds or local cluster of anchoring sites can mediate adhesion of yeast to glass. Indeed, this mobility allows the particle to be driven around a narrow surface, estimated to be less than 1 μm^2.

Figure 6(a) shows a series of snapshots of a single cultured yeast cell in 150 mM NaCl solution that has adhered for 1 h to a glass coverslip. A circular movement has been applied to the 95 mW optical trap, producing the same movement of the yeast cell. Although the yeast is still adhered, it is extremely mobile and moves 360° around an axis.

Similarly, Fig. 6(b) shows a series of snapshots of a single rehydrated yeast cell in 1.5 mM NaCl solution that has adhered for 1 h to a glass coverslip. The phenomenon of extreme mobility was again observed when we tried to detach the particle with the optical trap at 125 mW. Nevertheless, the ionic force is 100 times weaker than previously. Once again, with the rehydrated yeasts, if the ionic strength is increased, the mobility is considerably reduced. At 150 mM, the yeast remained completely fixed on the surface (these results are not depicted here).

2. Second method: Surface positioned vertically [Figures 1(a) and 3(b)]

It should be recalled that the yeasts used here are rehydrated in saline solution (NaCl, 1.5 and 150 mM).

Figure 7(a) shows four snapshots of a single rehydrated yeast cell in a 1.5 mM NaCl ionic solution, tethered to the glass fragment (surface positioned vertically) at a distance $z=60$ μm from the glass coverslip. With a similar ionic

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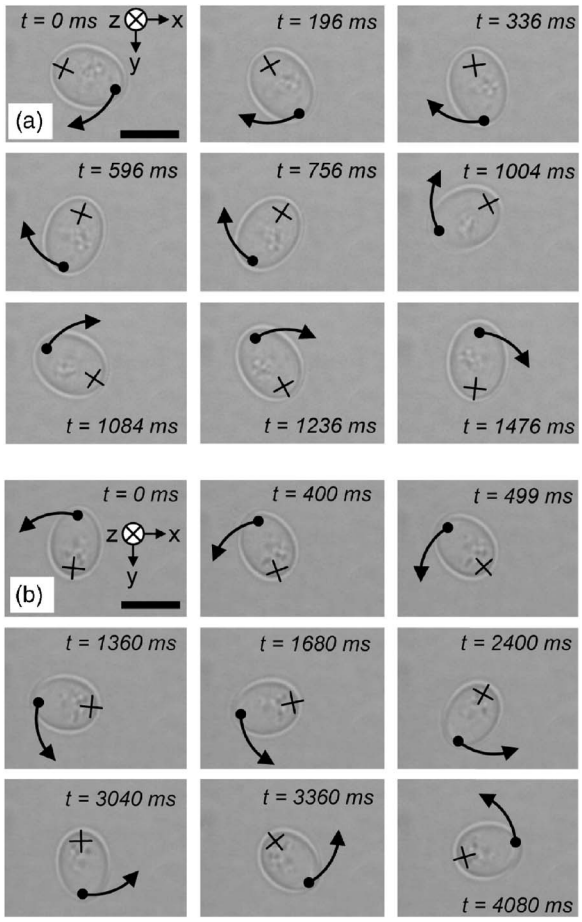

FIG. 6. (a) Images of a single rotating cultured yeast cell tethered to a glass coverslip for 1 h of contact time in a 150 mM, NaCl solution with 95 mW laser power. $T_{in\ situ}$=25.3 °C. (b) Images of a single rotating rehydrated yeast cell tethered to a glass coverslip with 1 h contact time in a 1.5 mM NaCl solution with 125 mW laser power. $T_{in\ situ}$=25.1 °C. The scale bars are 5 μm, the curved arrow indicates the direction of trap rotation, and the black cross shows the approximate center of rotation.

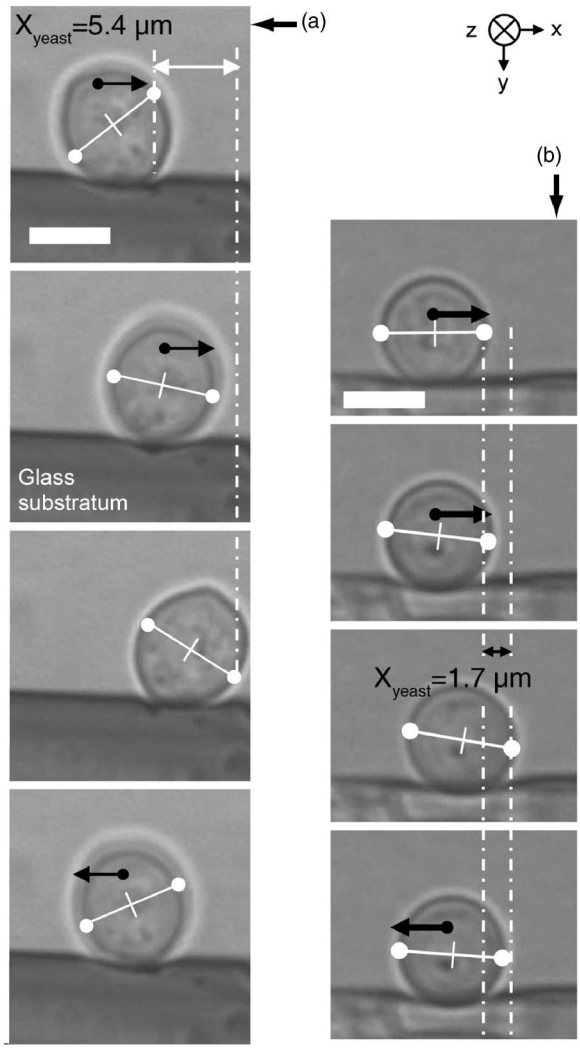

FIG. 7. (a) Images (160 ms apart) of a single 7.1 μm rehydrated yeast cell tethered to a glass fragment with 1 h contact time in a 1.5 mM, NaCl solution with 110 mW laser power at 60 μm from the coverslip. $T_{in\ situ}$=24.8 °C. (b) Images (160 ms apart) of a single 5.6 μm rehydrated yeast cell tethered to a glass fragment with 1 h of contact time in a 150 mM NaCl solution with 120 mW laser power at 55 μm far from the coverslip. $T_{in\ situ}$=24.9 °C. The scale bar is 5 μm and the black arrow indicates the direction of the trap.

strength, this yeast has the same mobility as a rehydrated yeast cell adhering to the glass coverslip [i.e., surface positioned horizontally, see Fig. 6(b)]. The same phenomenon of considerable mobility has thus been found again with this ionic strength and surfaces of a similar nature using different observation sights. A yeast cell displacement of 5.4 μm is observed when the optical trap is moved 10 μm along the x axis. The initial axis is indicated by the vertical white dotted lines.

Figure 7(b) shows a series of four successive snapshots of a single rehydrated yeast cell in a 150 mM NaCl solution, vertically tethered to the glass fragment 55 μm above the coverslip. When trying to remove it by moving the laser beam parallel to the wall, the particle shifts with a difficulty of 1.7 μm from its initial point (the vertical white dotted lines indicate the vertical axis). The low mobility of the adhering microorganism with this ionic strength of 150 mM is thus confirmed.

IV. DISCUSSION

Our experiments have revealed that yeast adhesion is strongly influenced by ionic strength and contact time. These results are in good agreement with those obtained in a shear stress flow chamber. Indeed, Mercier-Bonin *et al.*[5] carried out detachment tests on the same type of rehydrated yeast cells, initially tethered on glass, in NaCl solutions of 1.5 and 150 mM. These authors have confirmed the kinetic parameter of adhesion since the mean force needed to detach 50% of the yeast population has been enhanced by a factor of 5 with contact times increasing from 1 to 15 h. For the cultured yeast cells, Guillemot *et al.*[4] managed to detach 50% of the yeast at 150 mM NaCl, with a contact time of 1 h. The corresponding wall shear stress $\tau_{50\%}$ is 0.05±0.01 Pa, which corresponds to a detachment force of 14.4±2.8 pN for a mean cell radius of 3 μm. For more details about hydrodynamic force, refer to Lorthois *et al.*[22] and other previous works.[4,5] In the present study, under the same conditions of ionic strength and contact time, only 28% of yeast cells have been detached, with a mean force of 0.75 pN [Fig. 5(a)]. Under these experimental conditions, the mean detachment

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force using the shear stress flow chamber, in its present configuration, enables to evaluate detachment forces in the order of 10 pN. Hence, orders of magnitude are the same for the two length-scale observations. Consequently, it can be stated that the two methods of investigation are perfectly complementary.

These results allow us to determine reasonable boundaries for using the optical tweezer to quantify the removal forces of yeasts (about 5 μm in diameter) on the glass surfaces studied. In the case of removal forces above approximately 5 pN, it is not possible to quantify the effect of a given physicochemical condition on yeast adhesion. Nevertheless, for the removal forces below 5 pN, the optical tweezer is a noteworthy and accurate means of identifying differences in yeast adhesion properties, depending on the contact time and the ionic strength of the suspending medium. Indeed, this tool proves to be capable of measuring piconewton forces and therefore probing specific interactions as previously made with the AFM.[23,24] For example, Simpson *et al.*[1] brought a *S. aureus* bacterium into contact with a 10 μm polystyrene sphere coated with specific protein such as fibronectin or fibrinogen, which are often present in the extracellular matrix. The binding forces observed between fibrinogen or fibronectin and *S. aureus* proteins occurred as an approximate integer multiple of 20 or 25 pN, respectively. It could be concluded that N elementary forces were needed to break N anchoring bonds located in a definite zone.

What kind of adhesion mechanism can be produced for yeast cells?

Our experimental observations and force measurements converge towards the definition of an adhesion mechanism involving local binding domains mediating the interaction microorganism/surface. Indeed, Bowen *et al.*[25] have measured force-distance curves between yeast cells and surfaces with AFM. They have demonstrated that there is an adhesion component that is indicative of multiple bond breakage, cell stretching, and a "peeling" of the cell away from the surface. This work has suggested that specific interactions should be taken into account in adhesion processes.

In this study, the same phenomenon of discrete binding sites was assumed. Indeed, it has been seen that ionic strength greatly influenced the mobility of adhering yeast cells. Two ranges of ionic strength can therefore be distinguished, depending on the types of yeast.

(i) In the case of rehydrated yeast cells, it has been observed that, once they have adhered, they can be shifted at 1.5–150 mM NaCl. At 1.5 mM NaCl, the adhering yeasts were very mobile on the surface, so that they can be rotated around an axis by means of the optical tweezer. Figure 6(b) suggests that the existence of an anchoring site can be the basis of this freedom. This result has been confirmed with another glass surface of similar nature in terms of surface energy, but positioned differently [Fig. 7(b)]. The cell has been displaced like a "balloon" hung on the surface and attached by an area estimated to be less than 1 μm^2. A horizontal displacement of $X_p=5.4$ μm is then observed (corresponding to a mobility value non-dimensionalized with the average diameter of the yeast: $X_p^+=0.76$) when the optical trap was moved along the x axis. However, this phenomenon disappears when the ionic strength is increased up to 150 mM NaCl. Mobility becomes very slight and the Brownian movement motion of the cell is then hardly perceptible. This ionic contribution has led to a decrease in electrostatic repulsion, as predicted by the DLVO theory, and favored adhesion, as illustrated by Fig. 7(b). Once the yeast cell adheres, it sticks strongly to the substrate and the mobility is reduced to a displacement $X_p=1.7$ μm (corresponding to $X_p^+=0.30$ as dimensionless mobility using the average diameter) when the optical trap is moved along the x axis.

(ii) The cultured yeast cells do not adhere at 1.5 mM NaCl. Adhesion becomes easier when the ionic strength is increased, with a considerable mobility observed at 150–330 mM NaCl. Figure 6(a) highlights the phenomenon of anchoring sites or quasispecific adhesion zone since it was possible to rotate it around an axis. At 330 mM, no further mobility was detected, confirming the results of Fig. 6(a). By increasing the ionic strength of the medium, adhesion becomes stronger (see Fig. 4). Detachment force levels investigated with the optical tweezer confirms this result [Fig. 5(a)], demonstrating that the translation of the peak towards increasing ionic strength is possible. Thus, these results are in agreement with the DLVO theory, which describes the energy of interaction between two charged surfaces in a polar medium (such as water) 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 potential and of the electrostatic repulsion (for more details see Ref. 26). Increasing the ionic strength decreases the double-layer thickness, which results in lower electrostatic repulsion and significantly enhanced adhesion.

Regardless of the materials employed, various authors have highlighted the influence of the electrostatic contribution to the process of yeast adhesion. For example, while focusing on the adhesion of *Candida* species on polystyrene, charged negatively like glass, Klotz *et al.*[27] and Gallardo-Moreno *et al.*[28] have concluded that an increase in ionic strength leads to a rise in the percentage of adhering cells.

According to Fig. 4, adhesion is influenced both by contact time and ionic strength. As seen previously, yeasts control the osmotic pressure within the cell and consequently the solute concentration in the close vicinity of the cell wall, in the extracellular medium.[12] In addition, the yeast controls the selective transport of cations and anions by specific channels through the plasma membrane.[8,13,14] In this way, the ionic strength imposed on the suspending medium may be involved in ion transport. This phenomenon can be linked to zeta potential measurement carried out on the yeast cell wall by AFM.[23] Indeed, these authors were able to demonstrate the heterogeneous distribution of adhesion force and charge

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of the outer cell wall surface to within 15 nm, depending on the *p*H. In the present case, there was greater heterogeneity at a *p*H of about 5–6. The DLVO theory should be confirmed if the yeast cell transported ions and water favoring lower electrostatic repulsion (i.e., a reduction in the double-layer thickness). Moreover, Ahimou *et al.*[23] have shown that the outer surface of the cell wall is charged heterogeneously. There appear to be local zones where there is less repulsion, thus promoting adhesion.

Another observation deserves attention. Yeasts are living microorganisms that secrete extracellular proteins. Reynolds and Fink[7] have first demonstrated that *S. cerevisiae* required Flo11p, a member of a large family of fungal cell surface glycoproteins, in order to attach to plastic. Mercier-Bonin *et al.*[5] have provided possible information concerning the salting out of components, in particular, proteins, in the extracellular medium, due principally to cell lysis. These macromolecules thus appear to take part in the formation of a conditioning film that modifies the physicochemical properties and thus influences microbial adhesion. The possible secretion of macromolecules such as adhesins on the outer layer of the cell wall can contribute to the adhesion phenomenon. Various environmental factors may trigger this process. The yeast cell evolves according to external stresses. Flocculation is the best evidence[11,15] since it seems to protect yeasts within the floc against a hostile environment. The yeast cell can then mobilize these proteins to secure its own survival.

Then, binding site locations can be confirmed notably by combining adhesin secretion with local physicochemical interactions at the cell wall level. Decavé *et al.*[29,30] have applied a peeling model on nondeformable *S. cerevisiae* cell in hydrodynamic flow conditions. They described adhesive bonds distributed over the entire cell wall surface as springs with a stiffness k. At this level, adhesion is molecular and discrete.

These phenomena of glycoprotein secretion and solute transport partly explain the dynamic features of adhesion and especially its kinetics, as a characteristic time must exist. However, these processes, which are coupled, may also interfere or even compete with one another. It would be interesting to know whether they stimulate or inhibit one another when the ionic strength of the medium varies: Do aqueous solutes in the suspending medium play a part in stimulating or inhibiting secretion depending on their concentration?

This paper has demonstrated that ionic strength influence and kinetics parameters are determinant to yeast adhesion mechanisms. The use of an optical tweezer as a probe has revealed these influences when the first steps of adhesion are close to weak force levels. To focus on these experiments at the initial deposition, i.e., contact time below 1 h and close to a few seconds, theories as DLVO's and extended DLVO's can be implemented and described in a future study.

V. CONCLUSION

In this study, in the purpose of characterization of adhesion of an individual microorganism on a surface, an optical tweezer device has been constructed in order to handle baker's yeast during its adhesion to glass and to measure the force required to remove it.

This work, performed with rehydrated and cultured yeasts, has been focused on two key adhesion parameters: The ionic strength of the suspending medium and the yeast/glass contact time. Firstly, the percentage of cells of both types of yeast adhering to the glass was found to increase significantly with ionic strength, which can be explained by the fact that electrostatic repulsion weakened. Secondly, for long contact times (up to 17 h), the cells stuck more strongly to the glass plate which could be related to the release of macromolecules such as proteins into the extracellular medium.

In this study, two distinct experimental methods have been set up, referring to horizontal or vertical positioning of the glass surface. Both methods have led to similar conclusions concerning the yeast-surface anchoring mechanisms. The two methods have revealed various mechanisms that confirm the influence of ionic strength on the adhesion process. For short time periods (1 h) at weak ionic strength (1.5 mM for rehydrated yeasts and 150 mM for cultured yeasts), the yeast cells handled by the optical trap remained tethered by tiny cell wall surface areas ($<1\ \mu m^2$). Possibilities of movement of the particle are significant, as it could be seen when it was trapped. For higher ionic strength levels (150 mM for rehydrated yeasts and 330 mM for cultured yeasts), the particle was found to lose its freedom of movement and remained anchored to the surface.

The two matching methods should allow investigation of various metal surfaces, which are of course nontransparent for the profile sighting method, as well as polymeric surfaces, which are transparent and will replace the glass coverslip. The present study, performed with a simplified yeast/glass model system, can be extended to more realistic conditions concerning the choice of microorganisms and support media.

ACKNOWLEDGMENTS

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APPENDIX A: OPTICAL TRAPPING AS A NONINTRUSIVE TECHNIQUE FOR HOLDING MICRON-SIZED LIVING OBJECTS

Since light exerts a force on a refractive microsized particle, it is worth asking whether it could have secondary damaging effects on the particle in question. Laser-induced heating may occur, and also radiation, which is assumed to cause irreversible damage to the biological particle. These effects are related to the beam wavelength and also to the exposure time, the biological nature of the object, and its

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TABLE IV. Bibliographical review concerning irradiation effects on living/nonliving trapped objects.

Authors	Laser	Wavelength	Material	Laser power	Time of trapping	Effect
Vorobjev *et al.* (Ref. 31)	Argon ion	700–840 nm	Chromosomes in mitotic rat kangaroo *Potorous tridactylus* cells	130 mW	0.3 s–5 min	Minimal sensitivity to 700 and 800–820 nm
Liu *et al.* (Ref. 32)	Nd:YAG cw[a]	1064 nm	Chinese Hamster ovary cells (CHOs)/liposome vesicles	40–250 mW	10 s	$+(1.15\pm0.25)\,^{\circ}\mathrm{C}/100$ mW $/+(1.45\pm0.15)\,^{\circ}\mathrm{C}/100$ mW
Liu *et al.* (Ref. 33)	Nd:YAG cw/PM[b]	1064 nm	CHOs/motile human sperm cells	0–400 mW	10 min max (CW)/5 min max (PM)	Loss of viability >2 min (300 mW, cw) DNA denaturation-(PM, increase of 100 °C)
Neuman *et al.* (Ref. 34)	Nd:YAG cw	790–1064 nm	*E. coli*	100 mW	8 s/2 s (trap/release)	min at 830 and 970 nm max at 870 and 930 nm
Leitz *et al.* (Ref. 35)	Argon ion	700–850 nm	*Caenorhabditis elegans*	240–480 mW	30–240 s	min for 120 s and 240–460 mW max for 120 s at 240 mW and 810 nm
Peterman *et al.* (Ref. 36)	Nd: YVO_4	1064 nm	Silica and polystyrene micron-sized beads in glycerol or water	100 mW	···	$+0.8\,^{\circ}\mathrm{C}/100$ mW
Singh *et al.* (Ref. 37) Volpe *et al.* (Ref. 38)	···	785 nm	*Saccharomyces cerevisiae*	5 mW	3 h	Bud growth of trapped yeast Metabolic activity monitored by Raman microspectroscopy

[a]cw, continuous wave.
[b]PM, pulsed mode.

suspending medium. The nonintrusive optical tweezer technique is therefore discussed and the conditions for minimizing such effects have been highlighted here from the literature data,[31–38] see Table IV.

This nonexhaustive bibliographical review reported in Table IV enables us to venture some conclusions concerning laser irradiation effects on our system and noticeably to consider that heating and photodamage are trivial in the case of all the measurements made in the present study for the following reasons.

(i) The selected wavelength was tuned to minimize laser damage to the particle [up to $1.5\,^{\circ}\mathrm{C}/100$ mW (Ref. 32)] and aqueous suspending medium [approximately $0.8\,^{\circ}\mathrm{C}/100$ mW (Ref. 33)].
(ii) Laser power did not exceed 130 mW in our configuration.
(iii) The aqueous suspending medium allowed slight absorption of the radiation [$\alpha\sim0.1\ \mathrm{cm}^{-1}$ at 1064 nm and $0.0219\ \mathrm{cm}^{-1}$ at 810 nm (Ref. 39)] and generated negligible variations in viscosity.
(iv) The exposure time did not exceed 120 s.
(v) A yeast cell was trapped only once in each test. The same yeast cell was trapped again later.

The literature provided precious evidence for highlighting the damaging effects of the optical trap on a living cell and for creating all the necessary conditions for minimizing such damage. During the experiments, we did not observe any major qualitative changes in the trapped yeast cell or its environment. Moreover, a test was carried out with the yeast being held in the optical trap for an excessively long trapping time of 15 h. Throughout this test, no general modification in shape (budding, wall scission) was detected, nor was there any nucleation of air bubbles or increase in Brownian movement.

APPENDIX B: METHODS AND ASSUMPTIONS OF FORCE DETERMINATION

1. Trap force measurement technique

The purpose of this work is to determine precisely the force exerted by the laser on the yeast cell (F_{trap}) when removal occurs. To do this, when yeast is detached from the glass substratum, the living cell is moved away from it by displacing the laser's focal point along the z axis to a given distance z from the coverslip. This force is calibrated as follows:[1,40,41] the laminar flow is generated in the suspending medium around the yeast cell, which is kept motionless by the laser beam. In the case of micron-sized objects, the Reynolds number, defined by $\mathrm{Re}=VD\rho/2\eta$, was less than 10^{-2} [V is the fluid velocity ($\mathrm{m\,s}^{-1}$), D the mean diameter of the particle (m), ρ the density of the particle ($\mathrm{kg\,m}^{-3}$), and η the viscosity of the suspending medium (Pa s)]. The micropress maintaining the sealed chamber, in fact, received a triangular

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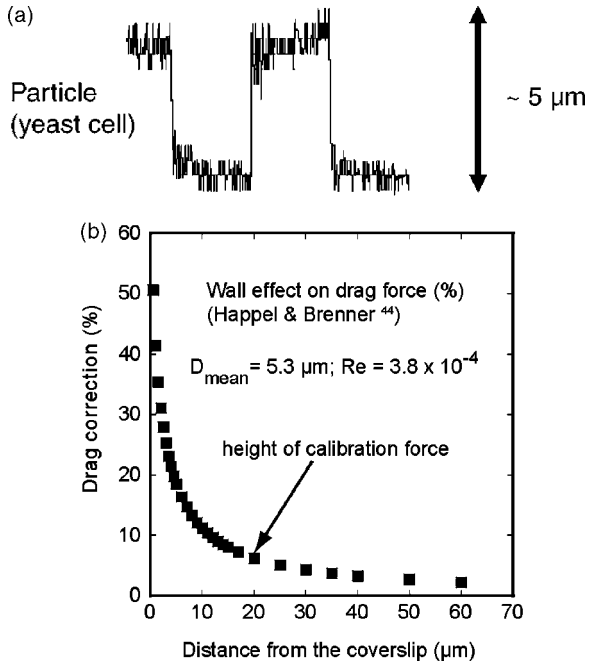

FIG. 8. (a) Response of a trapped bead to a triangular wave input to the microscope stage position. The bead shows a square wave response corresponding to the viscous force and thus to the constant velocity of the medium. (b) Wall effect on drag force in the case of a 5.5 μm mean diameter cultured yeast cell vs distance from the glass coverslip [see Happel and Brenner (Ref. 42)].

electric signal resulting in a single-direction movement of amplitude A_0 of 21.3±0.1 μm. The movement of the yeast cell induced by the flow of the suspending fluid was then located by the quadrant photodiode detector. The signals corresponding to the movement of the micropress-chamber system and of the yeast cell induced by the fluid flow are displayed in Fig. 8(a). According to this figure, the distance between the beam focal point and the yeast cell center is constant, so it may be concluded from each backward and forward motion that the steady flow was achieved. Indeed, the response of the particle to this triangular temporal wave was converted into a backward and forward motion such as a square temporal wave corresponding to the viscous force and thus a constant speed of the medium.

As soon as the steady flow is achieved, two forces are in opposition during displacement in a given direction: the springlike force F_{trap} of the optical trap and the drag force F_{drag} the fluid exerts on the yeast [see Fig. 2(a)]:

$$F_{\text{drag}} = \frac{6\pi\eta D A_0 f}{K(r,h)}, \tag{B1}$$

with f the frequency (Hz) of the movement applied by the micropress, η the dynamic viscosity of the medium (Pa s), D the mean diameter of the yeast (μm) measured directly on the screen with a calibrated slide, and K an adjustment factor due to wall effects, stemming from Faxén's laws.[42] Before carrying out the detachment experiments, it is advisable to explore the parameters that influence force measurement in the calibration. Optical force assessment depends only on the force measurement throughout the escape assay.

2. Force measurement conditions and validation of measurement assumptions

The assumption of rigid particles was checked according to Smith *et al.*[9] and our observations. Indeed, according to the latter, the cell wall of the yeast considered at the stationary growth phase presented an average surface modulus of 12.9±0.7 N/m (corresponding to 12.9×10^3 pN for 1 nm of deformation) and Young's modulus of 107±6 MPa. They provide us an order of magnitude of cell elasticity, which tends to be big enough to validate Stokes drag assumptions. In addition, the particle did not become visibly deformed during the handling and calibration assays. Consequently, the elasticity of the yeast cells is considered to be negligible in the case of the stresses imposed and is therefore not taken into account in this force calculation.

A further requirement was to work in a diluted medium (yeast cell concentration of 4×10^6 cells/ml). Indeed, if tethered yeast cells were present between the laser and the trapped yeast cell, the optical force could be somewhat affected. Besides, wall effects were significant: At less than one diameter from the wall (about 5 μm), measurement took into account the effects of depletion and surface flatness. Calculations were carried out on the basis of Faxén's law[42] and revealed [see Fig. 8(b)] that wall effects became negligible beyond 20 μm from the coverslip. Consequently, all force measurements will be gauged at a distance $z=20$ μm from the coverslip.

3. Escape force determination

With a given power P_{laser}, force calibration was carried out while varying the frequency of the imposed signal and thus the velocity of the suspending fluid around the yeast cell. The frequency was gradually increased up to a threshold value, beyond which the drag force became greater than the optical-trap force. The fluid ejected the particle from the trap. The expulsion was detected in two ways. (a) Visually, it was observed that beyond a certain fluid velocity, the particle was extracted from the trap by the fluid and left the camera field; this method is the most rapid and reliable. (b) The distance separating the optical-trap center and the yeast cell center during flow was measured with the quadrant photodiode detector. We saw previously that in steady flow, this distance was constant. The escape force was reached when the position of the particle, located by the quadrant photodiode sensor, left the geometrical limits of the diode surface. This escape force thus revealed the maximum elastic recall force which the laser exerts on the particle. Through these calibration tests, the optical force F_{trap} could be plotted according to the power P_{laser} of the laser beam. The maximum force exerted on the yeast cell by the laser depending on the laser power at the objective end is shown in Fig. 9(a) (rehydrated yeast cells) and Fig. 9(b) (cultured yeast cells). The error bars of these two graphs correspond to the uncertainty in measuring the mean diameter D of the yeast cell (0.3 μm), the amplitude A_0 of particle displacement (0.1 μm), and the threshold frequency f (0.05 Hz). This diagram is specific to a given suspending medium and type of yeast cell.

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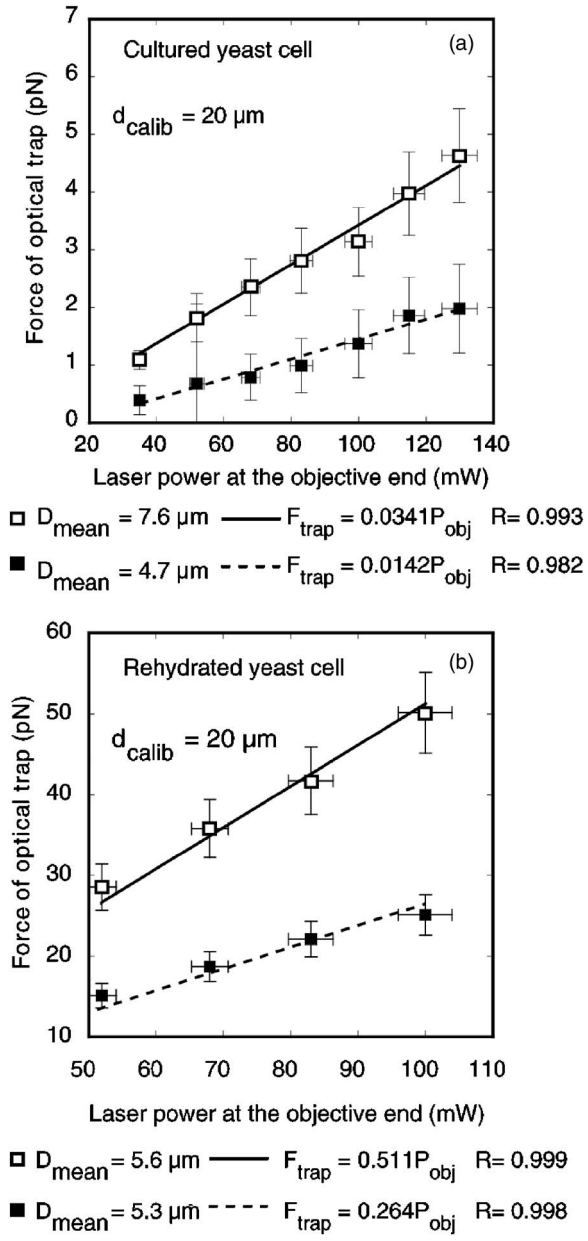

FIG. 9. Force of optical trap (pN) obtained with calibration measurement vs power at the objective end (mW) with (a) rehydrated yeast cells of 5.29 and 5.60 μm mean diameters respectively, $T_{in\ situ}$=25.2 °C and (b) cultured yeast cells of 4.7 and 7.6 μm mean diameters respectively, $T_{in\ situ}$ =24.9 °C. The height of calibration from the coverslip is fixed at 20 μm.

4. Dependence of trapping ability on type of yeast (rehydrated or cultured)

It was observed that the relations between the optical-trap force and laser power are linear. The directing coefficient of this line has often been quoted in the literature, in particular, by the optical tweezer pioneer Ashkin.[16] In the Mie regime ($D > \lambda$), optical forces are defined by the following relationship:

$$F_{\text{trap}} = \left(\frac{n_{\text{medium}} P_{\text{obj}}}{c} \right) Q, \tag{B2}$$

in which Q is a dimensionless efficiency depending on the optical parameters of the particle and the medium, n_{medium} is the refractive index of the suspending medium, c is the speed of light (m s^{-1}), and P is the incident laser power measured at the lens end (mW). The Q coefficient is also quoted by Svoboda and Block[40] as being a parameter that reveals the optical tweezer's ability to exert a force. Indeed, in his case, Ashkin[16] determined a factor Q of 0.30 for dielectric spheres such as polystyrene. The theoretical predictions of Wright *et al.*[43] on the other hand are dissimilar since they announce a value of Q=0.055 for 10 μm sized dielectric particles. In their work, Ghislain *et al.*[44] indexed Q values for polystyrene spheres of various diameters and silica spheres. For a diameter of 6.1 μm, they determined a Q value of 0.20 at a calibration distance of 8.3 μm from the wall and a power of 60 mW at the objective end.

In our case, the Q value, calculated with Eq. (2), Figs. 9(a) and 9(b), depends on the yeast preparation method. With rehydrated yeast cells, the Q value comes close to that given by Ghislain *et al.*[44] since it is 0.059–0.115, corresponding to forces of 0.26–0.51 pN/mW with particles of 5.3 and 5.6 μm in mean diameter, respectively. With cultured yeast cells, the Q value is 0.0032–0.0077, corresponding to forces of 0.014–0.034 pN/mW with particles of 4.7 and 7.6 μm in mean diameter, respectively. A factor of 10 is found between the optical forces for the two types of yeast. Indeed, this Q value encloses the optical parameters specific to the particle considered, in particular, its refraction index. The yeast population is thus dispersed in terms of size (a deviation of 4 μm maximum in both types of yeast) and also in terms of optical properties, conferring a singularity to the optical-trap/particle system.

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