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Full Length Research Paper

Study of a cleaner extraction of pyruvic acid from fermentation broth

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A new cleaner technology for extracting pyruvic acid (PA) from fermentative broth was explored. This process involves recycling waste residue combined with small amount of dispersant added to extract PA. Limited amount of dispersant in the process of rectification under vacuum (1.5 kPa) was found to inhibit the polymerization of PA and to enhance the extraction rate of PA from 20.5 to 92.3% when the volume of dispersant was 0.20 times that of preliminary extract liquid (PEL). Experimental results indicate that a cleaner extraction of PA might be achieved by circulation. The purity of PA still exceeded 96% after the waste liquid was used up to 30 times when the added amount of dispersant was 5% of the PEL. A mathematic model was developed to describe the relationship of purity of PA (P), the repetition times of waste residue (N) and the added amount of dispersant (M) during the first-round of waste liquid usage: $P = 105.84N^{-0.0251}M^{0.01955}$.

Key words: Pyruvic acid, extract, dispersant, rectification under vacuum, waste residue, circulation.

INTRODUCTION

Bioprocess engineering includes key steps such as selection of good living species, production of crude material by bioreaction and subsequent isolation, and purification of the final products. The importance of downstream processing mainly comes from the specialization, complexity and strict quality requirements of products. Therefore, the cost of downstream processing is always the major cost of the whole bioprocess. A reasonable design can help reduce production cost of the target products and achieve successful commercial production (Chu and Li, 2002).

Pyruvic acid, also known as 2-oxopropanoic acid, α-ketopropionic acid or acetylformic acid, is one of the most important α-oxocarboxylic acid (Zhang and Gao, 2006; Ma et a.l, 2006; Roufs et al., 1996; Uchio et al., 1976; Rosche et al., 2002). In recent years, biocatalytic reactions and fermentation technology have started to replace conventional synthesis (Ogawa et al., 2001; Li et al., 2001a; Yokota et al., 1994; Gu et al., 2005). Several methods have been suggested to recover pyruvic acid from fermentation broth. Commonly used methods include organic solvent extraction (Uchuio et al., 1976; Chen et al., 2000; Biwer et al., 2005), reverse osmosis method (Matsuno et al., 1994) and ion exchange process (Matsuno et al., 1994; Li et al., 2001b; Huang et al., 2007; Zhao et al., 2010).

As a result of its special molecular structure, pyruvic acid tends to participate readily in chemical reactions. Direct rectification can achieve relatively low purification cost because pyruvic acid is liquid under room temperature. But it is a difficult task to extract pyruvic acid from fermentation broth due to impurities such as sugar and proteins. Pyruvic acid at high concentration also undergoes condensation, polymerization and decomposition reactions under heating during rectification. An example of the reaction is described thus (Zhuge and Wang, 1994):

$$2\,CH_3COCOOH \xrightarrow{-CO_2} CH_3COCOHCOOHCH_3 \xrightarrow{-CO_2} CH_3COCHOHCH_3$$

As rectification proceeds, the condensation extent will increase and it will lead to lower yield and reduce purification of pyruvic acid in the final product. To minimize such reactions, this study examined the effect of dispersant addition on the extraction of pyruvic acid and dispersant recycling. A closed circulation cleaner

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extraction process of pyruvic acid from fermentation broth based on the findings was established.

MATERIALS AND METHODS

Microorganism and preparation of immobilized cells

A four-vitamin-auxotrophic yeast, *Torulopsia glabrata* TJ400 was used. And its growth culture contained 30 g glucose, 10 g peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$. *T. glabrata* TJ400 was cultivated in a 5 L fermentor by fed-batch fermentation at 30°C and pH 5.0 for 20 h. The cells were collected for immobilization as described by Lu (1990). This immobilized organism produces 65 g/L pyruvic acid in an optimized fermentation process.
Dispersant D was synthesized in our laboratory under an undisclosed process. Sulphuric acid, activated carbon and other chemicals were of analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd.

Analytical method of pyruvic acid

Pyruvic acid was determined by the method described by Li et al. (2000).

Closed circulation in cleaner extraction of pyruvic acid

Pyruvic acid in the fermentation broth was released by adding sulphuric acid followed by $CaSO_4$ which was removed by filtration. The solution was then concentrated to approximately 500 g/L under vacuum evaporation which avoided condensation reaction. Residual calcium sulphate was then further removed by filtration. The resulting solution was therefore a PEL. A certain amount of dispersant D was then added to the PEL in order to reduce condensation reaction and remove pyruvic acid. The cleaner recovery process was continued until pyruvic acid purity was decreased to a specified standard such as 96%, which was called one round of recovery. Activated carbon was added to the final waste liquid to adsorb humus and other impurities. After centrifugation, the treated waste liquid was applied to recover pyruvic acid for the second round (Figure 1).

RESULTS AND DISCUSSION

A one-step vacuum rectification method was used for pyruvic acid extraction from broth in this study. This method can greatly reduce the production cost and environmental pollution of organic solvent and wastewater produced by extraction method and ion exchange process.

Effect of dispersant amount on extraction

The greatest characteristic of direct vacuum rectification was found to be the addition of dispersant D to the PEL. Traditionally, the direct vacuum rectification is limited to the process of chemical synthesis of pyruvic acid. This method was made successful in this study for the system of fermentation because the reactions of condensation, polymerization and decomposition were prohibited by dispersant D. When the absolute pressure and temperature of rectification system were kept at 1.5 kPa and 78°C, respectively, the influence of dispersant amount on pyruvic acid extraction was significant (Figure 2).

When dispersant D was not supplied, the purity and extraction rate of pyruvic acid were only about 72 and 20%, respectively. Purity and extraction rate increased as the dispersant amount was higher. These two parameters reached 99.2 and 92.3%, respectively, when the ratio of dispersant D amount to PEL was 0.20.

The conventional production process of pyruvic acid includes distillation of pyruvic acid from a mixture of tartaric acid and potassium hydrogen sulfates at 220°C and then distillation of the crude acid obtained under vacuum. In recent years, biocatalytic reactions and fermentation technology have started to replace conventional synthesis (Li et al., 2001a) but it is rather difficult to obtain pyruvic acid from fermentation broth. Some researchers obtained sodium pyruvate rather than pyruvic acid. According to Zhao (2010), the purity and total extraction rate of sodium pyruvate from fermentation were 98 and 81%, respectively, through a series of steps, including treatment with ultra-filtration and nano-filtration, decoloration with HD-1 resin and addition of ethanol. Huang (2009) studied the solvent effect in the crystallization of sodium pyruvate in different solvents including methanol, ethanol, acetone and ether. Ma (2006) extracted pyruvic acid with tri-n-octanylamine from biotransformation solutions and a total recovery of 71 to 82% of pyruvic acid was obtained. The purity of pyruvic acid was 97%.

Closed circulation in cleaner extraction of pyruvic acid

In the extraction process by using closed circulation, the circulation cannot be carried on illimitably since the circulation may be broken down before all the components reach equilibrium. The major cause is always the over accumulation of impurities in the circulation system. The main aim of this study was to establish the circulation parameters while ensuring a minimal purity of pyruvic acid of 96%.

Effect of dispersant D on extraction in the process of waste liquid reuse

Determination of the amount of dispersant D in the first round of reuse

The influence of dispersant D amount on pyruvic acid purity in the first reuse of waste liquid is shown in Figure 3.

It is clear that the purity of pyruvic acid increased with the increase of dispersant amount but the extent of

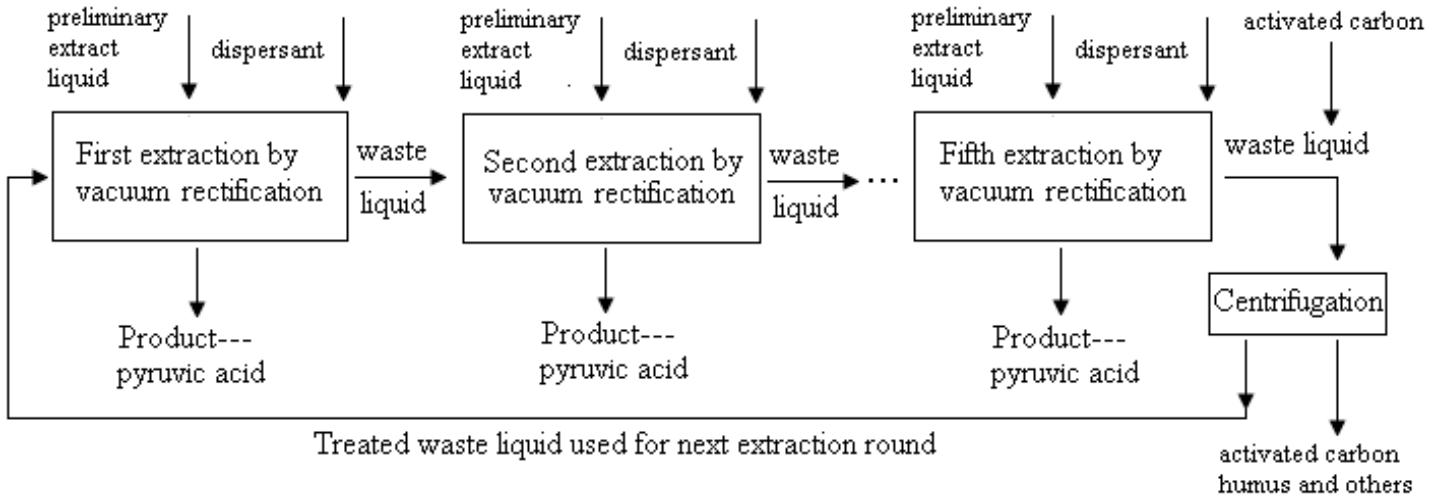


Figure 1. Closed circulation in cleaner extraction of pyruvic acid.

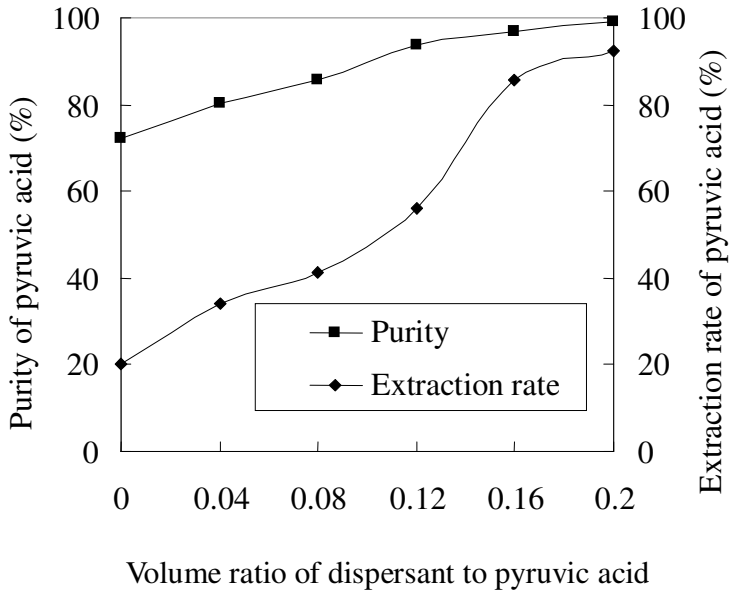


Figure 2. Effect of dispersant amount on pyruvic acid purity and extraction rate.

increase of purity became smaller when the volume ratio of dispersant to PEL reached 0.05. Therefore, the suitable amount of added dispersant was 0.05. A purity of 99.54% and an extraction rate of 93.1% were obtained under this condition. Then, the resulting waste liquid was used for the second extraction.

Results of second to fifth extractions

For second extraction, a similar way with first extraction was used and the result is illustrated in Figure 4A. The variation of pyruvic acid purity was in agreement with the results in Figure 3. The purity and extraction rate of pyruvic acid were 98.11 and 92.5%, respectively, when the volume ratio of dispersant D to PEL was controlled at 0.05. Similar changes were observed for the third, fourth and fifth extraction with the use of waste liquid (Figure 4 B, C and D) but the purity reached a slightly lower level at 96.05% after the fifth extraction.

Obviously, purity of pyruvic acid would fall below 96% when waste liquid is used beyond the fifth extraction. So, the waste liquid was treated with activate carbon and centrifugation and used for the second round cleaner extraction. The waste liquid was treated again when the purity fell to 96% and was used for the third round

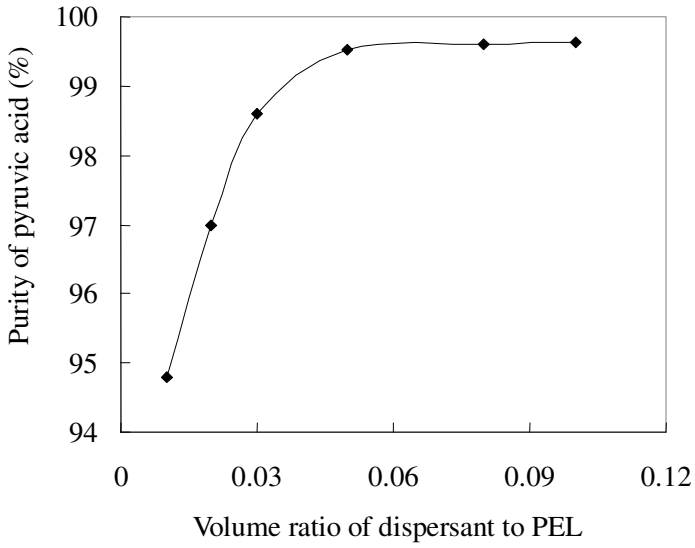
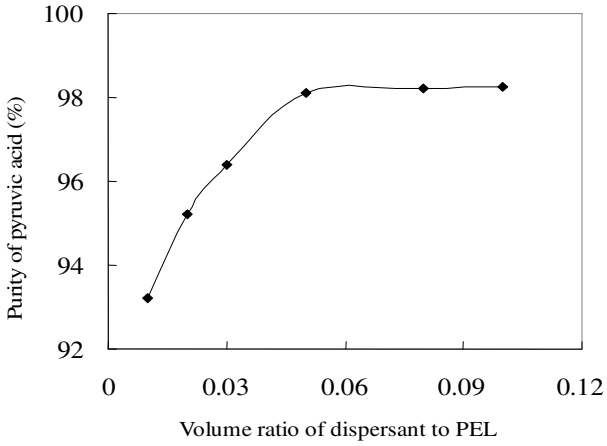
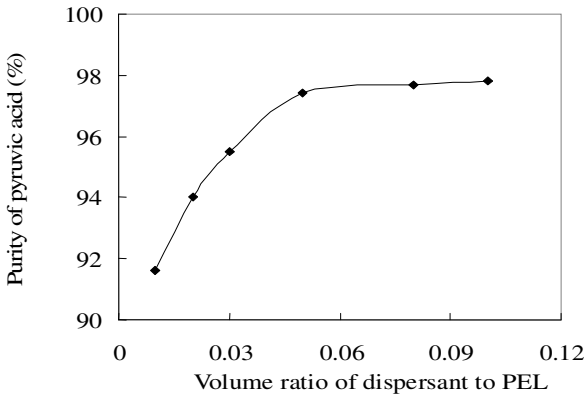


Figure 3. Effect of dispersant amount on pyruvic acid purity in first reuse of waste liquid.

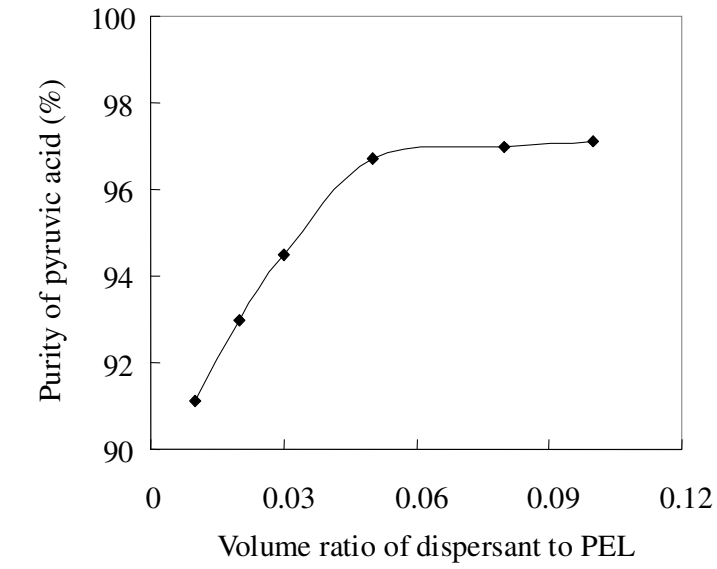


A



B

Figure 4. Effect of dispersant on purity of pyruvic acid in second to fifth cleaner extraction.



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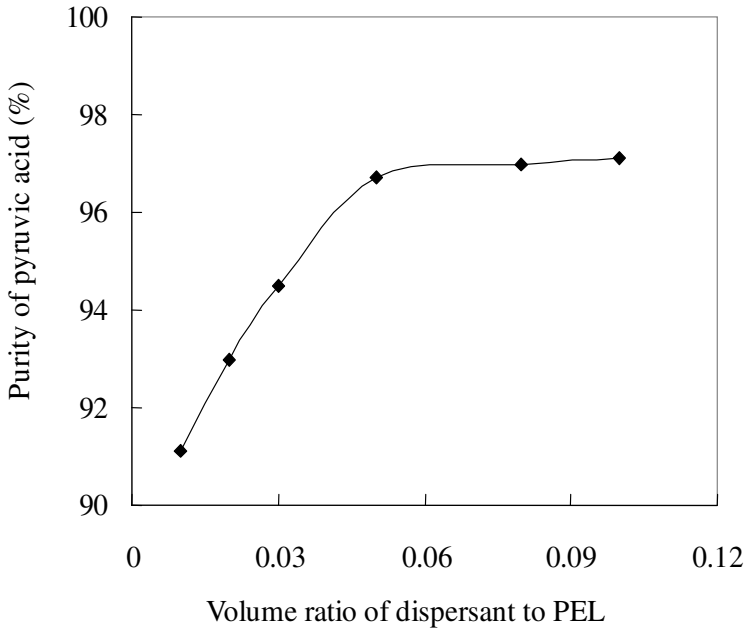

D

Figure 4. Contd.

extraction, fourth round, and so on.

Extraction of the second to seventh rounds

The second to seventh rounds of cleaner extraction were carried out under the same conditions as the first round. The fifth purity and extraction rate of every round are indicated in Figure 5.

The results show that purity and extraction rate decreased with an increase of extraction times in each round and the purity was 96.01 and 95.8%, respectively, at the end of the sixth and seventh rounds. It can be concluded therefore that optimal reuse rounds of waste liquid were six (a total of 30 times) in the process of cleaner extraction.

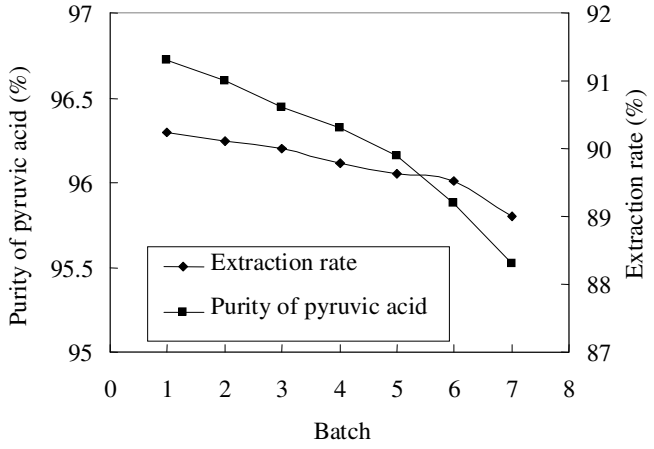

Figure 5. Variation of purity and extraction rate with rounds in the cleaner extraction process.

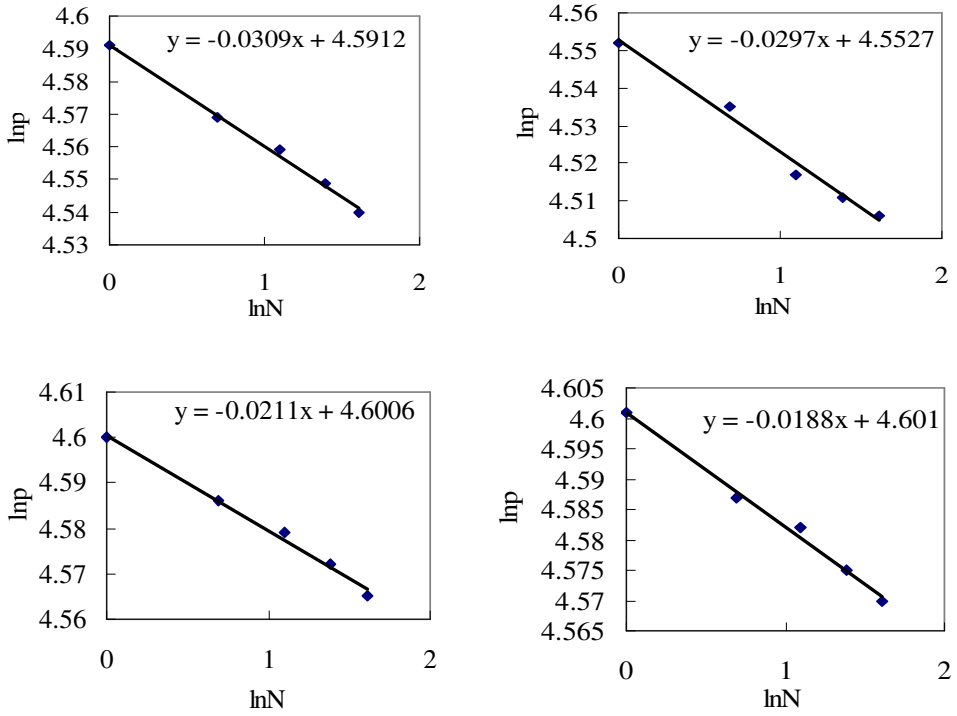

Figure 6. Relation of purity, dispersant amount and reuse time in the first round.

Mathematic model in closed circulation of cleaner extraction

Group number formulas are always found using experimental method in data processing and engineering technology (Chen et al., 1992). Purity of pyruvic acid, extraction time and dispersant amount fit certain mathematic relation in the process of cleaner extraction of pyruvic acid. Suppose purity of pyruvic acid, extraction time and dispersant amount fit the following power function relation:

$$P = kN^{\alpha}M^{\beta}$$

This formula can be converted to: lnp = lnk +αlnN +βlnM

Take the first closed circulation for example to calculate each parameter, series relation figures of lnp with lnN can be obtained by varying dispersant amount (Figure 6). Volume ratios of dispersant to PEL were 0.01, 0.03, 0.05

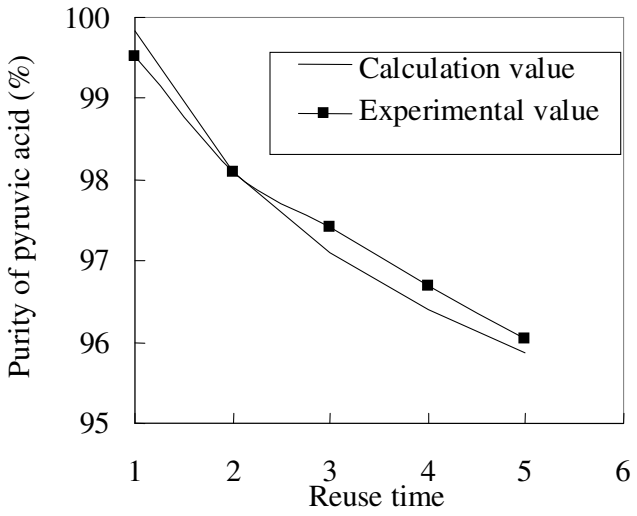

Figure 7. Model and experimental value of purity in the first round; M = 0.05.

and 0.08, respectively.

The relation of lnN and lnp was linearity for specific value of M. -0.0251 of average α value was obtained and the average values of β and k were calculated: 0.01955 and 105.84, respectively. So, the relationship of purity with dispersant amount and waste liquid reuse time can be expressed by the following mathematic model:

$$P = 105.84 N^{-0.0251} M^{0.01955}$$

Comparison of experimental value with calculation value of model is shown in Figure 7. It can be seen from Figure 7 that model value was almost identical to the experimental value and the deviation was within ±0.5%. So, this model can be used to describe the purity variation in the process of waste liquid reuse. Further study show that similar model can be used to describe the purity in circulation of later five rounds.

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