Kinetics for Citric Acid Production from the Concentrated Milk Factory Waste Water by *Aspergillus niger* ATCC 9142

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Abstract

The possible use of milk factory waste water as fermentation media for the production of citric acid by cells of *Aspegillus niger* ATCC 9142 has been investigated. The addition of Mn^{2+}, Fe^{2+} and Cu^{2+} to a medium promoted the citric acid production steadily, but addition of another metal ion Mg^{2+} decreased the citric acid production. The concentrations of citric acid were marked up to 7.2g/l and 16.5g/l in a batch bioreactor by *A. niger* ATCC 9142 with 50g/l and 100g/l of reducing sugar concentration in milk factory waste water, respectively.

Introduction

Recently as instant foods are popularized the consumption of alkaline foods has increased rapidly. Mass production of these foods in factories cause water pollution and the treatment of water pollution become a social problem. With the increasing cost of pollution abatement, there is a need for information on ways to reduce the strength of effluent from processing milk. The conversion of different organic wastes by microorganisms has been the subject of many research works. Most suitable substrates are food industry waste materials. Several investigators have reported the citric acid production using *A. niger* and different sources of carbohydrates. Most of researches has been directed to finding ways of utilizing waste water. Milk factory waste water is significant sources of high-strength waste streams in the dairy industry. For any fermentation process based on milk factory waste waters it is advantageous if the microorganism concerned is capable of utilizing all of the sugars present in the substrate, both on the basis of product yield and to minimize any waste disposal problem resulting from residual sugar. In spite of this reason, little information has been reported on the use of milk waste water as substrate. The purpose of this investigation was to explore the possible use of milk factory waste water instead of sugars for production of citric acid.

Materials and Methods

A citric acid producing strain of *A. niger* ATCC 9142 was obtained from ATCC. It was grown on a potato dextrose agar(PDA) slant at 30℃ for 7 days, and stored at 4℃. The agar slant renewed every two month. Milk factory wate water used in this study was obtained from the dairy industry. It contained 1.2%~3.5% reducing sugars, and most of them was lactose and its moisture content was 97%. So it was concentrated 5% concentration of total reducing sugar in experiment. 0.2% inoculum, containing 10^5~10^6 spores, in distilled water was used to inoculate the 100㎖ culture medium. Shake-flask culture experiments were performed in 250㎖ erlenmeyer flask containing 100㎖ medium. All media and equipment were sterilized at 121℃ for 20min. The flasks were incubated at 30℃ on a rotary shaking incubator Model KMC-8480SF and operating speed of 180rpm. Fermentation experiments were performed in a batch reactor. The aeration rate was 1vvm and agitation speed was 400rpm. The temperature was at 30℃ but the pH was not controlled. Mycelial dry weight was determined by filtering, washing with distilled water and drying at 105℃ overnight. Reducing sugar was measured by the method of dinitrosalicylic acid(DNS), and citric acid by the method of Marier and Boulet.

Results and Discussion

The effect of initial pH on the production of citric acid is shown in Fig. 1. Citric acid concentration increased steadily between upto pH 7.2 from pH 3, achieving a maximum concentration of 3.6g/l at pH 3. At pH 2 and pH values greater than pH 3 a loss of production occurred i.e., 1.12g/l at pH 2, 2.8g/l at pH 4 and 2.5g/l at pH 7.2. The results of steady-state cell and residual sugar concentrations as a function time are shown in Fig. 1. Between pH 2.0 and pH 7.0, the cell concentration increased to a maximum of 13.4g/l at pH 2.0/l. As shown in Fig. 1, the cell concentration at pH 2.0 was 65% higher than that at pH 7.2. As expected the concentration of residual sugars decreased between pH 2.0 and pH 7.2 coinciding with an increase in cell, citric acid production. Ammonium nitrate was used as a nitrogen source. pH is known to be very important in citric acid fermentation and can be maintained at a low level by using ammonium nitrate as a nitrogen source. A series of flask cultures are conducted with different initial ammonium nitrate concentrations from 0 to 3g/l. Effect of ammonium nitrate on citric acid conversion from the milk factory waste water is shown in Fig. 2. It is apparent that none of the added ammonium nitrate increased the formation of citric acid. This results show that the citric acid yield is inversely related to the nitrogen content of the culture medium. As the nitrogen content increases, so does the amount of sugar consumption, but this appears to be converted to storage carbohydrate, and possibly carbon dioxide, rather than to citric acid. Metal ions are the micronutrients which modulate the biochemical conversions, and direct the sequence of metabolic conversions to result in overproduction of desired secondary metabolites. *A. niger* accumulates large amounts of citric acid extracellularly when grown on a minimal salts medium, but an overabundance of the metal ions lead to excessive vegetative growth at the expense of citrate accumulation. Separate runs were carried out with various levels of Mn^{2+}, Zn^{2+}, Fe^{2+} and Cu^{2+} in milk factory waste water. It was observed that the yield of citric acid is enhanced by higher concentrations of Mn^{2+}, Zn^{2+}, Fe^{2+} and Cu^{2+} (table 1). In contrast, Mg^{2+} ion at higher

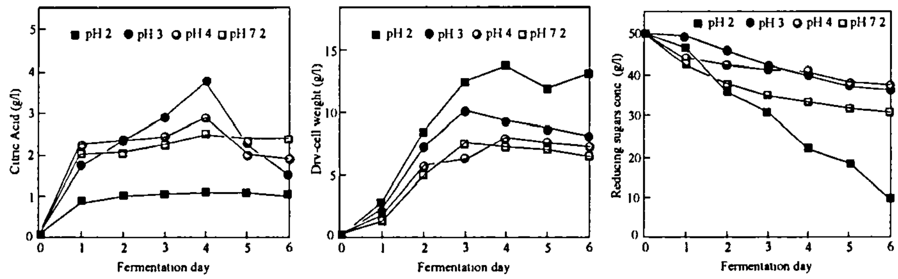

Fig. 1. Effect of initial pH on citric acid production, mycelial growth and total reducing sugars.

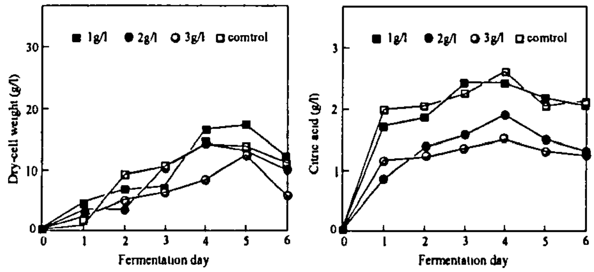

Fig. 2. Effect of nitrogen source as NH_4NO_3 on citric acid production and mycelial growth.

Table 1. Data of addition metal ions.

Metal ions	Addition Concentration (g/l)	Mycelial dry wt.(g/l)	reducing sugar consumption (g/l)	(%)	Yield of citric acid (g/l)	(%)
Mn^{2+}	0	8.1	25.5	51.0	2.7	10.6
	0.01	9.3	21.0	42.0	3.5	16.7
	0.02	9.0	24.0	48.0	3.6	15.0
	0.03	12.3	25.8	51.6	3.9	16.3
Mg^{2+}	0	8.1	25.5	51.0	2.7	10.6
	0.01	5.1	28.5	57.0	2.3	8.1
	0.02	4.1	26.9	53.8	2.3	8.6
	0.03	3.3	29.7	59.4	2.2	7.4
Zn^{2+}	0	8.1	25.5	51.0	2.7	10.6
	0.01	6.5	9.8	19.6	2.3	23.5
	0.02	6.0	7.9	15.8	2.7	34.2
	0.03	4.8	13.0	26.0	3.2	24.6
Fe^{2+}	0	8.1	25.5	51.0	2.7	10.6
	0.01	8.7	30.5	61.0	2.9	9.5
	0.02	11.7	28.6	57.2	3.4	11.9
	0.03	12.3	27.5	55.0	3.0	10.9
Cu^{2+}	0	8.1	25.5	51.0	2.7	10.6
	0.01	9.9	9.5	19.0	3.8	40.0
	0.02	10.5	15.0	30.0	4.2	28.0
	0.03	13.1	17.0	34.0	4.4	25.9

concentrations, shows an inhibitory effect on citric acid production. The optimum concentrations of metal ions were taken as the value above which no significant increase in the yield of citric acid was observed. Milk factory waste water contains about 12g/l~35g/l reducing sugar according to the process of milk production. In order to high concentration of citric acid, reducing sugars were used at different levels in batch bioreactor. This experiments were conducted 50g/l, 70g/l and 100g/l reducing sugar concentrations. Increasing the reducing sugar concentrations, the citric acid and cell mass increased(Fig. 3). The concentrations of citric acid were marked up to 7.2g/l, 11.4g/l and 16.5g/l in a batch bioreactor by *A. niger* ATCC 9142 with 50g/l, 70g/l and 100g/l of reducing sugar concentrations in milk factory waste water, respectively. From this result concentrated medium was increased the production of citric acid about 8.4~33 fold as compared with no concentrated. The model employs rate equations for cell(X), product([CIT]), and substrate([GLU]) to describe the fermentation process. For cell, the logisitic rate equation is used:

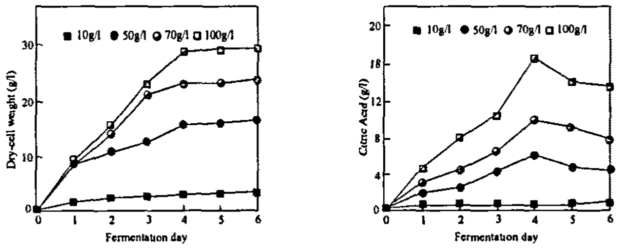

Fig. 3. Time course profiles of citric acid production and mycelial growth for *A. niger* ATCC 9142 cultures with concentrated milk factory waste water in a batch reactor.

$$dX/dt = \mu X(1 - X/X\mathrm{max}) \qquad (1)$$

where μ is the growth constant and X_{max} is the maximum attainable cell concentration. The product formation rate equation is taken as that of Luedeking-Piret,

$$d[CIT]/dt = a(dX/dt) + \beta X \qquad (2)$$

where a is experimental constant for growth relation, β is experimental constant for non growth constant. Equation (2) divided by X and substituting equation (1) into this expression gives

$$d[CIT]/Xdt = (a\mu + \beta)\{1 - a\mu X/(a\mu + \beta)X_{\mathrm{max}}\} \qquad (3)$$

Appling Luedeking-Piret equation to an stationary state of batch culture it follows that, since β=0,

$$d[CIT]/Xdt = a\mu(1 - X/X_{\mathrm{max}}) \qquad (4)$$

This means that the cell concentration increases with the fermentation time but production rate of citric acid decreases. Substrate consumption rate is shown below. Substrate consumption is taken to depend on the magnitudes of three terms: the instantaneous cell growth and product formation rates, and cell maintenance function.

$$-d[CIT]/Xdt = (dX/Y_{X/G}\ X\ dt + d[CIT]/Y_{A/G}\ X\ dt) + K_e = A\text{-}BX \qquad (5)$$

Since X increases during the fermentation, $-d[CIT]/Xdt$ decreases gradually, but the substrate can't increase. Thus, the substrate consumption term can't fall down below zero. In substrate consumption parameters were estimated Runge-Kutta-Gill method. And the results were represented Table 2. Methematical model was simulated for predictability of cell growth, citric acid production and substrate consumption and gave good agreement results with experimental data(Fig. 4).

Table 2. Estimated model parameters.

Initial concentration of reducing sugar milk factory waste water(g/l)	Parameter				
	Xmax(g/l)	$\mu(hr^{-1})$	α	A	B
50	18	0.048	3.281	8.677	0.320
70	26.1	0.053	4.774	7.851	0.362
100	30.4	0.056	8.963	11.616	0.39

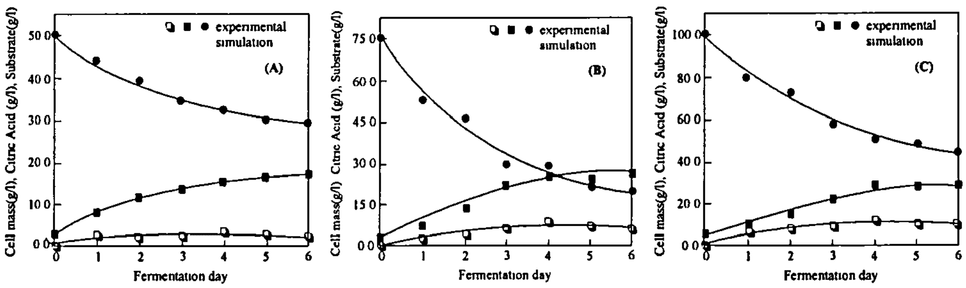

Fig. 4. Comparison of the experimental data and simulation results of milk factory waste water at (A) 50g/l, (B) 70g/l, (C) 100g/l reducing sugar concentration; □, citric acid; ■, cell mass; ●, substrate

Conclusion

Citric acid concentration increased steadily between pH 3 and pH 7.2, achieving a maximum concentration of 3.6g/l at pH 3. The yield of citric acid is enhanced by higher concentrations of Mn^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+}. But Mg^{2+} ion at higher concentrations shows an inhibitory effect on citric acid production. Mathematical model was develped and simulated for their predictability of strain growth, citric acid production and substrate consumption rate and gave good agreement results with experimental data.

Reference

Erkmen, O. and E. Alben, Mathematical modeling of citric acid production and biomass formation by *A. niger* in undersized semolina, *J. Food Eng.*, 52: 161-166, 2002.

Kim, B. S., M. G. Suh, K. S. Park, Z. S. Seo, and J. W. Hur, A study on treatment of milk waste water using on fluidized-bed bioreactor, *Kor. J. Env. Hlth. Soc.*, 25: 143-149, 1999.

Lee, Y. H., M. G. Suh, H. S. Roh, D. H. Lee, K. T. Chung, and Y, K Jeong, Optimal condition for citric acid production from milk factory waste water by using the immobilizes cells of *A niger*, *Kor J. Microbiol. Biotechnol.*, 32: 154-157, 2004.