

Reactive-extraction of 2,3-butanediol from fermentation broth by propionaldehyde: Equilibrium and kinetic study

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Abstract—An effective process was developed to separate 2,3-butanediol (2,3-BD) from fermentation broth (FB) by reactive-extraction. Propionaldehyde (PRA) was used as reactant and reaction product 2-ethyl-4,5-dimethyl-1,3-dioxolane (EDD) acted as extractant. HCl was selected as catalyst. Appropriate conditions were obtained by experiment as follows: 10 °C, $C_{HCl}=0.2 \text{ mol}\cdot\text{L}^{-1}$, two-stage cross-current extraction, reactant volume ratio ($V_{PRA} : V_{FB}$) for first stage and second stage is 0.10 and 0.05, respectively. The yield rate of 2,3-butanediol for the whole process can reach 90% w/w, and 2,3-butanediol in the final product can be more than 99% w/w. The novel process required less solution and especially had advantages in treating dilute fermentation broth. Furthermore, equilibrium and kinetic study were investigated on the reaction of propionaldehyde and 2,3-butanediol to provide basic data for process development. The results reveal that reaction enthalpy and activation energy of the reaction were $-21.84\pm 2.38 \text{ KJ}\cdot\text{mol}^{-1}$ and $51.97\pm 2.84 \text{ KJ}\cdot\text{mol}^{-1}$, respectively. Kinetics was well described by pseudo-homogeneous model.

Key words: 2,3-Butanediol, Reactive-extraction, Kinetics, Propionaldehyde, Fermentation Broth

INTRODUCTION

2,3-Butanediol (2,3-BD) is a chemical with high boiling point and high hydrophilicity [1], which can be used as raw material in chemical and rubber industry. It has the potential to be a new liquid fuel as its heating value is $27.19 \text{ KJ}\cdot\text{g}^{-1}$ [2-4]. The biological route of 2,3-butanediol production has many attentions and great progress has been made in this area, which makes it possible for biological route of 2,3-butanediol production to be commercialized [5-9]. However, recovery of 2,3-butanediol from fermentation broth is a expensive process for the special properties of fermentation broth, which may limit the commercialization of bio-based 2,3-BD's manufacture.

Separation processes have been reported in the literature, including steam stripping [10], solvent extraction [11], aqueous two-phase extraction [12] and reaction separation process [13]. All these processes can be used to separate 2,3-BD, but they still have their inherent problems such as high energy costs and high solution consuming.

Reactive-extraction, intensifying the efficiency of extraction by reaction, is an effective way to get a target compound from complicated mixture system [14]. Reactive-extraction processes have been reported to separate 1,3-propanediol from fermentation broth [15,16]. While the complete reactive-extraction processes for 2,3-butanediol are still a few [17].

In this paper, a complete reactive-extraction process was investigated. The reaction is shown in Fig. 1. Propionaldehyde (PRA), which was selected for its low boiling point, can reversibly react with 2,3-BD to form 2-ethyl-4,5-dimethyl-1,3-dioxolane (EDD), while some PRA may be oxidized to be propionic acid (PAD). In the process, 2,3-BD was first changed to hydrophobic material EDD by reacting with propionaldehyde (PRA). As the reaction has high selectiv-

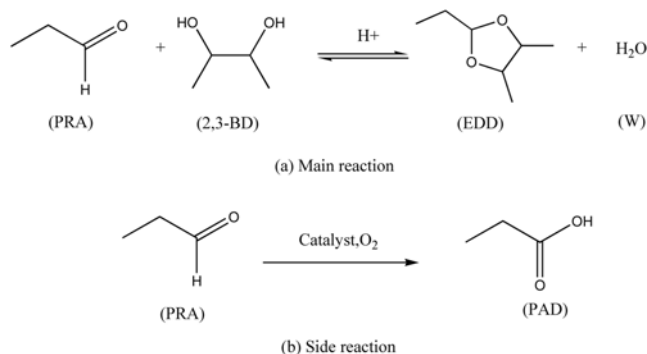


Fig. 1. Reaction equation of propionaldehyde and 2,3-butanediol system: (a) Main reaction; (b) side reaction.

ity and reaction product EDD has low solubility in water, reactive-extraction can separate 2,3-BD from fermentation broth effectively. Then the extract hydrolyzed and turned back to 2,3-BD. Feasible operation mode was chosen to reduce the solution consuming. These works would provide a simple and effective separation process to recover 2,3-BD from fermentation broth using less solution. Furthermore, equilibrium and kinetics study were investigated on the reaction of 2,3-butanediol and propionaldehyde (PRA) to provide basic data for process development.

MATERIAL AND METHODS

1. Materials

2,3-Butanediol (2,3-BD) was bought from the Sinopharm Chemical Reagent Co., Ltd. with a minimum purity of 98.2% w/w. Propionaldehyde (PRA) was purchased from the Sinopharm Chemical Reagent Co., Ltd with a minimum purity of 99.3% w/w. Acetoin was bought from the Sinopharm Chemical Reagent Co., Ltd. as a

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solid under room temperature with a minimum mass fraction purity of 99.0%. Anhydrous ethanol was provided by the Shanghai Chemical Reagent Co., Ltd. with a mass fraction of more than 99.5%. Propionic acid (PAD) was bought from Shanghai Chemical Reagent Co., Ltd. with a mass fraction of more than 99.0%. The standard of 2-ethyl-4,5-dimethyl-1,3-dioxolane (EDD), which cannot be bought from commercial source, was synthesized by our team and purified twice by distillation, with a mass fraction of more than 98.5%. All these chemicals were measured and assured by gas chromatography. Glucose was provided by Taizhou Changpu Chemical Reagent Co., Ltd. as a solid with a mass fraction of more than 98.0%. Bovine serum albumin (BSA) was provided by Shanghai Aibi Chemistry Preparation Co., Ltd. with BR level. Glucose reactant kit was provided by Shanghai Runcheng Biotechnology Co., Ltd. Coomassie brilliant blue was bought from Shanghai Siji Biological Product Co., Ltd. with BR level. Hydrochloric acid (HCl) was provided by the Sinopharm Chemical Reagent Co., Ltd. with a minimum fraction of 36% w/w. Sulfuric acid (H₂SO₄) was provided by the Sinopharm Chemical Reagent Co., Ltd. with a mass fraction of 95-98%. Tosylate acid was bought from the Shanghai Chemical Reagent Co., Ltd. with a mass fraction of more than 99.0%. Ion-exchange resin HZ732 was given by the Shanghai Huazhen Sci & Tech Co., Ltd.

Fermentation broth was provided by the National Key Lab of Bioreactor Engineering in East China University of Science and Technology with 2,3-butanediol (30.0-143.0 g·L⁻¹), acetoin (6.1-23.2 g·L⁻¹), cells (35.4-70.4 g·L⁻¹), glucose (2.3-5.2 g·L⁻¹), proteins (0.2-1.3 g·L⁻¹) and impurities, such as salts and surface active substances.

2. Methods

2-1. Equilibrium and Kinetic Study

Catalyst selection was first carried out to choose suitable catalyst from the reaction system. Reaction time to equilibrium (RT_{eq}), conversion ratio (η) and reaction selectivity (β) were used to measure the catalytic activity [18]. η and β can be calculated by Eq. (1) and Eq. (2).

$$\eta = \frac{n_{BD_o} - n_{BD_e}}{n_{BD_o}} \times 100\% \quad (1)$$

$$\beta = \frac{n_{EDD_e} - n_{EDD_o}}{n_{PRA_o} - n_{PRA_e}} \times 100\% \quad (2)$$

where n_{BD_o} is the mole amount of 2,3-BD in the reaction system before reaction, n_{BD_e} is the mole amount of 2,3-BD in the reaction system before reaction; n_{EDD_o} is the mole amount of EDD in the reaction system before reaction, n_{EDD_e} is the mole amount of EDD in the reaction system before reaction; n_{PRA_o} is the mole amount of PRA in the reaction system before reaction, n_{PRA_e} is the mole amount of PRA in the reaction system before reaction.

Equilibrium experiments were conducted in a beaker with a water jacket connected to a thermostat. Three samples were collected under one temperature to make sure the reaction reached equilibrium. As the reaction between PRA and 2,3-BD is reversible, reaction equilibrium constant K was used to describe the reaction limit of reaction (Eq. (3)) [19].

$$K = \frac{[W_{eq}][EDD_{eq}]}{[PRA_{eq}][BD_{eq}]} \quad (3)$$

And reaction enthalpy $\Delta_r H^\circ$ can be calculated by van't Hoff equation (Eq. (4)) [20]:

$$\ln K = -\frac{\Delta_r H^\circ}{RT} + C \quad (4)$$

The least square inversion method was used to analyze the experiment data.

Kinetic study was conducted in a beaker with a water jacket connected to a thermostat without diffusion impact. The beaker for the study was airtight with 1,000 ml in volume. The reaction system occupied more than 600 ml when the reaction occurred. The volume of the tested sample was 2 ml for each, the effect of which on the reaction system can be neglected considering its volume ratio to the whole reaction system.

As the reaction of 2,3-BD and PRA is a reversible reaction with two reactants, it was assumed as a second-order reaction. In the reaction system, there are two phases. The reaction rate equations can be presented as follows (Eq. (5)-Eq. (8)):

$$r_{BD,or} = -\frac{dC_{BD,or}}{dt} = f_{or}(C_{cat,or}) \times (k_{+or}C_{PRA,or} \times C_{BD,or} - k_{-or}C_{EDD,or} \times C_{W,or}) \quad (5)$$

$$r_{BD,aq} = -\frac{dC_{BD,aq}}{dt} = f_{aq}(C_{cat,aq}) \times (k_{+aq}C_{PRA,aq} \times C_{BD,aq} - k_{-aq}C_{EDD,aq} \times C_{W,aq}) \quad (6)$$

$$r_{BD,sys} = r_{BD,or} + r_{BD,aq} = -\frac{dC_{BD,sys}}{dt} = -\left(\frac{dC_{BD,or}}{dt} + \frac{dC_{BD,aq}}{dt}\right) = f_{or}(C_{cat,or}) \times (k_{+or}C_{PRA,or} \times C_{BD,or} - k_{-or}C_{EDD,or} \times C_{W,or}) + f_{aq}(C_{cat,aq}) \times (k_{+aq}C_{PRA,aq} \times C_{BD,aq} - k_{-aq}C_{EDD,aq} \times C_{W,aq}) \quad (7)$$

Where $f(C_{cat})$ is the effect of catalyst amount on reaction rate, it can be presented as:

$$f(C_{cat}) = aC_{cat}^2 + bC_{cat} + c \quad (8)$$

In a uniform distributed reaction system, organic phase and aqueous phase were evenly distributed in micro-level. The experiments were carried out with strong stirring to make sure the mixture was distributed uniformly in micro-level. The system can be considered as a pseudo-homogeneous system. In that case, a pseudo-homogeneous model [18,19] can be used to describe the reaction behavior, which was presented as follows (Eq. (9)):

$$r_{BD,sys} = -\frac{dC_{BD,sys}}{dt} = f_{sys}(C_{cat,sys}) \times (k_{+sys}C_{PRA,sys} \times C_{BD,sys} - k_{-sys}C_{EDD,sys} \times C_{W,sys}) \quad (9)$$

As

$$K = \frac{[W_{eq}][EDD_{eq}]}{[BD_{eq}][PRA_{eq}]} = \frac{k_{+sys}}{k_{-sys}} \quad (10)$$

Eq. (9) can be shown as:

$$r_{BD,sys} = -\frac{dC_{BD,sys}}{dt} = f_{sys}(C_{cat,sys}) \times k_{+sys} \times \left(C_{PRA,sys} \times C_{BD,sys} - \frac{1}{K} C_{EDD,sys} \times C_{W,sys} \right) \quad (11)$$

And activation energy of reaction can be calculated by Arrhenius

equation (Eq. (12)) [18]:

$$\frac{d \ln k}{dT} = \frac{E_a}{RT^2} \quad (12)$$

Model parameters including reaction rate constant $k_{r,sys}$ and catalyst concentration effect $f_{c,sys}(C_{cat,sys})$ can be obtained by fitting experimental data.

2-2. Reactive-extraction Process

A thorough process of separation 2,3-BD from fermentation broth is provided in this paper (Fig. 2). It consists of pretreatment, reactive-extraction, hydrolysis and purification.

Cells and proteins were removed by using membrane separation as pretreatment to reduce the emulsion for the following steps. And the yield rate of 2,3-BD in this step can reach 99% w/w.

Reactive-extraction occurred in the reactive-extraction unit. The reactive-extraction unit, constructed in the laboratory, consisted of airtight beakers with a water jacket connected to a thermostat. The size of the beaker was 3,000 ml for each. Reaction and extraction took place in the same unit. 2,3-BD reacted with PRA to produce EDD under acidic conditions, and then the reaction product EDD

accumulated to form organic phase and extracted PRA and 2,3-BD from fermentation broth in sequence. A distillation column was used as the hydrolysis unit. The column was 180 cm in height and its tower diameter was 4 cm. The reaction zone was filled with ion-exchange resin HZ732 and separation zone was using Cannon Ring. In the hydrolysis unit, the organic phase hydrolyzed to obtain crude 2,3-BD, which was enriched in the bottom. The PRA was collected at the top of the unit and the reactive-extraction step was reused. Finally, the vacuum distillation was used to purify the bottom liquid and obtain the final product. The vacuum distillation column was 120 cm tall and with 4 cm in tower diameter.

Reactive-extraction conditions, which included reactant ratio, reaction temperature and operation mode, were studied to obtain an operational reaction condition. Three operation modes were investigated in this paper (Fig. 3). Conversion ratio (η), extraction ratio (ER) [21], reactant ratio (RR) and distribution ratio (D) [22] were used to measure the efficiency of reactive-extraction.

$$ER = \frac{(C_{EDD,or} + C_{BD,or}) \times V_{or}}{(C_{EDD,or} + C_{BD,or}) \times V_{or} + (C_{EDD,aq} + C_{BD,aq}) \times V_{aq}} \times 100\% \quad (13)$$

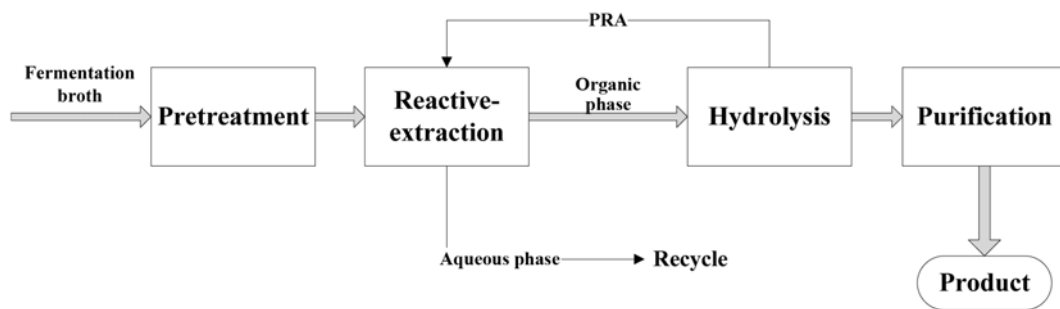


Fig. 2. Process of separation 2,3-butanediol from fermentation broth.

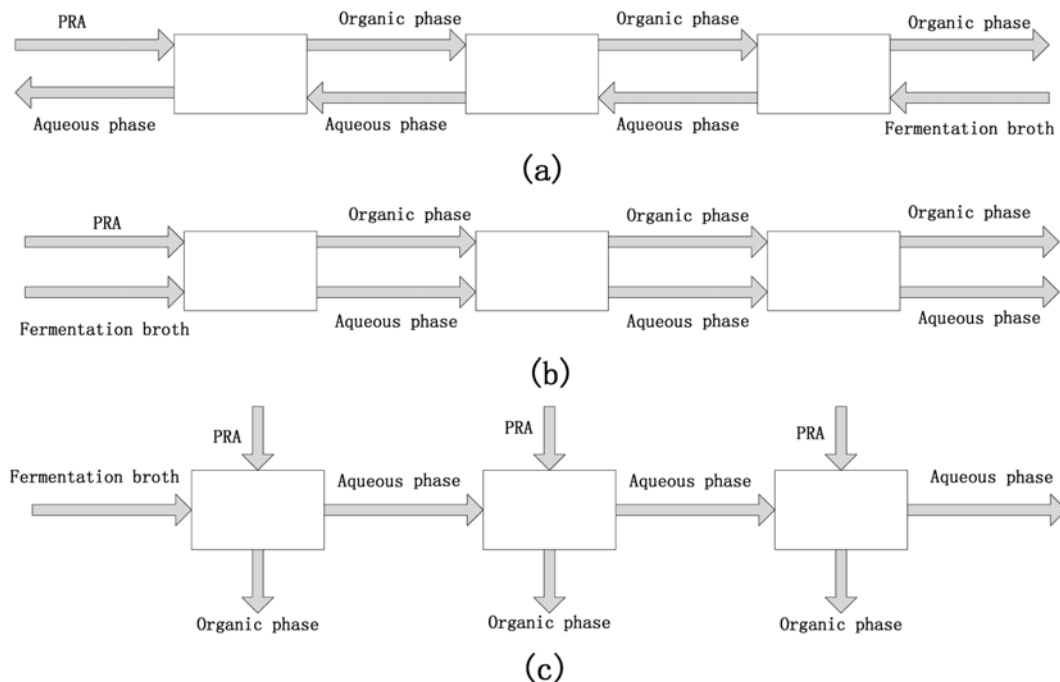


Fig. 3. Reactive-extraction operation modes. (a) Counter-current mode; (b) con-current mode; (c) cross-current mode.

$$PR = \frac{V_{PRA}}{V_{FB}} \quad (14)$$

$$D = \frac{C_{EDD,or}}{C_{EDD,aq}} \quad (15)$$

2-3. Analysis

The concentrations of 2,3-BD, EDD, PRA, PAD, acetoin and ethanol were analyzed by internal standard method with ethanol as standard by GC FULI GC9790J, FID-detector, PEG-20M 50 m×0.32 mm×0.5 μm capillary column and operated with N₂ as the carrier gas at flow rate of 50 mL·min⁻¹, detector temperature 200 °C and column temperature 140 °C. Three samples were collected for one condition and all samples were measured with three repeats. The concentration of proteins was measured by Coomassie brilliant blue method [23]. Glucose reactant kit was used to measure the concentration of glucose with water as blank and glucose standard solution as control group. The absorbency was measured by UV Spectrophotometer under 500 nm.

RESULTS AND DISCUSSION

1. Catalyst Selection

At the beginning, catalyst effect on reaction was investigated. Concentration data of PRA, 2,3-BD and EDD under different reaction time were collected. Analytic results are listed in Table 1. The results reveal that both sulfuric acid and tosylate acid can catalyze the reaction between PRA and 2,3-BD, but their β are lower than 90%. When hydrochloric acid and ion-exchange resin HZ732 are used as catalyst, both β is higher than 99%, which means side reaction is not easy to occur. The exchange capacity for ion-exchange resin HZ732 is 4.5 mmol/g (dry resin); thus catalytic activity of ion-exchange resin HZ732 (20 g·L⁻¹) is equal to that of HCl (0.09 mol·L⁻¹). With the same mass mount of catalyst, the reaction rate of hydrochloric acid system is faster than that of ion-exchange resin HZ732, so it takes less time to reach reaction equilibrium. According to the experimental results, hydrochloric acid is chosen as the catalyst for following experiments.

2. Reaction Equilibrium

In reaction equilibrium experiments, the temperature varied from 10 °C to 50 °C. The concentrations of PRA, 2,3-BD and EDD were tested. The regression results of reaction equilibrium are shown in Fig. 4. It reveals that the reaction enthalpy is Δ_rH_r^o = -21.84 ± 2.38 KJ·mol⁻¹, which means the reaction between PRA and 2,3-BD is an exothermic reaction. Increasing reaction temperature will benefit the counter reaction and cause η to decrease.

The definition of the reaction enthalpy Δ_rH^o [19] was used to meas-

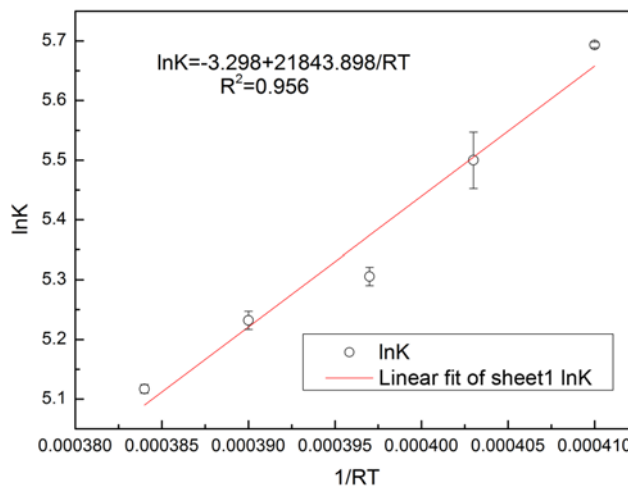


Fig. 4. Regression results of reaction equilibrium ($C_{BD}=100 \text{ g}\cdot\text{L}^{-1}$, $C_{PRA}=110 \text{ g}\cdot\text{L}^{-1}$, $C_{HCl}=0.2 \text{ mol}\cdot\text{L}^{-1}$).

ure the reliability of the experimental results, which can be calculated as follows (Eq. (16)):

$$\Delta_r H_{298}^o = \sum \gamma_i \Delta_f H_{298}^o(l)_i \quad (16)$$

In this paper, group additivity method is used to solve the problem of lacking the standard mole enthalpy of the chemicals' formation. The calculated result shows that the reaction enthalpy Δ_rH_r^o = -27.92 KJ·mol⁻¹. The regression result and calculated result of the reaction enthalpy are of the same order of magnitude. Thus, the experimental results are reliable and can be used to assess the process.

3. Kinetic Study

3-1. Effect of Diffusion on Reaction

To investigate the effect of diffusion on the reaction, mechanical agitation was used in the experiments. The stirring rate of the mechanical agitation was changed from 0 rpm to 500 rpm. The experiment results (Fig. 5(a)) show conversion ratio η changes with reaction time under different stirring rates, which reveals that diffusion has an effect on reaction rate. When there is no agitation in the system, η curve, as the bottom one, rises rapidly at the first 10 min and then grows linear with the reaction time increasing. While there is agitation in the system, curves are totally different from the bottom one. That means diffusion impact can be weakened by the increasing of stirring rate (STR). If the reaction rate is controlled by the diffusion, the reaction enthalpy obtained by experiment will be located around 10⁰ KJ·mol⁻¹. The reaction enthalpy obtained in the work is located in the region of 10¹ KJ·mol⁻¹, which means the reaction rate is not controlled by the diffusion but by the reaction. The curve in Fig. 5(b) illustrates that the diffusion impact can be weakened by improving stirring rate, while the improvement is not obvious. To make sure that the diffusion impact disappears, the kinetic study should be done with STR over 500 rpm.

3-2. Effect of Catalyst Concentration on Reaction

To test the effect of catalyst concentration on reaction, HCl in the reaction system was varied from 0.01 mol·L⁻¹ to 1 mol·L⁻¹. The conversion ratio η - reaction time graph (Fig. 6(a)) was drawn for a reaction system with different HCl concentration. The curves (Fig. 6(a)) reveal that reaction rate increases with the catalyst concentration increasing. When C_{HCl} is 0.01 mol·L⁻¹, only 10% w/w of 2,3-

Table 1. Effect of catalyst on reaction

Catalyst	RT _{eq} (min)	Selectivity β(%)	Conversion rate (%)
Sulfuric acid	4.0±0.3	89.3±0.3	85.2±0.3
Hydrochloric acid	6.5±0.3	99.3±0.8	96.3±0.3
Tosylate acid	45.5±1.3	88.4±0.3	84.1±0.3
Ion-exchange resin HZ732	312.0±4.3	99.2±0.4	96.2±0.1

*T=25 °C, C_{BD}=100 g·L⁻¹, C_{PRA}=60 g·L⁻¹, C_{cat}=20 g·L⁻¹

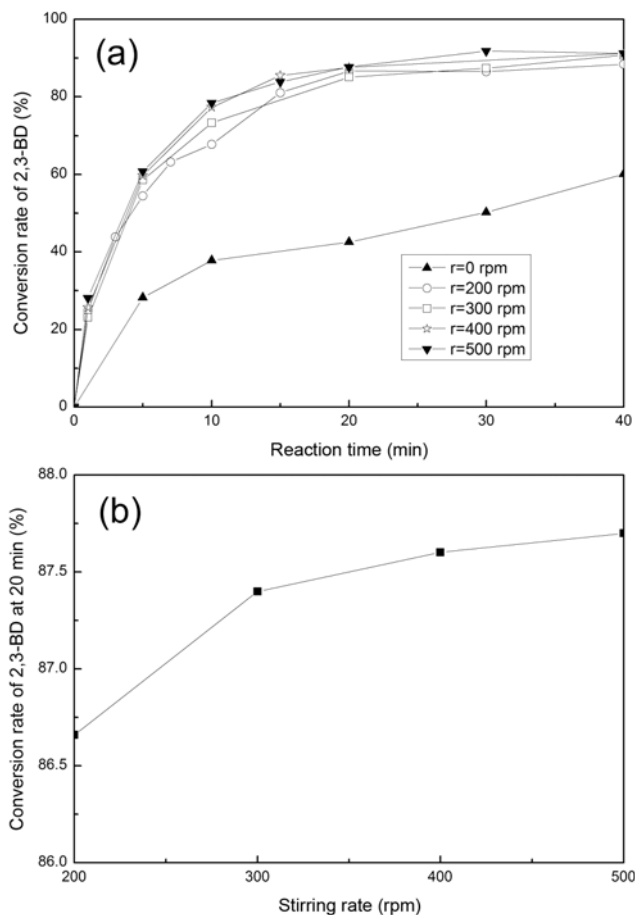


Fig. 5. Diffusion impact on reaction ($T=25\text{ }^{\circ}\text{C}$, $C_{BD}=100\text{ g}\cdot\text{L}^{-1}$, $C_{PRA}=160\text{ g}\cdot\text{L}^{-1}$, $C_{HCl}=0.1\text{ mol}\cdot\text{L}^{-1}$).

BD can alter to be EDD in 15 min. Increasing the C_{HCl} to $0.1\text{ mol}\cdot\text{L}^{-1}$, more than 70% w/w of 2,3-BD will convert to EDD in 15 min. With $C_{HCl}=0.2\text{ mol}\cdot\text{L}^{-1}$, it takes 10 min to reach the equilibrium. And the time to reaction equilibrium (R_{TEq}) for $C_{HCl}=0.5\text{ mol}\cdot\text{L}^{-1}$ and $C_{HCl}=1\text{ mol}\cdot\text{L}^{-1}$ is 5 min and 2 min, respectively. From Fig. 6(b) the reaction rate has a linear correlation with C_{HCl} and the square of linear correlation coefficient R^2 is 0.996. Thus, the experimental results are reliable. And the conversion rate increases linearly with C_{HCl} increasing. According to the above, the appropriate concentration for subsequent experiments is $0.2\text{ mol}\cdot\text{L}^{-1}$.

3-3. Kinetic Model

A kinetic study of the reaction between PRA and 2,3-BB was carried out; concentrations of PRA, 2,3-BD and EDD were tested. Fig. 7 is the concentration-time plots under different temperature and Table 2 contains reaction rate constants which are obtained by fitting the experiment data. The results under different temperature (Fig. 7 and Table 2) reveal that increasing reaction temperature will accelerate reaction rate for both positive reaction and reverse reaction. The regression results of data reveal that the activity energy of the positive reaction and hydrolysis are $51.97\pm 2.84\text{ kJ}\cdot\text{mol}^{-1}$ ($R^2=0.985$) and $79.50\pm 6.37\text{ kJ}\cdot\text{mol}^{-1}$ ($R^2=0.969$), respectively. It indicates that a positive reaction is easier to occur than hydrolysis. Putting model parameters into Eq. (4) and Eq. (10), the reaction rate can be presented as:

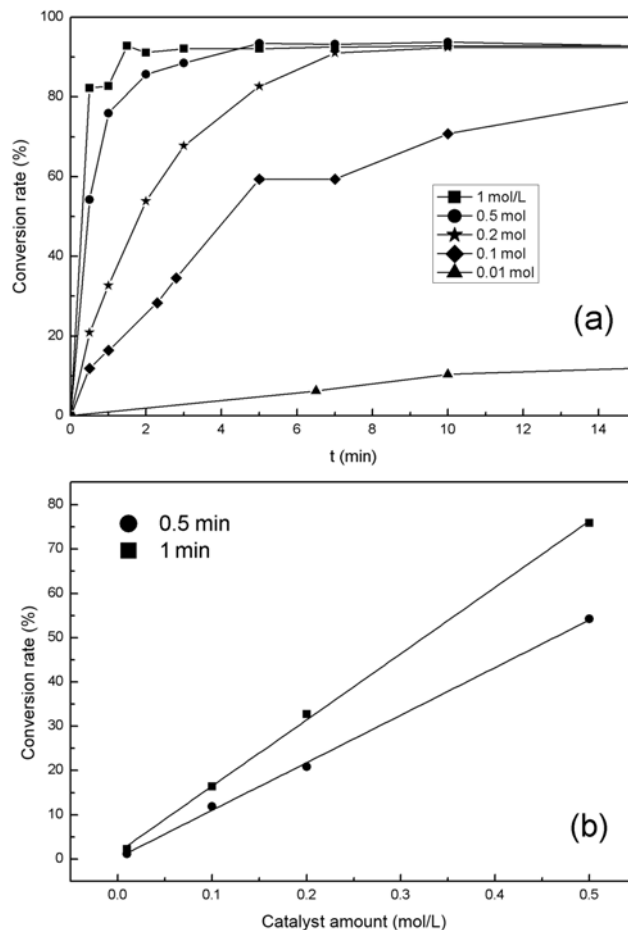


Fig. 6. Effect of catalyst amount on reaction ($T=25\text{ }^{\circ}\text{C}$, $C_{BD}=100\text{ g}\cdot\text{L}^{-1}$, $C_{PRA}=160\text{ g}\cdot\text{L}^{-1}$, $\text{STR}=500\text{ rpm}$).

$$r_{BD\text{sys}} = -\frac{dC_{BD\text{sys}}}{dt} = 5.418 \times 10^8 \exp\left(\frac{-6251.04}{T}\right) C_{HCl\text{sys}} \left(C_{PRA\text{sys}} \times C_{BD\text{sys}} - \frac{1}{K} C_{EDD\text{sys}} \times C_{W\text{sys}} \right) \quad (17)$$

$$K = 0.03695 \times \exp\left(\frac{2627.36}{T}\right) \quad (18)$$

4. Reactive-extraction Process

In this part, the post-treated fermentation broth used as raw material. The concentration of 2,3-BD in the post-treated broth varies from $30\text{ g}\cdot\text{L}^{-1}$ to $140\text{ g}\cdot\text{L}^{-1}$.

4-1. Effect of Reactant Ratio on Reactive-extraction

Reactant ratio (RR, $V_{PRA} : V_{FB}$) effect on reactive-extraction was investigated. The reaction ratio was changed from 0.05 to 0.40. Concentrations of PRA, 2,3-BD and PRA of the system were tested. Conversion rate η and concentration of PRA left in aqueous phase were used to present the efficiency of reactive-extraction. Results in Table 3 indicate that η increases with reactant ratio increasing. When RR is 0.05, η is 21.1%, far from its calculated result 49.4%. Under the RR, PRA is not enough to make all 2,3-BD to form EDD. 2,3-BD is left in the aqueous phase, which may cause EDD hard to form organic phase and limit the η to reach its calculated value. With the increasing of RR, η grows rapidly until the RR reaches 0.25, at which point η is 97.2% w/w, which is over its calculated

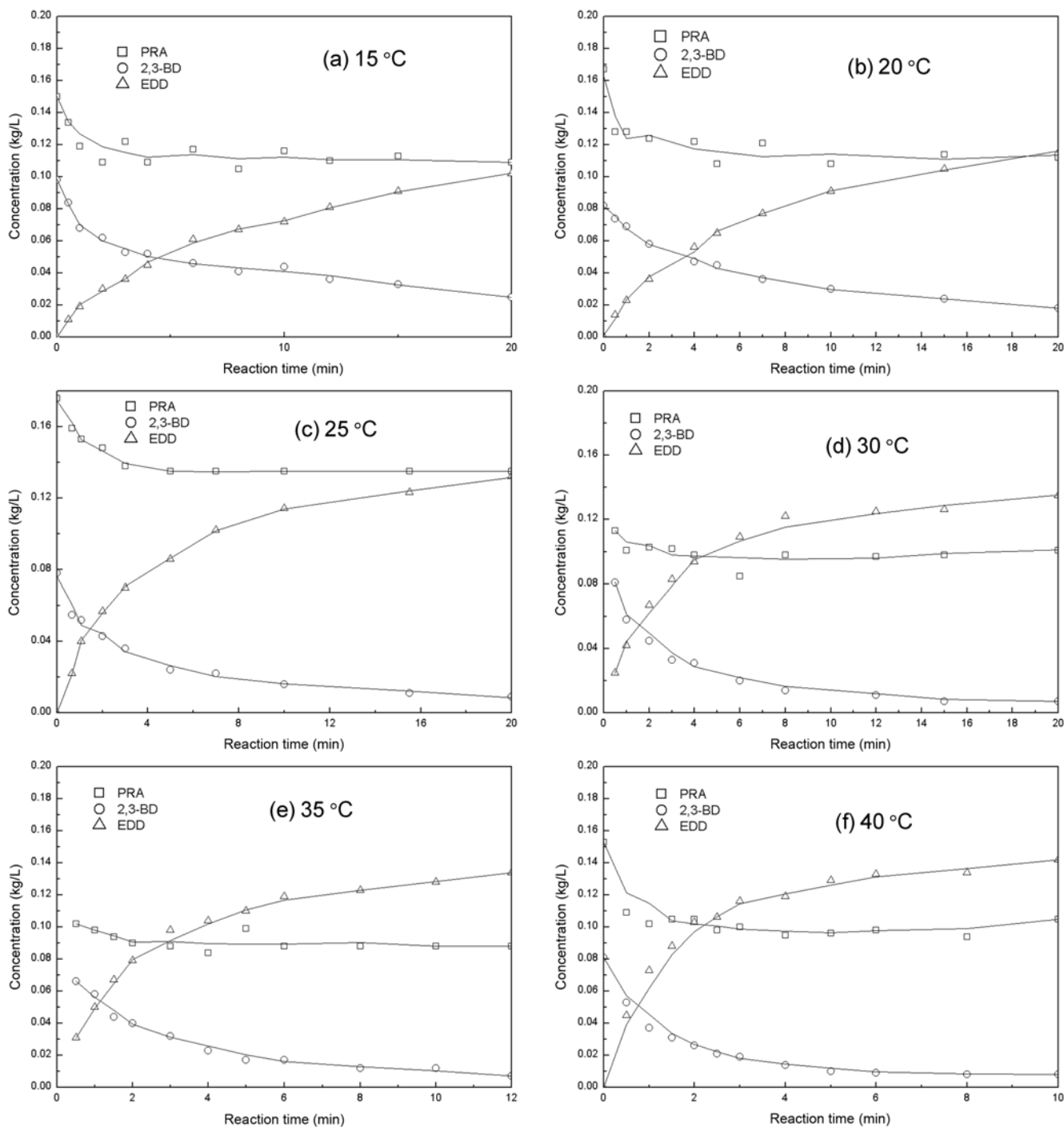


Fig. 7. Concentration of PRA, 2,3-BD and EDD changes with reaction time under different temperature (STR=500 rpm).

value. When RR is over 0.25, PRA is excess for reaction, so the 2,3-BD left in the aqueous phase has little effect on forming the organic phase, while the existence of the organic phase may improve the η by removing the reaction product EDD from aqueous phase. The differences illustrate that large RR has a positive effect on reactive-extraction. After RR reaches 0.25, increasing PRA amount in the system can improve the conversion ratio, but the improvement is very small. It can be seen that there are differences between the experiment results of η with the calculated η by Eq. (4). When RR is under 0.19, experiment η values are smaller than the calculated

ones, but when RR is over 0.25, experiment η values are larger than the calculated ones.

The concentration of PRA goes up with RR. Before RR reaches 0.05, most of PRA reacts with 2,3-BD to form EDD, and the left one is extracted into the organic phase, so the C_{PRA} in aqueous can't be measured by the equipment. And when RR is between 0.05 and 0.25, the upward trend of PRA concentration is gentle. In this period, reaction and extraction occur in sequence. Then the PRA concentration in aqueous phase goes up sharply. From the data in Table 3, RR should be over 0.25 to recovery 97% of 2,3-butanediol from

Table 2. The calculated results of reaction rate constant

Temperature (°C)	k_{+sys} ($L^2 \cdot mol^{-2} \cdot min^{-1}$)	k_{-sys} ($L^2 \cdot mol^{-2} \cdot min^{-1}$)
15	0.234 ± 0.048	$(4.926 \pm 0.065) \cdot 10^{-4}$
20	0.315 ± 0.020	$(1.061 \pm 0.032) \cdot 10^{-3}$
25	0.509 ± 0.064	$(2.086 \pm 0.097) \cdot 10^{-3}$
30	0.740 ± 0.044	$(3.675 \pm 0.063) \cdot 10^{-3}$
35	0.987 ± 0.029	$(5.063 \pm 0.031) \cdot 10^{-3}$
40	1.363 ± 0.065	$(8.172 \pm 0.089) \cdot 10^{-3}$

* $C_{BD}=100 \text{ g} \cdot \text{L}^{-1}$, $C_{PRA}=190 \text{ g} \cdot \text{L}^{-1}$, $C_{HCl}=0.2 \text{ mol} \cdot \text{L}^{-1}$, STR=500 rpm
 k_{+sys} is the reaction rate constant for positive reaction; k_{-sys} is the reaction rate constant for counter reaction

fermentation broth with $C_{BD}=100 \text{ g} \cdot \text{L}^{-1}$ through one-stage reactive-extraction.

The analysis results of reactive-process reveal that acetoin and ethanol can react with PRA to form corresponding acetal and can be extracted into organic phase. The PRA for reacting with acetoin and ethanol to form corresponding acetal is about 1.1 to 1.3 times of the total mole amount of acetoin and ethanol. The recovery ratio of acetoin and ethanol is 93.2% and 64.2%, respectively. While more than 90% of glucose is left in the aqueous phase and the extraction rate of proteins is less than 3%. It can be concluded from the results that reactive-extraction has a good selectivity to impurities of fermentation broth.

4-2. Temperature Effect on Reactive-extraction

Experiments were done in the region of 10 °C to 50 °C. The data collected under different temperature were listed in Table 4 which can show the temperature effect on reactive-extraction. The experimental results reveal that temperature is an influence impact on reactive-extraction. It will affect the conversion ratio (η) and distribution ratio (D) of EDD, both of which can change the extraction ratio (ER). From the data (Table 4), η , ER and D decrease with the temperature increases. When the temperature rises from 10 °C to 50 °C, η drops from 98.3% to 93.2%, ER decreases from 97.6% to 88.8%, D goes down as the same from 33.8 to 20.8. High reaction temperature can increase the reaction rate, but it will benefit the hydrolysis. Furthermore, the evaporation of PRA will be increased with the operation temperature. Based on the experimental results, when operation temperature increases 10 °C, the reaction doubles the rate. But the loss ratio of PRA increases sharply from 1.2% at 10 °C to 11.2% at 50 °C and the ER decreases about 2% every 10 °C. To reac-

Table 4. Temperature effect on reactive-extraction

T (°C)	η (%)	ER (%)	D	Loss of PRA (%)
10	98.3 ± 0.3	97.6 ± 0.2	33.8 ± 0.3	1.2 ± 0.3
20	97.9 ± 0.3	95.5 ± 0.2	31.3 ± 0.2	2.3 ± 0.4
30	96.4 ± 0.4	93.2 ± 0.2	28.9 ± 0.1	4.3 ± 0.2
40	94.8 ± 0.4	92.6 ± 0.1	24.5 ± 0.2	7.6 ± 0.4
50	93.2 ± 0.3	88.8 ± 0.1	20.8 ± 0.2	11.2 ± 0.5

* $C_{BD}=100 \text{ g} \cdot \text{L}^{-1}$, $C_{PRA}=190 \text{ g} \cdot \text{L}^{-1}$, $C_{HCl}=0.2 \text{ mol} \cdot \text{L}^{-1}$, STR=500 rpm

Table 5. Counter-current operation results

Stage (n)	Reactant ratio ($V_{PRA} : V_{FB}$)	η (%)	ER (%)	C_{PRA} in final aqueous phase (g/L)
2	0.10	81.4 ± 0.5	80.7 ± 0.3	1.5 ± 0.4
2	0.15	89.4 ± 0.3	88.3 ± 0.3	2.3 ± 0.3
2	0.20	93.2 ± 0.2	92.5 ± 0.2	4.5 ± 0.3
3	0.10	91.5 ± 0.3	90.5 ± 0.3	1.3 ± 0.4
3	0.15	96.5 ± 0.2	94.8 ± 0.2	1.9 ± 0.3
3	0.20	98.0 ± 0.1	97.3 ± 0.2	4.3 ± 0.3

*T=10 °C, $C_{BD}=100 \text{ g} \cdot \text{L}^{-1}$, $C_{HCl}=0.2 \text{ mol} \cdot \text{L}^{-1}$

tive-extraction operation, recovery of as much as 2,3-BD with low consumption of PRA is required, so we finally chose 10 °C as the feasible operation temperature.

4-3. Reactive-extraction Operation Mode

Reactive-extraction is an operation which combines the reaction and extraction into one. So the operation mode could affect the efficiency of reactive-extraction. In the work, three operation modes were studied to select the feasible one for the PRA-2,3-BD system.

Counter-current operation results (Fig. 3(a)) are listed in Table 5. With the increasing of RR in the system, η , ER and C_{PRA} are also increasing. It can be concluded from the data that results of three-stage counter-current operation are better than those of two-stage counter-current operation. To recover 97% of 2,3-butanediol from fermentation broth with $C_{BD}=100 \text{ g} \cdot \text{L}^{-1}$, minimum RR should be 0.20 through three-stage counter-current extraction.

Concurrent operation results (Fig. 3(b)) are listed in Table 6. It can be concluded that concurrent operation can improve the η , while the solution amount is the same as one-stage reactive-extraction.

Cross-current operation results (Fig. 3(c)) are listed in Table 7. η , ER and C_{PRA} are increasing with the total RR. When the total RR

Table 3. The effect of reactant on reactive-extraction

Reactant ratio ($V_{PRA} : V_{FB}$)	Experimental results of η (%)	Calculated results of η (%)	Difference (%)	Concentration of PRA in aqueous phase (g/L)
0.05	21.07 ± 0.34	49.39	-57.33 ± 0.69	0 ± 0.2
0.10	59.54 ± 0.21	76.85	-22.52 ± 0.27	2.5 ± 0.3
0.14	72.78 ± 0.54	86.45	-15.81 ± 0.62	4.9 ± 0.4
0.19	81.90 ± 0.43	90.73	-9.73 ± 0.47	6.1 ± 0.2
0.25	97.20 ± 0.12	93.01	$+4.50 \pm 0.13$	13.4 ± 0.3
0.29	98.20 ± 0.07	94.21	$+4.24 \pm 0.07$	20.6 ± 0.4
0.40	98.90 ± 0.06	95.58	$+3.47 \pm 0.06$	45.8 ± 0.4

*T=25 °C, $C_{BD}=100 \text{ g} \cdot \text{L}^{-1}$, $C_{HCl}=0.2 \text{ mol} \cdot \text{L}^{-1}$, STR=500 rpm

Table 6. Concurrent operation results

Stage (n)	Reactant ratio ($V_{PRA} : V_{FB}$)	η (%)	ER (%)	C_{PRA} in final aqueous phase (g/L)
2	0.05	37.1±0.5	35.5±0.3	0±0.3
2	0.15	71.8±0.3	68.3±0.2	4.5±0.5
2	0.25	97.7±0.2	96.5±0.2	13.4±0.4
3	0.05	40.1±0.3	38.5±0.2	0±0.2
3	0.15	72.5±0.2	70.1±0.3	4.3±0.2
3	0.25	97.8±0.1	96.5±0.1	13.2±0.2

* $T=10\text{ }^{\circ}\text{C}$, $C_{BD}=100\text{ g}\cdot\text{L}^{-1}$, $C_{HCl}=0.2\text{ mol}\cdot\text{L}^{-1}$

is 0.10, ER is just over 74% through two-stage cross-current operation. As the total RR becomes 0.15, with the operation condition $RR_{n1}=0.10$ and $RR_{n2}=0.05$, more than 98% of 2,3-butanediol can be separated from fermentation broth with $C_{BD}=100\text{ g}\cdot\text{L}^{-1}$. When the total RR reaches 0.20, the recovery rate of 2,3-butanediol does not have a big improvement but causes an increasing of C_{PRA} in final aqueous phase. So the minimum amount required by cross-current operation is 0.15 to recover 97% of 2,3-butanediol from fermentation broth with $C_{BD}=100\text{ g}\cdot\text{L}^{-1}$.

4-4. Reactive-extraction Results

Based on the previous experiments, feasible operation conditions are obtained as follows: $10\text{ }^{\circ}\text{C}$, $C_{HCl}=0.2\text{ mol}\cdot\text{L}^{-1}$, two-stage cross-current extraction, total reactant ratio ($V_{PRA} : V_{FB}$) is 0.15, reactant ratio for first stage and second stage are 0.10 and 0.05, respectively. In practical conditions, C_{BD} , which changes with fermentation conditions, varies from $40\text{ g}\cdot\text{L}^{-1}$ to $140\text{ g}\cdot\text{L}^{-1}$. So the amount of PRA required may vary from $60\text{ ml}\cdot\text{L}^{-1}$ to $210\text{ ml}\cdot\text{L}^{-1}$, by mass is $48\text{ g}\cdot\text{L}^{-1}$ to $168\text{ g}\cdot\text{L}^{-1}$. And C_{EDD} in the final organic phase is over $600\text{ g}\cdot\text{L}^{-1}$, equal to $C_{BD}=400\text{ g}\cdot\text{L}^{-1}$.

These results obtained in our study are much better than those ($C_{BD}=200\text{ g/L}$ in final organic phase, the amount of solvent required is $240\text{ g}\cdot\text{L}^{-1}$) by using aqueous two-phase extraction [12] and those ($C_{BD}=80\text{ g/L}$ in final organic phase, the amount of solvent required is $800\text{ g}\cdot\text{L}^{-1}$) by using solvent extraction [11]. Especially when the C_{BD} in fermentation is lower than $50\text{ g}\cdot\text{L}^{-1}$, reactive-extraction has its advantages in reducing solution consumption while other pro-

cesses will require more. So the process can be a promising method in industrial application.

4-5. Hydrolysis

It can be concluded from the kinetic experimental result that the hydrolysis is harder than EDD forming reaction. To improve the hydrolysis ratio, distillation is used to remove PRA, which is a hydrolysis product. Distillation with hydrolysis is carried out in a distillation column at $70\text{ }^{\circ}\text{C}$. PRA is cooled by water and collected at the top, while ethanol, acetoin and 2,3-BD are obtained at the bottom. The yield of 2,3-BD is $93.4\pm 0.5\%$ w/w. And the yields of ethanol and acetoin are 46.5% and 91.2%, respectively.

4-6. Purification and Recycle

2,3-BD solution, obtained by hydrolysis, is purified in vacuum rectifying apparatus with a vacuum degree of 0.07 Mpa. The distillation cut between $140\text{ }^{\circ}\text{C}$ and $143\text{ }^{\circ}\text{C}$ is collected.

PRA, which is left in aqueous phase during the reactive-extraction, should be recycled. In this study, distillation is used to recover PRA from the aqueous phase. And the final yield rate of PRA is $86.5\pm 0.4\%$ w/w.

With the complete separation process described above, the mass fraction of 2,3-BD as the final product is over 99% w/w and the total yield rate of 2,3-BD from fermentation broth by reactive-extraction process is 90% w/w.

CONCLUSION

Equilibrium and kinetics of the reaction between propionaldehyde and 2,3-butanediol were investigated. The results reveal that it is an exothermal reaction with reaction enthalpy of $-21.84\pm 2.38\text{ KJ}\cdot\text{mol}^{-1}$. The activation energy of reaction and hydrolysis is $51.97\pm 2.84\text{ KJ}\cdot\text{mol}^{-1}$ and $79.50\pm 6.37\text{ KJ}\cdot\text{mol}^{-1}$, respectively. Kinetics was well described by pseudo-homogeneous model. The data can be used in process development. A complete separation process was achieved; with feasible operation conditions, the total yield of 2,3-butanediol is over 90% w/w and 2,3-BD in final product is over 99% w/w. The process required less solution, especially owning advantages in treating dilute fermentation broth.

As hydrochloric acid is used as catalyst in the process, the catalyst cannot be recycled and has to be neutralized. Hydrochloric acid owns

Table 7. Cross-current operation results

RR_{total}	RR_{n1}	RR_{n2}	RR_{n3}	η (%)	ER (%)	C_{PRA} in final aqueous phase (g/L)
0.10	0.05	0.05	-	76.5±0.3	74.3±0.2	1.3±0.2
0.15	0.05	0.10	-	85.4±0.3	83.2±0.2	4.2±0.4
	0.10	0.05	-	99.3±0.1	98.2±0.1	4.3±0.3
	0.05	0.05	0.05	98.5±0.1	97.6±0.1	5.1±0.4
	0.05	0.15	-	97.6±0.1	96.1±0.1	7.4±0.3
0.20	0.10	0.10	-	99.5±0.1	98.3±0.1	7.2±0.2
	0.15	0.05	-	99.7±0.1	98.3±0.1	6.9±0.2
	0.05	0.05	0.10	99.6±0.1	98.3±0.1	10.6±0.5
	0.05	0.10	0.05	99.7±0.1	98.3±0.1	12.9±0.4
	0.10	0.05	0.05	99.7±0.1	98.3±0.1	14.9±0.5
	0.05	0.10	0.05	99.7±0.1	98.3±0.1	14.9±0.5

* $T=10\text{ }^{\circ}\text{C}$, $C_{BD}=100\text{ g}\cdot\text{L}^{-1}$, $C_{HCl}=0.2\text{ mol}\cdot\text{L}^{-1}$

RR_{total} : the total reactant ratio ($V_{PRA} : V_{FB}$) for the process; RR_{n1} : the reactant ratio ($V_{PRA} : V_{FB}$) for the first stage; RR_{n2} : the reactant ratio ($V_{PRA} : V_{FB}$) for the second stage; RR_{n3} : the reactant ratio ($V_{PRA} : V_{FB}$) for the third stage

the best catalytic activity in our research, but it will corrode equipment. If catalyst with better catalytic activity can be found, the process can have a promising future in industrial application.

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NOMENCLATURE

β	: reaction selectivity
η	: conversion ratio
r	: reaction rate
K_{eq}	: reaction equilibrium constant
k	: reaction rate constant
A	: absorbency
C	: concentration
$\Delta_r H^\circ$: reaction enthalpy
$\Delta_f H^\circ$: standard mole enthalpy of the formation
T	: temperature
E_a	: activity energy
RT	: reaction time
V	: volume
a, b, c	: coefficient

Subscripts and Superscripts

eq	: equilibrium
o	: beginning
e	: end
or	: organic phase
aq	: aqueous phase

Abbreviations

2,3-BD	: 2,3-butanediol
D	: distribution ratio
EDD	: 2-ethyl-4,5-dimethyl-1,3-dioxolane
ER	: extraction ratio
FB	: fermentation broth
PRA	: propionaldehyde
RR	: reactant ratio
STR	: stirring rate

W : water

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