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Reactive extraction of 2,3-butanediol from fermentation broth

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Abstract−Biochemical 2,3-butanediol is a renewable material, but the lack of an effective separation process limits its industrial application. We developed an effective separation process to recover 2,3-butanediol from fermentation broth by reactive-extraction with ion-exchange resin HZ732 as catalyst. n-Butylaldehyde was used as both reactant and extractant. Feasible operation conditions were obtained as follows: room temperature, C_{cat}=200 g·L^{-1}, three-stage cross-current extraction, with reactant ratio ($V_{Butylaldehyde}$: $V_{fermentation\ broth}$) 0.05 for each stage. Reactive-extraction can recover over 98% of 2,3-butanediol in the form of 2-propyl-4,5-dimethyl-1,3-dioxolane from fermentation broth. Then 2,3-butanediol was obtained by hydrolyzing 2-propyl-4,5-dimethyl-1,3-dioxolane and purified by vacuum distillation. The total yield rate of 2,3-butanediol through the process was over 94% and purity of final product reached 99%.

Key words: 2,3-Butanediol, Reactive-extraction, Fermentation Broth, Ion-exchange Resin, Cross-current

INTRODUCTION

2,3-Butanediol (2,3-BD), a chemical with two -OH groups, has a high boiling point and high hydrophilicity [1,2]. It has been used in the chemical and rubber industries as a raw material. Bio-based 2,3-BD has been regarded as a potential alternative energy for its high combustion value [3-5]. Present biotechnology developments make it possible to manufacture 2,3-BD through biological route [6,7]. However, the low concentration of 2,3-BD in fermentation broth and its strong interaction with water make its separation costly by traditional distillation [8].

Separation processes have been reported to recover 2,3-BD from fermentation broth with low energy consumption. Liquid-liquid extraction has been investigated by using different organic solutions [9-12]. The high hydrophilicity of 2,3-BD and the complex of fermentation broth force liquid-liquid extraction process to require a large amount of solution. Aqueous two-phase extraction [13] and salt out separation [14] are low in energy and solution consuming while high in salt requirement. A separation process combining extraction and membrane distillation [15,16] can effectively recover 2,3-BD from fermentation broth with low energy requirement. However, its industrial application still has not been reported.

Reactive-extraction, intensifying the efficiency of extraction by reaction, is an effective way to get target compound from complicated mixture system [17]. Reactive-extraction has been reported to recover 1,3-propanediol from fermentation broth using aldehyde [18,19]. As 1,3-propanediol can react with aldehyde to form its corresponding acetal, a similar reaction can also occur between 2,3-BD [20,21] and aldehyde.

In this work, a reactive-extraction process was investigated to recover 2,3-BD from fermentation broth. n-Butylaldehyde (BA) acted as both reactant and extractant in the study for its low solubility in aqueous solution. Ion-exchange resin HZ732 was used as catalyst to avoid the drawbacks like equipment corrosion. In the reactive-extraction unit, 2,3-BD and BA reversibly reacted to form 2-propyl-4,5-dimethyl-1,3-dioxolane (PDD) (Fig. 1) and then PDD was extracted into organic phase in sequence. Then the organic phase, collected in the reactive-extraction unit, entered the hydrolysis column in which PDD turned back to 2,3-BD. Operation conditions were selected for the process to get higher recovery. Finally, an effective process was achieved to treat 2,3-BD fermentation broth by combining reactive-extraction and hydrolysis. The results would provide basic elements in design and operation for industrial application.

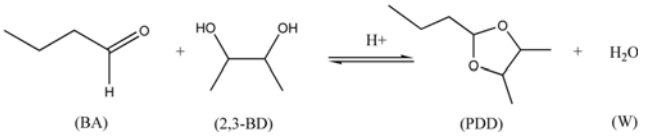

Fig. 1. Reaction equation of 2,3-butanediol and n-butylaldehyde.

MATERIALS AND METHODS

1. Materials

All the chemicals were purchased from a commercial source in the study. 2,3-Butanediol (2,3-BD) was bought from the Sinopharm Chemical Reagent Co. Ltd. with a minimum mass fraction purity of 98.2%. n-Butylaldehyde (BA) was purchased from the Sinopharm Chemical Reagent Co. Ltd. with a minimum mass fraction purity of 98.5%. Activated carbon was bought from the Sinopharm Chemical Reagent Co. Ltd. All these chemicals were measured and assured by gas chromatography. Ion-exchange resin HZ732 was given 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. 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

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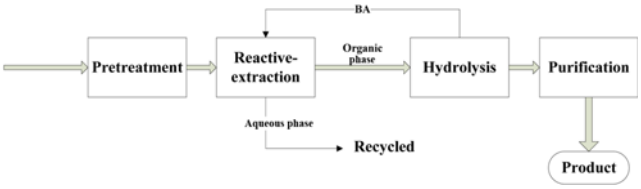

Fig. 2. Process of separation 2,3-butanediol from fermentation broth by coupling reactive-extraction and hydrolysis.

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.

2. Methods

The separation process (Fig. 2) consisted of membrane separation, reactive-extraction, hydrolysis and purification.

Cells and proteins were removed from fermentation broth by membrane separation as pretreatment to reduce the emulsion for the following treatments. And the yield rate of 2,3-BD in this step could be over 99.5%.

In reactive-extraction unit, the reaction between 2,3-BD and BA occurred in the post-treated broth with ion-exchange resin HZ732 as catalyst. Reaction product PDD was extracted by BA into the organic phase and separated from fermentation broth. Then the organic phase, obtained by reactive-extraction, went into the hydrolysis unit. In the hydrolysis column, PDD was hydrolyzed in the middle to form 2,3-BD, while 2,3-BD was enriched in the bottom and BA, which was reused in reactive-extraction unit, was collected at the top. Finally, the vacuum distillation was used to purify 2,3-BD solution and to obtain the final product.

Reaction conditions, including reactant ratio and reaction temperature and operation mode, were studied in this work. The efficiency of reactive-extraction were measured by conversion ratio (CR), distribution ratio (D) and extraction ratio (ER), which can be calculated by the following equations [22,23]:

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

$$D = \frac{C_{PDDor}}{C_{PDDaq}} \quad (2)$$

$$ER = \frac{(C_{PDDor} + C_{BDor}) \times V_{or}}{(C_{PDDor} + C_{BDor}) \times V_{or} + (C_{PDDaq} + C_{BDaq}) \times V_{aq}} \times 100\% \quad (3)$$

The concentrations of 2,3-BD, PDD, ethanol, acetoin, and BA were analyzed using internal standard method with acetone as stan-

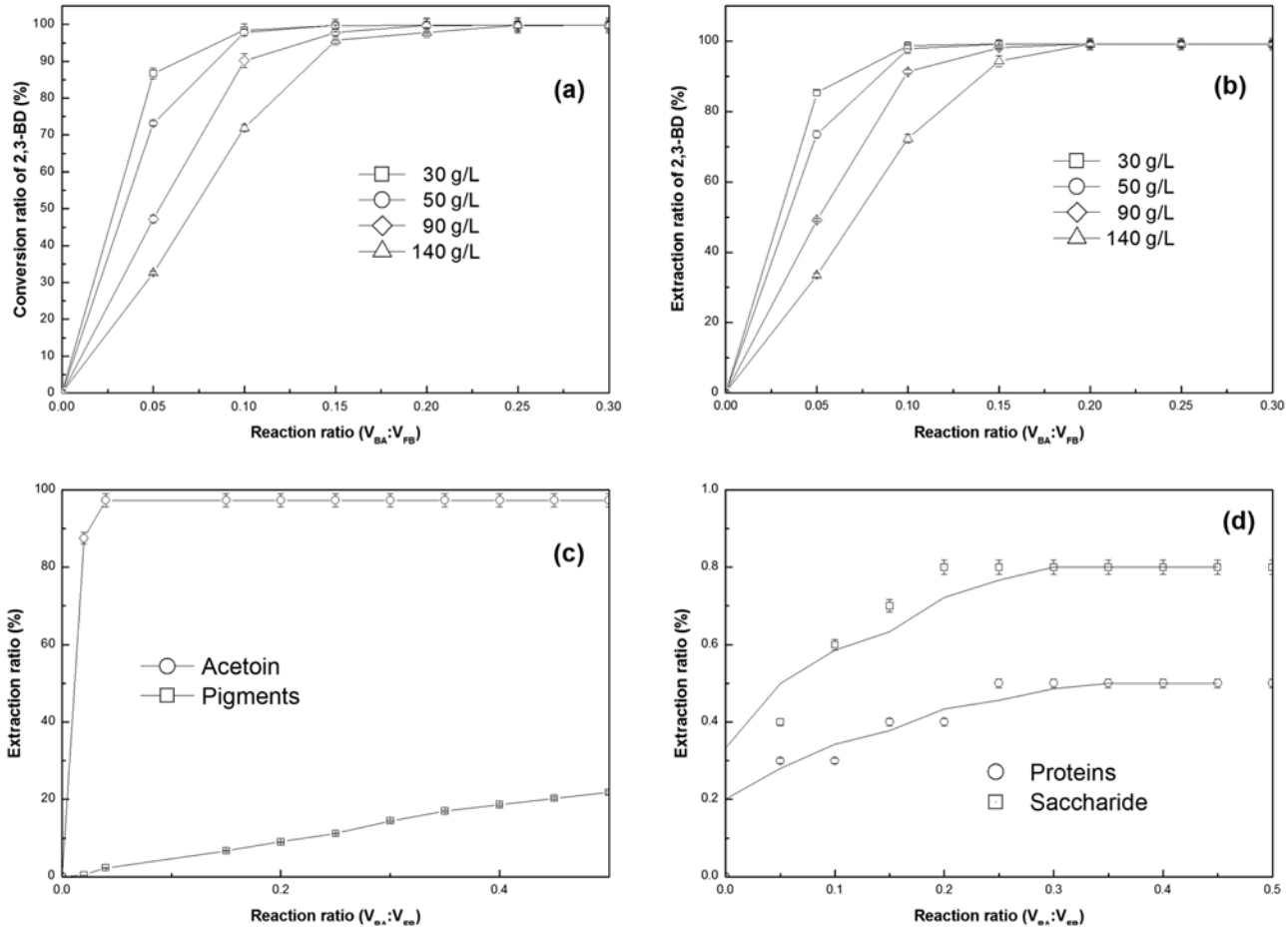

Fig. 3. Effect of reactant ratio on reactive-extraction. (a) The conversion ratio of 2,3-BD under different reactant ratio, (b) The extraction ratio of 2,3-BD under different reactant ratio, (c) The extraction ratio of acetoin and pigments under different reactant ratio, (d) The extraction ratio of proteins and saccharide under different reactant ratio.

dard 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 flow rate of 50 mL·min^{-1}, detector temperature 200 °C and column temperature 140 °C. The concentration of proteins was measured by Coomassie brilliant blue method [24]. 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. Pigments were measured by UV spectrophotometer.

RESULTS AND DISCUSSION

1. Reactive-extraction Experiments

Post-treated fermentation broth is used in this work. The broth contains 2,3-BD, acetoin and impurities like saccharide, proteins and pigments. The concentration of 2,3-BD in the post-treated broth varies from 30 g·L^{-1} to 140 g·L^{-1}.

1-1. Reactant Ratio

At the beginning, the effect of reactant ratio (RR, V_{BA} : V_{FB}) on reactive-extraction was investigated. The concentration of 2,3-BD, acetoin, pigments, proteins and saccharide was tested. The results are shown in Fig. 3. From the curves in Fig. 3(a) and Fig. 3(b), both CR and ER of 2,3-BD will grow with the increasing use of BA in the reaction system. When the C_{BD} is less than 50 g·L^{-1}, 100 ml·L^{-1} BA is enough to make sure CR of 2,3-BD is over 98% and ER of 2,3-BD reach 98%. When C_{BD} increases the required amount of BA also increases; for the fermentation broth with C_{BD}=140 g·L^{-1}, the required amount of BA is 250 ml·L^{-1}. Fig. 3(c) is about the extraction ratio of acetoin and pigments under different RR. Extraction ratio curve of acetoin reveals that acetoin can be extracted into organic phase. While, the extraction ratio curve of pigments indicates that reactive-extraction does not have a good selectivity on them. Fig. 3(d) illustrates that the reactive-extraction has a good selectivity on proteins and saccharide. Over 99% of proteins and saccharide can be separated from 2,3-BD by the method.

1-2. Effect of Temperature on Reactive-extraction

To study the temperature effect, operation temperature of reactive-extraction was changed from 20 °C to 60 °C. The results are given in Fig. 4. Increasing operation temperature has a negative impact on reactive-extraction. CR, ER and D decrease with the temperature increases. The decrease of CR and ER is not obviously in the region of 20 °C to 40 °C, but after the temperature reaching 40 °C, decreases are shapely for both CR and ER. Based on the results, the operation temperature should not be higher than 40 °C; it could be set at room temperature.

1-3. Effect of Catalyst amount on Reaction

To investigate the effect of catalyst amount on reaction, the catalyst amount in the reaction system was varied from 20 g·L^{-1} to 500 g·L^{-1}. The time to reaction equilibrium (RT_{eq}) under different catalyst amount is shown in Fig. 5. Fig. 5 reveals that increasing the catalyst amount reduces RT_{eq}. When the catalyst amount increases from 20 g·L^{-1} to 200 g·L^{-1}, the RT_{eq} goes down sharply from 480 min to 90 min. Then increasing the catalyst amount can still accelerate the reaction, but the decrease of RT_{eq} in the region of 200 g L^{-1} to 500 g·L^{-1} is not as obvious as that in the region of 20 g·L^{-1} to 200 g·L^{-1}. The curve indicates that a suitable catalyst amount is 200 g·L^{-1}.

1-4. Reactive-extraction Mode

In the reactive-extraction unit, reaction and extraction occur in sequence; a positive factor for reaction may have a negative effect on extraction. Three operation modes (Fig. 6) were investigated in this work, including the countercurrent extraction (Fig. 6(a)), concurrent extraction (Fig. 6(b)) and crosscurrent extraction (Fig. 6(c)).

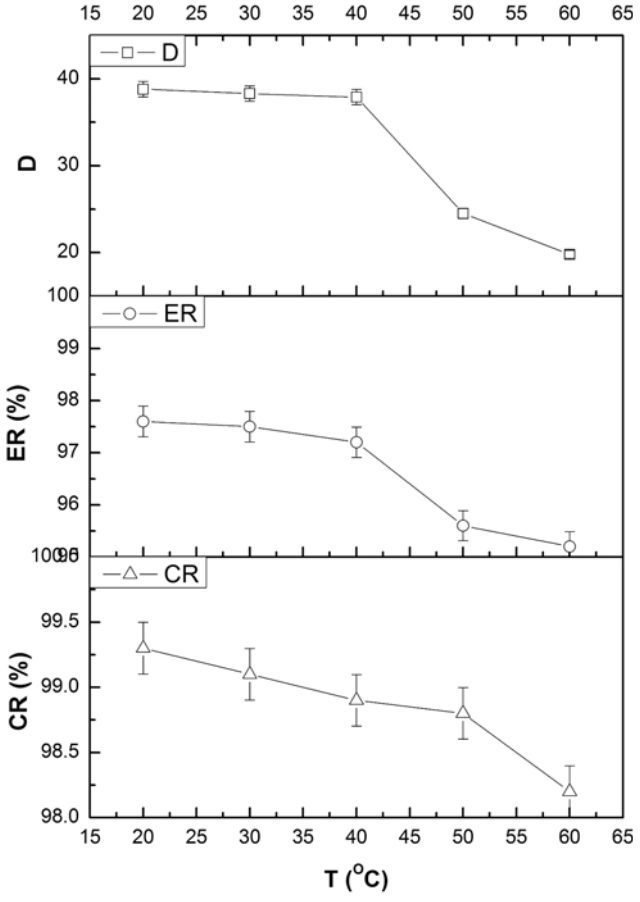

Fig. 4. Effect of temperature on reactive-extraction (C_{BD}=100 g·L^{-1}, C_{BA}=100 g·L^{-1}).

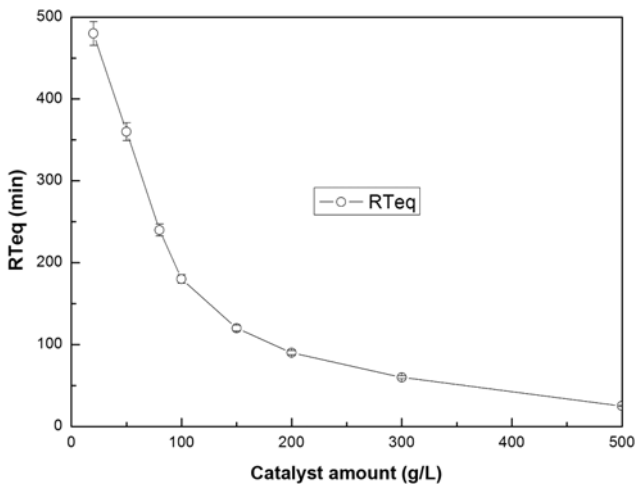

Fig. 5. Time to reaction equilibrium (RT_{eq}) under different catalyst amount (T=25 °C, C_{BD}=100 g·L^{-1}, C_{BA}=140 g·L^{-1}).

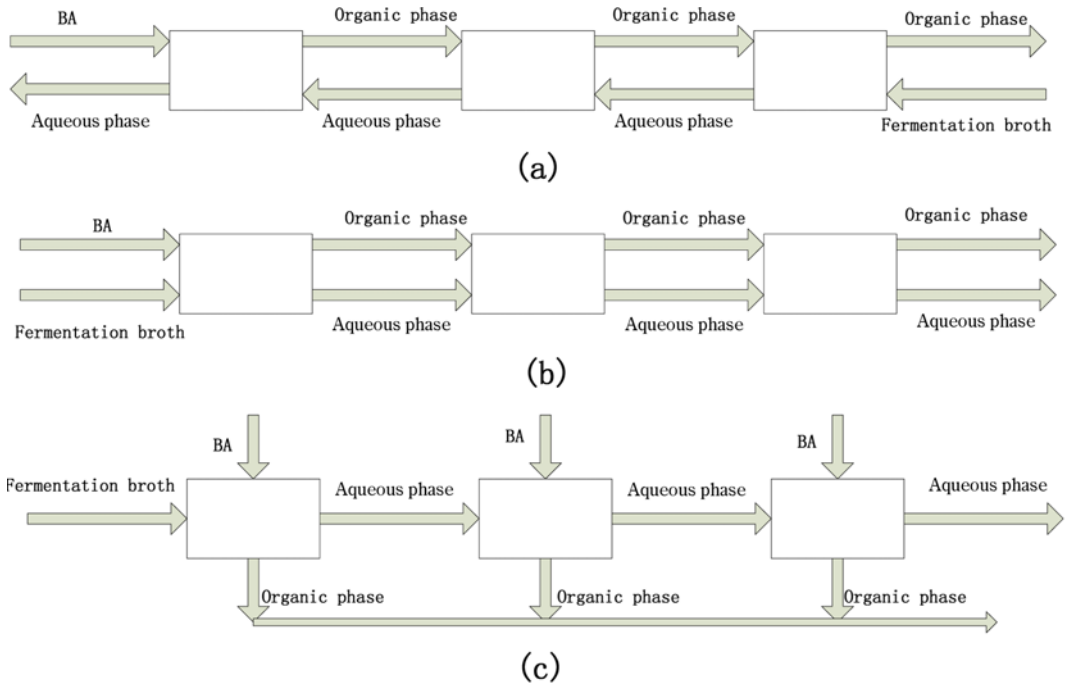

Fig. 6. Reactive-extraction operation modes (a) Counter-current extraction, (b) Con-current extraction, (c) Cross-current extraction.

Experiments of operation mode selection were carried out in funnels connected in series [25]. To measure the efficiency of extraction mode, different reactant ratios were used in the experiment.

The countercurrent extraction has been widely used in industrial application to intensify the extraction. The results (Table 1) reveal that increasing extraction stage can improve the efficiency of reactive-extraction. When the reactant is 0.2, the CR and ER of 2,3-BD for three-stage countercurrent extraction are 99.8% and 98.7%, respectively. BA, which is left in the aqueous phase, is 4.3 $g \cdot L^{-1}$. While for the two-stage countercurrent extraction under the same RR, CR and ER of 2,3-BD are 97.2% and 96.2%, respectively. The amount of BA in aqueous phase is 4.5 $g \cdot L^{-1}$. The results indicate that efficiency of three-stage countercurrent extraction is better than that of two-stage.

The results (Table 2) illustrate concurrent extraction has an effect on intensifying the reaction. Increasing extraction stage numbers

Table 1. Counter-current operation results

Stage (n)	Reactant ratio (V_{BA} : V_{FB})	Residence time (min)	CR (%)	ER (%)	C_{BA} in final aqueous phase ($g \cdot L^{-1}$)
2	0.10	87.5±0.5	85.4±0.5	83.7±0.3	1.5±0.2
2	0.15	74.3±0.4	93.4±0.3	91.2±0.3	1.7±0.3
2	0.20	68.4±0.7	97.2±0.2	96.2±0.2	4.5±0.3
3	0.10	97.2±0.5	91.5±0.3	90.5±0.3	1.3±0.4
3	0.15	85.7±0.4	96.5±0.2	94.8±0.2	1.6±0.3
3	0.20	76.6±0.3	99.8±0.1	98.7±0.2	4.3±0.3

*T=25 °C, C_{BD}=100 $g \cdot L^{-1}$, C_{cat}=200 $g \cdot L^{-1}$

Table 2. Con-current experimental results

n	Residence time (min)	Reactant ratio	C_{BD} in final aqueous phase ($g \cdot L^{-1}$)	CR (%)	ER (%)
1	60±2.8	0.25	1.4±0.2	98.6±0.3	97.9±0.3
2	40±3.1	0.15	4.5±0.3	95.5±0.2	94.8±0.3
2	35±2.3	0.20	2.3±0.1	97.7±0.3	96.7±0.2
2	30±1.3	0.25	0.7±0.1	99.3±0.1	98.4±0.1
3	24±1.5	0.15	4.4±0.3	95.6±0.1	94.9±0.1
3	20±0.8	0.20	2.3±0.3	97.7±0.2	97.3±0.2
3	18±1.3	0.25	0.5±0.1	99.5±0.1	98.7±0.2

*T=25 °C,C_{BD}=100 $g \cdot L^{-1}$, C_{cat}=200 $g \cdot L^{-1}$

Table 3. Cross-current experimental results

RR_{total}	RR_{n1}	RR_{n2}	RR_{n3}	Residence time (min)	CR (%)	ER (%)	C_{BD} in final aqueous phase ($g \cdot L^{-1}$)
0.10	0.05	0.05	-	127.4±0.3	67.5±0.3	67.3±0.2	47.9±0.2
0.15	0.05	0.10	-	89.5±0.4	83.4±0.3	83.2±0.2	25.8±0.4
	0.10	0.05	-	80.4±0.6	98.6±0.1	98.2±0.1	1.3±0.3
	0.05	0.05	0.05	167.4±0.3	99.0±0.1	98.2±0.1	1.2±0.4
0.20	0.05	0.15	-	84.2±0.3	97.6±0.1	96.1±0.1	1.4±0.3
	0.10	0.10	-	78.2±0.3	99.5±0.1	98.3±0.1	1.2±0.2
	0.15	0.05	-	40.3±0.4	99.7±0.1	98.3±0.1	0.9±0.2
	0.05	0.05	0.10	84.2±0.2	99.6±0.1	98.3±0.1	0.6±0.2
	0.05	0.10	0.05	84.2±0.2	99.7±0.1	98.3±0.1	0.6±0.1
	0.10	0.05	0.05	69.3±0.2	99.7±0.1	98.3±0.1	0.6±0.1

*T=25 °C, C_{BD}=100 $g \cdot L^{-1}$, C_{cat}=200 $g \cdot L^{-1}$

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

cannot improve ER of 2,3-BD obviously, but it can reduce the operation time. In one stage extraction with RR=0.25, it costs 60 min to recover 98% of 2,3-BD. While under the same RR, the operation times for two-stage-extraction and three-stage-extraction are 30 min and 18 min, respectively. Using the concurrent extraction, to make sure the recovery of 2,3-BD can reach 98%, the reactant ratio should be 0.25.

Crosscurrent extraction is an effective way to recover 2,3-BD from fermentation broth. According to Table 3, when the total RR is 0.2, more than 96% of 2,3-BD can be recovered from broth. But using three-stage extraction with RR=0.05 for each stage, as the total reactant ratio is 0.15, more than 98% of 2,3-BD can be separated from fermentation broth by crosscurrent operation. Based on the results in Table 3, the feasible operation condition for crosscurrent extraction is three-stage with RR=0.5 for each stage.

1-5. Catalyst Reuse

As a solid catalyst, ion-exchange resin HZ732 is easy to be separated and reused. To estimate its reuse time, its catalytic activity under different used time is tested. Setting the catalytic activity for the first time as 100%, the results (Fig. 7) reveal that the catalytic activity goes down with the used times. To keep the operation stable, the catalytic activity should keep at least 80%. That means the resin can be used for four times.

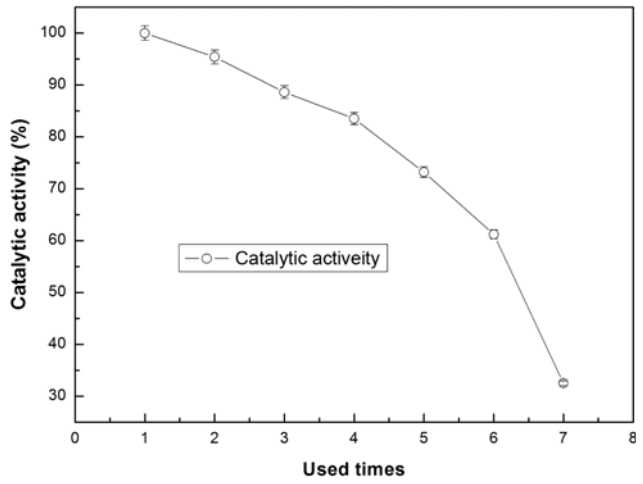

Fig. 7. Catalytic activity of catalyst under different reused times (T=25 °C, C_{BD}=100 $g \cdot L^{-1}$, C_{BA}=140 $g \cdot L^{-1}$).

1-6. Results of Reactive-extraction

According to the experimental results of all the above, the feasible operation conditions are obtained as follows: room temperature, C_{cat}=200 $g \cdot L^{-1}$, three-stage crosscurrent extraction, with reactant ratio 0.05 for each stage.

As the C_{BD} changes with the fermentation conditions, varying from 30 $g \cdot L^{-1}$ to 140 $g \cdot L^{-1}$, the required amount of BA would change from 45 $ml \cdot L^{-1}$ to 150 $ml \cdot L^{-1}$, equal to 36 $g \cdot L^{-1}$ to 120 $g \cdot L^{-1}$. That means C_{PDD} in the final organic phase could be over 750 $g \cdot L^{-1}$, equal to C_{BD} 430 $g \cdot L^{-1}$.

These results obtained in this work are better than those (C_{BD}= 200 $g \cdot L^{-1}$ in final organic phase; the amount of solvent required is 240 $g \cdot L^{-1}$) of aqueous two-phase extraction [13] and those (C_{BD}=80 $g \cdot L^{-1}$ in final organic phase, the amount of solvent required is 800 $g \cdot L^{-1}$) of solvent extraction [15]. Especially, when the C_{BD} in fermentation is lower than 50 $g \cdot L^{-1}$, reactive-extraction has its advantages in low solution consumption. So the process can be a promising method in industrial application. Ion-exchange resin is easy to recycle and catalyst drawbacks such as equipment corrosion can be avoided. It can be used in a fluidized bed, fixed bed and simulated moving bed, which may be developed in the future research. Whereas, reactive-extraction using ion-exchange resin requires long operation time and the catalyst can only used for four times which will increase the cost.

2. Hydrolysis and Purification

The liquid obtained by reactive-extraction should be treated by activated carbon to remove pigments first and then enter the reactive-distillation column for hydrolysis.

To improve hydrolysis efficiency, hydrolysis distillation is carried out in a distillation column. Hydrolysis temperature is set at 90 °C. BA are cooled by water and collected at the top, while the 2,3-BD is obtained at the bottom. The recover rate of 2,3-BD and BA in the hydrolysis is 96.1% and 95.4%, respectively. And the yield of acetoin and 2,3-BD is 96.8% and 97.4%, respectively.

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

and 143 ºC is collected.

Distillation is used to recycle BA in the aqueous phase. The bottom temperature of the distillation column is 95 ºC, and BA is collected at the top with the temperature between 75 ºC and 78 ºC. The recovery rate of BA is over 85%.

With the separation process described above, the mass fraction of 2,3-BD in the final product is more than 99% and the total yield rate of 2,3-BD from fermentation broth by reactive-extraction process can be over 94%.

CONCLUSION

A novel process of separation 2,3-butanediol from fermentation broth was achieved in this work. Reactive-extraction was effective in 2,3-butanediol separation. With appropriate operation conditions (room temperature, C_{cat}=200 g·L^{-1}, three-stage cross-current extraction, with reactant ratio 0.05 for each stage), the reactive-extraction can recover over 98% of 2,3-butanediol from fermentation broth. The total yield rate of 2,3-butanediol through the process was over 94% and the purity of the final product reached 99%.

ACKNOWLEDGEMENT

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NOMENCLATURE

C : concentration
T : temperature
t : time

Subscripts and Superscripts

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

Abbreviations

2,3-BD : 2,3-butanediol
FB : fermentation broth
V : volume
CR : conversion ratio
D : distribution ratio
PDD : 2-propyl-4,5-dimethyl-1,3-dioxolane
ER : extraction ratio
BA : n-butylaldehyde
RR : reactant ratio
RT_{eq} : time to reaction equilibrium
STR : stirring rate
W : water

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