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Sustainable approach in recycling of phase components of large scale aqueous two-phase flotation for lipase recovery

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ABSTRACT

Recently, aqueous two-phase flotation system has become a powerful method for separation of biomolecules. However, the application of this technique for large scale operation elevates a concern on the environmental issue. This is due to the large amount of chemical consumption for phase components formation. Recycling of hydrophilic organic solvent and inorganic salt in aqueous two-phase flotation is an efficient and green technique for biomolecules recovery. Up to date, there is limited research concerning the recycling of the phase components. In this present work, recycling capability of pilot scale aqueous two-phase flotation composing alcohol/salt was employed to separate lipase from fermentation broth. The drive of this research is to economize the usage of chemicals, to reduce environmental pollution and to establish this approach for sustainable recovery of lipase. The aqueous two-phase flotation system investigated was composed of 1-propanol and ammonium sulphate whereby both phase components went through complete recycling process. Recycling of entire phase components is found to be a sustainable approach as it able to reduce the consumption of chemicals and it is an eco-friendly technique. From the result obtained, it was exhibited that by reusing the bottom phase, separation efficiency was sustained beyond 70%. As for the lipase yield, 80% of yield was achieved from recycling the crystallized salt that was recovered by dilution crystallization with methanol.

1. Introduction

The massive growth of biotechnology over the past decade has initiated bio-based production that provides us with a great array of beneficial products. Many industries are utilizing biological products in their production (Jegannathan and Nielsen, 2013). The apprehension concerning pollution issues on the current global situation due to processing industries lead to the need for a replacement of the traditional multistep process with a single stage process that has revolutionary economic effect (Pietruszka et al., 2000) and cleaner manufacturing process (Kanth et al., 2009).

For the past two decade, researchers have been interested on lipase enzyme due to their growth in industrial applications. Lipase also known as triacylglcerol acylhydrolases, is a ubiquitous enzyme that has substantial physiological importance and industrial potential. The function of lipase is to catalyse the hydrolysis of triacylglycerol into monoglycerides, diglycerides, free fatty acids and glycerols. In addition, lipase able to catalyse extensive range of reactions such as esterification, inter-esterification, alcoholysis, acidolysis and aminolysis (Abdelmoez et al., 2013). The wide range of catalytic ability of lipase is due to their high level of specificity that lead lipase to be a major biocatalyst in several oleochemicals production (Mustafa et al., 2016). For these reasons, many studies have been done relating to recovery and purification of lipase enzyme in the past few decades.

Various purification techniques are employed in the downstream processing of lipases which includes ultrafiltration, precipitation, partitioning via liquid-liquid and chromatography.

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List of abbreviation

ATPF	Aqueous Two-Phase Flotation
ATPS	Aqueous Two-Phase System
PEG	Polyethylene glycol
P_{FT}	Purification factor
EOPO	Ethylene oxide–propylene oxide
OVAT	One Variable at a time
S	Selectivity
E	Separation Efficiency
Y	Lipase yield

However, these methods are laborious and the product obtained is low in purity. As for the chromatographic technique, it is costly, multistep and it is not suitable for large scale and continuous process (Show et al., 2015). Sustainability and economic concerns of lipase separation have directed intense exploration in unconventional solvents and extraction processes.

Aqueous two-phase system (ATPS) has shown to be an alternative method for the separation and purification of biomolecules from crude streams that is partitioning via two aqueous liquid phases. Various approaches of ATPS has been studied for lipase purification such as utilizing polymer-polymer (Ooi et al., 2011), polymer-salt (Padilha et al., 2012), ionic liquid (Deive et al., 2012) and alcohol-salt (Ooi et al., 2009b). Recently, the method of ATPS has been extended to a technique known as aqueous two phase flotation (ATPF) system. ATPF is an effort to attain effective and instantaneous recovery of the biomolecules in the combined unit operations. This system is an integration of separation technique grounded on solvent sublation (SS) and aqueous two-phase system (ATPS), where the target biomolecules with surface-active sites from salting-rich aqueous phase (bottom phase) are selectively adsorbed onto the surface of bubbles created by ascending nitrogen gas stream and transferred to hydrophilic organic phase (top phase) (Lee et al., 2016).

Alcohol/salt system has few advantages over polymer/polymer, polymer/salt and surfactant such as they are lower in cost, low viscosity, simple recovery of alcohol by evaporation and it is simple for scaling up in industrial production (Tan et al., 2013). Despite these advantages, concern on the large scale application of ATPF due to the high chemical demand and the environmental problems that may arise from the elevated salt concentration in waste disposal (Yang et al., 2013). The concentration of salt in two-phase system has a major effect on the partition coefficient (Iqbal et al., 2016). Most of the ATPF system requires at least 200 g/L of salt in their bottom phase for biomolecules separation (Md Sidek et al., 2016). Each time of separation process, large amount of salts is required thus, there is a need to establish an effective method of ATPF application for sustainable recovery of biomolecules.

Phase forming polymer such as polyethylene glycol (PEG) which is not recyclable set a limitation to the ATPF process, whereas the use of ethylene oxide–propylene oxide (EOPO) copolymer in ATPF results in high cost and slow separation of two-phase which subsequently limit the usage of ATPF at large-scale. To the authors' knowledge there is no extensive research done concerning the recycling of the large scale phase components for ATPF method. In a previous study (Show et al., 2013a), lipase was recovered using recycled hydrophilic organic solvent/inorganic salt ATPF. However, recycling of component was done separately. The top organic phase was recycled five successive cycles utilizing fresh aqueous phase each time. The fourth and fifth ATPF cycle utilized aqueous phase that was recovered from the previous ATPF (Show et al., 2013a). The waste water produced in each ATPF cycle causes environmental concern that leads to the need for a sustainable ATPF recycling technique to reduce the amount of waste water produced and the usage of salt. In the direction to improve the established ATPF system, an extra economic and eco-friendly ATPF with the ability to recover phase components for large scale has been proposed in this study.

The aim of this study is to investigate the effect of full and continuous recycling of both alcohol and salt phase components using ATPF system. The objectives of this study are to reduce waste disposal and high salt consumption in the lipase separation. The bottom aqueous phase was reused for eleven successive times to find the optimised condition for recycling, whereas the alcohol was recycled by evaporation. The salt in bottom phase was then crystallized through dilution crystallization by methanol and was tested for the possibility of recycling crystallized salt. The design of experiment in this study is focused on the mono-dimensional approach, whereby each parameter was studied based on one factor-at a time. In this study, the proposed design of operating conditions was taken based on few literatures related to lipase separation method using ATPF (Mathiazakan et al., 2016; Show et al., 2013b). These literature focused on the operating conditions for lipase separation process. Since this study emphasizes on the recycling, ranges of operating conditions from previous literatures were selected to perform recycling study.

2. Material and methods

2.1. Material

Olive oil, ammonium sulphate [(NH4)2SO4], trisodium citrate [$Na_3C_6H_5O_7$], magnesium sulphate [$MgSO_4$], di-potassium hydrogen phosphate (K_2HPO_4), monopotassium phosphate (KH_2PO_4), sodium carbonate (Na_2CO_3), Bradford reagent, glucose, Triton X-100, ethanol, methanol, 1-propanol, 2-propanol, butanol, were purchased from R&M Chemicals, 4-nitrophenyl dodecanonate (p-NPL), calcium chloride [$CaCl_2$], gum arabic and nutrient broth were acquired from Sigma-Aldrich (St. Louis, USA). All the chemicals and solvents were of analytical grade.

2.2. Apparatus

The ATPF system consists of glass column of 0.12 m (i.d.) diameter, 0.25 m height and capacity of 2.5 L. The bottom of the column is equipped with a sintered disk of G4 porosity (size of pore approximately 10 μm) attached to a compressed air system that is used to source the bubbles to the column. The flowmeter used to measure the flowrate is a rotameter RMA-26-SSV with a range of 0.5–5 LPM (Dweyer, USA) connected to the compressor by a regulator. Fig. 1, illustrates the equipment set up that was used in this study.

2.3. Microorganism and fermentation

B. *cepacia* strains ST8 (a kind gift from Prof. Ling Tau Chan) obtained from Laboratory of Biological Science, Science Faculty, University of Malaya was used in this research for producing lipase. This strain was selected for this study because lipase from B. *cepacia* has high tolerance towards organic solvents and aliphatic alcohol. In addition, lipase derived from that strain has higher thermal stability and wide substrate specificity. For the inoculum, the bacteria were incubated in a nutrient broth 1% (w/v) for 16 h. Fermentation was conducted in a 250 ml shake flask that consisted 150 ml of medium. Fermentation medium comprised of nutrient