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Pilot-scale aqueous two-phase floatation for direct recovery of lipase derived from *Burkholderia cepacia* strain ST8

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ABSTRACT

The efficient and economical pilot-scale production of lipase is necessary for meeting the growing demand of lipase especially in bioprocessing industry. Aqueous two-phase flotation (ATPF) is a purification technique that can be applied in the recovery and purification of biomolecules. ATPF is based on a combination of aqueous two-phase systems (ATPS) and solvent sublation (SS). In this report lipase was recovered and purified from the fermentation broth of *Burkholderia cepacia* (*B. cepacia*) using an alcohol/salt ATPF system on a pilot scale. The working parameters of ATPF, including concentration of crude lipase feedstock, types of alcohol and salt, concentrations of alcohol and salt, volumes of buffer solution and alcohol, were investigated for their effects on the partitioning behavior of lipase in ATPF. ATPF comprised of 1-propanol and ammonium sulphate was successfully established for feasible and cost effective separation of *B. cepacia* ST8 lipase from liquid fermentation broth. The alcohol/salt ATPF system showed a purification factor of 12.2, a separation efficiency of 93% and a selectivity of 40. Furthermore, a comparison has been made between small-scale and large-scale ATPF production. Our results showed that this novel pilot-scale ATPF has the potential to be applied at industrial scale.

1. Introduction

Bio-based production is an emerging field in the bioprocessing industry as it represents a greener and an environmental friendly alternative to synthetic-based production. Lipases (E.C.3.1.1.3) are catalysts that hydrolyses triacylglycerol to glycerol and free fatty acids [1]. Lipases originate from plants, animals and microorganisms. Among these, microbial lipases are commercially preferred due to their high productivity under mild operating conditions which demand less energy [2]. The extracellular bacterial lipases are found in various genera such as *Pseudomonas*, *Bacillus* or *Burkholderia* [3]. Lipase derived from *Burkholderia* have desirable traits like high alkalinity, thermal stability, organic solvent tolerance, enantioselectivity, effective enzyme activity at different pH, and non-toxicity to the environment [4]. The enzyme is well known for its exceptional catalytic activity in different substrates, as well as its partial solubility [5]. Due to the biodegradable and non-toxic properties, lipases can be produced with less raw material and less waste generation than other processes [6]. It is commonly applied in the food [7], detergents [8,9], pharmaceuticals [10], paper [11], cosmetics [12], pesticides industries [13], biomedical [14], biosensors [15], and environmental management applications [16]. These applications create a strong demand for lipase in industry and highlight the necessity for the mass production of lipase.

Aqueous two-phase system (ATPS) is an effective liquid-liquid separation technology that has proven to be effective in biomolecules recovery [17]. ATPS is popular due to its low energy consumption, capability for industrial scale up, high yield and

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rapid partition of molecules between phases [18–21]. ATPSs are usually formed by the combination of either polymer/polymer or polymer/salt as phase-forming components [22]. However, the large amount of polymer used in the scaling up of ATPS could substantially affect the overall efficiency of ATPS. Moreover, the phase segregation is rather time consuming. The recycling of the expensive phase-forming polymer (Table 1) may be able to resolve the cost issue, but it has also raised environmental concerns [20,23]. For instance, the usage of PEG that could cause equipment corrosion and precipitation of target product [24]. Another weakness of using polymer as phase-forming component is that the separation performance of ATPS can be influenced by the polarity difference between the two phases [25]. In addition, some researchers also suggested that alcohol may affect the microbial activity of lipase [21].

Originally introduced by Sebba [26], solvent sublation (SS) serves as an alternative for ion flotation. SS utilises the principle of effective adsorption of surface-active material on the bubbles in aqueous phase. Ascending bubbles carrying the target component will enter the immiscible top phase composed of organic solvent. The rupturing of bubbles at the surface of top phase will permit the surface-active material to be collected in the organic solvent in the column upper zone [27].

Aqueous two-phase flotation (ATPF) is a recent technology that merges ATPS and SS. It is capable of giving a high concentration coefficient at the reduced expense of organic solvents hence improving biocompatibility and economy compared to standalone ATPS. The main working basics of ATPF process is the absorption of surface-active components on the surface of air bubbles of an ascending gas stream in aqueous solution or salt solution. The air bubbles are then dissolved in an organic solvent phase, which is hydrophilic in nature and is located above the aqueous, or inorganic salt phase. These surface-active compounds on the air bubbles will be collected in the top phase (shown in Fig. 1). Recently, many applications of ATPF have been reported, covering the separation and purification of penicillin G [28], lincomycin [23], puerarin [29], baicalin [30], chloramphenicol [31], tetracycline [32], and lipase [5,33]. ATPF offers advantages such as high concentration efficiency, soft separation, user-friendly operation, low environment impact, low cost and convenient recycling of the phase-forming chemicals [33].

Presently, to the author's knowledge, no research has been done on the recovery and purification of lipase based on a pilot-scale alcohol/salt ATPF system. The objective of this work is therefore to examine the feasibility of recovering lipase by ATPF from the fermentation broth of *Burkholderia cepacia* (*B. cepacia*) on a pilot scale.

Table 1
Comparison of price of phase-forming agents.

Phase-forming agents	Price (USD)
Ethylene oxide-propylene oxide (EO/PO) random copolymer (1 L)	99.80
Methanol (1 L)	59.80
Ethanol (1 L)	91.00
1-Propanol (1 L)	74.90
2-Propanol (1 L)	63.40

In this study, a large-scale ATPF equipment was fabricated with low-cost and recyclable material to improve sustainability. Lipase derived from *B. cepacia* was selected for this research due to their high tolerance towards organic solvents and aliphatic alcohol, superior thermal stability, and wide substrate specificity [5]. The effect of concentration of crude lipase, type of alcohol, volume of alcohol, concentration of alcohol, the volume of crude lipase + salt solution, concentration of salt and type of salt were investigated. The performance of lipase separation in ATPF was evaluated by indicators like the separation efficiency (E), selectivity (S) and purification factor (P_{FT}) of lipase.

2. Material and method

2.1. Material

Ethanol, methanol, 1-propanol, 2-propanol, ammonium sulphate [$(NH_4)_2SO_4$], trisodium citrate [$Na_3C_6H_5O_7$], magnesium sulphate [$MgSO_4$], di-potassium hydrogen phosphate (K_2HPO_4), potassium di-hydrogen phosphate (KH_2PO_4) were purchased from Merck (Darmstadt, Germany), 4-nitrophenyl dodecanonate (*p*-NPL), calcium chloride [$CaCl_2$], gum arabic and nutrient broth were acquired from Sigma-Aldrich (St. Louis, USA), Bradford reagent and Triton X-100 were obtained from R&M Chemicals. Olive oil was sourced from Bertolli (USA). All the chemicals utilized were of analytical grade.

2.2. Apparatus

The lab-scale ATPF apparatus is made of a filtration assembly incorporating a sintered glass disk that generates gas bubbles during the upflow of gas from the bottom, as shown in Fig. 2(a). However, there is no commercial ATPF equipment that can support the operation of ATPF up to 5-L capacity. As an alternative, an ATPF

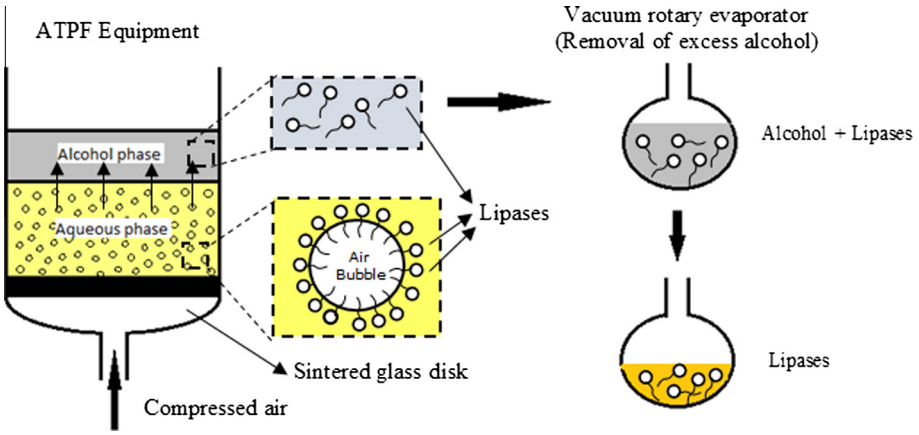

Fig. 1. Schematic diagram describing the recovery of lipase using ATPF. The lipases in the bottom phase would attach themselves on the surface of the gas bubbles produced by the ascending gas stream, and would accumulate in the top phase (alcohol-rich phase).