Ultrasonically Enhanced Settling: Influence of process parameters on separation efficiency of yeast cells at 2.2 MHz

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ABSTRACT

Ultrasonic resonators are successfully used for the separation of suspended particles or bio-cells, e.g. as cell filters in biotechnology. The scope of this paper is to investigate the separation efficiency over time using yeast suspensions and its dependencies on true electrical power input, liquid medium throughput and initial cell concentration. Data of systematic measurements at different values of these process parameters will be shown and an attempt to model the development of the number of cells passing the filter over time will be presented.

INTRODUCTION

The principle of ultrasonic particle manipulation (Gröschl 1998) is successfully applied, e.g. in cell filtration (Doblhoff-Dier, Gaida et al. 1994; Gröschl, Burger et al. 1998) and rapid particle agglutination (Coakley 1997). Cell filtration systems (Gröschl 1998; Benes, Gröschl et al. 2001) exploiting the principle of ultrasonically enhanced settling are commercial available (BioSep, Applikon Dependable Instruments BV, The Netherlands) with capacities of 10-250 L/day.

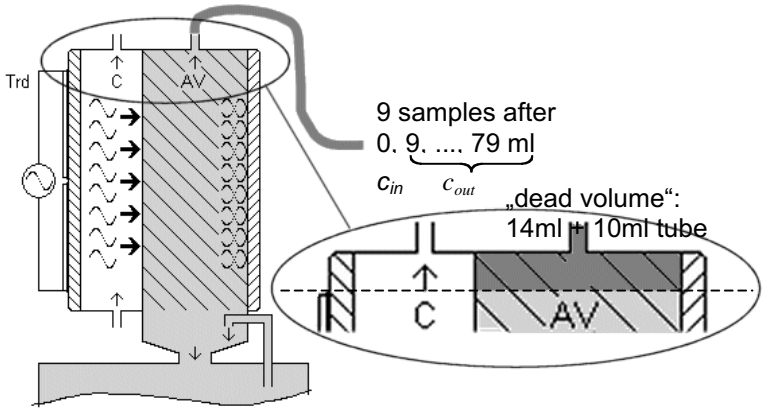

Figure 1 Two chamber separation system used; samples of one ml were pulled just before the ultrasound was switched on and after 9, 19 … 79 ml of suspension were pumped through the system. Cell concentration was measured using a haemocytometer.

These systems consist mainly of an ultrasonic resonator (see Figure 1). A piezo-glass-composite transducer emits an ultrasonic wave through the suspension (and a cooling medium)

onto a glass reflector. The superposition of the incident wave and its reflection leads to a standing wave. The primary radiation force drives suspended particles into pressure nodal or anti-nodal planes depending on the sign of the acoustic contrast between the liquid medium and the suspended particles. The acoustic contrast between particles and suspension medium is a function of particle diameter, mass density ratio and speed of sound ratio between the liquid and the particle, respectively. In case of cells as particles, the acoustic contrast value is such that the cells are driven towards the pressure nodal planes. Suspended cells are therefore concentrated in these planes and subsequently the resulting aggregates settle. This happens much faster than for a single cell or particle due to the decrease of the Stoke's drag force. The following sets of experiments were conducted to further (see also Keijzer, Trampler et al. 2001) examine the development in the beginning of the process of settling after the ultrasound is switched on.

METHOD

Separation efficiency refers to the ability of the system to reduce the concentration of cells or particles of a given suspension. To ensure that all settling effects were induced by the ultrasonic field a non-flocculating type of yeast (Class 1 after Gilliland 1951) was used. Values will be given as relative separation efficiency C_{rel}

$$C_{rel} = \left(1 - \frac{C_{out}}{C_{in}}\right) \cdot 100\ \% \quad . \tag{1}$$

The concentration C_{out} of the sample after treatment, e.g. pulled from the outlet is compared to the concentration C_{in} of the original suspension. The resulting percentage ranges from 0 % - no cells were retained - to 100 % where all cells have been removed from the liquid. Some process parameters have been varied to assess dependencies of the separation efficiency. The cell concentration C_{in} or bio-mass level and the true electrical power input P_{rms} of the driving electronics have been set to 0.5, 5 and 50 g/L and 10, 20 and 30 W respectively. Each of bio-mass/ P_{rms} combinations 0.5/10, 5/10, 5/20, 5/30 and 50/30 were carried out at throughputs of 5.6, 20.0 and 46.2 L/d.

RESULTS

Separation Efficiency Measurements: Figure 2 shows the result for 20 W and 5 g/L yeast cells for throughputs 5.6, 20.0 and 46.2 L/d over volume. In all cases a rapid increase of separation efficiency started immediately after the ultrasound has been switched on, though ending at different levels for different throughputs. As well the final concentration was reached approximately after 30 ml for 5.6 L/d and after about 50 ml for 20.0 L/d. The highest throughput of 46,2 L/d clearly showed more instability as reflected by the higher error bars, which indicate the respective standard deviation. The relative concentrations of the in-going suspension C_{in} as well showed a high standard deviation.

In Figure 3 P_{rms} was varied for 5 g/L and 5.6 L/d. Though the use of 20 W showed a steeper slope and a higher final separation efficiency than that of 10 W, however a further increase was not achieved when 30 W were applied. The development for 20 and 30 W was very similar. The final percentage was reached earlier at 30 W. Bio-mass levels of 0.5 g/L and 5 g/L are put opposite to each other in Figure 4 for P_{rms} = 10 W and 20.0 L/d throughput. The difference for measured means was high, and therefore the results were discernible, although high error bars indicated some instability. The final concentration for the lower solid fraction reached not even 50 %. At the higher concentration of cells a rise of the separation efficiency was detected not before 20 ml of liquid had left the system.

Model: A closer look at the graphs shown in Figure 2 to Figure 4 suggests that a mathematical description - a model - capable to describe the development exists and therefore an attempt of curve fitting was made. To avoid a loss of information due to normalisation[1] the absolute cell concentration over time was used here. Furthermore as it was found that corresponding samples, i.e. samples taken after the same volume pumped through the system showed higher

[1] division by C_{in} and by the throughput

standard deviations than e.g. the minimum cell concentration regardless of the actual sample the data of single runs were chosen for this research.

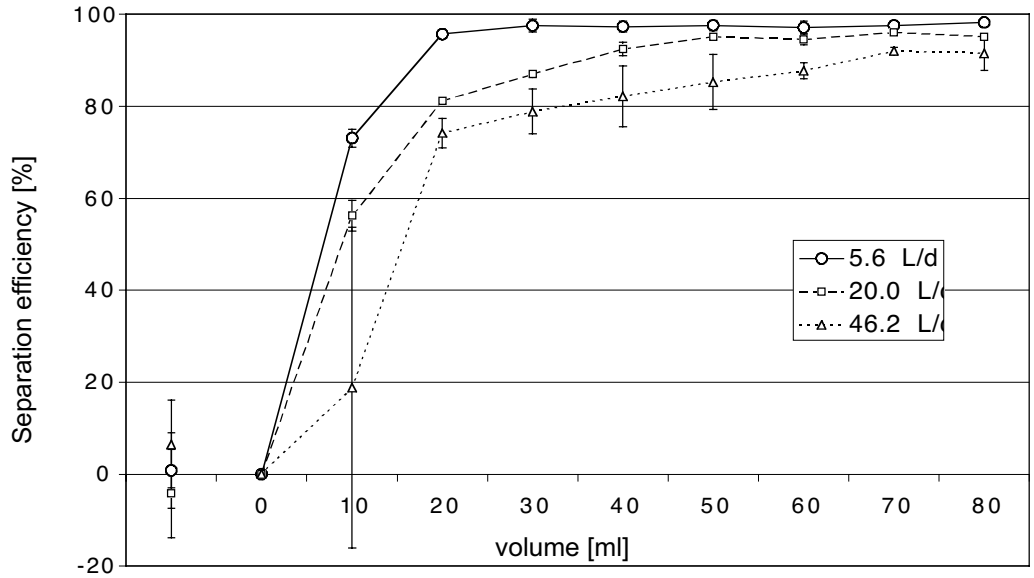

Figure 2 Development of the separation efficiency over volume at throughputs 5.6, 20.0 and 46.2 L/d. Process parameters were P_{rms} = 20 W and bio-mass level C_{in} = 5 g/L.

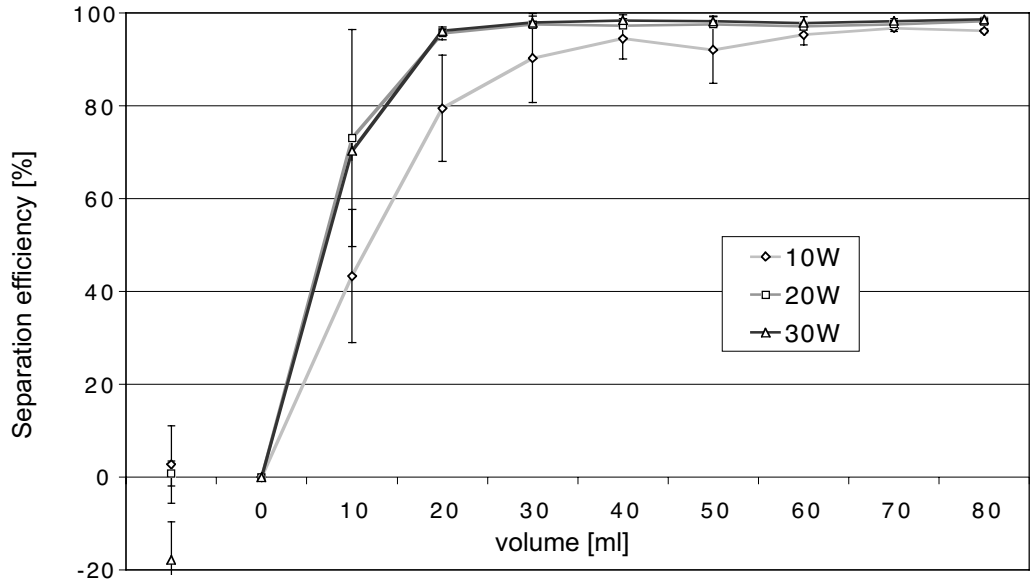

Figure 3 Development of the separation efficiency over volume at P_{rms} = 10, 20 and 30 W. Process parameters bio-mass level and throughput were 5 g/L and 5.6 L/d, respectively. Data for 20 W repeated from Figure 2.

The quality of the fit was examined using the measure of determination R^2. Examination of certain results prohibited to use a simple biased exponential $a_0 + a_1 \cdot \exp(-b_1 \cdot t)$. No set of parameters $\{a_0, a_1, b_1\}$ could be found to fit the steep decrease in the beginning of the run for almost all measurements. A solution to this was to add a bell-shaped function

$$a_2 \cdot \exp\left(-b_2 \cdot (t-c)^2\right) \tag{2}$$

yielding

$$C_{mdl}(t) = a_0 + a_1 \cdot e^{-b_1 \cdot t} + a_2 \cdot e^{-b_2 \cdot (t-c)^2} \quad . \tag{3}$$

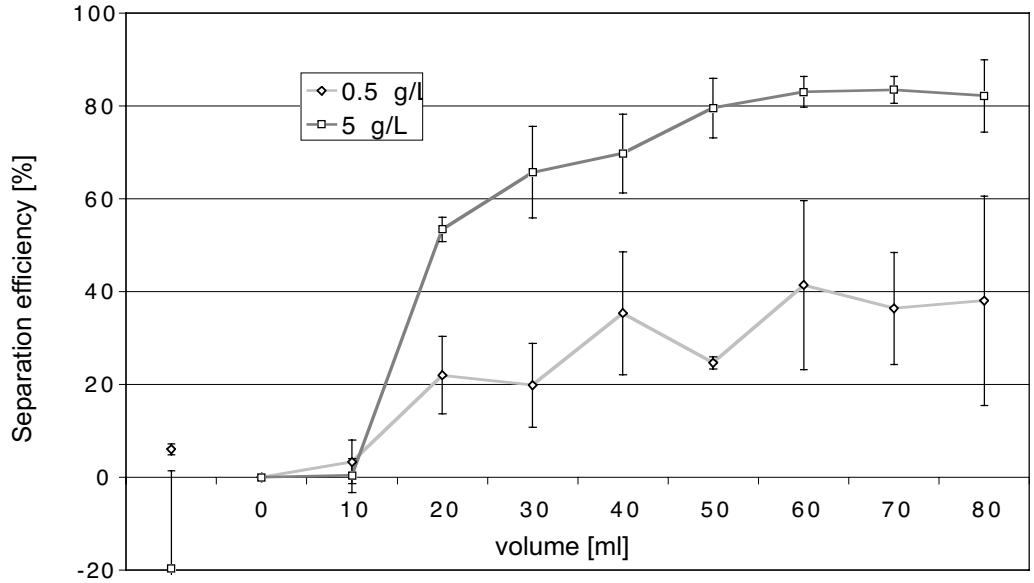

Figure 4 Development of the separation efficiency over volume at 0.5 g/L and 5 g/L. Process parameters P_{rms} and throughput were 10 W and 20.0 L/d.

The non-linear regression equation (3) for the modelled separation efficiency $C_{mdl}(t)$ has six free parameters $\{a_0,a_1,b_1,a_2,b_2,c\}$ embraced in three terms of a sum. The bias a_0 is the absolute separation efficiency maximum, $a_1 \cdot \exp(-b_1 \cdot t)$ describes the decrease of the absolute separation efficiency increased in the beginning of the run by (2). The parameter c enables one to define the centre of the bell-shape. Two basic assumptions decreased the number of free parameters. Firstly the measured absolute separation efficiency C_{in} had to be equal $C_{mdl}(0)$ leading (4) to a dependency of a_2.

$$\begin{aligned} &C_{mdl}(0) = C_{in} \quad , \\ &C_{in} = a_0 + a_1 \cdot e^{-b_1 \cdot 0} + a_2 \cdot e^{-b_2 \cdot (0-c)^2} = a_0 + a_1 + a_2 \cdot e^{-b_2 \cdot c^2} \quad , \\ &\rightarrow a_2 = (C_{in} - a_0 - a_1) \cdot e^{b_2 \cdot c^2} \quad . \end{aligned} \tag{4}$$

The second demand was, as no change of the cell concentration at the outlet was expected before the ultrasound was switched on, that the gradient of $C_{mdl}(t)$ to be 0 for $t = 0$. This condition lead (5) to a dependency of b_1

$$\begin{aligned} &\left.\frac{\partial C_{mdl}}{\partial t}\right|_{t=0} = C_{mdl}(0) = 0 \quad , \\ &C_{mdl}(t) = -a_1 b_1 \cdot e^{-b_1 \cdot t} - 2a_2 b_2 (t-c) \cdot e^{-b_2 \cdot (t-c)^2} \quad , \\ &C_{mdl}(0) = -a_1 b_1 + 2a_2 b_2 c \cdot e^{-b_2 c^2} \quad , \\ &\rightarrow b_1 = \frac{2a_2 b_2 c \cdot e^{-b_2 c^2}}{a_1} \quad . \end{aligned} \tag{5}$$

The substitution of (4) and (5) into (3) resulted in the final regression equation

$$C_{mdl}(t) = a_0 + a_1 \cdot e^{-\frac{2b_2 c \cdot (C_{in} - a_0 - a_1)}{a_1} \cdot t} + (C_{in} - a_0 - a_1) \cdot e^{-b_2 \left((t-c)^2 - c^2 \right)} \quad . \tag{6}$$

Figure 5 shows the exploitation of (6). The exponential and the bell-shaped term were added to the bias a_0 resulting in a fit of high quality $R^2 > 0.96$. It should be mentioned that the increase in the beginning of the run would prohibit the fitting without the bell-shaped term.

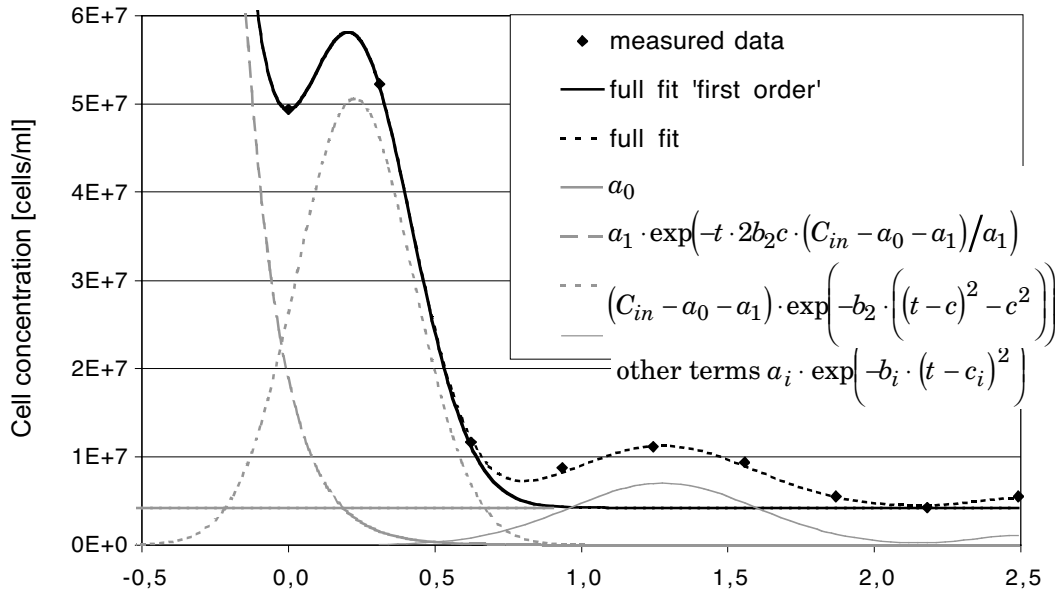

Figure 5 Model of the development of the absolute separation efficiency over time as of (6). A simple biased exponential did not completely describe the data, so the bell-shape was added.

Another point was the behaviour beginning just before one minute run-time in this particular measurement: an additional bell-shaped term could be used to fit this increase-decrease (black dashed line), resulting in a significant rise of the quality of the fit $R^2 > 0.999$. Although (6) reflected the behaviour very well the results were not clear-cut. Figure 6 shows three fits for one measurement. Although they were of comparable high quality $R^2 > 0.999$ the result was unstable, i.e. there were three different sets of parameters $\{a_0, a_1, b_2, c\}$ which could be used to describe the runs. This over-parameterisation prevented to find direct relations between the fitting parameters and the three process parameters that have been changed during this study. Furthermore this was the reason, why the fitting had to be done by hand instead of using a statistical algorithm. Table 1 shows the maximum of the separation efficiency measured and calculated from the fitting parameters as means of corresponding measurements. Only such curve fittings have been accepted where $R^2 > 0.95$. This was not possible for all measurements with 0.5 g/L bio-mass and the high-concentration/high-throughput measurement. The direct comparison reveals the high agreement, however the model tended to be slightly higher than the measured data, as no fluctuations after the main increase were taken into account. Again for reasons of comparability those values were the means of the lowest cell concentrations measured in each run, regardless if they were reached in corresponding samples. A significant trend of lower separation efficiency caused by increasing throughput for a given set of P_{rms} and bio-mass was measured. The first line of Table 1 shows that P_{rms} = 10 W resulted in significant low separation efficiencies for medium and high throughput. The picture is different when a bio-mass of 5 g/L was used, separation efficiency not just increased for higher P_{rms}, but as well the influence of rising throughput decreased, i.e. the difference between corresponding results was lower for higher P_{rms} The effect of higher cell concentration leading to higher separation efficiencies could be shown for 5.0 L/d in comparison with results for 50 g/L as well. In case of 20.0 and 46.2 L/d throughput, however, a significantly lower separation efficiency was found.

The presented data rise some questions which are to be answered in the future: Firstly: All results showed some increase in separation efficiency at volumes that should not be directly affected by the ultrasonic field. A more or less “dead volume” (Figure 1) of altogether 24 ml of suspension can be identified. Secondly: Although working well the model does not allow to conclude the separation efficiency directly from a given set of process parameters. And finally: Do the bell-shaped terms describe the partial re-suspension of particles when the aggregates slip down?

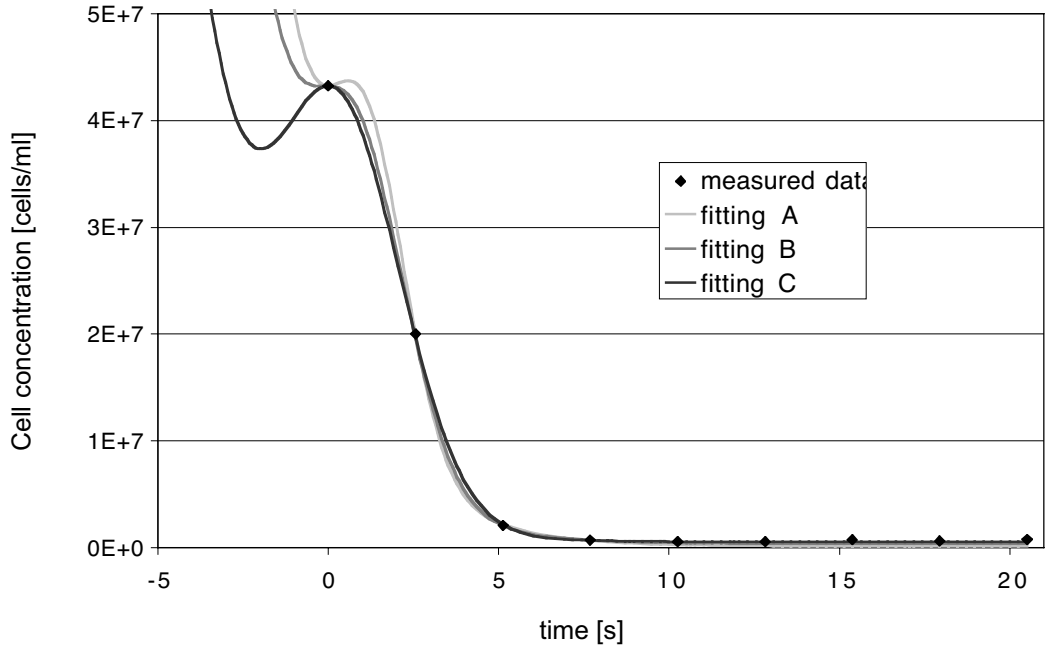

Figure 6 Over-parameterisation; the model allows fittings of the same set of measured data with different sets of parameters. Process parameters were 30 W, 5 g/L and 5.6 L/d.

Table 1: separation efficiency maximum, measured and delivered by the fitting [%]

throughput [L/d]						process parameters	
5.6	5.6 fit	20.0	20.0 fitted	46.2	46.2 fitted	bio-mass [g/L]	P_{rms} [W]
69.2 ± 6.0	-	41.4 ± 18.2	-	17.5 ± 0.1	-	0.5	10
97.0 ± 0.8	97.5 ± 0.3	84.6 ± 4.4	85.4 ± 3.5	72.3 ± 1.2	74.9 ± 0.3		10
98.2 ± 0.5	98.7 ± 1.1	96.0 ± 0.2	96.4 ± 0.3	92.8 ± 2.0	93.8 ± 3.3	5.0	20
98.9 ± 0.1	98.9 ± 0.2	97.5 ± 0.1	97.2 ± 0.4	94.1 ± 2.5	94.9 ± 2.8		30
99.6 ± 0.1	99.6 ± 0.1	94.4 ± 1.7	96.7 ± 2.3	66.0 ± 6.7	-	50.0	30

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