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.

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**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...
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 surface of air bubbles will enter the immiscible top phase (alcohol-rich phase).

Table 1 Comparison of price of phase-forming agents.

<table>
<thead>
<tr>
<th>Phase-forming agents</th>
<th>Price (USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene oxide-propylene oxide (EO/PO) random copolymer (1 L)</td>
<td>99.80</td>
</tr>
<tr>
<td>Methanol (1 L)</td>
<td>59.80</td>
</tr>
<tr>
<td>Ethanol (1 L)</td>
<td>91.00</td>
</tr>
<tr>
<td>1-Propanol (1 L)</td>
<td>74.90</td>
</tr>
<tr>
<td>2-Propanol (1 L)</td>
<td>63.40</td>
</tr>
</tbody>
</table>

In this study, a large-scale ATPF equipment was fabricated with low-cost and recyclable material to improve sustainability. Lipase derived from Burkholderia 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 was investigated. The performance of lipase separation in ATPF was evaluated by indicators like the separation efficiency (E), selectivity (S) and purification factor (PRT) of lipase.

2. Material and method

2.1. Material

Ethanol, methanol, 1-propanol, 2-propanol, ammonium sulphate [(NH4)2SO4], trisodium citrate [Na3C6H5O7], magnesium sulphate [MgSO4], di-potassium hydrogen phosphate (K2HPO4), potassium di-hydrogen phosphate (KH2PO4) were purchased 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).](image-url)
The apparatus prototype was fabricated in-house by using the water tank made of high density polyethylene (HDPE), as shown in Fig. 2(b). HDPE is a stable compound that is inert to alcohol. The water tank is 0.25 m in diameter and 0.4 m in height. The bottom of an empty water tank was first cut and removed. The water tank was positioned in a metal holder at which the neck of the tank was facing downward. A rubber gas hose was then connected to the neck of the water tank. A porous media made of double woven fabric was used as the generator of air bubbles. The thickness and the porosity grade of the porous media were approximately 0.05 m and G4, respectively. The media was fitted at a slightly higher position from the neck of water tank, in order to allow the uniform pressure applied to the surface of porous media.

2.3. Fermentation culture of B. cepacia

B. cepacia ST8 strain is a gram-negative bacteria that secretes extracellular lipase and has been reported to have an effective growth rate [4]. Moreover, it can tolerate aliphatic alcohol. This bacterium was originally isolated from the soils in Sarawak and was cultivated in batches. The basic medium of the fermentation contained nutrient broth, 0.325% (w/v); CaCl₂, 0.1% (w/v); olive oil, 1% (v/v); and gum arabic, 1% (w/v) with the pH adjusted to 7.0. The olive oil served as the carbon source to boost the production of lipase [34], while the presence of CaCl₂ could intensify and extend the enzymatic activity for 30 days [35]. Fermentation was conducted in 250 mL conical flasks. The culture was subjected to agitation speed of 105 rpm at 37 °C in a shaking water bath. After a period of 72 h, the fermented culture was collected for the ATPF experiments [19].

2.4. ATPF

The experiment was conducted in a series of batches. For each batch, a volume of 1.4 L of crude lipase (fermentation broth containing lipase) was used. 10 mL of the total crude lipase solution was kept aside for lipase and protein analyses. Table 2 shows the design of experiments for the pilot-scale recovery of lipase with ATPF. One variable at a time (OVAT) approach was applied to investigate the effects of the parameters on the recovery of lipase from fermentation broth. The sequence of parameters being investigated was arranged specifically to save cost and to overcome the time constraint. Two assumptions were made: (1) the concentration of lipase in alcohol-rich top phase and salt-rich bottom phase were homogenous; (2) the volume of the aqueous phase remained constant throughout the experiment.

The compressed air was fed at 0.5 bar to the neck of the flotation tank. The stock solutions of lipase and salt were poured into the flotation tank, followed by the addition of alcohol. The top section of flotation cell was covered with aluminium foil to limit the alcohol evaporation. Fig. 1 shows a diagram depicting the lipase recovery with ATPF.

The experiment was carried out at room temperature, and the duration of ATPF experiment was set at 30 minutes. The collection of samples from both phases was performed after the experiment. For the alcohol-rich top phase containing the recovered lipase, the alcohol can be removed from the solution via rotary evaporation.

2.5. Analytical methods

2.5.1. Lipase assay

The lipase enzyme activity was determined using the methods described by Gupta et al. with minor changes. 9.8% (v/v) of 0.02 M

Table 2

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Initial setting</th>
<th>Variables</th>
<th>Unit</th>
<th>Justification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Concentration of crude feedstock</td>
<td>N/A (1st parameter)</td>
<td>10, 15, 20, 25 [33]</td>
<td>% (w/w)</td>
<td>i. Chosen as the 1st parameter due to the limited supply of the crude feedstock prepared by lab-scale apparatus &lt;br&gt; ii. The optimum value was to be determined first, in order to identify the maximum processing volume of crude feedback in pilot-scale operation</td>
</tr>
<tr>
<td>2</td>
<td>Type of hydrophilic organic solvent</td>
<td>2-propanol [5]</td>
<td>Methanol, ethanol, 1-propanol, 2-propanol</td>
<td>N/A</td>
<td>i. Type of hydrophilic organic solvent is the 2nd parameter to be tested so that volume and concentration of hydrophilic organic solvent can be optimized readily based on the chosen type of hydrophilic organic solvent</td>
</tr>
<tr>
<td>3</td>
<td>Volume of hydrophilic organic solvent</td>
<td>0.5</td>
<td>0.3, 0.5, 0.7, 0.9</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Concentration of alcohol</td>
<td>50</td>
<td>40, 50, 60, 70 [5]</td>
<td>% (w/w)</td>
<td>i. Ammonium sulphate was used in the initial setting because it was available at a large amount in our lab, as compared to other salts&lt;br&gt; ii. The influence of parameters to each other was assumed to be independent in this case</td>
</tr>
<tr>
<td>5</td>
<td>Volume of salt</td>
<td>1.5</td>
<td>1, 1.5, 2.0, 2.5</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Concentration of salt solution</td>
<td>250</td>
<td>200, 250, 300, 350 [5]</td>
<td>g/L</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Type of inorganic salt</td>
<td>Ammonium sulphate</td>
<td>Ammonium sulphate, magnesium sulphate, trisodium citrate, potassium phosphate (K₂HPO₄ + KH₂PO₄)</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>
p-NPL (dissolved in ethanol), 1.96% (v/v) Triton X-100 and 78% (v/v) of 0.05 M phosphate buffer (di-potassium hydrogen phosphate and potassium di-hydrogen phosphate) were added to 9.8% (v/v) sample. Triton X-100 was added to reduce interference caused by turbidity during UV-Vis spectrophotometric measurement [36].

The assay mixture was incubated for 30 min (200 rpm) at 37 °C and the lipase activity was measured using a UV–vis spectrometer (model UV-1800, Shimadzu, Japan) at 25 °C and wavelength λ = 405 nm. The p-nitrophenol (p-NP) released was determined using the standard curve of p-NP [33,37,38]. The regression equation obtained was \( y = 89.467x \) (\( R^2 = 0.96 \)), where \( y \) represents the absorbance (nm) and \( x \) represents the lipase activity (U). A unit (U) of lipase activity refers to the amount of lipase needed for releasing 1 \( \mu \)mol of p-NP per minute. Triplicate readings were acquired with an estimated error of ±0.05.

2.5.2. Protein assay

The total protein concentration in the samples were analysed using the Bradford method [39]. 1.5 mL of sample and 1.5 mL of Bradford reagent were mixed in a cuvette. After 10 min, the absorbance at \( \lambda = 595 \) nm was measured and compared against a reagent blank that contains an appropriate diluted phase solution without protein sample [40].

2.6. Selectivity, purification factor and separation efficiency

The partition coefficient is the coefficient of protein, denoted as \( K_P \), or lipase activity, \( K_E \), in the top phase over the corresponding concentrations in the bottom phase. Eqs. (1) and (2) describes the partition coefficient equation.

\[
P_T = \frac{P_T}{P_B} \tag{1}
\]
\[
K_E = \frac{E_T}{E_B} \tag{2}
\]

where \( P_T \) and \( P_B \) are the protein concentrations (mg/mL) at the top and bottom phases, respectively. \( E_T \) and \( E_B \) are the lipase concentrations (U/min) at the top and bottom phases, respectively.

Selectivity (\( S \)) is the ratio of partition coefficient of lipase activity (\( K_E \)) to the partition coefficient of protein (\( K_P \)). The selectivity describes parting effect on the lipase relative to that of the total protein in the bottom phase. In another words, a high selectivity is preferred as to minimize the amount of unwanted proteins collected in the top phase. Eq. (3) shows the equation.

\[
S = \frac{K_E}{K_P} = \frac{E_T}{E_B} \times \frac{P_B}{P_T} \tag{3}
\]

The purification factor (\( P_{FT} \), as shown in Eq. (4)) is the ratio of specific activity (\( SA \)) of lipase in the top phase to the \( SA \) of lipase in crude feedstock. Fig. 3 shows that a higher \( P_{FT} \) of lipase can be achieved after the ATPF process, where the content of impurities in lipase solution was reduced.

\[
P_{FT} = \frac{SA \ of \ lipase \ in \ top \ phase \ sample}{SA \ of \ crude \ lipase \ feedstock} \tag{4}
\]

Whereas \( SA \) is defined as the ratio of enzyme activity of lipase to the protein concentration. Eq. (5) describes the equation of \( SA \).

\[
SA = \frac{Enzyme \ activity \ (U)}{Protein \ (mg)} \tag{5}
\]

The separation efficiency (\( E \)) is given by Eq. (6), where \( C_{w} \) and \( C_{w} \) represent the concentrations of lipase in the bottom phase before and after the ATPF process, respectively. The \( E \) value determines the concentration of lipase being successfully recovered in alcohol-rich top phase.

\[
E = \left( 1 - \frac{C_{w}}{C_{m}} \right) \times 100 \tag{6}
\]

3. Results and discussions

3.1. Effect of concentration of crude feedstock

The experiment was carried out by varying the concentration of the crude feedstock from 10–25% (w/w). Fig. 4 shows the \( P_{FT} \) of lipase recovered from different concentrations of crude feedstock in ATPF systems. Previous studies have shown that the concentration of loaded crude feedstock will affect the ATPF performance due to the specific partition behaviour of the targeted protein [41]. Based on Fig. 4, it can be seen that the \( P_{FT} \) increased with the increase in crude feedstock concentration, peaking at 20% (w/w) of crude concentration (\( P_{FT} = 5.1 \)). However, a further increase in the concentration of crude feedstock was unfeasible as it could reduce the performance of ATPF. This was due to the increased amount of contaminants and impurities in the system when the loading of crude feedstock was increased. The increment of lipase and other contaminants in the system might result in a decline in the ATPF performance [37]. Also, the increase in crude load changed the volume ratio between two phases. Consequently, the excluded volume of alcohol at the top phase decreased and affected the partitioning of lipase to the top phase. Similarly, the precipitates built up at the interface of the both phases, leading to an inefficient purification process [20,41]. Therefore, 20% (w/w) of crude lipase was opted as the optimum concentration of crude feedstock to be used in the subsequent experiments.
3.2. Effects of the type, volume and concentration of alcohol

The selection of an appropriate alcohol phase is essential as different types of alcohols have different interaction with lipase. Previous studies have proven that lipase is stable in the presence of alcohols such as ethanol, 1-propanol and 2-propanol [38]. For this work, methanol, ethanol, 1-propanol and 2-propanol were chosen for investigation. Our observation showed that methanol was unfit for the ATPF process due to its high evaporation rate that resulted in a rapid loss of solvent from the top phase and restricted the amount of lipase to be accommodated in the free volume of alcohol-rich top phase. Moreover, a higher concentration of methanol was needed to form the two phases with inorganic salts. In the same way, ethanol demonstrated the undesirable results. Ooi et al. reported that ethanol/salt ATPS could be hardly formed, owing to the high solubility of salts in ethanol [38]. On the other hand, a longer carbon chain in alcohols facilitated the formation of two-phase system since a lower concentration of the less-polar alcohol was sufficient to form the two phases [42]. From Fig. 5, it can be seen that 1-propanol showed the highest $E$ value (53.61%) compared to other alcohols. Propanol has the tendency to form biphasic system with the lesser amounts of salt and alcohol [43]. When compared to 2-propanol, 1-propanol could form the biphasic system easily due to its relatively high level of hydrophobicity [38]. Therefore, 1-propanol is an ideal alcohol used for the ATPF process. The ATPF composed of 1-propanol recorded the optimum results of $S$ value (2.7) and $E$ value (54%) and was therefore chosen for further studies.
a volume lesser than 0.3 L would cause the difficulty in sampling the top phase because the layer of top phase in the flotation tank was too thin. From Fig. 6, it was noted that the \( S \) and \( E \) values increased along with the increasing volume of alcohol. As the volume of alcohol-rich top phase in ATPF system increased, the free volume available would be correspondingly increased [38,41]. However, beyond a threshold of alcohol volume of 1.9 L, the \( S \) value would be decreased (data not shown) because of the accumulation of impurities in the alcohol-rich top phase. The optimum volume of 1-propanol in ATPF system was determined to be at 0.9 L, where the \( E \) and \( S \) values achieved were 88% and 13.4.

As for the effect of alcohol concentration on the performance of ATPF process, the range of the 1-propanol concentration was fixed at 40–70% (w/w). From Fig. 7, the optimum concentration of alcohol was found to be at 50% (w/w), where the highest \( E \) value (i.e., 16.2) was achieved. This result was consistent with that obtained from a past study [21]. At concentration below the 50% (w/w) of alcohol, the biphasic system might be easily reverted to the homogenous system [21]. In fact, in this study, we noted that the biphasic system could not be formed when the concentration of 1-propanol was reduced to a value below 40% (w/w).

### 3.3. Effects of the volume, concentration and type of salt solution

The effect of volume of salt solution on the performance of ATPF process was examined. The range of tested volume of salt solution was 1.0–2.5 L, and the results are shown in Fig. 8. The optimum volume of salt solution was determined to be at 1.5 L, where the \( P_{FT} \) achieved was 8.58. A volume of salt solution lower than 1.5 L was not favorable due to the reduction in the volume ratio of the formed biphasic system. As a result, the free volume in the alcohol-rich top phase was reduced, thereby limiting the amount of lipase to be accommodated in the top phase.

The formation of biphasic system in the ATPF system depends on the concentrations of salt solution and alcohol. In this section, the concentration of \((\text{NH}_4)_2\text{SO}_4\) ranging from 200 g/L to 350 g/L were examined. Fig. 9 shows the effect of concentration of salt solution on the performance of ATPF system. As the concentration of salt increased to 250 g/L, both \( S \) and \( P_{FT} \) values of lipase were also increased. Beyond the salt concentration of 250 g/L, the \( S \) and \( P_{FT} \) values of lipase were found to be decreased.

The ability of a salt to induce biphasic system with hydrophilic alcohol in ATPF also depends on the Gibbs free energy of hydration of salts [21]. In this section, \((\text{NH}_4)_2\text{SO}_4\), Na$_3$C$_6$H$_5$O$_7$, MgSO$_4$, K$_2$HPO$_4$, and KH$_2$PO$_4$ salts were experimented for the recovery of lipase in ATPF.

#### Table 3

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pilot-scale ATPF</th>
<th>Small-scale ATPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production</td>
<td>Direct recovery of lipase</td>
<td>Direct recovery of lipase</td>
</tr>
<tr>
<td>System</td>
<td>1-propanol/((\text{NH}_4)_2\text{SO}_4)</td>
<td>EOPO/((\text{NH}_4)_2\text{SO}_4)</td>
</tr>
<tr>
<td>Bacteria strain</td>
<td>\textit{B. cepacia}</td>
<td>\textit{B. cepacia}</td>
</tr>
<tr>
<td>( P_{FT} )</td>
<td>12.2</td>
<td>12.2</td>
</tr>
<tr>
<td>( E )</td>
<td>93%</td>
<td>76%</td>
</tr>
<tr>
<td>( S )</td>
<td>40</td>
<td>13</td>
</tr>
<tr>
<td>Reference</td>
<td>Obtained from this research</td>
<td>[33]</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Type of salt</th>
<th>Purification factor</th>
<th>Selectivity</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tri-sodium citrate</td>
<td>7.18</td>
<td>5.07</td>
<td>2.06</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>11.89</td>
<td>2.41</td>
<td>7.49</td>
</tr>
<tr>
<td>Potassium phosphate</td>
<td>5.86</td>
<td>11.72</td>
<td>14.82</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>10.58</td>
<td>5.07</td>
<td>39.67</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Table 3</th>
<th>Comparison on the recovery of lipase on pilot-scale and small-scale ATPF processes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td>Pilot-scale ATPF</td>
</tr>
<tr>
<td>Production</td>
<td>Direct recovery of lipase</td>
</tr>
<tr>
<td>System</td>
<td>1-propanol/((\text{NH}_4)_2\text{SO}_4)</td>
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<td>Bacteria strain</td>
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</tr>
<tr>
<td>Reference</td>
<td>Obtained from this research</td>
</tr>
</tbody>
</table>
Based on Fig. 10, ATPF system composed of potassium phosphate solution showed the highest $P_T$ (14.82), while the ATPF system composed of $(NH_4)_2SO_4$ solution showed the highest values in both $E$ (93.27%) and $S$ (39.67). The $S$, $P_T$, and $E$ values of lipase obtained from the ATPF system composed of MgSO$_4$ were relatively insignificant.

4. Comparison of pilot-scale ATPF and lab-scale ATPF

A comparison has been made between pilot-scale and small-scale ATPF processes for the purification of lipase. As can be seen in Table 3, the past study using ATPF system made of EPOO/ammonium sulphate recorded a $P_T$ of 12.2, an $E$ value of 76% and a $S$ value of 13. In contrast, the $E$ and $S$ values of lipase obtained from the large-scale ATPF system were relatively higher (i.e., 93% and 40, respectively). Thus, the the purification of lipase with the 1-propanol/ammonium sulphate-based ATPF was more robust and efficient than that with the EPOO/$(NH_4)_2SO_4$ ATPF system.

Moreover, a comparison has been made between the small-scale purification of lipase derived from Escherichia coli (E. coli) and pilot-scale recovery of lipase obtained from this research. It can be seen that the $P_T$ of lipase extracted from E. coli was low (4.05). In conclusion, the large-scale ATPF based on alcohol/salt system was proven to be suitable for the purification of lipase.

5. Conclusions

The lipase derived from B. cepacia was successfully separated and purified from fermentation broth by using pilot-scale alcohol/salt ATPF. The optimized operating conditions of 1-propanol/$(NH_4)_2SO_4$ ATPF system were: (1) crude feedstock concentration: 20% (w/w), (2) type of hydrophilic organic solvent: 1-propanol, (3) type of inorganic salt: ammonium sulphate, (4) concentration of hydrophilic organic solvent solution: 50% (w/w), (5) concentration of inorganic salt solution: 250 g/L, (6) volume of hydrophilic organic solvent phase: 0.9 L, (7) volume of inorganic salt phase: 250 g/L, (8) concentration of inorganic salt solution: 250 g/L, (9) volume of hydrophilic organic solvent phase: 0.9 L, (10) volume of inorganic salt phase: 250 g/L, (11) concentration of inorganic salt solution: 250 g/L, (12) temperature of enzyme cocktail: 25°C, (13) volume of hydrophilic organic solvent phase: 0.9 L, and (14) volume of inorganic salt phase: 250 g/L.

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