



## Recovery of lipase derived from *Burkholderia cenocepacia* ST8 using sustainable aqueous two-phase flotation composed of recycling hydrophilic organic solvent and inorganic salt

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### ABSTRACT

Recycling hydrophilic organic solvent/inorganic salt aqueous two-phase flotation (ATPF) is a novel, low cost, green and high efficient technique for recovery of biomolecules. Recycling ATPF composed of 2-propanol and potassium phosphate was developed for sustainable separation, concentration and purification of *Burkholderia cenocepacia* ST8 lipase from liquid fermentation broth. Thirteen parameters upon recycling hydrophilic organic solvent/inorganic salt ATPF performance were investigated. The optimum conditions for this recycling ATPF were determined to be 40 mL volume of 50% (w/w) 2-propanol, 1.0 L of 250 g/L of potassium phosphate, pH 8.5, 100% (v/v) of crude feedstock, 30 mL/min of N<sub>2</sub> flow rate for 30 min in a 8 cm radius of colorimeter tube with G4 porosity (5–15 μm) sintered glass disk. A purification factor of 14.4 ± 0.04 and a lipase yield of 99.2 ± 0.03% were achieved in this optimized ATPF. The recycling of phase-forming components employed at the end of recovery process was based on the principals of green chemistry, with high efficiency and economical viability. There was no gross variation of results during the process of scaling-up. Therefore, this novel recycling ATPF is feasible to be applied at industrial-scale.

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### 1. Introduction

Aqueous two-phase system (ATPS) has been proven to be an effective, environmental friendly, economically viable and biocompatible method for separation, concentration and purification of biological material, particularly for macromolecules like protein, enzyme and DNA [1]. ATPS is predominantly based on polymer/salt system, such as polyethylene glycol (PEG)/potassium phosphate, or polymer/polymer system, such as PEG/dextran, which have been well-developed and documented for the recovery of biomolecules. In recent years, conventional ATPS has been enhanced by the utilization of random copolymers of ethylene oxide propylene oxide (EOPO), which can be recovered through thermo-induction. However, the challenges to apply this thermoseparating copolymer in biotechnological industry are still remained unresolved, owing to the high cost of the polymer and the difficulties in isolating

the target biomolecules from polymer phase. Furthermore, there are several limitations in polymer/salt ATPS and polymer/polymer ATPS. These include slow segregation of two-phase in polymer/polymer ATPS, time consuming and complications associated with the recycling of phase-forming components. ATPS consists of a hydrophilic organic solvent and an inorganic salt solution has been reported for the use in recovery of protein [2], amino acid [3], and other natural products [4]. Hydrophilic organic solvent/inorganic salt ATPS has many advantages, which include rapid phase-separation, high extraction efficiency, low viscosity, high polarity, gently environment, inexpensiveness of phase-forming chemicals and facile recycling of the hydrophilic organic solvent and inorganic salt [5].

Solvent sublation (SS) is an adsorptive bubble mass transfer technique. In SS, surface-active compounds in aqueous phase will be adsorbed and attached on the bubble surfaces of an ascending gas stream flowing to the top of bulk aqueous phase in the column. These surface-active compounds will be collected in an immiscible liquid layer on the top of aqueous phase (Fig. 1). The advantages of

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