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Harvesting and dewatering yeast by microflotation

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ABSTRACT

Microbubble has been applied for the recovery of yeast cells from their growth medium using the bioflocculant–chitosan. Results reaching 99% cell recovery were obtained under various conditions examined. The result of bubble size distribution showed that mean bubble size increased as microbubble diffuser pore size was increased. Also, cell recovery efficiency was a function of both bubble size and particle size (cell size). For smaller particles (<50 μm), relatively smaller bubbles (<80 μm) were found to be more effective for recovery, otherwise, relatively larger bubbles (80–150 μm) proved to be efficient in recovering larger particles (particle size: ~250 μm). Acidic and neutral pHs were effective in separation as hydrophobic particles were formed. As pH tends toward alkalinity, flocs become more hydrophilic, leading to low recovery from the aqueous solution. In addition, separation efficiency was dependent on flocculant dose as increase in concentration improved flocculation and consequently, yeast recovery. However, above a critical concentration, overdosing occurred and inadvertently, recovery efficiency decreased. The application of chitosan as a bioflocculant and the subsequent application of microflotation for the separation of yeast cells proved effective and promises several advantages over non-bubble based separation techniques that preclude continuous industrial-scale production.

1. Introduction

Yeast is one of the most well studied microorganisms and has been long used as the main ingredient in many food products [7]. Their application in the production of bioethanol, as solvent for pharmaceutics, a renewable source of energy [21], feedstock in alcoholic beverage production [7] or animal feed has also become widespread. Bioremediation of wastewaters containing heavy metals can also be achieved using yeast cells, owing to their ability to remove a wide range of metals [15] with fast separation of biomass upon treatment [15]. It has also been exploited for the production of heterologous products such as vaccines and human hormones [4] and they offer apart from their abundance, a cost effective option [23].

One of the most important stages in the utilization of yeast in any of the above operations is the harvesting and dewatering of the yeast biomass. Yeast must be harvested from culture medium for further processing. Several methods have been designed and developed for the recovery of yeast from culture medium. However, their small size and relatively high cell density pose numerous separation difficulties. Some of the traditional techniques employed such as filtration and sedimentation are time consuming and relatively inefficient, preventing their continuous large-scale application in industries [12].

Given these challenges, yeast harvesting and dewatering has become a significant concern across several overlapping industries and could contribute substantially to the total production cost with potential impacts on food prices. More efficient techniques must be sought to overcome the challenge with yeast separation from culture medium. Bubble based separation techniques provide an advantageous approach to harvesting yeast because flotation is a rate intensifying separation process by enhancing buoyancy force over sedimentation (Molina et al. [17]). Dissolved air flotation (DAF) and Jameson's cells are industry's most employed bubble based separation techniques [6] but yet untried in yeast cell recovery from medium. Their application however, is likely unfeasible largely due to their intrusive nature but also the high energetic consumption associated with their operations. Another main reason for their unsuitability is the high shear from the exit nozzle due to the high operating conditions (pressure ~6 bar) [6,9].

The key challenge is to change from high power consumption regimes to low power consumption methodologies without sacrificing performance and the yeast biomass quality. Here, we address all these concerns with microflotation [9] that applies fluidic oscillation induced microbubble clouds [26], already applied for gas exchange in accelerating algal biomass growth [27] and

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in microalgae harvesting and dewatering. Microflotation is one approach that achieves low power consumption with desirable bubble size and flux under laminar flow regime. In DAF systems the energetic release of supersaturated liquid in the flotation cell for microbubble generation results in high turbulent flow and inadvertently floc break-up. This counter-productive behavior is not experienced in microflotation, as only gas is injected to produce bubble under atmospheric pressure. Thus the study aims to investigate the application and performance of fluidic oscillator generated microbubbles in yeast separation using standard methodologies for flotation separations as well as the effect of chitosan on recovery efficiency. In addition, the study explores the effect of varying bubble sizes on flotation of particles. This paper is organized as follows: In Section 2, the materials and methods are presented followed by the results and discussions in Section 3.

2. Materials and methods

2.1. Material preparation

Sterile Yeast Peptone Dextrose (YPD) medium was made using yeast broth and yeast extract (Sigma Aldrich, UK). 8.5 g of the yeast broth and yeast extract respectively were added to 1 L distilled water and mixed until dissolved before sterilizing with high pressure saturated steam for about 15 min. Meanwhile, chitosan (Sigma Aldrich, UK) stock was made by dissolving 5 g of dry chitosan (Sigma Aldrich, UK) in 150 mL 0.5 M HCl (Sigma Aldrich, UK) which gives a viscosity of 0.9 Pa s.

2.2. Experimental procedure

After pH adjustment of the growth medium, 1 g of dried yeast (*Saccharomyces cereviseae*, Lallemand, UK) was reconstituted into 1 L of growth medium and mixed for 1 min to form a homogenous dispersion before chitosan was added. Rapid coagulation with a motorized stirrer at 3500 rpm followed for 1 min before the mixture was stirred for a further 1 min under low speed at 75 rpm to promote floc growth. After flocculation, the microbubble generator was turned on and the mixture was gradually introduced into the flotation rig where cells were harvested for 20 min. Samples were collected every 2 min for optical density measurements. Biomass concentration correlates with optical density (OD) and was measured by spectrophotometer DR 2800 (Hach Lange, UK) to determine OD at 660 nm.

The experimental rig is shown in Fig. 1. The rig consists of a fluidic oscillator, a microbubble diffuser and a microflotation column. Bubbles were generated by fluidic oscillation [9,25]. For each run, the microbubble diffuser was fitted with different membranes with pore size: 25, 50, 75, 100, 125 μm respectively. Also, the chitosan concentration was varied (0.2; 0.4; 0.6; 0.8; 1%, v/v) as was the pH of the growth medium (pH 5, 7 and 9). All experiments were conducted under room temperature (21 °C). Recovery efficiency was determined using the equation below:

$$R = \left(\frac{C_i - C_f}{C_i}\right) \times 100.$$

where C_i and C_f are the initial and final yeast concentrations at mid-depth respectively.

2.3. Bubble size measurement

The measurement of the size distribution of gas bubbles was carried out by high-speed photography and image analysis according to the method described by Hanotu et al. [10,11].

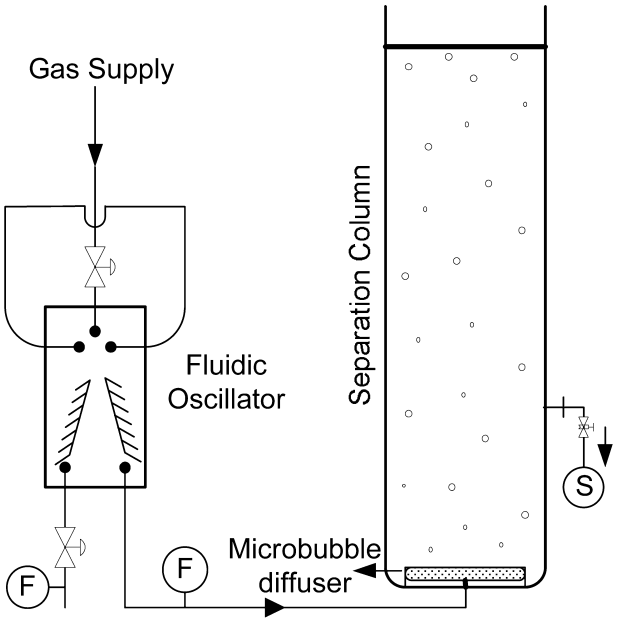

Fig. 1. Schematic representation of the experimental set-up. Compressed air (0.8 bars) is fed into the oscillator, which then feeds the microbubble diffuser with a portion of the air bleed-off or channeled otherwise to another set of diffuser. In this study, a portion of the air was bled off downstream of the fluidic oscillator.

2.4. Zeta potential measurement of yeast cells

Dry yeast was reconstituted in 10 mL YPD medium and placed in an incubator at 30 °C and 300 rpm to keep cells from settling. Following that, 3.7 g agar was made up in 250 mL YPD medium to a concentration of 1.5% (w/v) before pouring into plates and allowed to set. Then, 20 μL of the reconstituted yeast was cultured in the YPD agar medium for 24 h. Zeta potential was measured with the zeta potential analyzer (Brookhaven ZetaPALS, UK) using the phase amplitude light-scattering method. Samples were centrifuged at 3000 × *g* for 5 min. After which cells were washed and re-suspended twice in 100 mM potassium chloride (KCl) before centrifugation at 3000 × *g* for 5 min. An electric field of ~2.5 V/cm was used during zeta potential measurements [18]. Triplicate measurements of samples of cells were done for reproducibility. Results represent the average of ten successive runs.

2.5. Yeast particle size measurement

The particle size distribution of the yeast cells/flocs was measured with the Mastersizer *S* (Malvern Instrument, UK). Cells were measured with and without the addition of a chitosan. Yeast cells (1 g) were reconstituted in growth medium and immediately dispersed by stirring for 1 min. Under no coagulant conditions, cells were measured immediately. Otherwise, chitosan was added at varying concentrations (0.2; 0.4; 0.6; 0.8; 1%, v/v) and the mixture rapidly mixed (coagulation) for 1 min. Next the sample was gently added to the Mastersizer until an obscurity of 15–20% was attained with the dispersion unit stirring at 1200 rpm. The stirring rate was chosen in order not to cause floc breakage but also to facilitate good dispersion around the measuring device. Samples were taken for measurement soon after rapid mixing to avoid floc settling.

3. Results and discussion

3.1. Bubble size measurements

Bubble size was measured in water as well as in the yeast culture medium with the mean bubble size, a function of the diffuser