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# Extractive bioconversion of poly-ϵ-caprolactone by *Burkholderia cepacia* lipase in an aqueous two-phase system

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ABSTRACT

In this study, the optimum condition for extractive bioconversion of poly-ϵ-caprolactone using *Burkholderia cepacia* lipase in an aqueous two-phase system (ATPS) was established. The optimum condition was found using a combined approach of full factorial design and conventional one-factor-at-a-time studies. Optimum extractive bioconversion was achieved at pH 7.0, 40 °C and composed of 19% (w/w) PEG 3000, 8.1% (w/w) potassium phosphate, with TLL of 28% (w/w) and $V_R$ of 80:20. Under this condition, 79.8% products and 42.0% lipase was recovered from the upper and lower phases, respectively. The products consisted of both the monomeric and dimeric forms of ϵ-caprolactone. An inverse relationship was observed between the temperature of bioconversion and the melting, crystallization as well as initial decomposition temperatures of the residual reactant. The molecular weight of the residual reactant decreased with increasing bioconversion temperature whereas the opposite trend was observed for its polydispersity index.

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## 1. Introduction

Aqueous two-phase system (ATPS) is a method used for recovery and purification of biological products [1–3]. Extractive bioconversion integrates biotransformation into ATPS to achieve the *in situ* isolation of bio-products immediately after they are formed [4]. Such an integrative approach not only saves time and cost, but also reduces the risk of product loss during individual separation and purification processes. Extractive bioconversion has been used for the simultaneous production and isolation of xylo-oligosaccharides formed from hydrolysis of xylan [5], and cyclodextrins from the starch bioconversion of sago [4].

Poly-ϵ-caprolactone (PCL) is biodegradable polymer that finds applications in a diverse range of industries. PCL is readily miscible with a variety of other compounds; hence, its major use is to improve, through chemical modifications, the characteristics and/or the quality of the compounds with which it blends [6,7]. Hydrolysis of PCL represents an important step for producing shorter subunits with tailored properties. Currently, hydrolysis of PCL is achieved by using free radicals as initiators and catalysts for the degradation process [8]. However, this process requires generation of free radicals into the system, which is laborious, time-consuming and not cost-effective. Alternatively, thermal degradation can be employed to hydrolyze the polymer [9]. Nonetheless, thermal degradation can potentially alter the structure of the products. A fast, simple, cost-effective and gentle method is therefore needed for PCL hydrolysis.

Recently, hydrolysis of PCL using lipase as a catalyst has been actively investigated. However, presently, the method of extractive bioconversion of PCL in an ATPS is not well-established. In addition, the properties of the products obtained have not been characterized. The present study was therefore conducted to screen for the ATPS parameters that could increase the partitioning efficiency of hydrolyzed PCL and lipase following the hydrolysis of PCL by *Burkholderia cepacia* lipase in PEG/phosphate-based ATPS, as well as to characterize the products obtained. Selected parameters

*Abbreviations:* ANOVA, analysis of variance; ATPS, aqueous two-phase system; DSC, differential scanning calorimetry; FFD, full factorial design; GC–MS/MS, gas chromatography-tandem mass spectrometry; GPC, gel permeation chromatography; PCL, poly-ϵ-caprolactone; PEG, poly(ethylene glycol); TGA, thermogravimetric analysis; TLL, tie-line length; VR, volume ratio.

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that affect the partitioning process significantly were optimized for maximal recovery of the hydrolyzed PCL and lipase in a systematic order. Six process variables were identified to be significant based on previous literatures, namely molecular weight of PEG, pH, bioconversion temperature, tie-line length (TLL), volume ratio ($V_R$) and NaCl additive [10–12]. The molecular weight of PEG influences not only the polymeric conformation (and therefore, intramolecular hydrophobic interactions) of the biomolecule, but also interfacial tension, which has been reported to play an important role in influencing the partitioning behavior of biomolecules in ATPS [13]. On the other hand, pH could alter the charge and structure of the biomolecules in the system [3], while bioconversion temperature directly influences the rate of bioconversion (and hence the initial amount of hydrolyzed PCL) as well as stability of the lipase. Besides, TLL corresponds positively to the concentrations of phase-forming components in both phases of ATPS [14], while $V_R$ affects the free volume available for accommodating the solute molecules in both phases and NaCl additive can present long range effects to the characteristics of aqueous solutions [13]. Therefore, it is justifiable to investigate the significance of the six process variables in influencing the partition behavior of hydrolyzed PCL and lipase in the present study.

## 2. Materials and method

### 2.1. Materials

All the reagents used in this study were of analytical grade. Poly-$\epsilon$-caprolactone (PCL), poly(ethylene glycols) (PEGs) with average molecular weights of 3000, 6000 and 8000, 4-nitrophenyl palmitate, 4-nitrophenol, sodium deoxycholate and Triton X-100 were purchased from Sigma–Aldrich (USA). Chloroform, tetrahydrofuran, dichloromethane, 2-propanol and *n*-hexane were obtained from Merck (Germany). Dipotassium hydrogen phosphate, potassium dihydrogen phosphate, and acacia gum were acquired from Systerm (Malaysia). *B. cepacia* lipase was obtained from Sigma–Aldrich (USA).

### 2.2. Extractive bioconversion

Phase diagrams of PEG/salt ATPS were modified from [14]. The ATPS was prepared from 50% (w/w) PEG stock solutions with different molecular weights (3000, 6000 and 8000) and 40% (w/w) potassium phosphate stock solutions. Each ATPS was subsequently topped up with appropriate amounts of distilled water, 3% PCL and 30 mg of *Burkhoderia* sp. lipase in order to obtain an ATPS with a final mass of 10 g. There were two controls used in this experiment, an ATPS without lipase and another with deactivated lipase. The ATPSs were then incubated in an incubator shaker at a specified temperature for 72 h. All experiments were conducted in triplicates. Subsequently, the systems were centrifuged at 5000 × *g* for 5 min to allow complete phase separation. The top and bottom phases were taken out separately for analyses of hydrolysed PCL and enzyme activity. The mass concentration of the hydrolyzed PCL was determined by using a density meter, with the initial mass concentrations of the top and bottom phases as the blank reference. The lipase activity was measured by using colorimetric method with UV/VIS Spectrophotometer V-630 (Jasco, Japan). The rate of reaction was calculated by using Spectra Measurement software. The yield of hydrolyzed PCL in the top phase was calculated using Eq. (1).

$$\text{Yield of hydrolyzed PCL}\,(\%) = \frac{100}{1 + \left(\frac{1}{V_R} * \frac{1}{K}\right)} \quad (1)$$

**Table 1**
Factors and value levels used in FFD.

| Variables (Factors) | Low value (−1) | Centre value (0) | High value (+1) |
|---|---|---|---|
| Molecular weights of PEG | 3000 | 6000 | 8000 |
| pH | 6 | 7 | 8 |
| Temperature (°C) | 40 | 49 | 58 |
| Tie-line length | 28 | 32 | 38 |
| Volume ratio (PEG:Salt) | 30:70 | 50:50 | 70:30 |
| Additive (NaCl) (%) | 0 | 0.5 | 1 |

where $V_R$ represents the volume ratio of the top phase to the bottom phase and $K$ is the ratio of the hydrolyzed PCL concentration in the two phases of ATPS.

The yield of lipase in the bottom phase was determined using Eq. (2).

$$\text{Yield of lipase} = \frac{100}{1 + (V_R * K)} \quad (2)$$

where $V_R$ represents the volume ratio of the top phase to the bottom phase of the ATPS. The $K$ is the fraction of the lipase concentration in the two phases of ATPS.

### 2.3. Screening of significant process variables

Full factorial design (FFD) was employed to screen for the effects of several process variables towards the efficiency of ATPS partitioning. The process variables examined included the molecular weights of PEG, tie-line length (TLL), pH, bioconversion temperature, volume ratio ($V_R$), and the presence of a neutral salt additive (NaCl) in the system (Table 1). The levels of the central (0), low (−1) and high (+1) values of the six factors were selected based on the preliminary and published data of a related study in our laboratory [10]. Analysis of variance (ANOVA) was used to determine the significance of each process variable in affecting the partitioning of the biomolecules. $P$ values of $< 0.05$ were assumed to be significant in all analyses.

### 2.4. Optimization

Temperature, TLL and $V_R$ were selected for further optimization using the conventional method of one-factor-at-a-time to determine the optimum operating conditions of extractive bioconversion of PCL. During the optimization process, the other variables were fixed at the following conditions: PEG 3000 at pH 7 and no NaCl additive. The range of temperatures tested were 31, 33, 35, 37, 40, 49, and 58 °C; whereas that of tie line lengths were 18, 23, 28, 32 and 38 (% w/w); and that of volume ratio were 30/70, 50/50, 70/30, 80/20 and 90/10.

### 2.5. Qualitative analysis

Qualitative analysis of the hydrolyzed PCL was performed by employing gas chromatography-tandem mass spectrometry (GC–MS/MS), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and gel permeation chromatography (GPC).

#### 2.5.1. Gas chromatography–tandem mass spectrometry (GC–MS/MS)

Gas chromatography-tandem mass spectrometry (GC–MS/MS) analysis was carried out for identification of hydrolysed products formed after PCL hydrolysis. The hydrolyzed PCL was subjected to silylation to form stable sily derivatives which were more suitable for analysis using GC–MS/MS. The analysis was carried out in Agilent Triple Quadrapole 7000B GC–MS System (Agilent, USA)

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