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FERMENTATION OF PULLULAN USING AN OSCILLATORY BAFFLED FERMENTER

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The oscillatory baffled reactor offers enhanced and uniform mixing at very low shear rates compared to conventional mixing devices. This is advantageous for biochemical and biomedical applications where shear sensitive cultures are involved. The work in this paper explores the way in which *Aureobasidium pullulans*, which produces the versatile biopolymer pullulan, behaves in the low and uniform shear environment of an oscillatory baffled fermenter (OBF), and compares its growth rate and pattern to those in traditional fermenters. A series of pullulan fermentation experiments were performed in the OBF with a particular emphasis placed on the influence of aeration on pullulan and biomass production. The results show that at the optimal volumetric airflow rate of 1 vvm (volume of air per volume of liquid per min) the performance of the OBF is significantly better than the traditional stirred tank fermenter.

Keywords: oscillatory baffled fermenter; fermentation; pullulan; biopolymer; mass transfer.

INTRODUCTION

Considerable interest has been shown in the production and use of microbial biopolymers, as these are generated from renewable natural sources, and are often biodegradable, and generally non-toxic. Pullulan is one of such biopolymers and is a viscous, water-soluble neutral exopolysaccharide secreted by the polymorphic fungus *Aureobasidium pullulans* (*A. pullulans*). Pullulan itself consists of either linear chain of glucopyranose units with regular alteration of two $\alpha(1-4)$ and one $\alpha(1-6)$ linkages or a linear polymer of maltotriose units connected by $\alpha(1-6)$ linkages (Boa and LeDuy, 1984). The regular introduction of $\alpha(1-6)$ linkages in pullulan interrupts what would otherwise be linear amylose chain. This difference is thought to impart structural flexibility and enhance solubility, resulting in distinct film- and fibre-forming characteristics that allow pullulan to mimic synthetic polymers derived from petroleum (Wallenfels *et al.*, 1961; Bouveng *et al.*, 1962; Sowa *et al.*, 1963). In the form of a thin film, it is biodegradable, transparent, oil-resistant and impermeable to oxygen. Pullulan can also be used as a material for coating and packaging, as a sizing agent for paper, as a starch replacer in low-calories food formulations, in cosmetic emulsions and in other industrial and medicinal applications (Childers *et al.*, 1991; Deshpande *et al.*, 1992).

A. pullulans has a number of different morphological forms, with filamentous and yeast-like cellular (or unicellular) structures being the two main types, as shown in Figure 1. There have been however contradictory literature on which type is responsible for pullulan synthesis; some reported for the former (Ono *et al.*, 1977; Bermenjo *et al.*, 1981; Simon *et al.*, 1993), while the others, the majority, for the latter (Catley, 1971; Heald and Kristiansen, 1985; McNeil *et al.*, 1989; Madi, 1995). Pullulan is synthesised intracellularly but relatively little is understood about the mechanism of the bioprocess. *A. pullulans* cells produce pullulan by receiving nutrients and oxygen from the medium and deposit it on the periphery of the cell as shown in Figure 1 and then release it into the medium. Although the factors affecting pullulan intracellular synthesis and subsequent release are still unclear, the mechanism of other microbial systems, e.g., exopolysaccharide synthesis, can generally be used to describe the pullulan synthesis (Sutherland, 1977).

As first noted by Catley (1971) and subsequent confirmed in studies by McNeil and Harvey (1993) and Leathers (2003) synthesis of pullulan commences typically in the late exponential phase and continues into the stationary phase. Thus, it is important to minimize the 'non-productive' process phase, the culture exponential growth phase, by maximization of growth rate, in order to reach the stage of pullulan synthesis as quickly as possible. This implies a bioreactor with effective mixing and

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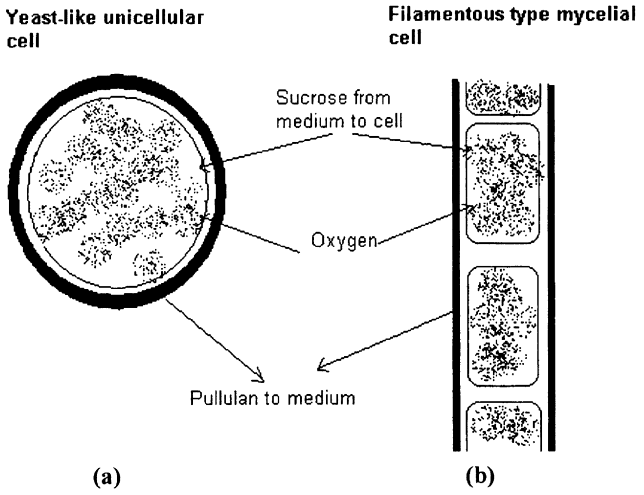

Figure 1. A. pullulans cells and the mechanism of pullulan formation.

oxygen transfer. However, both these processes become increasingly difficult as the viscosity of the process fluid increases from mid-exponential phase onwards. Late exponential phase culture broths, e.g., *A. pullulans*, become highly viscous and exhibit marked non-Newtonian behaviour (pseudo-plasticity), which limits mass (oxygen, nutrient), momentum and heat transfer (Rho *et al.*, 1988; McNeil and Harvey, 1993), leading to stagnant zones and impeller flooding in the standard stirred tank reactor (STR) type used (Rau *et al.*, 1992). It is clear that process control in such poorly mixed systems will be suboptimal, and since mixing in conventional fermenters profoundly influences pullulan yield, productivity and quality (essentially molecular weight distribution) (Bouveng *et al.*, 1962; Catley, 1971; Bermenjo *et al.*, 1981; Boa and LeDuy, 1984), a fermentation system offering good bulk mixing of viscous fluids is desirable. Additionally, since extended (fed) batch processing has been proposed as a route to higher pullulan productivity in the STR (Catley, 1971), the ability to monitor and control such fermentation systems implies the requirement for good bulk mixing.

Fermenters utilizing STR technology have been the workhorse of fermentation and indeed bioprocessing industries worldwide. Such fermenters, although generally considered well-characterized types, are acknowledged to have a number of drawbacks and limitations (Popovic and Robinson, 1993; Leib *et al.*, 2001; Rossi, 2001), including poor bulk mixing and the tendency of gas (air) channelling, leading to inadequate gas dispersion for viscous systems. Generally, these drawbacks worsen as the scale of operation increases, and the process fluid viscosity rises. Thus, there is a pressing need to develop alternatives to the conventional STR for processing viscous fermentation products, such as biopolymers, like pullulan.

We have now developed a robust fermenter using oscillatory baffled reactor (OBR) technology capable of providing much more uniform global mixing at significantly lower levels of shear strain rate for a similar power input to traditional STRs (Harrison and Mackley, 1992; Ni *et al.*, 2000). The fluids mixing in the OBR is achieved by the generation and cessation, as well as the interaction of eddies, within each baffled cavity, leading to similar orders of magnitude in axial and radial velocities. Six times higher mass transfer coefficients were reported in an oscillatory baffled column in comparison with a bubble column (Hewgill *et al.*, 1993) and 75% higher with a stirred tank involving yeast culture (Ni *et al.*, 1995). These are contributed by the enhanced gas hold-up due to prolonged residence times of bubbles retained in each baffled cavity and more uniform size distributions of bubbles (Oliveira and Ni, 2001, 2004). The encouraging scientific results in applications of OBR in gas–liquid systems prompted this study investigating the fermentation of pullulan using an oscillatory baffled fermenter.

MATERIALS AND METHODS

Microorganism

The *A. pullulans* (IMI 145194) was supplied as a lyophilized culture. The culture was resuscitated by addition of sterile diluent and then plated out onto potato dextrose agar (Oxoid Ltd, Basingstoke, UK) plates, and incubated for 5 days at 30°C.

Inocula were produced by adding 0.005 dm^3 of sterile medium consisting of sucrose, 30.0 (g l); $(NH_4)_2SO_4$, 0.6; KH_2PO_4, 5.0; $MgSO_47H_2O$, 0.2; NaCl, 1.0; yeast extract (Oxoid Ltd), 0.4, and by scraping culture from agar plate to generate a suspension. This suspension was aseptically transferred to 0.125 dm^3 sterile medium in a 0.5 dm^3 Erlenmeyer flask which was subsequently incubated in an orbital shaker at 30°C, 200 rpm for 24 h. The inoculum's volume in fermenter culture was 5% v/v.

Experimental Methods

The oscillatory baffled fermenter consists of a cylindrical stainless steel column of 100 mm in internal diameter and 420 mm in height, giving a total liquid capacity of 3.3 dm^3 with a working volume of 2.5 dm^3 (75% of the total capacity). A set of three orifice baffles were used in this study, and these baffles were made of 3 mm thick stainless steel plate and designed to fit closely to the wall of the fermenter. The baffles were equally spaced at 150 mm apart, and supported by two 6.35 mm diameter stainless steel rods. The orifice diameter was 46 mm, creating a baffle free area of 22%. The baffles were oscillated by the means of a motor and a frequency inverter, which allows frequencies of 0.2 to 10 Hz. Oscillation amplitudes of 5 to 30 mm (centre-to-peak) were generated by adjusting the preset distance between the linkage and the fly arm, as shown in Figure 2. The framework supporting the column was made of mild steel, minimizing vibration during operation.

The pH in the OBF was maintained at 4.5 throughout the experiments by automatically adding 1 mol l NaOH or 10% (v/v) H_2SO_4, depending on the pH of the medium. An Anglicon Solo series 2 pH controller was used for controlling and recording the pH data. The temperature of the fermenter was controlled at 28°C using a temperature-indicating controller with the sensing element located inside the fermenter. Air was fed at the base of the vessel after passing through a sterile filter. The gas flow rate was measured and controlled via a mass flow meter. The dissolved oxygen (DO) concentration was measured by a Vernier DO probe. For the fermentation experiments