The continuous citric acid production from milk-wastewater used the immobilized Aspergillus niger

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Abstract

The study was carried out immobilized Aspergillus niger used of milk-wastewater. The purpose of investigation is to optimize the ermentational conditions of milk-wastewater. The optimal pH, temperature and dilution rate were 3.0, 30℃ and 0.025 h^{-1}. The maximum amount and yield of citric acid produced by immobilizes Aspergillus niger ATCC 9142 were 4.5g/*l* and 70.3%. Compared to shake-flask culture, yield was increased about 20%.

Introduction

The citric acid is not only widely useful for the food industry with the distinguished taste but also worthy enough in the pharmacy with the low toxicity. The citric acid is mainly produced from the fermentation by a microorganism, such as mold, yeast, and bacteria etc. Among them, the method by the Aspergillus niger is one of the most famous methods. The milk-wastewater contains much sugar and protein to make the pollution level high that it costs a lot to discharge the milk-wastewater that is diluted by water. According to this reason, if we use a plenty of sugar in the milk-wastewater as a culture medium for the citric acid fermentation, there will be two effects, which are an antipollution and use of a fermentation culture medium. This experiment is performed with the milk-wastewater as the fermentation culture medium and Aspergillus niger ATCC 9142 as a citric acid production strain. We entrap the strain, which was cultured for 3 days, with Ca-alginate and then bead about 2.5-3.5㎜. The dilution rate is 0.045h^{-1}. The purpose of this experiment is to optimize the condition of the pH, temperature, and dilution rate for the continuous citric acid production by using the immobilized Aspergillus niger. In addition, we will discuss a result of the continuous experiment with a result of the shake-flask fermentation.

Material and method

We used Aspergillus niger ATCC, which is obtained from ATCC, for the citric acid production strain. To maintain the activity of bacteria, with keeping this at the temperature 4°C, we use it after we take it subculture in the PDA culture medium once a two months and then culture it in the temperature 30°C for a week. The PDA culture medium consists of potato 200g, dextrose 20g, and agar 15g. The milk-wastewater of which the ingredients are in the table 1 is used for the culture medium. We add 4N-HCl to the milk-wastewater and then filter protein, which is precipitated at pH=4.3, from that mixture. We mix it with active carbon and then filter this with the Watman paper NO.1. After keeping the limit condition of nitrogen component, we control the sugar concentration to be 5% and then fix it at the suitable pH. We sterilize this at the temperature 121°C for 15 minutes and use it. In this experiment, we put the sterilized milk-wastewater 100㎖ in a 500㎖ Erlenmeyer flask and then inoculate a spore from the PDA culture medium. Next, we culture it in a shake-incubator with the temperature 30°C for 72 hours and then centrifuge strain from that. After we mix that strain with 4% Na-alginate(bacteria : alginate=1:10, w/w), with an injector we drop it on 2% $CaCl_2$solution and then bead it diameter 2.5-3.5㎜. We charged the immobilized bacteria to be 30% of reactor volume. Figure 1 is a continuous culture that is used in this experiment. The citric acid is measured by the method of Mairer and Boulet. We mix sample 1㎖ with pyridine 1.3㎖ and then put acetic anhydride 5.7㎖. After agitating this, we react it in a thermostatic watertank for one hour and then developed. The reducing sugar is measured with the DNS method. We add 3,5-dinitrosalicylic 0.25g and Rochelle salt 75g to 2M-NaOH 50㎖ and then melt them. Subsequently, we dilute that with distilled water to make 250㎖ dintrosalicylic acid(DNS) reagent. We developed the mixture of sample 1§¢ and the DNS reagent 1㎖ at the temperature 100°C for 10 minutes. After cooling it at the room temperature, we find the absorbency with the ultraviolet spectrophotometer(Shimadzu, Japan, Model U-3210) and fix its quantity with a calibration curve.

Table 1. Typical compositions of milk-waste water.

Itrms	Waste water
PH	7.0~7.2
BOD5 (mg/*l*)	20,000
COD (mg/*l*)	17,000
Total reducing sugars (g/*l*)	10
Total nitrogen (mg/*l*)	575
Total phosphorus (mg/*l*)	2.1

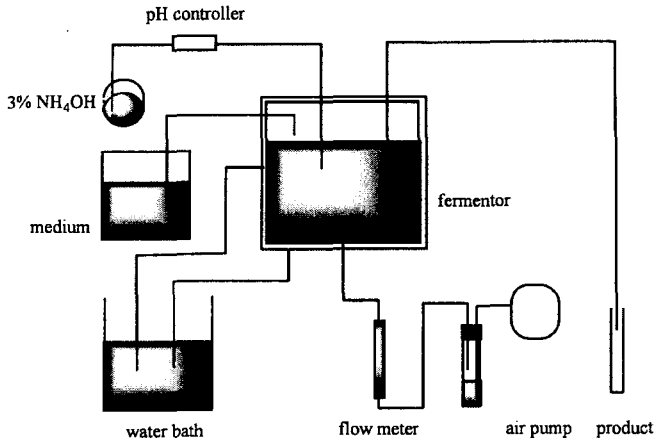

Fig. 1. Diagram of continuous reactor

Table 2. Nitrogen concentration of milk-waste water.

	Nitrogen (mg/l)
milk-waste water	575
after pH precipitation	320
After activating charcoal	24.5

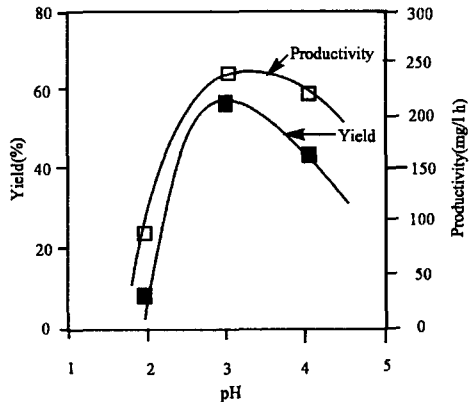

Fig. 2. Effect of pH values in citric acid production

Result and discussion

In this experiment, we pass the culture medium through active carbon to reduce nitrogen source density of the milk-wastewater. Table 2 that is result of the nitrogen source density after taking every treat states that the nitrogen source density of the culture medium decreases about 1/13 of that as compared with pH precipitation. We vary pH as 2,3, and 4 and maintain dilution rate as $0.045h^{-1}$ in this experiment. After 9^{th} day when the citric acid production reaches at the stable level, the highest amount of the citric acid production is

3.86g/*l* and yield is 58.9% at pH=3(Figure2). Particularly, sugar is consumed too much at pH=2. We guess that it is because sugar can penetrate into the cell membrane at low level of pH, or because the activity of β-galactosidase in ATCC 1942 is big about at pH=2. In this experiment, as a result of the test with the temperature range 25~35°C, the yield is the highest at the temperature 30°C (Figure3). Figure 4 shows the yield and productivity at each dilution rate on fermentation for 9 days. The more the citric acid production increases, the more the dilution rate decreases. Finally, the yield is maximum at $0.025h^{-1}$. At low dilution rate ($0.01h^{-1}$), the yield decreases. It might be because a little amount of sugar influx makes coefficient of utilization go down at low dilution rate. Figure 5 shows the result of shake-flask and continuous bioreactor last for 20 days in the citric acid production experiment by using the milk-wastewater. In the Table 3, which compares the shake-flask productivity with the continuous one, shows that the volumetric productivity(Dividing the density of the citric acid by the retention time) of the continuous culture(63.3㎖/*l* h) is two and half times as small as the continuous cultures one.

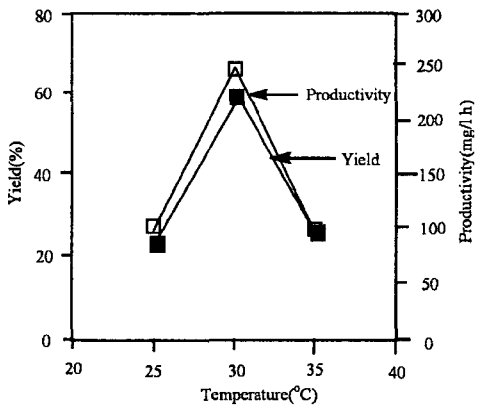

Fig. 3. Effect of Temperature on citric acid production

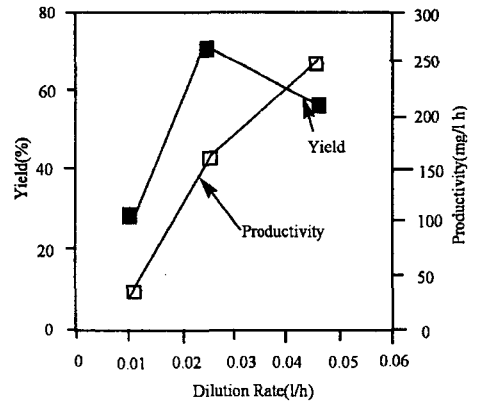

Fig. 4. Effect of dilution rate on citric acid production

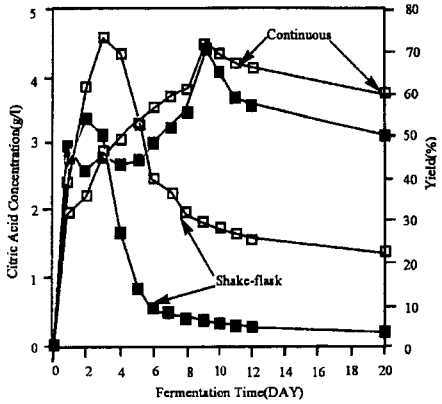

Fig. 5. Comparison of continuous culture and shake flask culture on citric acid production in citric acid

Table 3. Comparison of shake-flask, continuous culture for citric acid production

Culture	Volumetric Productivity (mg/*l*)
Shake-flask	63.3
Continuous	160

Conclusion

In the continuous citric acid production by using the milk-wastewater, the variety of the pH, the temperature and the dilution rate varies the yield. The optimal conditions are The optimal conditions are pH=3.0, 30°C, and 0.25h^{-1}. At this point, the maximum amount of the citric acid production is 4.5g/*l* and the yield is 70.3%. Hence, the milk-wastewater is worthy to be used for the citric acid fermentation substrate.

Reference

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