Effect of high shear stress on microbial viability

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Abstract: Escherichia coli and Saccharomyces cerevisiae suspensions were submitted to controlled shear stress. Above a threshold value shear stress induced a decrease in micro-organism viability. The threshold of shear stress efficiency depended on the micro-organisms, being between 1292Pa and 2770 Pa for S cerevisiae, and about 1250 Pa for E coli. Above 1810 Pa, Ecoli cells were disrupted whereas the S cerevisiae cells remained intact. The higher the cellular concentration, the greater the rate of decrease in viability. Viability loss was influenced by the number of passages through the experimental shear stress device and by exposure time.

Keywords: shear stress; viability; *Saccharomyces cerevisiae*; *Escherichia coli*

NOTATION

D	Inner diameter of the capillary (m)
L	Capillary length (m)
N_n	Number of passages through the capillary
ΔP	Pressure drop over the capillary ends (Pa)
Q	Volumetric flow rate ($m^3 s^{-1}$)
Δt_i	Time between two samplings (s)
Δt_i^{exp}	Duration of exposure to shear stress between two samplings (s)
T_n	Total exposure time (s)
V_{cap}	Inner volume of the capillary (m^3)
V_c	Circulating volume of the suspension (m^3)
μ	Dynamic viscosity (Pas)
τ_{MAX}	Maximal shear stress (Pa)

1 INTRODUCTION

Large scale cultivation of micro-organisms is becoming increasingly important for a variety of purposes. In order to process microbial cells, it is necessary to provide agitation to maintain them in suspension. The major drawback of such handling is that cells inevitably encounter stresses due to fluid dynamic effects. The influence of hydrodynamic forces on micro-organisms is of great interest in the development of scaled up bioreactor systems.

Shear stress may induce morphological variations,[1,3] lysis,[3,4] and changes in metabolism[5,6] in both animal and plant cells.[7] A review of the existing literature shows that the threshold of tolerance to shear stresses is not well understood for micro-organisms: in the range from 0 to 80 Pa, some morphological changes have been observed for fungi and bacteria,[5,8] and reduced resistance to freezing has been observed for bacteria.[9]

There are no studies on the effect of controlled shear stresses on yeasts. The available data only describe damage occurring to budding yeast cells under mechanical stress such as that provided by agitation.[10,11] The mechanism of killing of yeasts in suspension by ultrasound was also studied.[12]

The main purpose of the present work was to determine the resistance to shear stress of two micro-organisms (the Gram negative bacterium, *Escherichia coli* and the yeast, *Saccharomyces cerevisiae*), under controlled shear conditions. The study also assessed the effects of high values of controlled shear stress, which has not been previously reported.

2 MATERIALS AND METHODS

2.1 Micro-organisms and growth conditions

Chemicals and other media components were purchased from Prolabo (Fontenay-sous-Bois, France).

2.1.1 *The yeast* Saccharomyces cerevisiae

S cerevisiae (strain UG5, Institut National Sciences Appliquées Collection, Toulouse, France) was grown in Erlenmeyer flasks in 200 cm^3 of the following medium: yeast extract, 5 g dm^{-3}; $MgSO_4$, 0.4 g dm^{-3}; $(NH_4)_2SO_4$, 2 g dm^{-3}; KH_2PO_4, 5 g dm^{-3}; glucose, 50 g dm^{-3}. The pH was adjusted to 4.0 with H_3PO_4 (15 mol dm^{-3}). Cultures were incubated for 22 h at 30 °C with stirring (250 rpm).

2.1.2 *The Gram negative bacterium* Escherichia coli

E coli (stain 188, Institut National Sciences Appliquées Collection, Toulouse, France) was grown in Erlenmeyer flasks in 200 cm^3 of the following medium: yeast

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extract, 5 g dm^{-3}; $MgSO_4$, 1 g dm^{-3}; NaCl, 5 g dm^{-3}; glucose, 50 g dm^{-3}; papaïn1c peptone soya, 10 g dm^{-3}. Cultures were 1ncu1ated for 22 h at 30 °C w1th st1rr1ng (250 rpm).

2.2 Determination of cell numbers and viability

Cell num1ers were measured 1y count1ng under a m1croscope us1ng two d1fferent haemocytometers: a Thoma sl1de for yeast and a Pet1t Salum1en1sl1de for 1acter1a.

The 11a1111ty of yeasts was determ1ned us1ng the methylene 1lue sta1n1ng method[13] and the1acter1a were o1ser1ed us1ng ep1fluorescent m1croscopy.[14]

2.3 Viscosity measurements

The 11scos1ty of m1cro-organ1sms suspens1ons was measured 1y us1ng a Couette11scometer (Epprech-Rheomat 15).

2.4 Shear stress device

2.4.1 Principle

Cell suspens1ons were su1m1 tted to shear stress1 y flow1ng through a narrow cap1llary.1 ns1de the cap1llary, shear stresses are generated1 y the flu1d flow as a rad1al funct1on: shear stress1 s n1l along the long-1tud1nal ax1s and1 s max1mal near the cap1llary wall. The max1mum1 ntens1ty of shear stress, τ_{max} can1 e expressed us1ng the follow1ng formula:

$$\tau_{max} = \frac{D}{4}\frac{\Delta P}{L} \quad (1)$$

where ΔP is the pressure drop over the capillary ends, L the capillary length and D the capillary diameter.

2.4.2 Shear stress device

The shear stress device used, based on Poiseuille laminar flow, has been described elsewhere.[15] Stirred cell suspensions (20 cm^3) were pumped (Gilson: piston pump: 100 SC and manometric module: 805) through the capillary. The pump characteristics were: maximum volumetric flow rate of 1.67×10^{-7} m^3 s^{-1} and maximum pressure set up at 6×10^7 Pa.

Three capillaries were obtained from Leguellec (BP 134, 29174 Douarnenez, France). The determination of their average inner diameter was carried out according to the Poiseuille law:

$$D^4 = \frac{128\mu QL}{\pi \Delta P} \quad (2)$$

where μ is the dynamic viscosity of the suspension and Q the volumetric flow rate.

The calculated diameters of the capillaries were: 183×10^{-6} m, 108×10^{-6} m and 96×10^{-6} m, and the length of the capillaries was 5×10^{-2} m, 20×10^{-2} m or 40×10^{-2} m.

In order to expose the micro-organisms to shear stresses during a significant period of time, the cell suspension was returned to the Erlenmeyer flask at the exit of the capillary; hence the cells were recycled. Experiments were performed at 20 °C. The repeated sampling during each experiment decreased circulating volume. Data from these experiments are presented in Table 1.

2.4.3 Calculation

Between two successive samples $(i-1)$ and (i) separated by duration Δt_i, the total volume (V_i) of the suspension remained constant. Thus, between these two samples, cells were submitted to shear stresses for a duration (Δt_i^{exp}) given by:

$$\Delta t_i^{exp} = \frac{V_{cap}}{V_i}\Delta t_i \quad (3)$$

where V_{cap} is the inner volume of the capillary.

The total duration of the exposure (T_n) to shear stresses for the cells in the sample (n) was:

$$T_n = V_{cap}\sum_{i=1}^{n}\frac{\Delta t_i}{V_i} \quad (4)$$

The average passage number (N_n) through the capillary for the sample (n) was:

$$N_n = \frac{T_n}{V_{cap}/Q} \quad (5)$$

Run	Micro-organism	Capillary diameter ($\times 10^{-6}$ m)	Volumetric flow rate ($\times 10^{-8}$ m^3 s^{-1})	Capillary length ($\times 10^{-2}$ m)	Cell number ($\times 10^6$ cells cm^{-3})	Shear stress (Pa)
1	*S c*	183	11.7	20	161	290
2	*S c*	108	11.7	20	153	1292
3	*S c*	96	15.0	20	190	2770
4	*S c*	96	15.0	20	101	2620
5	*S c*	96	15.0	20	186	2770
6	*S c*	96	16.2	5	119	2450
7	*S c*	96	13.3	5	288	2350
8	*E c*	183	16.7	40	1200	450
9	*E c*	96	8.3	5	1200	1250
10	*E c*	96	11.7	5	1200	1810

Table 1. Exposure of micro-organisms to shear stress

Saccharomyces cerevisiae: S c; Escherichia coli: E c.

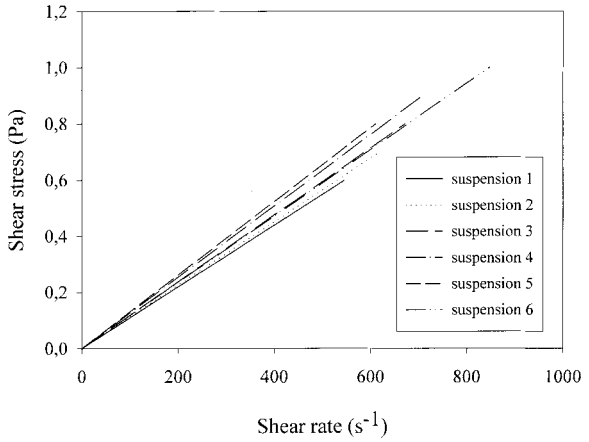

Figure 1. Rheogram of the micro-organisms' suspensions. 1, *S cerevisiae* at 19×10^6 cells cm^{-3}; 2, *S cerevisiae* at 143×10^6 cells cm^{-3}; 3, *S cerevisiae* at 185×10^6 cells cm^{-3}; 4, *S cerevisiae* at 228×10^6 cells cm^{-3}; 5, *S cerevisiae* at 281×10^6 cells cm^{-3}; 6, *E coli* at 1200×10^6 cells cm^{-3}.

3 RESULTS AND DISCUSSION

3.1 Preliminary results

3.1.1 Rheology of the cellular suspensions

The Poiseuille formula is valid only under specific conditions and it can be applied only to Newtonian fluids. As the temperature may influence the flow characteristics, each rheogram was established at 20 °C (Fig 1). All the suspensions tested behaved as Newtonian fluids, whatever the concentration.

All suspension viscosities are presented in Table 2. Measured values ranged from 1.10×10^{-3} Pa s to 1.31×10^{-3} Pa s, close to the viscosity of water.

3.1.2 Validation of the experimental set-up

Experiments were also performed without any capillary in order to determine the effect of pumping and flowing on the two micro-organisms. The highest flow and the highest cellular concentration studied by stress experiments were tested. It appeared that neither lysis nor decrease of viability occurred for *S cerevisiae* after 3 h, which corresponded to 90 passages through the pump.

Similar tests showed a non-significant 2% drop of *E coli* viability (compared with the experimental error of the method used for the determination of viability[14]). Neither lysis nor morphological change occurred during this preliminary experiment.

Table 2. Viscosities of micro-organisms suspensions

Rheogram number	*Micro-organism*	*Cell concentration* ($\times 10^6$ *cells cm*$^{-3}$)	*Viscosity* ($\times 10^{-3}$ *Pa s*)
1	*S c*	19	1.10
2	*S c*	143	1.13
3	*S c*	185	1.19
4	*S c*	228	1.27
5	*S c*	281	1.31
6	*E c*	1200	1.18

Saccharomyces cerevisiae: S c; Escherichia coli: E c.

According to these results, it was considered for the following experiments that only a viability decrease higher than 2%, clear cut lysis or morphological changes would be significant and could be related to shear stress effect. The number of passages through the pump was less than 90 for all experiments.

3.2 Effect of high shear stress on *Saccharomyces cerevisiae*

3.2.1 Effect on the cellular concentration

Shear stress did not affect cell numbers. Yeast cells were not disrupted, even at the highest shear stress value (2770 Pa).

3.2.2 Effect on the viability of yeasts suspensions

The influence of the exposure time (T_n) at three intensities of shear stress is illustrated in Fig 2.

Up to shear stress values of 1292 Pa there was no significant effect on yeast viability.

When *S cerevisiae* was submitted to 2770 Pa shear stress, viability decreased rapidly as the duration of exposure to shear stress increased. Replicate experiments (duplicate), showed good reproducibility. There was clearly an intensity threshold above which the generated stress affected the viability of yeasts. The experiments described above indicate that the threshold should be set above 1292 Pa and below 2770 Pa but the experimental conditions did not allow the refining of its value.

The concentration of yeast cells was an important factor in the decrease of viability (Fig 3). The higher the cell concentration, the greater the decrease in viability, for a quasi-constant intensity of shear stress.

To study the effect of the repeated passages of microbial cell suspension through the capillary, experiments were performed with two capillaries of different length, for a constant intensity of shear stress and a constant cellular concentration (Fig 4). For the same exposure time, the number of passages can be modified by changing the capillary length (ie: $T_n = 100$ ms, $N = 10$ for $L = 20 \times 10^{-2}$ m and $N = 40$ for $L = 5 \times 10^{-2}$ m). For the same exposure time (T_n)

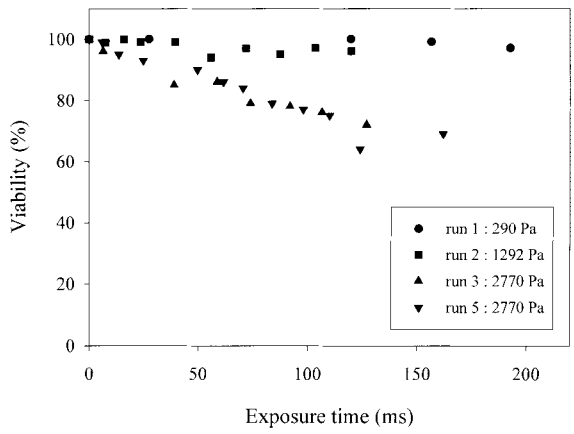

Figure 2. Effect of shear stress intensity on the viability of *S cerevisiae* for a quasi-constant concentration 177×10^6 cells cm^{-3}.

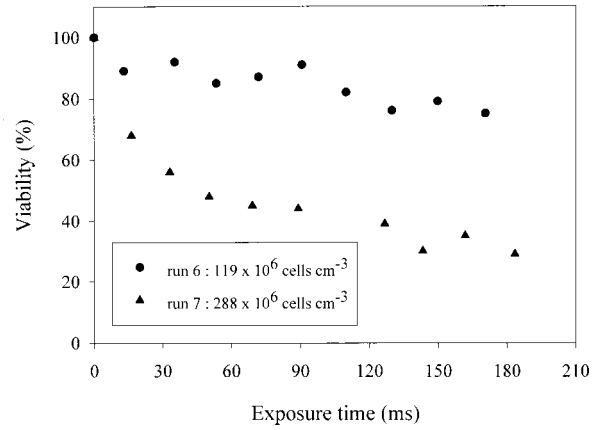

Figure 3. Effect of cell number on the viability of *S cerevisiae* for a constant shear stress intensity (2770 Pa).

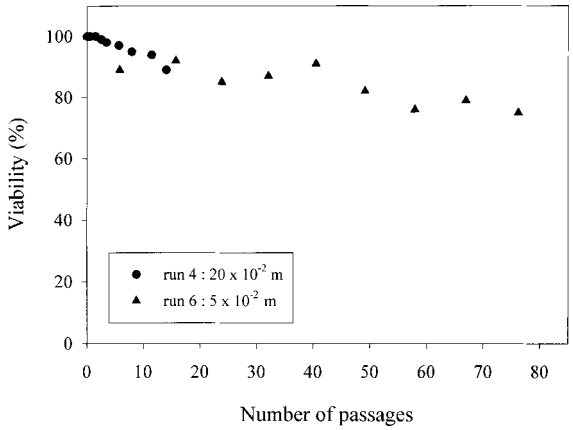

Figure 5. Effect of the number of passages on *S cerevisiae* viability at quasi-constant shear stress (2620 Pa and 2450 Pa) and quasi-constant cellular concentration (101×10^6 cells cm^{-3} and 119×10^6 cells cm^{-3}).

the viability was significantly higher within the capillary of 20×10^{-2} m length than within the 5×10^{-2} m capillary. According to these results, the number of passages through the capillary seemed to be more important than the total exposure time. The relationship between loss in viability and number of passages is shown in Fig 5. For a fixed number of passages, the loss of viability was constant, whatever the length of the capillary.

3.3 Effect of high shear stress on *Escherichia coli*

The viability of three suspensions of *E coli* submitted to shear stress was studied (Fig 6).

When *E coli* was submitted to 450 Pa shear stress, no decrease in viability was observed. For 1250 Pa shear stress, a decrease in viability of 10% was observed after 30 passages through the capillary, but there was no lysis. Nevertheless, after the same number of passages at 1810 Pa, viability decreased by 80% with cell lysis.

These experiments showed, as previously observed for *S cerevisiae*, that there was a threshold above which shear stress affected the viability of *E coli*. This value was probably above 1250 Pa since only a small decrease in viability was observed during the exposure of *E coli* to such shear stress.

4 CONCLUSION

The intensity threshold of shear stress was greater than 1292 Pa for *S cerevisiae* and close to 1250 Pa for *E coli*. The shear stress threshold was characteristic of the micro-organism.

Lysis of yeast cells was not observed even at the highest shear stresses of 2770 Pa. *S cerevisiae* was more resistant than *E coli*, in confirmation of data already published.[16] This study, however, determined the ranges of tolerance to shear stress for the two micro-organisms studied. The tensile strength of walls or membranes confers rigidity to the cells and thus tolerance to shear stress. *E coli*, as a Gram negative bacterium, has only a thin peptidoglycan layer of 2×10^{-9} m whereas the wall of *S cerevisiae* has a relatively thick layer of glucan and mannan. It is then not surprising that *E coli* is less resistant to shear stress than *S cerevisiae*.

The same concept of intensity threshold was illustrated in studies on the use of ultrasound to

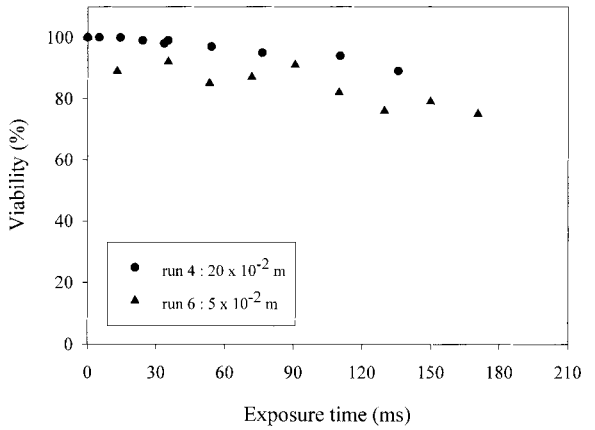

Figure 4. Effect of the capillary length on *S cerevisiae* viability at quasi-constant shear stress (2620 Pa and 2450 Pa) and quasi-constant cell number (101×10^6 cells cm^{-3} and 119×10^6 cells cm^{-3}).

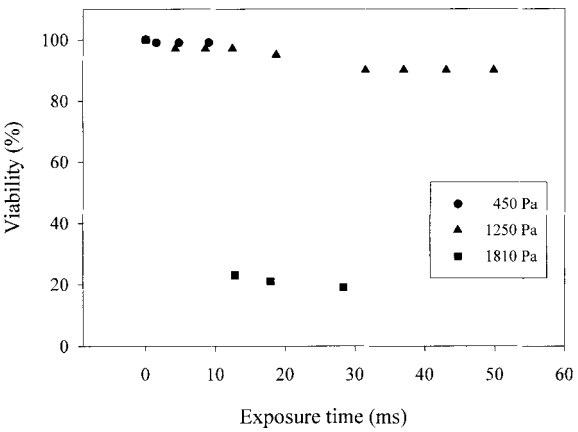

Figure 6. Effect of shear stress intensity on *E coli* viability.

disrupt yeast cells.[12] The velocity gradient derived from ultrasonically stimulated bubble motion seemed to be responsible for the destruction of yeast cells. Ultrasound corresponding to shear rates greater than $10^6 s^{-1}$ led to yeast destruction. In this study, the shear stress threshold was between 1292 Pa and 2770 Pa which corresponds to an approximate shear rate of $10^6 s^{-1}$. A shear rate of $10^6 s^{-1}$ led to cell death, but not to cell disruption.

The data also showed that both the number of passages through the capillary and exposure time affected microbial viability.

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