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Enhanced recovery of lipase derived from *Burkholderia cepacia* from fermentation broth using recyclable ionic liquid/polymer-based aqueous two-phase systems

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ABSTRACT

In this work, a microbial lipase recovery scheme based on aqueous two-phase systems (ATPS) formed by a biocompatible ionic liquid (IL) and a thermo-sensitive polymer was studied. The IL composed of naturally derived cholinium cation and anion obtained from biological buffer, i.e. BES, designated as [Ch][BES], was used to prepare ATPS with polypropylene glycol with an average molecular weight of 400 g mol^{-1} (PPG 400). In conjugation with centrifugation step as pre-purification, the IL/polymer-based ATPS achieved an enhanced recovery of lipase from fermentation broth with a purification factor of 17.96 ± 0.32 and a recovery yield of 99.30% ± 0.03, after evaluating several phase compositions and feedstock loads. The recycling use of both phase-forming components was investigated, and there was no significant difference in the purification results with the use of recycled components compared to the systems using fresh chemicals. The proposed system is associated with many advantageous properties such as fast, simple, high biocompatibility, marginally toxicity, non-volatility, buffering capacity and recyclability, supporting its potential as a viable and sustainable platform for lipase purification.

1. Introduction

Lipases (triacylglycerol acylhydrolases, E.C.3.1.1.3) are hydrolases which catalyse the hydrolysis of carboxyl ester bonds present in triglycerides under aqueous conditions to yield fatty acids and glycerol moieties. The catalysis reaction of lipases can be reversible under micro-or non-aqueous conditions, and therefore the diverse functionality of lipases promises a wide array of industrial applications, such as organic synthesis, oleochemistry, food, pharmaceutical and detergent [1,2]. For mass production to meet industrial demands, lipases from bacterial and fungal origins are the prominent source [3]. However, commercially available microbial lipases are highly expensive, impeding the growth of lipases as biocatalysts in biotechnological applications, primarily due to the lack of cost-effective schemes for recovery and purification of lipase from fermentation broth. There are no set protocols that will ensure the purification of lipase from a cell extract [4]. Typically, these procedures consist of multiple steps, i.e. removal of biomass and insoluble compounds, concentration, purification by chromatographic techniques and polishing [5,6]. Each step is requisite to gradually enhance the purity level until the desired specification. Despite several classic methods are available, lipase purification remains a major challenge for any method adopted, associated with the drawbacks concerning the complexity of protein mixtures, enzyme deactivation and high consumption of time, energy and chemicals. Therefore, there is an urge demand to develop sustainable lipase purification schemes.

Aqueous two-phase systems (ATPS) are proposed by Albertsson [7] as an alternative bioseparation tool for biomolecules. The extraction based on ATPS is principally dependent on the selective distribution of the product of interest in two immiscible aqueous two phases [8], which are formed when two polymers or one polymer and a salt present beyond certain concentrations in an aqueous medium [9]. Due to the attractive properties such as simplicity, time saving, high biocompatibility and amenability to scale-up, ATPS have soon recognized as a feasible technique to fill the inadequacies related to traditional methods in protein separation technology [10,11]. Particularly, in view of the relatively low yields obtained using lengthy conventional purification strategies

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[12–14], ATPS have studied widely as alternatives in lipase purification strategies [15,16]. Several studies demonstrated the successful use of ATPS for direct recovery of lipase derived from microbial origin such as *Burkholderia pseudomallei* [17,18], *Burkholderia cepacia* [19], *Rhodotorula glutinis* [20] and *Aspergillus niger* [21,22]. Beside of being investigated for their feasibility as primary recovery operation, ATPS are integrated with conventional concentration/purification techniques to further improve the purity level of lipase after pre-purification process (e.g. ammonium sulfate precipitation method) [23,24]. Promising results are attained, particularly with the use of ATPS with ionic liquids (ILs) as phase constituents [25,26] or adjuvants [27]. Amongst the phase-forming components, ILs offer additional advantage, that is their tunable physicochemical properties by a proper manipulation of IL cation and anion combination, which allows the tighter control of two-phase polarities [28–30].

In lipase purification studies, imidazolium-based ILs with halogens and inorganic salts are usually used in ATPS formulations [25,31]. These systems however might raise some issues regarding the biocompatibility and environmental impact. Additionally, several studies reported that imidazolium family has deleterious effect to catalytically active lipases [32,33]. Moreover, the partition behaviour of lipase in ATPS is dramatically affected by the change in pH in the extraction system, largely due to the alteration of lipase's surface properties [23]. Aiming at getting over these shortcomings, an IL with high biocompatibility, low toxicity and buffering capability is a good candidate in ATPS formulations. Besides, the recovery of lipase by selective partitioning in two aqueous phases necessitates the optimization of key parameters in IL-based ATPS for effective purification, so that the subsequent processing steps can be reduced or eliminated [9]. Furthermore, the recycling of phase-forming constituents such as ILs is important for economical viability, and however yet to be explored in lipase purification studies.

In this work, a purification scheme using IL-based ATPS approach was studied for the recovery of an extracellular lipase produced by submerged fermentation of *B. cepacia*. Species of *B. cepacia* is one of the most important lipase-producing bacteria genera [34]. The IL studied was cholinium 2-[bis(2-hydroxyethyl)amino]ethanesulfonate ([Ch][BES]), which belongs to the group of Good's buffer ionic liquids (GBILs) that first introduced in 2014 [35,36]. Amongst the GBILs reported [35–38], the [Ch][BES] was chosen majorly due to its low toxicity, maximal buffering capacity at neutral pH region (pH 7.1) and good enzyme stabilization ability [36]. The use of the [Ch][BES] allows the creation of adequately neutral pH-controlled ATPS that are able to retain the surface properties of lipase and thus optimal partition [39]. Besides, polypropylene glycol with a molecular weight of 400 g mol^{-1} (PPG 400) was used as second phase-former owing to their high biodegradability, almost null toxicity nature and ease of recyclability [40,41]. Their chemical structures are presented in Fig. 1. This work concentrates efforts on the development of the adequately neutral pH-controlled ATPS composed of [Ch][BES] and PPG 400 for efficient recovery of lipase from fermentation broth. The key process parameters, namely the two-phase compositions and the feedstock loads, were investigated in terms of their effect on the system's purification capability. After selecting the optimal process parameters, an attempt has been made to recycle both phase-formers, i.e. [Ch][BES] and PPG 400, and then reuse in lipase purification studies, aiming at assessing the purification capability of the recycled phase-formers.

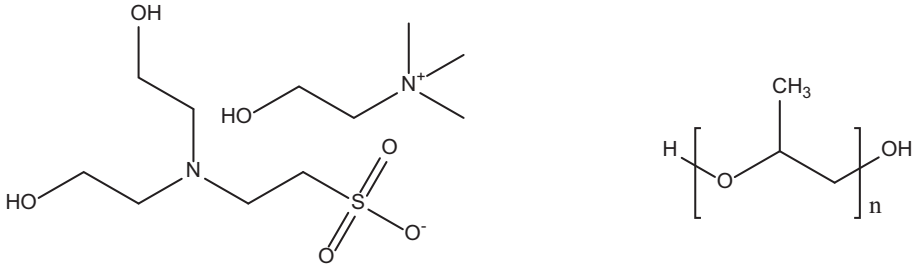
2. Experimental Section

2.1. Materials

2-[bis(2-hydroxyethyl)amino]ethanesulfonic acid (BES, purity ⩾99%), choline hydroxide solution (46 wt% in H_2O), poly(propylene glycol) P400 with an average molecular weight of 400 g mol^{-1} (PPG 400), potassium phosphate monobasic (KH_2PO_4, purity ⩾99.5%), potassium phosphate dibasic (K_2HPO_4, purity ⩾99%), 4-nitrophenyl laurate (*p*-NPL, purity ⩾98.0%), 4-nitrophenol (*p*-NP, spectrophotometric grade), brilliant blue G-250 (microscopy grade), bovine serum albumin (BSA) protein standard, trichloroacetic acid (TCA, purity ⩾99.0%), gum arabic from acacia tree, olive oil (highly refined, low acidity grade) and calcium chloride (CaCl, purity ⩾97.0%) were supplied by Sigma-Aldrich. Methanol (purity ⩾99.9%), hydrochloric acid (HCl), orthophosphoric acid and ethanol were purchased from Fisher Scientific. Nutrient broth was acquired from Becton Dickinson. The chemicals and protein standard for polyacrylamide gel electrophoresis were obtained from Bio-Rad, and silver stain kit were purchased from Sigma-Aldrich. All the materials were used without further purification. The water used throughout the work was ultrapure water treated by a Milli-Q integral water purification system.

2.2. Synthesis and characterization of IL

In this work, [Ch][BES] was synthesized by neutralization reaction of choline hydroxide and BES. The purity of the [Ch][BES] was checked by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy (Bruker, model AMX 300) operating at (300 and 75) MHz and shown to be 0.97 in mass fraction, as described in our earlier work [42].



cholinium 2-[bis(2-hydroxyethyl)amino]ethanesulfonate
[Ch][BES]

polypropylene glycol
PPG

Fig. 1. Chemical structures of compounds used in the formation of ATPS in the present work.