On a Dual-Chamber Ultrasonic Separator for the Filtration of Suspensions Containing Yeast Cells and Oil

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The principle of ultrasonically enhanced settling (UES) is successfully applied in biotechnology for filtration purposes, e.g. as cell-filter. Particles (biological cells) are concentrated in certain regions of a sonicated volume by an ultrasonic standing wave field (~2 MHz). Due to the dependence of the final settling velocity on the diameter of an object (Stoke's law), the agglomerates formed by sonication settle more quickly than single cells. This principle, which relies on a sufficiently large difference in mass densities of the particles and the host liquid, is also applicable to dispersed material lighter than the liquid. Up to now, no feasible solution was at hand when a suspension contained both heavier and lighter particles. For this demand, a novel setup, the UES dual-chamber separator, was tested on suspensions of yeast cells and oil droplets as a model system. Here the volume exposed to the standing wave field is divided into two parts by an ultrasonically transparent thin foil oriented perpendicularly to the direction of sound propagation. Experiments have shown that the UES dual-chamber separator was able to deliver oil-enriched medium at an upper collecting outlet of the first chamber and a cell-enriched medium at a lower collecting outlet of the second chamber. Data for the over-all separation efficiency of above 90 % at inlet throughputs of approximately 18 L/d will be shown and discussed.

1 Introduction

The principle of ultrasonically enhanced settling (UES) utilises radiation forces that act on suspended particles in an ultrasonic standing wave [1]. Consequently the particles are concentrated in certain regions within a resonator. Thus particles are immobilised, e.g. versus an up-flowing liquid phase or, alternatively, the sound field can be switched off from time to time, which leads to acoustical enhanced sedimentation or settling because of the decreased surface to volume ratio of the aggregates. In either case significantly less particles can be found in the outlet compared to the original suspension.

This principle is exploited effectively in suspensions carrying biological cells [2, 3], a filter performance of 99.9% and above were achieved with mammalian cell cultures [4].

The radiation forces are strongly dependent on the size of the particles for a given frequency [5]. This was utilised in even more sophisticated applications like the discrimination between viable and non-viable cells [6] or for the removal of small bacteria from a yeast suspension [7].

The UES technique is applied in industrial environments successfully [8, 9], which results in the identification of new demands. One of them being the filtration of biological cells with a mass density higher than the host liquid (dense particles) in dispersions additionally containing particles lighter than the host liquid (buoyant particles). Such would be for instance oil droplets, which are of course supposed to be retained by the acoustic filter too. Here the standard set-up fails as agglomerates of buoyant particles rise rather than sediment. However the radiation forces act similar if not stronger on them [10] and consequently this material is found in the filtrate. The scope of this paper was it to address this problem in lab scale, hence to find a way to successfully apply the technique of UES on dense and buoyant particles in suspension.

2 Materials and Methods

2.1 Suspensions

Cell and oil free supernatant filtered out of a production broth was used to test the dual-chamber ultrasonic separation. This liquid was enriched with common bakery yeast 5% (w/v) and surfactant stabilised plant oil. The specific oil content [% (v/v)] will be given in the result section at the particular experiment.

2.2 Sonication

The presented experiments were performed in a standard and two modified separation systems USSD-05 (Paar, Graz, Austria). The modifications will be described in detail. The electrical driving signal was generated by a synthesizer-amplifier combination USCS-05 (Paar, Graz, Austria) at a frequency of approximately 2.2 MHz. All experiments were conducted at flow rates of 5.5-6 L/d at the outlets. Therefore the inlet throughput for the experiments employing one outlet was 11-12 L/d, and for the dual-chamber separator experiment 16.5-18 L/d,
respectively. The amplifier was driving the transducer at 20-30 W true electrical power input.

2.3 Assessments

Numbers representing the filter performance will be expressed as separation efficiency (S.E.), a relative value calculated from the total number of cells per ml of a given inlet \( c_{\text{ini}} \) and a given outlet \( c_{\text{out}} \) using:

\[
S.E. = \left( 1 - \frac{c_{\text{out}}}{c_{\text{ini}}} \right) \times 100 \% 
\]  

The measurement of cell concentration, i.e. the total number of yeast cells per ml (milliliter) was performed using a Neubauer improved haemocytometer.

The attenuation of an ultrasonic wave was used to evaluate the entire population of dispersed “particles”, which included yeast cells and oil-droplets in this context. The device used was a electro-acoustic particle spectrometer (DT-1200, Dispersion Technologies, USA) utilising an ultrasonic propagating wave at several frequencies ranging from 1 to 100 MHz.

3 Ultrasonic Separator Set-Up

In the presence of an ultrasonic wave field so-called radiation forces are exerted on suspended particles. The primary radiation forces – in contrary to the secondary radiation force, which is of no relevance here – are the result of the incident wave being scattered at the particles. Depending on their orientation in relation to sound propagation direction the primary radiation forces are signified axial or transversal primary radiation force [11]. The axial primary radiation force is exerted in direction of sound propagation. Depending on the acoustic contrast – a function of the speed of sound ratio and the mass density ratio of particle and liquid, respectively – particles are driven in direction of increasing or decreasing sound pressure amplitude [10]. Hence, for a (quasi) standing wave, in which the amplitude of the ultrasonic field varies in space but not in time, particles are concentrated in the nodal regions of the wave field.

For a plane ultrasound wave the amplitude distribution in direction of sound propagation leads to nodal planes. Figure 1 depicts the arrangement of suspended particles before and after an ultrasonic standing wave has been applied. Firstly freely suspended particles (left) are arranged in said nodal planes (middle). Particles with a negative acoustic contrast – generally buoyant particles – are concentrated in the displacement nodes, i.e. regions of vanishing displacement, a positive acoustic contrast leads to an aggregation in the pressure nodes of the quasi standing wave. It takes typically one to a few seconds until this spatial ordering is established.

The transversal primary radiation force is brought about by the uneven distribution of excitement amplitude over the surface of the transducer due to border effects. Here the force exerted points towards higher amplitudes for both lighter and heavier particles. The resulting agglomeration of particles is shown in Figure 1 (right).

![Figure 1: The effect of an ultrasonic standing wave on a dispersion containing particles with positive and negative acoustic contrast.](image1)

An application of this principle of arranging particles by ultrasonic radiation forces is the ultrasonic enhanced settling. The agglomerates represent bigger “super-particles” which settle more rapidly than single corpuscles. According to Stokes' law of final settling velocity, the larger diameter leads to a decrease in surface to volume ratio and thus to a decrease of the friction at the particles surface per volume.

![Figure 2: Common set-up of an ultrasonically enhanced settler in biotechnology.](image2)
The UES technique is applied in biotechnology for purposes of the filtration of biological cells. The exploitation of such acoustic filters allow for so-called perfusion type fermentations [12], the common set-up is shown in Figure 2. The ultrasonic filter is mounted on top of the bio-reactor through a fitting in the head plate. Fermentation broth is fed from the bio-reactor below into the separation system. The cell-agglomerated are built up in the system and settle towards the bottom of the vessel. This biomass is subsequently recycled back into the fermenter. The clarified culture medium carrying the product is harvested at the top during the whole production phase. The amount of broth leaving the bio-reactor for downstream processing is replaced by fresh feed.

Typical advantages of a fermenter equipped with an acoustic filter are high cell densities, high viability and therefore high productivity. It has been shown, that the cells which are exposed to the ultrasonic field only for a few tens of seconds do not show changes in viability or their ability to reproduce [13, 14]. The ultrasonic device does not express fouling or clogging as there are no moving parts and can be hot steam sterilised in place.

Figure 3: Ultrasonically enhanced settler for dense particles. The filtrate leaves the system upwards.

Figure 4: “Banding” of particles in a sonicated volume (left). Agglomerates of buoyant particles are concentrated at the top (right, black) while dense particles are enriched at the bottom (right, red).

The investigations presented here were triggered by a measurement of the performance of an acoustic filter on a dispersion containing buoyant and dense particles (the first experiment in the result section). It was found, that this could not be accomplished in a standard separator like shown in Figure 3. However the technique should be applicable to lighter material. For a multi-wavelength resonator the arrangement after the ultrasound has been applied for a while the pattern - also referred to as “banding” - looks like Figure 4 on the left hand side. Depending whether one deals with buoyant or dense particles the agglomerates will be concentrated at the bottom (Figure 4, right, black particles) or the top of the volume (Figure 4, right, red particles).

Figure 5: Dual-chamber ultrasonic separator set-up for suspensions carrying buoyant and dense particles.
Following this a separation system shown in Figure 5 was constructed for the concentration of additionally present buoyant particles like oil droplets in a second active volume (AV I). Here the inlet (Feed) and the outlet for the oil-enriched retentate (Retentate I) was located at the top, while the “clarified” outlet at the bottom was connected to the inlet of a standard active volume (AV II). The outlet for cell-enriched retentate (Retentate II) was at the bottom and the outlet for the finally clarified liquid (Filtrate) at the top.

Figure 6 shows the principle in detail. The full dispersion containing both buoyant (red) and dense (black) particles is filled into the additional sonication chamber (Feed) on the left hand side. Lighter particles are agglomerated there by the ultrasonic standing wave and retrieved at an upper outlet for recirculation. Subsequently the resulting suspension – now containing mainly the heavier-than-water yeast cells - is fed into the second active volume by a bottom outlet. Again the ultrasonic standing wave leads to a particle-enriched suspension which is taken off at the bottom (Retentate heavier particles). The highly cleaned permeate is to be picked up at a the upper outlet.

Furthermore accumulated oil was found at the top of the chamber and the region of “banding” was clearly smaller than usual.

Figure 6: Flow diagram of the dual-chamber separator.

4 Result

Typically the S.E. for yeast in aqueous host liquids can be expected at 99% and above [4, 6, 14]. In Figure 7 the results of the S.E. of the acoustic filtration of cell free production supernatant enriched with 5% (v/v) yeast and 5% (v/v) emulsified plant oil over time are shown. During 25 minutes (30 W, 12 L/d) during which the ultrasonic field was applied, not even a S.E. of 50% was reached in a standard separation system (Figure 3). Furthermore the S.E. was highly unstable throughout the experiment.

During the ultrasonic treatment aggregates in the inlet region of the separators active volume were observed. The S.E. for yeast cells measured at an initial experiment with the dual-chamber separator measurement (30 W) was encouraging (see Table 1). It was possible to reach S.E. between 70 and 80%. The rather low value of S.E. at 0% oil was presumably brought about by the used production supernatant being somewhat more damping than water. Moreover a slight tendency of decreased S.E. at increased oil content was observed.

Table 1. S.E. of yeast cells in the dual-chamber ultrasonic separator using dispersions containing different fractions of oil.

<table>
<thead>
<tr>
<th>Oil content [% (v/v)]</th>
<th>S.E. [%]</th>
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<tbody>
<tr>
<td>0.0</td>
<td>85.4</td>
</tr>
<tr>
<td>1.0</td>
<td>82.3</td>
</tr>
<tr>
<td>2.5</td>
<td>78.4</td>
</tr>
<tr>
<td>5.0</td>
<td>71.7</td>
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Attenuation data of this experiment showed good agreement with the oil content (Figure 8). The damping by the ingoing dispersion was the higher the more oil it contained. Therefore the data could be interpreted as measurement of oil content. The separation of oil was shown by the significantly different levels of damping of the ingoing dispersion (in, blue) compared to the similar attenuation of the respective filtrate data (out, green). The measurement of the filtrate suggested a stable separation, comparable levels of damping were found in the outlet independent of how much oil the dispersion contained initially.
As a first optimisation step the AV I for the oil-retention (Figure 5) was reduced in size in direction of the sound propagation to one third of the original length. With this set-up driven at 20 W the original investigation of the dual-chamber ultrasonic separator was repeated.

Table 2. S.E. of yeast cells in the dual-chamber ultrasonic separator with reduced length of AV I using dispersions containing different fractions of oil.

<table>
<thead>
<tr>
<th>Oil content [% (v/v)]</th>
<th>S.E. [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>94.0</td>
</tr>
<tr>
<td>1.0</td>
<td>93.2</td>
</tr>
<tr>
<td>2.5</td>
<td>93.2</td>
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<tr>
<td>5.0</td>
<td>93.3</td>
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The S.E. in Table 2 was above 90% for all oil concentrations. A very mild dependency on the oil content was suggested, however this was not significant.

Figure 9 shows the attenuation of the ingoing dispersion (in, blue) and the filtrate (fil, green) measured with the dual-chamber ultrasonic separator with shorter AV I. Almost no dependence of the filtrates attenuation on the ingoing suspension was measured. Independently of the initial oil content – depicted by the different levels of the data of the ingoing dispersion – equally low oil content was suggested by the damping data.

5 Discussion

The reason for the insufficient S.E. (see Figure 7) of yeast cells when UES was applied on a dispersion containing small oil-droplets as well might be a combination of two circumstances. Firstly additional streaming is brought about by the rising agglomerates disturbing the settling clusters of cells. This induces a fair amount of re-suspension making the yeast available to be dragged away into the upper outlet for the filtrate. Secondly the biomass very well might have been attached to the surface of oil droplets due to the presence of surfactant.

The improvement of the situation regarding the S.E. (see Table 1) by the application of a modified separation system, the dual-chamber ultrasonic separator with an additional active volume was brought about by the reduction of the oil content prior to the yeast filtration. The measurement of the attenuation of a propagating ultrasonic wave (Figure 8) showed this decrease of oil.

It is not sensible to increase the true electrical input power too much as e.g. heat problems may arise. Therefore the active volume for the enrichment of buoyant particles was shortened to improve the separation performance in respect to emulsified oil. The success of this measure was confirmed by the attenuation measurement obtained by the dual-chamber separation data shown in Figure 9. The attenuation of the ingoing dispersions was well discernible in respect
to the oil content and the damping of the filtrates were found to be in a very narrow range. Moreover the filtrate was less damping compared to the result obtained with the dual-chamber separator with a long active volume for buoyant particles (Figure 8).

Furthermore Table 2 shows that the influence of the oil content on the separation performance of the yeast cells was decreased as well resulting in a more satisfactory S.E. of 93% and above. Further optimisation steps as the tuning of the throughputs might very well lead to S.E. values close to 99%.

Finally it shall be mentioned, that the different attenuation data for a dispersion containing 5% (v/v) oil (see Figure 8 and Figure 9) did not slip the attention of the authors. As this work was performed with real-life fermentation supernatant this presumably was a result of other contents like protein or similar.

6 Conclusion

The dual-chamber ultrasonic separation has shown to solve the problem of the application of UES in the case a dispersion contains dense and buoyant particles at the same time. The buoyant fraction – emulsified plant oil was used here – could be successfully removed by a second active volume with bottom outlet for the filtrate and reduced length. The S.E. for yeast cells was measured to be 93% and above.

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References