Regular article

Biobutanol recovery from model solutions/fermentation broth using tripotassium phosphate

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ARTICLE INFO

Article history:
Received 19 July 2016
Received in revised form 28 August 2016
Accepted 31 August 2016
Available online 1 September 2016

Keywords:
Biobutanol
Recovery
Model solutions/fermentation broth
Salting-out
Tripotassium phosphate

ABSTRACT

The acetone + 1-butanol + ethanol (ABE) fermentation has a long history but still faces the challenge of enhancing the low ABE concentration to reduce production cost. Nowadays there is an unprecedented resurgence of interest in separation and purification technology to recovery ABE from fermentation broth. Here we describe a simple salting out procedure for extracting ABE fermentation products efficiently from model solutions/fermentation broth by employing tripotassium phosphate (K₃PO₄). Increasing the K₃PO₄ content permits the liquid-liquid splits and enables the recovery of ABE. The liquid-liquid equilibria were mainly determined by the K₃PO₄ content and slightly affected by temperature and original solvents level. The correlation between the solubility of ABE and the molality of K₃PO₄ demonstrated this. More than 90 wt% of ABE was recovered from the model solutions/fermentation broth and more than 99.75% of water was removed. This study provides a means to reduce the energy demand of the subsequent distillation process for ABE purification.

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1. Introduction

The research into the sustainable fuels becomes a hot field nowadays. On the one hand, the oil crisis gives impetus to the diversity of energy. On the other hand, the fossil fuels, such as coal and oil, have constituted significant sources of contamination of environments. Thus it is prudent to start a transition to cleaner fuels. Biofuels aim at cutting down the emission of carbon dioxide and other exhaust emissions into the atmosphere due to the source of biofuels, including sustainable biomass such as corn grain, corn stovers, and other feedstocks. 1-Butanol and ethanol, for example, are alternative fuels because they are renewable bio-based resources [1,2].

1-Butanol can be produced by the acetone + butanol + ethanol (ABE) fermentation [3]. After the fermentation, acetone, 1-butanol, and ethanol are recovered and purified by the multi-distillation method [4]. The separation process is unattractive because of the large energy consumption [5]. That’s why 1-butanol is mainly obtained via chemical synthesis. The large energy consumption is attributed to the dilute fermentation broth and the binary heterogeneous azeotropic 1-butanol + water system. At the end of the ABE fermentation, the broth contained ABE with different total solvents (ABE) levels. The total solvents levels of the fermentation broth varied from 18 g L⁻¹ to 30.1 g L⁻¹ because of the 1-butanol tolerance [6,7]. A prefractionator was always used to enrich the fermentation broth, and then the concentrated ABE solution was obtained in the top of the prefractionator [4]. But the water content of this solution was 50%–60%. So the binary heterogeneous azeotropic 1-butanol + water system formed in the downstream manipulations [8]. The aforementioned factors make the ABE fermentation inferior to chemical synthesis. Therefore, various in-situ techniques for the recovery of acetone, 1-butanol, and ethanol from the fermentation broth get rapid developments in solving these problems.

The energy requirement for distillation was dependent of the ABE feed concentration [9]. By removing the solvents from the fermentation broth with other energy-saving methods, the energy requirement for the biobutanol separation fell sharply. These in-situ recovery methods include adsorption [10], pervaporation [11], extraction [12], and gas stripping [13]. All the methods hold advantages and disadvantages, especially with regard to selectivity, regeneration, cost, and fouling. However, this concept of in-situ enrichment makes perfect sense to reduce the energy requirement.

Acetone and 1-butanol from a dilute aqueous solution can be extracted by the extractants of cyclopentanol, n-ualeraldehyde, tert-amyl alcohol, and Adol BSNF [14]. Moreover, the extraction efficiency was significantly affected by the addition of a few strong electrolytes. The solvents (ABE) from the fermentation broth can

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http://dx.doi.org/10.1016/j.bej.2016.08.010
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2.1. Materials and methods

2.1. Materials

Acetone (99.5 wt%) and tripotassium phosphate trihydrate (K$_3$PO$_4$·3H$_2$O, 99.0 wt%) were supplied by Guangzhou Chemical Reagent Factory, (Guangzhou, China). 1-Butanol (99.5 wt%) was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd., (Shanghai, China). Ethanol (99.7 wt%) was obtained from Guangdong Guanghua Sci-Tech Co., Ltd., (Shantou, China). These chemicals were used without further pretreatment. Dilute model solutions were prepared by using acetone, 1-butanol, ethanol and distilled deionized water with electrical conductivity of lower than 1.5 × 10⁻⁴ S·m⁻¹. Fermentation broth was purchased from Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences (Qingdao, China). It was produced in a fed-batch fermentation system under anaerobic conditions using C. beijerinckii NCIMB8052.

2.2. Salting-out experiments

The variable dilute feed for the salting-out experiments could contain the solvents (ABE) at different concentrations. In terms of the typical compositions of the fermentation broths, two dilute model solutions were selected. According to a typically approximate ratio of A:B:E = 3:6:1 (ω/ω) [18,19], model solution A is adopted, as shown in Table 1. Model solution B is featured with higher concentration of the total solvent and a higher relative ethanol concentration [15]. These model solutions are homogeneous because of the low concentration of 1-butanol and the cosolvents of acetone and ethanol. The acetone, 1-butanol, and ethanol contents of the fermentation broth were 0.39 wt%, 1.00 wt%, and 0.04 wt%, respectively. The contents of butyric acid and acetic acid were ignored in this recovery study.

Tripotassium phosphate trihydrate was dissolved in the model solution A for phase separation. The initial water-based concentrations of K$_3$PO$_4$ in the salting-out system varied from 100 g·kg⁻¹ to 650 g·kg⁻¹. The water-based initial concentration of K$_3$PO$_4$ was given as,

$$C_i = \frac{m_{salt}(g)}{m_{salt}(g) + m_{water}(g)} \times 1000$$

where $C_i$ represented the water-based initial concentration of K$_3$PO$_4$ in the unit of g·kg⁻¹, $m$ represented the mass of water or salt in the unit of gram (g).

The salting-out system was shaken thoroughly and then settled in a thermostatic bath until the salting-out phase equilibria were achieved at a given temperature (298.15 K, 310.15 K, 323.15 K). The partition coefficients of ethanol, acetone, and 1-butanol between the organic phase and the aqueous phase were given as,

$$D_i = \frac{\omega_{i2}}{\omega_{i3}}$$

2.3. Analysis

The mass fraction of K$_3$PO$_4$ in the salting-out agent was determined by flame atomic absorption spectrometry (FAAS), at the sub-sensitive resonance line (404.5 nm) of potassium [20]. A calibration curve was created for the calculation. Nitric acid (1 ml of 50% V/V nitric acid) and cesium nitrate (3 ml of 10 g/L cesium nitrate

## Notation

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABE</td>
<td>Acetone + 1-butanol + ethanol</td>
</tr>
<tr>
<td>$D_i$</td>
<td>Partition coefficient for $i$ component, $i=1, 2, 3, 4$</td>
</tr>
<tr>
<td>$R$</td>
<td>Recovery</td>
</tr>
<tr>
<td>$\omega_{ij}$</td>
<td>Mass fraction of one component in one phase (subscript $i = 1,2,3,4,5$ represent water, ethanol, acetone, and 1-butanol, and $j$ represents salt), respectively; subscript $j = 1,2$ represents aqueous phase and organic phase, respectively, e.g., $\omega_{011} + \omega_{021} + \omega_{031} + \omega_{041} + \omega_{051} = 1$</td>
</tr>
<tr>
<td>$\omega_{ijf}$</td>
<td>Mass fraction of one component in one phase under salt-free condition (subscript $i = 1,2,3,4$ represent water, ethanol, acetone, and 1-butanol, respectively; subscript $j = 1,2$ represent aqueous phase and organic phase, respectively; $F$ represents salt-free condition, e.g., $\omega_{11F} + \omega_{21F} + \omega_{31F} + \omega_{41F} = 1$)</td>
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$m_1$ Mass of the aqueous phase

$m_2$ Mass of the organic phase

$m_0$ Mass of model solution

$\omega_{0i}$ Mass fraction of each component in model solution

$M$ Molar mass of salt (g/mol)

$b$ Molality of salt (mol/kg)

$s$ Solubility of ABE in the aqueous phase (g/100 g water)

$\alpha$, $\beta$ Constants

Also be extracted by themselves after sufficient addition of salts [15]. It appeared that the acetone/K$_3$HPO$_4$ system was more favorable. These two methods enriched model solutions/fermentation broths, which reduced the energy requirement for distillation. But the extra additions of organic extractants would greatly complicate the subsequent separation process. Compared with the hydrophobic organic solvent extraction and the salting-out extraction, the salting-out technique seems to be more efficient for the recovery of acetone, 1-butanol, and ethanol from the fermentation broth, in which no extra extractant is needed.

The ABE fermentation broth contained proteins, remaining starches, remaining sugars, cells, and other impurities. Therefore, the model solutions only containing acetone, 1-butanol, ethanol, and water were used instead of the ABE fermentation broth to investigate the salting-out behavior. The salting-out abilities of the anions are in the order of PO$_4^{3-}$ > HPO$_4^{2-}$ > CO$_3^{2-}$ [16]. When (ethanol + water) mixture was salted out, K$_3$PO$_4$ showed relatively better performance among the investigated salts [17]. Thus, K$_3$PO$_4$ was selected as the salting-out agent. In this research project, the salting-out effect of K$_3$PO$_4$ on the separation of acetone, 1-butanol, and ethanol from the model solutions/fermentation broth was investigated. Potassium phosphate trihydrate or its solution was added to the model solutions/fermentation broth to achieve the phase separations. The purpose of this study was to increase the concentration of the solvents (ABE) and remove the water from the model solutions/fermentation broth. Then the recoveries of acetone, 1-butanol, and ethanol were calculated.
aqueous solution) were added to the samples in a 50-mL volumetric flask. Cesium nitrate was used as an ionization buffer.

After leading to phase separation at equilibrium, the mass fractions of acetone, 1-butanol, ethanol, and water in both phases were measured by gas chromatography (GC 7900, TECHCOMP, China) equipped with a thermal conductivity detector (TCD) and a packed column (2 m(L) × 3 mm(ID) × 5 mm(OD), Porapak Q, 80–100 mesh, TECHCOMP, China). The carrier gas was hydrogen (99.999%) with at a flow rate 30 ml/min. The dead cells arising from osmotic shock in the fermentation broth was filtered out and only the liquid passed into the GC analysis system. The salt contents in both phases were also measured by FAAS. Samples were pretreated as previously described. All the analytical experiments were duplicated.

3. Results and discussion

3.1. Effect of salt concentration

When an adequate amount of K₃PO₄·3H₂O was added into the dilute model solution A at 298.15 K, the solvents(ABE) were found to separate from the model solution and formed a new immiscible organic phase. The initial concentration of K₃PO₄ needed to cause phase separation was between 200 g kg⁻¹ and 250 g kg⁻¹, as shown in Table 2. Control represented the model solution A. The new phase would not appear if the initial concentration of K₃PO₄ was lower than 200 g kg⁻¹. K₃PO₄ gave the charged ions to attract the water molecules [21]. But a small amount of charged ions was insufficient to repel the solvents. The salting-out system was still homogeneous. Therefore, a certain amount of ions was needed for phase separation.

Table 2 shows the partition coefficients of ethanol, acetone, and 1-butanol at different initial concentrations of K₃PO₄. When the initial concentration of K₃PO₄ was higher than the critical point, the partition coefficients increased as the initial concentration of K₃PO₄ increased. As a result, more solvents were repelled into the upper phase. The partition coefficients also indicated that the charged ions showed a stronger salting-out effect on 1-butanol with lower polarity index [22]. When the initial concentration of K₃PO₄ was 500 g kg⁻¹, 1-butanol could not be detected by us. After the addition of salt, the characteristic tetrahedral structure of water had been disrupted by the ions [23]. The water-mediated interactions between the solvents (ABE) and charged ions are closely tied to the extent of hydration of the ions [24]. Therefore, the ions at high concentrations showed strong salting-out effects through nonspecific electrostatic interactions and increased the recovery of the solvents.

After the phase equilibrium at the initial concentration of K₃PO₄ of 250 g kg⁻¹ at 298.15 K, the organic phase contained 13.87 wt% water, with most water remained in the bulk aqueous phase. The mass fraction of water in the organic phase decreased as the initial concentration of K₃PO₄ increased, as shown in Fig. 1(a). It was 6.57 wt% under salt-saturated condition and much lower than 98.20 wt%. Thus, the model solution was highly concentrated.

![Figure 1](image-url)  
Fig. 1. The mass fraction of water in the organic phase (a), the salt-free condition mass fraction of water in the aqueous phase (b), as a function of different initial concentrations of K₃PO₄.
Moreover, the 1-butanol + water azeotrope would be reduced in quantity. The mass fraction of water in the aqueous phase under salt-free condition increased as the initial concentration of K3PO4 increased, as shown in Fig. 1(b). “Salt-free” means the compositions of ABE and water in the aqueous phases if the salt mass is ignored. We imagined $w_{11F} + w_{21F} + w_{31F} + w_{41F} = 1$. Then we had these equations:

$$w_{11F} \times (1 - w_{01}) = w_{11F} \times (1 - w_{51}) = w_{21F} \times (1 - w_{01}) = w_{31F} \times (1 - w_{01}) = w_{41F} (1 - w_{51}) = w_{41F}. \text{At last, we had this equation:}\ w_{11} + w_{21} + w_{31} + w_{41} = w_{41}. \text{The mass fractions under salt-free conditions were imaginary. It also indicated that the recovery of ABE gradually increased. The water content in the aqueous phase under salt-free condition was 98.84 wt% at the initial K3PO4 concentration of 250 g kg$^{-1}$ at 298.15 K. The mass fraction of water in the organic phase and the recovery of ABE were closely tied to the initial K3PO4 concentration. Thus, the salting-out phase separation should be performed at a high initial K3PO4 concentration.}

3.2. Effect of temperature

The solubility of K3PO4 in pure water increased as temperature increased [25], Fig. 1 also shows the effect of temperature on the salting-out process. The mass fraction of water in the organic phase at the same initial concentration of K3PO4 decreased slightly as temperature increased from 298.15 K to 323.15 K. A much more significant reduction of it under salt-saturated condition was shown due to the increasing solubility of K3PO4 at higher temperatures. It decreased from 6.57 wt% at 298.15 K to 3.31 wt% at 323.15 K. Meanwhile, the water content in the aqueous phase under salt-free condition increased slightly as temperature increased.

3.3. Using K3PO4 solution to recover ABE from model solutions

Besides the solid salt, a 63 wt% aqueous solution of K3PO4 was also introduced into the model solutions. Fig. 2 shows the variations of the mass fractions of ethanol, acetone, or 1-butanol in the aqueous phase with increasing the salting-out factor for the model solutions. The salt concentration in the aqueous phase increased as the salting-out factor increased. It is postulated that the mass fractions of the solvents decreased. When the salting-out factor was greater than 0.8, the mass fractions of 1-butanol was lower than that of ethanol or acetone. Though the model solution contained more 1-butanol, the salt solution gave priority to the recovery of 1-butanol.

After the K3PO4 solution was introduced into the model solution B, the mass fractions of ethanol, acetone, and 1-butanol in the aqueous phase decreased, as shown in Fig. 2(b). Comparing with the model solution A, as shown in Fig. 3, the mass fraction of 1-butanol from model solution B dipped to a low point significantly at a lower salting-out factor. By contrast, the mass fraction of ethanol from the model solution B was higher than that from the model solution A at the same salting-out factor. Based on the acetone content close to each other, it indicated that the 1-butanol molecules were replaced by the ethanol molecules in the aqueous phase. The model solution B containing the higher ethanol content availed the recovery of 1-butanol.

After the phase equilibrium, an individual recovery was calculated as,

$$R_i = \frac{m_2 \omega_2}{m_0 \omega_0} = \frac{m_0 \omega_0 - m_1 \omega_1}{m_0 \omega_0}$$

where $R_i$ represented the recovery of acetone, 1-butanol or ethanol, $m_1$ was the mass of the aqueous phase, $m_2$ was the mass of the organic phase, $\omega_1$ was the mass fraction of each component in the aqueous phase, $\omega_2$ was the mass fraction of each component in the organic phase, $m_0$ is the mass of the model solution/fermentation broth, $\omega_0$ is the mass fraction of each component in the model solution/fermentation broth.

To evaluate the salting-out effect of the K3PO4 solution on the model solutions, the individual recovery was shown in Fig. 4. The sequence of the individual recovery was $R_4 > R_3 > R_2$ at a certain salting-out factor because of the polarity indexes of ethanol (5.2) > acetone (5.1) > 1-butanol (4.0) [22]. It indicated that higher salting-out factors could achieve the greater individual recovery. When the salting-out factor was 1.8, 1-butanol from the model solution A was fully recovered. By contrast, the salting-out factor that needs to fully recover 1-butanol from model solution B was 1.5 because of the higher level of total solvents. The total recovery of ABE was calculated as,

$$R = \frac{\sum m_2 \omega_2}{\sum m_0 \omega_0} = \frac{\sum m_0 \omega_0 - \sum m_1 \omega_1}{\sum m_0 \omega_0}$$

The total recovery of ABE was greater than 92% at the salting-out factor of 2.0.
3.4. Effect of total solvents level on the recovery of ABE

Fig. 5 shows the effect of solvents level on the recovery of acetone, 1-butanol, or ethanol. The individual recovery increased significantly when the solvents level increased, suggesting that there was a significant improvement in the total recovery of ABE from the model solution B. Though the aqueous phases in Fig. 3 contains different contents of ABE, the higher total solvents level is just naturally more inclined to contribute to the molecular aggregation behaviors, resulting in the formation of a new phase. It indicates that the fermentative production of ABE by organism in higher solvents level and yield will result in higher ABE recovery.

3.5. Using K$_3$PO$_4$ solution to recover ABE from fermentation broth

Using the K$_3$PO$_4$ solution that had been tested in the model solutions to salt out ABE from fermentation broth, we successfully employed the salting-out method to promote the liquid-liquid phase splitting. It can be seen from Fig. 6(a) that acetone and 1-butanol contents decrease significantly when the salting-out factor increases. The much lower content of ethanol led to the slow trend of decline, suggesting the lower recovery of it, as shown in Fig. 6(b). The high selectivity for 1-butanol over acetone and ethanol led to the same trend of recovery. The sequence of the individual recovery was also $R_4 > R_3 > R_2$ at a certain salting-out factor because of the polarity indexes of ethanol (5.2) > acetone (5.1) > 1-butanol.
However, the individual recovery of ethanol, acetone, 1-butanol, and total recovery from fermentation broth were all lower than those from the model solutions at the same salting-out factor due to the lower solvents level. Namely, it demonstrated the previous-mentioned conclusion that higher solvents level resulted in higher ABE recovery.

3.6. Removal of water

The dehydration ratio (DR) is determined by

$$DR = \frac{m_{10} - m_{2} \omega_{12}}{m_{10}}$$

where $m_{2}$ is the mass of the organic phase, $m_{10}$ is the mass of water in the model solutions/fermentation broth.

The organic phase becomes less attractive for water, thus there is a dramatical reduction in the water content of the organic phase. It was foreseeable that the mass fraction of water in the organic phase decreased as the salting-out factor increased, as shown in Fig. 7(a). The mass fraction of water in the organic phase of the (model solution $A + \text{K}_3\text{PO}_4$ solution) system was lower than that of the (model solution $B + \text{K}_3\text{PO}_4$ solution) system at the same salting-out factor. A larger amount of ethanol in the organic phase, which was salted out from the model solution $B$, had a significant effect on it. The higher ethanol concentration made this phase more hydrophilic. For the salting-out factor of 2.0, the mass fraction of water in the organic phase of the (model solution $A + \text{K}_3\text{PO}_4$ solution) system and the (model solution $B + \text{K}_3\text{PO}_4$ solution) system was 7.29% and 7.54%, respectively. The ethanol and acetone contents in the fermentation broth were much lower than those in the model solution $A$. The
salting-out organic phase was more hydrophobic, resulting in the lower water content. Consequently, the removal of water from the fermentation broth was easier, as shown in Fig. 7(b). Fig. 7(b) was calculated according to Fig. 7(a), where the errors were included in the plots. It can be seen that more than 99.75% of water from the model solutions/fermentation broth can be removed by the salting-out procedure.

A lower concentration of ethanol in model solution A makes the mass fraction of water in the organic phase lower. But it causes 1-butanol to be fully recovered at a higher salting-out factor. A higher salting-out factor increased the salt-recovery difficulty. Thus the preferable ABE fermentation broth should have a higher solvents concentration to achieve the salting-out recovery of ABE.

### 3.7. Solubility correlation

The data for ABE in the aqueous phase were mainly determined by the salt content, which can be fit by Eq. (6)

$$\log s = ab + \beta$$

The solubility of ABE in the aqueous phase (s) measured in the units of g per 100 g water is given by,

$$s = \frac{\omega_{21} + \omega_{31} + \omega_{41}}{\omega_{11}} \times 100$$

The molality of salt (b) in units of mol per 1 kg water is given by,

$$b = \frac{\omega_{51} \times 1000}{M \times \omega_{11}}$$

where $M$ is the molar mass, for K$_3$PO$_4$, $M = 212.27$ g/mol, $\alpha$ and $\beta$ are constants.

Results presented in Fig. 8 demonstrate that there is a linear relation between logarithm of the solubility of ABE and the molality of K$_3$PO$_4$ in the aqueous phase. It can be seen that the ABE content of the aqueous phase is mainly determined by the K$_3$PO$_4$ content and slightly affected by the original solvents level. Increasing the molality of K$_3$PO$_4$ resulted in lower solubility of ABE and achieved higher recovery of ABE. This finding suggests that the values of $\alpha$ and $\beta$ obtained from a linear regression can show the salting-out behavior of K$_3$PO$_4$, as shown in Table 3. Considering the model solution A, we can get,

$$\log s = -0.412b + 0.675$$

Considering the model solution B, we can get,

$$\log s = -0.404b + 0.679$$

Considering the model solution A and the model solution B together, we can get,

$$\log s = -0.411b + 0.686$$

Considering fermentation broth, we can get,

$$\log s = -0.414b + 0.682$$
The experimental points fall upon a straight line, no matter a model solution or the fermentation broth was used, suggesting that the linear relation between logarithm of the solubility of ABE and the molality of $K_3PO_4$ may be of general application. The agreement on the results of total linear regression and that of the fermentation broth is very satisfactory.

In processing for the separation, actual fermentation residues possess problems. The insoluble residues were filtered out before salting-out. The dead cells arising from osmotic shock and other salted-out macromolecules were also filtered out after salting-out. The soluble residues in the organic phase and the aqueous phase may cause some problems in the subsequent distillation and the salt recovery.

4. Conclusions

Using this salting-out of acetone, 1-butanol and ethanol induced by $K_3PO_4$, we developed a high-enrichment method for dilute aqueous ABE solutions (ABE in water). By gradually increasing the $K_3PO_4$ concentration we are able to obtain higher distribution coefficients of ABE and achieve higher ABE contents of the organic phase. The effect of temperature was very small except at the highest initial concentration. The water content of the organic phase below 10 wt% meant that most of the water from the model solution was removed.

1-butanol was recovered preferentially and achieved total recovery at a lower salting-out factor. When the $K_3PO_4$ solution was twice model solution/fermentation broth, more than 90 wt% of ABE could be recovered and more than 99.75% of water was removed. A higher level of ethanol made the organic phase more hydrophilic, resulting in a higher water content of the organic phase and the lower dehydration ratio. A higher solvents level contributes to the higher recovery of ABE.

The difference in the composition between model solutions A&B had a significant effect on the compositions of the aqueous phases. However, there is a linear relation between logarithm of the solubility of ABE and the molality of $K_3PO_4$ in the aqueous phase. The salting-out effect of the $K_3PO_4$ solution on the model solutions/fermentation broth was mainly determined by the molality of $K_3PO_4$.

The energy and ecological problem associated with the salting-out method is the salt recovery. Future work will concentrate on the improvements in the salt recovery from the aqueous phase, and the design of downstream distillation unit for the ABE purification. The evaporation may be involved in the salt recovery from the aqueous phase if the fermentation broth is pretreated by a prefractionator [26]. But it was impractical in terms of the bulk aqueous phase in this study. Therefore, other alternatives such as the breakthrough of the membrane science to the enrichment of the concentrated salt solution should be exploited to make the salt recovery more energy-saving. With the salt recovery from the aqueous phase solved, the new design of the downstream distillation unit for the purifications of ABE will be more accessible to the plant operation.

Acknowledgements

This work was funded by the Fundamental Research Funds for the Central Universities of China (2015ZM169), the International S&T Cooperation Program of China (2013DFA41670), and the National High-tech R&D Program (863 Program) (No. 2012AA021202).

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