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Extractive fermentation for improved production and recovery of lipase derived from *Burkholderia cepacia* using a thermoseparating polymer in aqueous two-phase systems

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ABSTRACT

An extractive fermentation technique was developed using a thermoseparating reagent to form a two-phase system for simultaneous cell cultivation and downstream processing of extracellular *Burkholderia cepacia* lipase. A 10% (w/w) solution of ethylene oxide–propylene oxide (EOPO) with a molecular mass of 3900 g/mol and pH 8.5, a 200 rpm speed, and 30 °C were selected as the optimal conditions for lipase production (55 U/ml). Repetitive batch fermentation was performed by continuous replacement of the top phase every 24 h, which resulted in an average cell growth mass of 4.7 g/L for 10 extractive batches over 240 h. In scaling-up the process, a bench-scale bioreactor was tested under the conditions that had been optimized in flasks. The production rate and recovery yield were higher in the bioreactor compared to fermentation performed in flasks.

1. Introduction

An aqueous two-phase system (ATPS) has been proposed as an ideal purification technique for the separation, extraction and concentration of biomolecules because of the system's high productivity, simplicity, short processing time, cost effectiveness, scalability and versatility. The ATPS method has been applied in downstream processing for compounds like enzymes (Ashipala and He, 2008; Kulkarni et al., 1999), biopharmaceuticals products (Wyman et al., 2005) and other natural products (Chavez-Santoscoy et al., 2010; Sinha et al., 1996). The use of ATPSs can be extended to direct purification during the fermentation process, called extractive fermentation. Extractive fermentation, or *in situ* product recovery, provides a scientific technological solution that overcomes the problems of low volumetric productivity characteristic of biotechnological operations due to product inhibition (Paquet et al., 1994). The concept of this *in situ* purification process involves integration of an extractive step as the first stage of downstream processing to simultaneously synthesize and remove the product. This is not only to ensure the primary recovery, but it also increases the rate of product formation by minimizing inhibition by the end-product during fermentation. Additionally, an ATPS provides a non-denaturing environment for labile bio-compounds (Sinha et al., 2000).

ATPS extractive fermentation predominantly uses PEG and high molecular weight dextran or a polymer-salt system. However, one of the restrictions of these systems is that most phase-forming components cannot be effectively recycled. This lack of recyclability results in an expensive process and environmental pollution, which has become an obstacle to the applications of ATPSs in the biotechnology industry. Ethylene oxide–propylene oxide (EOPO) is a water-solute copolymer and is capable of thermoseparating into two phases at a temperature greater than the lower critical solution temperature (LCST) (Johansson et al., 1997; Show et al., 2011). Conventionally, the target proteins are generally removed from the polymer phase by ultra-filtration, diafiltration, crystallization, a chromatographic step and back extraction into a new salt-phase (Alred et al., 1992). With the addition of EOPO to the fermentation process and manipulation of the temperature above the LCST when the fermentation is harvested, two phases can be formed. The top phase contains harvested products that are mostly depleted of EOPO, and the bottom phase contains concentrated EOPO and cells that can be reused in new extractive fermentation procedures (Fig. 1) (Show et al., 2011). In this paper, we report a novel extractive fermentation process that allows the polymer and cells to be

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