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RESEARCH PAPER

Separation of 2,3-Butanediol from Fermentation Broth by Reactive-extraction Using Acetaldehyde-cyclohexane System

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Abstract Biochemical 2,3-butanediol is a renewable material with the potential to be used as an alternative fuel. However, in the lack of an effective separation process has limited its industrial application. In this paper, an effective process was achieved to separate 2,3-butanediol by reactive-extraction. Acetaldehyde and cyclohexane were chosen as the reactant and extractant, respectively. Ion-exchange resin HZ732 was used as the catalyst. Reaction equilibrium and a kinetic study on the reaction between 2,3-butanediol and acetaldehyde were investigated to provide basic data for process development. The reaction enthalpy and activation energy of reaction of 2,3-butanediol and acetaldehyde were −30.05 ± 1.62 KJ/mol and 45.29 ± 2.89 KJ/mol, respectively. Feasible conditions were obtained as follows: operating temperature = 20°C, acetaldehyde: 2,3-butanediol = 0.5:1 (w/w), cyclohexane: fermentation broth = 0.5:1 (w/w), catalyst amount = 100 g/L, stirring rate = 500 rpm and three-stage counter-current extraction method was used. Under these conditions, the total yield rate of 2,3-butanediol from fermentation broth was over 90% and the mass fraction of 2,3-butanediol in the final product reached 99%.

Keyword: reactive-extraction, 2,3-butandiol, ion-exchange resin, kinetic, equilibrium

1. Introduction

Bio-based 2,3-butanediol (2,3-BD), which can be used as raw material in chemical industry, food industry and many other fields, is a renewable material with a promising future [1]. It is also considered as a possible alternative fuel since its combustion value is 27.19 KJ/g [2-4], and much attention has been given to this area of research. Biochemical 2,3-BD can be produced by fermentation broth on a commercial scale, however the bottleneck for bio-based 2,3-BD industrial application centers around the lack of an effective separation method, due to the combined complexity of fermentation broth and high hydrophilicity of 2,3-BD [5-9].

A few separation processes [10-14] were reported in the literature including steam stripping, liquid-liquid extraction, aqueous two-phase extraction and the reaction separation process. These processes can separate 2,3-BD from fermentation broth, but as 90 wt% of fermentation broth is water, these methods still retain inherent problems such as high energy costs and high solution consuming.

Reactive-extraction is one of the most effective ways to separate special material from complicated systems by improving its solubility in the organic phase through reversible reaction [15], especially with advantages in separating materials from dilute solutions. The difficulties of separating 2,3-BD from fermentation broth are low concentration, high hydrophilicity of 2,3-BD and complexity of fermentation broth. So transforming 2,3-BD to a hydrophobic material and then extracting it with a feasible solution may be an effective way to separate 2,3-BD from fermentation broth. Reactive-extraction has been used to separate 1,3-propanediol and 2,3-butanediol fermentation broth using sulfuric acid as a catalyst [16,17]. While sulfuric acid would not only cause corrode equipment, it also can not be recycle and needs to be neutralized, and therefore it is not the best catalyst for 2,3-BD separation process.

In this paper, a novel process was proposed to separate 2,3-BD from fermentation broth by reactive extraction. Acetaldehyde (AA) was chosen as the eactant, as it can react with 2,3-BD to form 2,4,5-trimethyl-1,3-dioxolane

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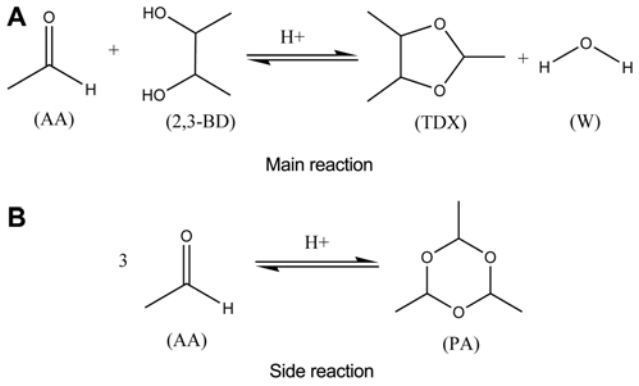

Fig. 1. Reaction equations for the main and side reaction. (A) Main reaction: 2,3-butanediol (2,3-BD) reacted with acetaldehyde (AA) under acidic condition to form 2,4,5-trimethyl-1,3-dioxolane (TDX) and water. (B) Side reaction: reaction between acetaldehyde (AA) to form Paraldehyde (PA).

(TDX) reversibly and is easily recycled through hydrolysis (Fig. 1). Cyclohexane (CYHX) was used as the extractant for its stable structure and low solubility in water. In the reactive-extraction unit, the reaction and extraction took place in sequence within the same unit, which as of yet had ever been reported in the literature. Reaction product TDX was extracted into the organic phase and separated from the fermentation broth. Due to the high selectivity of the reaction and low solubility of water and impurities in CYHX, a complete and effective separation process was achieved.

2. Materials and Methods

2.1. Materials

All the chemicals used in this study were purchased from commercial sources. 2,3-Butanediol (2,3-BD) was bought from the Sinopharm Chemical Reagent Co., Ltd. with a minimum mass fraction purity of 98.2%. Acetoin was bought from the Sinopharm Chemical Reagent Co., Ltd. as a 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%. Acetaldehyde (AA) was purchased from the Sinopharm Chemical Reagent Co., Ltd. as a solution with a mass fraction of more than 40%. Cyclohexane (CYHX) was provided by the Shanghai Chemical Reagent Co., Ltd. with a mass fraction of more than 99.0%. Paraldehyde (PA) was supplied by the Shanghai Chemical Reagent Co., Ltd. with a mass fraction of more than 95.0%. All these chemicals were measured and confirmed 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 Biological reagent (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. Sulfuric acid (H_2SO_4) 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%. Hydrochloric acid (HCl) was provided by the Sinopharm Chemical Reagent Co., Ltd. with a minimum mass fraction of 36%. And ion-exchange resin HZ732, a strong acidic styrene resin with exchangeability of 4.5 mmol/g, was provided by the Shanghai Huazhen Sci & Tech Co., Ltd. Water was distilled twice before utilization.

Fermentation broth was provided by the National Key Lab of Bioreactor Engineering in East China University of Science and Technology. Serratia marcescens was used as fermenting microbial species and sucrose was used as fermenting solution. The fermentation broth contained 2,3-butanediol (30.0 ~ 143.0 g/L), acetoin (6.1 ~ 23.2 g/L), Serratieae cells (35.4 ~ 70.4 g/L), glucose (2.3 ~ 5.2 g/L), proteins (0.2 ~ 1.3 g/L) and additives, such as salts and surface active substances.

2.2. Methods

2.2.1. Kinetics study

As the catalyst should be selected first, in our catalyst selection experiments, sulfuric acid, tosylate acid, hydrochloric acid and ion-exchange resin HZ732 were investigated. Catalyst activity can be presented by the time to reaction equilibrium (RT_{eq}), conversion rate of 2,3-BD (CR) and reaction selectivity (β). CR and β can be calculated by following equations [18]:

$$CR = \frac{n_{BDo} - n_{BDe}}{n_{BDo}} \times 100\% \quad (1)$$

$$\beta = \frac{n_{TDXe} - n_{TDXo}}{n_{AAo} - n_{AAe}} \times 100\% \quad (2)$$

With a suitable catalyst, equilibrium was investigated where experiments were conducted in a beaker with a water jacket connected to a thermostat. Both the main reaction (Fig. 1A) and side reaction (Fig. 1B) were reversible. The equilibrium constants K_{meq} and K_{seq}, which can be calculated by the following equations [19], are used to describe the limit of the reactions.

$$K_{meq} = \frac{[W_{eq}][TDX_{eq}]}{[BD_{eq}][AA_{eq}]} \quad (3)$$

$$K_{seq} = \frac{[PA_{eq}]}{[AA_{eq}]^3} \quad (4)$$

The reaction enthalpy $\Delta_r H^o$ has the interaction with the reaction equilibrium constant. According to the Van't Hoff equation, the equation can be deduced as

$$\ln K = -\frac{\Delta_r H^o}{RT} + C \quad (5)$$

This demonstrates that the reaction enthalpy $\Delta_r H^o$ is linearly related to lnK. The least square inversion method is adopted to analyze the experimental data.

Kinetic studies on the reaction between 2,3-BD and AA were carried out in a beaker with a water jacket connected to a thermostat without diffusion impact. The ideal kinetic model for the experimental data was provided and the reaction mechanism was discussed. Reaction parameters were obtained by fitting experimental data to kinetic model.

2.2.2. Process of separation 2,3-BD from fermentation broth

As shown in Fig. 2, a complete process of 2,3-BD separation from the fermentation broth, which consisted of membrane separation, reactive-extraction, hydrolysis and purification, was provided in this study.

Using directly the 2,3-BD fermentation broth as a raw material, the pretreatment was done before any further treatments to avoid emulsion. Membrane separation was used to remove cells and proteins. Recovery of 2,3-BD in this step could be over 98.3%.

There were two steps in the reactive-extraction unit. First, 2,3-BD reacted with AA to form TDX and extraction took place in sequence instantly. Then the organic layer was collected for the following process while the aqueous layer was reused in the fermentation unit.

Within the hydrolysis unit, TDX reacted with water to form 2,3-BD, which was enriched in the aqueous phase. Meanwhile AA evaporated into the vapor phase and was collected at the top of the unit. CYHX in the organic phase was removed from system after hydrolysis.

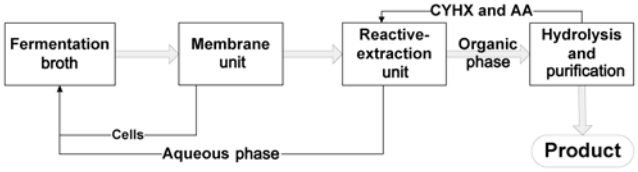

Fig. 2. Process of reactive-extraction of 2,3-BD from fermentation broth. The process consists of three parts: membrane unit, reactive-extraction unit, and hydrolysis and purification unit. In membrane unit, cells are removed and turned to fermentation unit to reuse. In reactive-extraction unit, 2,3-BD is extracted into organic phase in the form of TDX and separated with fermentation broth, the aqueous phase is reused in fermentation unit. And then TDX transforms to 2,3-BD by hydrolysis. Finally, vacuum distillation is used to purify the product. The fermentation go through the whole process to get pure 2,3-BD products.

Vacuum distillation was finally used to purify the liquid which was obtained by hydrolysis resulting in the final product.

2.2.3. Selection of reactive-extraction conditions

Reactive-extraction conditions including reactant ratio, extractant phase ratio and stage number of multistage counter-current extraction [20] were optimized in this study. The reactant ratio and extractant phase ratio experiments were carried out in a beaker with a water jacket connected to a thermostat and multistage counter-current extraction was simulated in funnels connected in series.

The reactant ratio (m_{AA}: $m_{2,3\text{-BD}}$, RR) was the mass ratio of AA and 2,3-BD while the extractant phase ratio (m_{CYHX}: m_{FB}, EPR) was the mass ratio of CYHX and the fermentation broth (FB). CYHX was added into the system before reaction and samples were collected at the equilibrium state. The extraction rate (ER) and distribution ratio (DR) can be calculated by the following equations [19]:

$$ER = \frac{n_{TDXor}}{n_{TDXor} + n_{TDXaq}} \times 100\% \quad (6)$$

$$DR = \frac{C_{TDXor}}{C_{TDXaq}} \quad (7)$$

2.2.4. Analytical methods

Three samples were collected for one condition and all samples were measured with three repeats. The concentrations of 2,3-BD, acetoin, ethanol, AA, TDX and CYHX were analyzed by GC FULI GC9790J, FID-detector, PEG-20M 50 m × 0.32 mm × 0.5 μm capillary column and operated with N_2 as the carrier gas at a flow rate of 50 mL/min, detector temperature at 200°C, and column temperature at 140°C. The concentration of proteins was measured using the Coomassie brilliant blue method [21]. Glucose reactant kit was used to measure the concentration of glucose, which can be calculated by Eq. (8), with water as a blank and glucose standard solution as the control group. The absorbency was measured by a UV spectrophotometer at a wavelength of 500 nm.

$$C_{sample}(\text{mmol/L}) = \frac{A_{sample}}{A_{standard}} \times C_{standard}(\text{mmol/L}) \quad (8)$$

3. Results and Discussion

3.1. Equilibrium and kinetic study

3.1.1. Catalyst selection

The results reveal that when sulfuric acid, hydrochloric

Table 1. Effect of catalyst on reaction

Catalyst	RT_{eq} (min)	Selectivity β (%)	Conversion rate (%)
Sulfuric acid	3.0 ± 0.3	15.3 ± 0.6	15.2 ± 0.3
Hydrochloric acid	5.5 ± 0.3	18.9 ± 0.8	20.3 ± 0.3
Tosylate acid	24.0 ± 1.3	18.9 ± 0.3	20.1 ± 0.3
Ion-exchange resin HZ732	240.0 ± 4.3	56.5 ± 0.4	98.6 ± 0.1

*T = 25°C, C_{BD} = 100 g/L, C_{AA} = 90 g/L, C_{cat} = 20 g/L.

acid or tosylate acid is used as a catalyst, CR and β values are less than 25 and 20%, respectively. This suggests that when one of them is used as catalyst, the side reaction is easier to occur compared to the main reaction. Though the reaction system takes more than 240 min to reach reaction equilibrium with ion-exchange resin HZ732, it retains both high CR and β as shown in Table 1, which are 97.6 ± 0.1% and 56.5 ± 0.4%, respectively. The differences in catalytic activity between different batches of resin are less than 6.0%. A possible reason for ion-exchange resin HZ732 having a better selectivity is that 2,3-BD can be more easily adsorbed by resin than AA. Therefore the main reaction has a greater chance to occur than the side reaction at the catalytic center. Based on the largest CR and β values, the ion-exchange resin HZ732 was chosen to be the catalyst for the subsequent experiments.

3.1.2. Diffusion effect on reaction

As ion-exchange resin HZ732 is a solid catalyst, the main and side reactions take place in the following steps:

a. External diffusion (2,3-BD and AA diffuse to resin surface);

b. Internal diffusion (2,3-BD and AA move to the acid centers of the resin and are absorbed by the resin);

c. Reaction (2,3-BD and AA react at the acid centers of the resin to produce TDX and PA);

d. External diffusion (TDX and PA diffuse into the solution).

With the stirring rate of the mechanical stirrer varying from 0 to 600 rpm, the experimental results of external diffusion are shown in Fig. 3.

The curves of Fig. 3A reveal that the external diffusion is an influencing factor of the reaction. The effect of external diffusion is apparent when there is no agitation in the reaction system, it can influence the reaction rates. The reaction rate under 0 rpm is much slower than that under 600 rpm. However the effect can be weakened by increasing stirring rate (STR) of the mechanical agitation for the reaction system. When the stirring rate reaches 440 rpm, the external diffusion impact disappears. It can be concluded from Fig. 3B that when reaction time is 15 min, CR increased with increasing STR. When STR was greater than 440 rpm, the increment of CR was not obvious. The results indicate that experiments of kinetic study should be carried out with STR over 440 rpm. From the plot we deduced CR is more stable when STR is in the region of 500 to 600 rpm.

Results of internal diffusion impact are shown in Fig. 4. Cracked and uncracked resins are used as the catalysts in the experiments, respectively. The two curves of the conversion rate coincide with each other, which suggest the

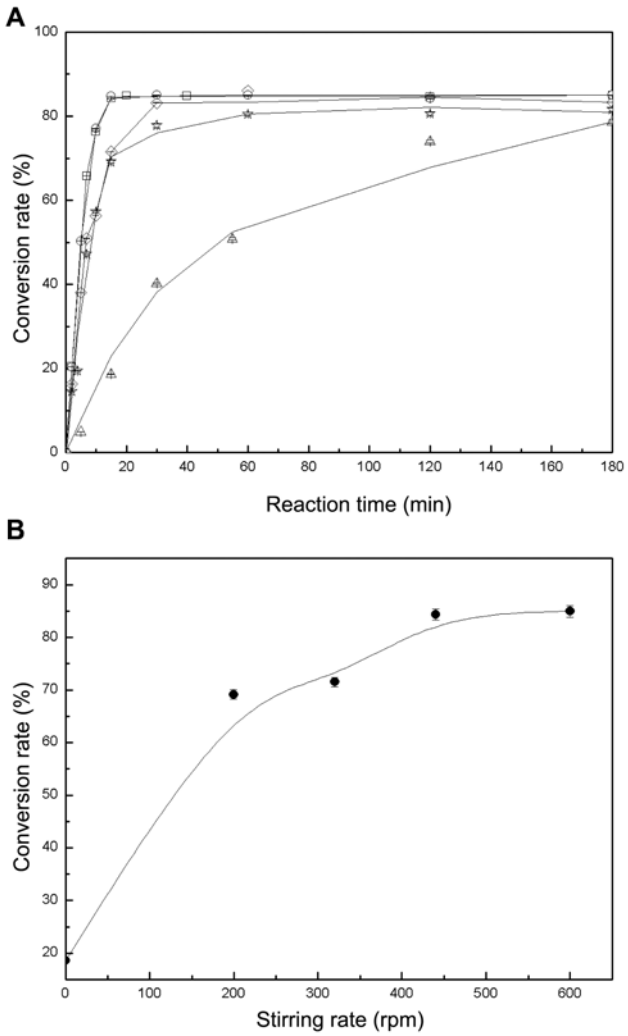

Fig. 3. External diffusion impact on the main reaction. (A) Conversion rate of 2,3-BD under different reaction time (T = 25°C, C_{BD} = 100 g/L, C_{AA} = 90 g/L, C_{cat} = 90 g/L). △; 0 rpm, ☆; 200 rpm, ◇; 320 rpm, □; 440 rpm, ○; 600 rpm. (B) Conversion rate of 2,3-BD under different stirring rate (T = 25°C, RT = 15 min, C_{BD} = 100 g/L, C_{AA} = 90 g/L, C_{cat} = 90 g/L). —; fit curve, ●; experimental results.

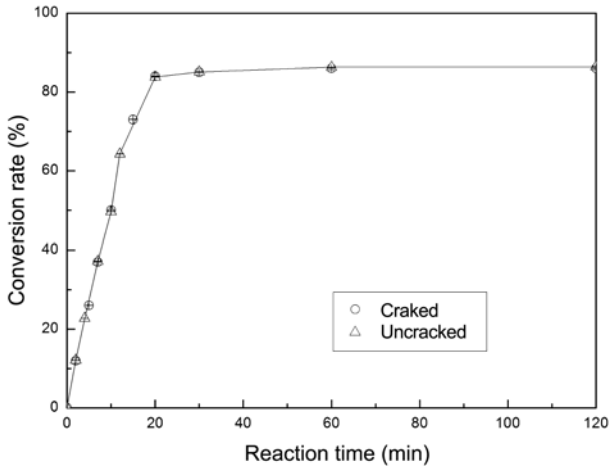

Fig. 4. Internal diffusion impact on the main reaction (T=25°C, C_{BD} = 100 g/L, C_{AA}= 90 g/L, C_{cat} = g/L, STR = 440 rpm). ○; cracked resin, △; uncracked resin.

internal diffusion impact on the reaction is negligible for ion-exchange resin HZ732.

3.1.3. Effect of catalyst amount on reaction

The data from Fig. 5 demonstrates that catalyst amount has an effect on reaction. Reaction rate increased with the increase of the catalyst amount. But after the catalyst amount reached 105 g/L, increasing catalyst amount in the system did not have an obvious effect on improving the reaction rate. Using the data in Fig. 5, the calculated results revealed that reaction rate has a linear correlation with catalyst amount (R^2 = 0.987). According to experimental results, the feasible catalyst amount was found to be 100 g/L.

3.1.4. Reaction equilibrium

The regression results of the reaction equilibrium are shown in Figs. 6 and 7. Analysis revealed that the reaction enthalpy of the main and side reactions were $\Delta_r H^o_{fm}$= −30.05 ± 1.62 KJ/mol and $\Delta_r H^o_{fs}$ = −79.01 ± 5.20 KJ/mol, respectively, and that both the main and side reaction were exothermic reactions. $\Delta_r H^o_{fs}$ was higher than $\Delta_r H^o_{fm}$, indicating the side reaction was more sensitive to temperature than the main reaction. Increasing reaction temperature can cause a decrease in CR value of 2,3-BD but improve the ß value.

The definition of the reaction enthalpy $\Delta_r H^o_f$ can be used to measure the reliability of the experimental results. It can be calculated by following equations:

$$\Delta_r H^o_{298} = \Sigma \gamma_i \Delta_f H_{298}{}^o(l)_i \quad (9)$$

In this paper, Universal Functional Activity Coefficient

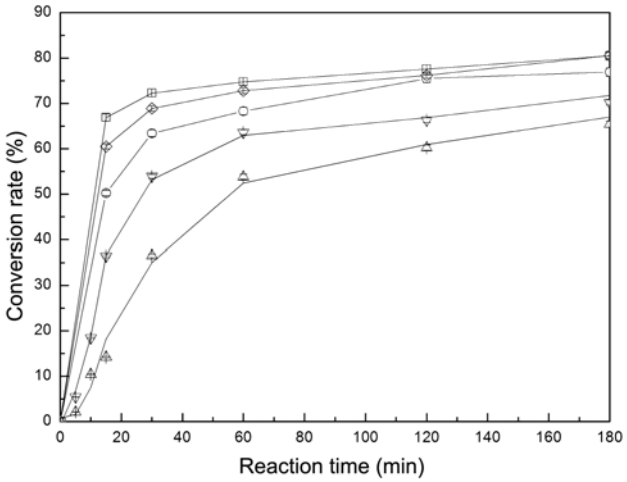

Fig. 5. Effect of catalyst amount on reaction rate of the main reaction (T=25°C, C_{BD} = 100 g/L, C_{AA}= 90 g/L, STR = 440 rpm). △; 20 g/L; ▽; 52 g/L; ○; 86 g/L; ◇; 105 g/L; □; 350 g/L.

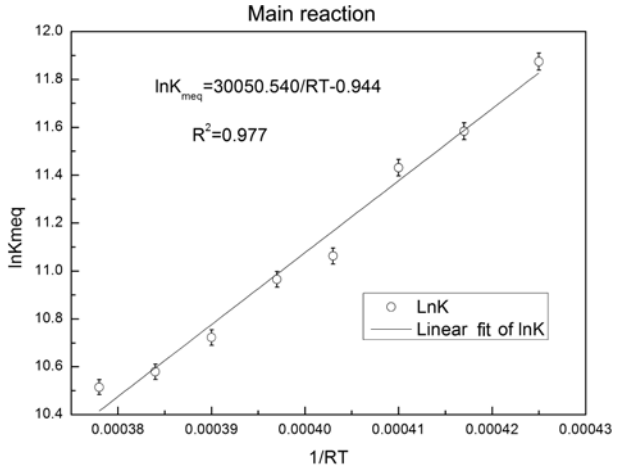

Fig. 6. Reaction equilibrium constant of main reaction by fitting reaction data under different temperature (C_{BD} = 100 g/L, C_{AA}= 90 g/L, C_{cat} = 100 g/L, STR = 500 rpm).

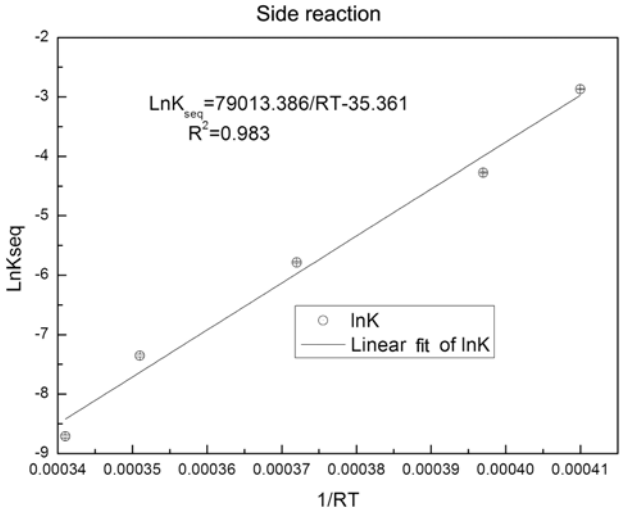

Fig. 7. Reaction equilibrium constant of side reaction by fitting reaction data under different temperature (C_{BD} = 100 g/L, C_{AA}= 90 g/L, C_{cat} = 100 g/L, STR = 500 rpm).

(UNIFAC) method is used to solve the problem of lacking the standard mole enthalpy of the chemicals' formation. The calculated results were as follows: $\Delta_r H^o_{fm} = -37.47$ KJ/mol and $\Delta_r H^o_{fs} = -94.56$ KJ/mol. The regression results and calculated results are on the same order of magnitude confirming that the experimental results are reliable and UNIFAC method can be used to assess the process.

3.1.5. Kinetic study

The stirring rate of kinetic experiments was set at 500 rpm. Under this condition, the diffusion impacts disappear and the catalyst is uniformly distributed. Thus the reaction system can be considered as a pseudo-homogeneous system.

The pseudo-homogeneous model is used as the kinetic model for the main reactions. As the main reaction is a reversible reaction with two reactants, it was assumed to be a second order reaction under our experimental conditions. The reaction rate r_{BD} was presented as follows [19]:

$$r_{BD} = -\frac{dC_{BD}}{dt} = f(C_{cat})(k_+ C_{AA} C_{BD} - k_- C_{TDX} C_W) \quad (10)$$

As

$$K_{meq} = \frac{[W_{eq}][TDX_{eq}]}{[BD_{eq}][AA_{eq}]} = \frac{k_+}{k_-} \quad (11)$$

The model can be shown as:

$$r_{BD} = -\frac{dC_{BD}}{dt} = k_+ f(C_{cat})\left(C_{AA} C_{BD} + \frac{1}{K_{meq}} - C_{TDX} C_W\right) \quad (12)$$

As reaction rate has linear correlation with C_{cat}, so the function can be presented as:

$$r_{BD} = -\frac{dC_{BD}}{dt} = k_+ C_{cat}\left(C_{AA} C_{BD} + \frac{1}{K_{meq}} - C_{TDX} C_W\right) \quad (13)$$

According to Arrhenius equation

$$k = A \times \exp\left(\frac{-Ea}{RT}\right) \quad (14)$$

Using the data in Table 2, the activation energy of the main reaction and its counter reaction was 45.29 ± 2.89 KJ/mol (R^2 = 0.988) and 73.75 ± 8.39 KJ/mol (R^2 = 0.974), respectively. The results of the kinetics study prove that the reaction between AA and 2,3-BD is in fact a second order reaction and a positive reaction to form TDX is easier to occur relative to its counter reaction. The reaction rate can be presented as

$$r_{BD} = -\frac{dC_{BD}}{dt} = 3.45 \times 10^5 \exp^{\frac{-5447.44}{T}} C_{cat}\left(C_{AA} C_{BD} - \frac{1}{K_{meq}} C_{TDX} C_W\right) \quad (15)$$

$$K_{meq} = 0.3892 \times \exp\left(\frac{3614.45}{T}\right) \quad (16)$$

Table 2. Reaction rate constant under different temperature

Temperature (°C)	k_+ (L^2/mol/min/g)	k_- (L^2/mol/min/g)
10	$(6.881 \pm 0.013)\cdot10^{-5}$	$(5.909 \pm 0.005)\cdot10^{-8}$
15	$(1.062 \pm 0.021)\cdot10^{-4}$	$(1.220 \pm 0.013)\cdot10^{-7}$
20	$(1.398 \pm 0.008)\cdot10^{-4}$	$(1.870 \pm 0.037)\cdot10^{-7}$
25	$(2.026 \pm 0.034)\cdot10^{-4}$	$(3.919 \pm 0.057)\cdot10^{-7}$
30	$(2.427 \pm 0.053)\cdot10^{-4}$	$(5.175 \pm 0.023)\cdot10^{-7}$

*C_{BD} = 100 g/L, C_{AA} = 100 g/L, C_{cat} = 1 00 g/L, STR = 500 rpm.

3.2. Reactive-extraction condition selection

3.2.1. Reactant ratio

Experimental results of the reactant ratio (RR, m_{AA}: $m_{2,3\text{-BD}}$) are shown in Fig. 8. The conversion rate of 2,3-BD increased as the amount of AA increased. When the reactant ratio was 0.5, CR and β of 2,3-BD were found to be 93.3 and 93.4%, respectively. When the reactant ratio was 0.75, the conversion rate reached 97.2%, but the selectivity dropped sharply to 70.2%. This phenomenon is indicative of the fact that the main reaction occurs more easily than the side reaction when the concentration of AA is low in the system. When the concentration of AA is high, the 2,3-BD conversion rate can be larger but the concentration of PA is also high. Accordingly, the suitable phase ratio was found to be 0.5:1.

3.2.2. Reactant phase ratio

As shown in Fig. 9, the general trend of the extraction rate (ER) of TDX increased as extractant phase ratio (EPR, m_{CYHX}: m_{FB}) increased. When EPR increased from 0 to 0.5, ER also increased sharply from 0 to 70%. When EPR was between 0.5 and 1.1, the increasing tendency of ER

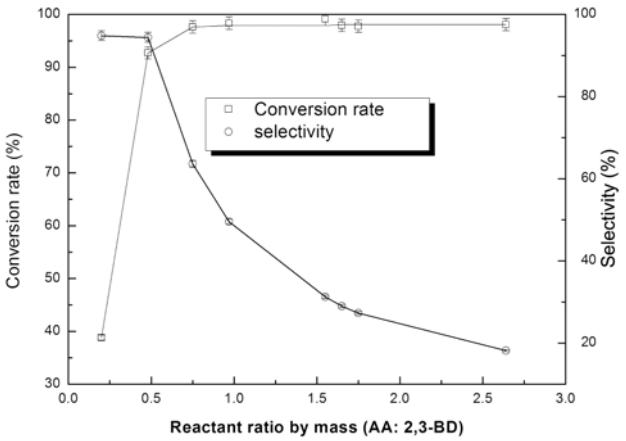

Fig. 8. Effect of the reactant ratio on conversion ratio of 2,3-BD and the reaction selectivity (T = 25°C, C_{BD} = 100 g/L, C_{cat} = 100 g/L, STR = 500 rpm). □; conversion rate, ○; selectivity.

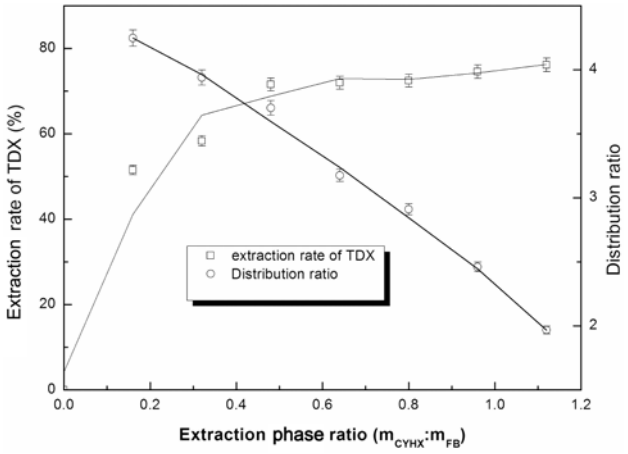

Fig. 9. Effect of extractant phase ratio on extraction rate and distribution ratio of TDX (T = 25°C, C_{BD} = 100 g/L, C_{AA} = 50 g/L,C_{Cat} = 100 g/L,STR = 500 rpm). □; extraction rate of TDX, ○; distribution ratio.

was gentle, while ER climbed slightly from 70 to 74%. As revealed by the graph, the distribution ratio (DR) dropped steadily as EPR increased, and decreased from 4.27 to 1.93 while EPR increased from 0.16 to 1.12. According to the experiment results, the feasible EPR was determined to be 0.5.

3.2.3. Effect of reactive-extraction

The results of effect of reactive-extraction are listed in Table 3. When there was no extractant in the system, the CR of 2,3-BD was 93.3%. When 500 g CYHX was added into the system, CR rose to 99.1% and at the same time selectivity also climbed from 93.4 to 98.2%. The possible reason for this may be that CYHX extracts TDX into the organic phase and results in a decline of TDX concentration in the aqueous phase, thus the reaction equilibrium in the aqueous phase moves in the direction to produce more TDX. According to the results of the reaction equilibrium, the main reaction can occur more easily than the side reaction, so selectivity goes up as well.

3.2.4. Effect of temperature on reactive-extraction

As shown in Table 4, temperature has an effect on reactive-extraction. Both CR and ER decreased when temperature increased, while the loss of AA increased with the increase in temperature. When temperature varied from 15 to 35°C,

Table 3. The effect of extraction on the reaction

	CR (%)	β (%)
0 g CYHX	93.3 ± 0.7	93.4 ± 0.3
500 g CYHX	99.1 ± 0.2	98.2 ± 0.2

*T = 25°C, C_{BD} = 100 g/L, C_{AA} = 50 g/L,C_{cat} = 100 g/L, STR = 500 rpm.

Table 4. Effect of temperature on reactive-extraction

Temperature (°C)	CR (%)	ER (%)	Loss of AA (%)
15	99.1 ± 0.2	75.3 ± 0.3	2.3 ± 0.3
20	99.1 ± 0.1	75.3 ± 0.2	2.4 ± 0.3
25	99.1 ± 0.1	75.2 ± 0.3	5.3 ± 0.4
30	98.7 ± 0.3	74.9 ± 0.2	6.4 ± 0.3
35	98.3 ± 0.3	74.6 ± 0.3	7.1 ± 0.2

*C_{BD} = 100 g/L, C_{AA} = 50 g/L, C_{cat} = 100 g/L, STR = 500 rpm, EPR = 0.5.

the changes in CR and ER values were small, but the loss of AA increased sharply after the temperature reached 25°C. As the boiling point of AA is 20.8°C, it becomes volatile when the temperature is higher than its boiling point. Accordingly, the feasible operating temperature was determined to be 20°C.

3.2.5. Multistage counter-current extraction of 2,3-BD from fermentation broth

Multistage counter-current extraction was carried out with a fixed EPR of 0.5. Concentration of TDX varied from 54.68 to 158.98 g/L in the aqueous phase (Table 5). When there was only one stage, TDX achieved saturated concentration in the organic phase and the extraction yield was only 73.21%. As the stage number changed to 2, more than 83% of TDX was recovered from the aqueous phase. In three-stage extraction, more than 99% of TDX was separated from the fermentation broth. Thus, three-stage extraction was chosen to recover as much TDX as possible.

As acetoin and ethanol can react with AA to form corresponding acetals, they can be extracted by CYHX into the organic phase in the form of acetals. The recovery rates of acetoin and ethanol were 97.6 and 88.9%, respectively. Though glucose contains hydroxyl groups, its structure does not promote acetal formation when resin is used as a catalyst. Proteins are too large to enter the reaction center and so the solubility of proteins in CYHX is low. The extraction rate of glucose and proteins were 1.2 and 0.4%, respectively. The proteins and salts in the fermentation broth will cause the ion-exchange resin HZ732 lose its

Table 5. Multistage current-counter extraction

Extraction stage number	Concentration in feed C_{TDX} (g/L)	ER (%)
3	158.98 ± 0.27	99.13 ± 0.17
2	158.69 ± 0.35	83.23 ± 0.27
2	128.81 ± 0.21	96.63 ± 0.16
2	54.56 ± 0.13	98.02 ± 0.08
1	54.68 ± 0.16	73.21 ± 0.17
1	127.81 ± 0.23	34.55 ± 0.07

*T=20°C, EPR = 0.5, C_{cat} = 100 g/L.

catalytic activity. After four times reuse, the resin's catalytic activity is about 80% of that for the new one. While only 34% of catalytic activity is left for the resin which is used for seven times. So the feasible used time for ion-exchange resin is no more than seven times.

After three-stage extraction, C_{TDX} in the aqueous phase could not be measured by equipment and C_{AA} was less than 0.6 g/L. As a result, the aqueous phase can be reused in the fermentation unit. So the aqueous phase can be reused in the fermentation unit. That can help reduce the pollution as the fermentation may cause the eutrophication of water.

3.3. Hydrolysis

The hydrolysis experiment was carried out at 50°C in a beaker with a water jacket connected to a thermostat. The mole ratio of water and TDX is 1 mol: 1 mol. AA was cooled by a cold trap at 10°C and collected at the top, while the 2,3-BD was enriched in the aqueous phase. After hydrolysis, CYHX was removed from the system and C_{BD} in the aqueous phase was over 930 g/L.

The experimental results showed that the yield of 2,3-BD was 94.8 ± 0.3%. The recovery rate of AA and CYHX was 80.2 ± 1.2 and 98.1 ± 0.2% respectively.

3.4. Purification

Finally, 2,3-BD was purified in a vacuum rectifying apparatus with a vacuum degree of 0.07 MPa. The distillation cut between 140 and 143°C was collected.

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

Acetion and ethonal in the fermentation broth can also react with AA to form corresponding acetal and extracted into organic phase. As the reactions require AA, so more AA will be required when the acetion and ethonal existing in the fermentation broth. The increasing mole amount of AA is about 1.1 to 1.3 times of the total mole amount of acetion and enthanol. The total recover rate of acetoin and ethanol from fermentation broth are 93.2 ± 0.6% and 67.4 ± 0.2%, respectively.

4. Conclusion

Effective separation of 2,3-BD by reactive-extraction process was achieved in this series of experiments. Acetaldehyde and cyclohexane were chosen as the reactant and extractant, respectively. Ion-exchange resin HZ732, which can be recycled easily, was selected as the catalyst. Upon determining feasible conditions, the process was able to effectively separate 2,3-butanediol from fermentation broth, where the total yield rate of 2,3-butanediol was greater than 90% and the mass fraction of 2,3-butanediol in final product was greater than 99%.

Kinetics was studied and well described by the Pseudo-Homogeneous model. According to the results of the reaction equilibrium and kinetics study, increasing reaction temperature can accelerate reaction rate and improve reaction selectivity. While the boiling point of AA is only 20.8°C, high reaction temperature will cause the AA to separate from the system easily. Thus hydrolysis can easily recover 2,3-butanediol from TDX solution.

As ion-exchange resin HZ732 is a solid catalyst, it is possible for the reaction to take place in a packed bed, fluidized bed and moving bed. Continuous separation of 2,3-butanediol from fermentation broth by reactive-extraction may be developed in the future. Our work investigated the equilibrium and kinetics of the reaction so that these data can be used as a basis for future process development. The proteins and salts in the fermentation broth will cause the ion-exchange resin HZ732 to lose its catalytic activity. After reusing four times, the resin's catalytic activity was approximately 80% of that for the new resin. After reusing for a total of seven times, only 34% of catalytic activity was left for the resin. Hence the feasible used time for ion-exchange resin is no more than seven times.

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Nomenclature

r	: Reaction rate
K_{eq}	: Reaction equilibrium constant
k	: Reaction rate constant
A	: Absorbency
C	: Concentration
$\Delta_r H^o$	: Reaction enthalpy
$\Delta_f H^o$	: Standard mole enthalpy of the formation
T	: Temperature
Ea	: Activity energy
RT	: Reaction time
n	: Number of moles
m	: Mass amount
β	: Reaction selectivity
m	: Main reaction
s	: Side reaction

eq : Equilibrium
o : Beginning
e : End
AA : Acetaldehyde
2,3-BD : 2,3-butanediol
cat : Catalyst
CR : Conversion rate
CYHX : Cyclohexane
DR : Distribution ratio
EPR : Extractant phase ratio by mass
ER : Extraction rate
FB : Fermentation broth
PA : Paraldehyde
RR : Reactant rate
STR : Stirring rate
TDX : 2,4,5-trimethyl-1,3-dioxolane
W : Water

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