

Citric acid production from cheese whey by *Aspergillus niger*

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Chen, A., Liao, P.H. and Lo, K.V. 1990. Citric acid production from cheese whey by *Aspergillus niger*. Can. Agric. Eng. 32:329-330. Filtered cheese whey with 2% methanol added was used as substrate for *Aspergillus niger* fermentation. Mycelial biomass and citric acid were produced, both of which have considerable commercial value. A citric acid concentration of 0.32 g/L, representing a yield of 1.5% (w/w) based on sugar consumed, was obtained. The maximum mycelial dry weight obtained was 12.0 g/L and the COD reduction of the cheese whey was 35.5%.

INTRODUCTION

British Columbia produces an estimated 15.8 million litres per year of cheese whey as a by-product of the cheese manufacturing process. Due to the high chemical oxygen demand (COD) of cheese whey (60-80 g/L), disposal of this by-product causes considerable difficulty. The transformation of this waste into valuable commercial products would be of obvious benefit to the industry. Given that lactose is the major component of cheese whey, the possibility of utilizing the raw whey as a fermentation substrate for the production of mycelial mass and citric acid was investigated.

MATERIALS AND METHODS

Substrate

The cheese whey was supplied by a local cheese manufacturer and stored at 4°C. It contained 44.7 g/L sugar and the pH measured 4.4. Prior to use, the cheese whey was heated for 20 minutes and then filtered to yield a clear filtrate.

Culture

A citric acid-producing strain of *A. niger*, ATCC 12846, was used. The culture was grown on potato dextrose agar slant at 30°C for 5 to 7 days. A spore inoculum was then prepared by adding 5 ml of sterilized distilled water to each slant followed by vigorous shaking for one minute.

Fermentation

The experiments were carried out in sixteen 250 ml Erlenmeyer flasks containing 100 ml of heated and filtered cheese whey. The following nutrients and trace elements (g/L) were added to each flask prior to autoclaving at 121°C for 15 minutes: NH_4NO_3 (2.5); KH_2PO_4 (2.5); $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.25); $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (60×10^{-6}); ZnSO_4 (0.25×10^{-3}); $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (1.3×10^{-3}). After cooling, each flask was inoculated with 1.0 ml spore inoculum and incubated at 30°C on a G24 Environmental Incubator shaker at an operating speed of 115 rev/min. Methanol to final con-

centration of 2 percent was added to the flasks after inoculation.

Analytical Procedures

At appropriate time intervals, fermentation samples of 15 ml or total flask contents were removed and analysed for citric acid, residual sugars, COD and pH. The mycelial dry weight was determined by filtering through Whatman 934-AH microfibre filters, washing three times with distilled water and drying to constant weight at 105°C (ca 24 hr). Citric acid content in the filtrate was determined by the colorimetric method of Marier and Boulet (1958) and the sugar was analysed as glucose by the phenolsulfuric acid method of Dubois et al. (1956). The filtrate COD was measured colorimetrically according to the Knechtel Method (1978).

RESULTS AND DISCUSSION

An increase in citric acid yields with the addition of methanol appears to be a general phenomena with *A. niger* strains. It is likely that methanol affects the permeability properties of the fungi and enables greater excretion of citric acid (Kapoor et al. 1982). In preliminary experiments to those reported here, tests were made on the addition of 1, 2 and 4% w/v methanol to the cheese whey prior to fermentation. Inhibition of mycelium growth was observed in the 4% methanol addition, and consequently the 4% methanol addition was deleted from subsequent experiments. The citric acid production for 0, 1, and 2% methanol additions were 0.11, 0.11 and 0.31 g/L, respectively. Similar results were observed in the solid fermentations of grape pomace and kiwifruit peel (Hang and Woodams 1986; Hang et al. 1987). In keeping with these results, 2% methanol was therefore added before fermentation for the batch of experiments reported here.

The mycelial growth and citric acid production of *A. niger* with an initial concentration of 44.7 g/L of sugar are summarized in Fig. 1. The lag phase in citric acid production lasted for three days. This was reflected in the initially small sugar uptake. Mycelial growth and citric acid production were parallel. In addition, citric acid production ceased when mycelial growth reached a plateau, despite the presence of about 24 g/L of residual sugar. The pH of the cheese whey measured 4.38 at the start of the experiment without any pH adjustment and during fermentation the medium decreased continuously to pH 2.0. The maximum observed citric acid concentration of 0.32 g/L was achieved on the tenth day, after which it slowly declined. This concentration represented a yield of 1.5% (w/w) based on sugar utilized. The decline in productivity may have been due to a decay in the enzyme system responsible for citric acid production.

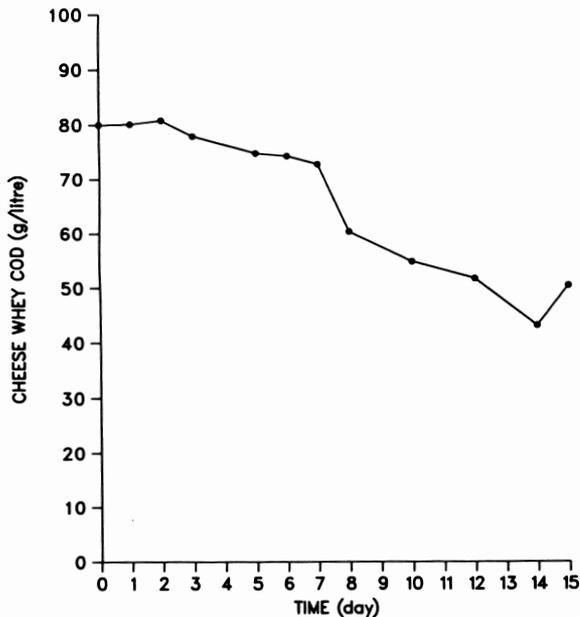


Fig. 1. The production of citric acid from filtered cheese whey with 2% methanol.

(Citric acid, \square ; Mycelial dry weight, \times ; Sugar \circ ; pH, \triangle)

The maximum yield of 12.0 g/L obtained in this study represents a good production rate of biomass. The maximum biomass yield reported by Hossain et al. (1983) from cheese whey permeate was 12.5 g/L at an initial lactose concentration of 43 g/L. The data also indicate a direct correlation between the decrease in COD (g/L) of the fermentation medium and the increase in mycelial dry weight (Fig. 2). The COD content of the cheese whey decreased from an original concentration of 80 g/L to an average of 51.6 g/L. The COD of cheese whey was thus reduced by 35.5%, a significant reduction from the stand-

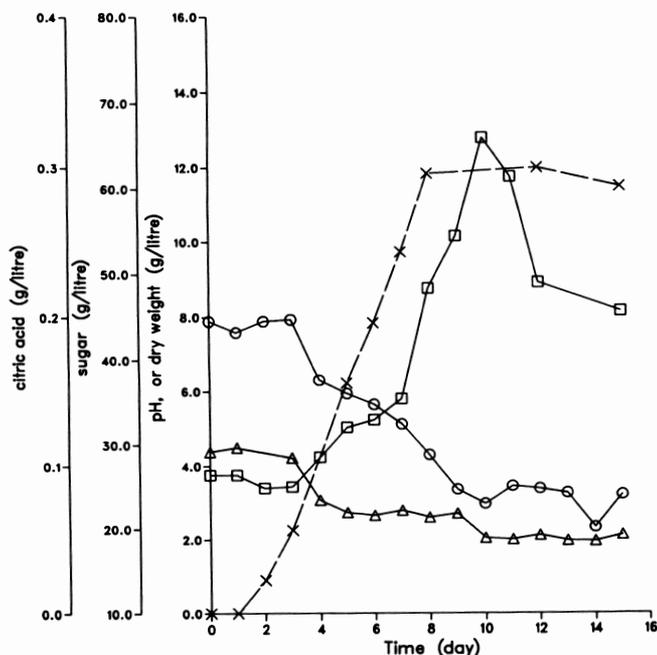


Fig. 2. Cheese whey COD versus fermentation time.

point of waste treatment and pollution control. In addition, both the substantial amount of mycelial biomass and the residual solubles resulting from the fermentation process could be directly utilized as animal feeds, fertilizers or soil conditioners (De Roo 1975). This may not be applicable if filter aid has to be added to harvest mycelia.

Despite the good mycelial growth on the cheese whey substrate, further investigations are warranted to improve the yields of citric acid. The yield of 1.5% (w/w) from the fermentation process was relatively low compared to the best results (up to 7.5%), obtained by Hossain et al. (1983) using cheese whey permeate and a mutant strain of *A. niger*. In the current system it is therefore obvious that more detailed studies are needed to improve the citric acid yield of the fermentation process used here. Significant increases in yield could be made by selection of a specific strain/mutant able to efficiently use lactose as a C-source for citric acid production. Cheese whey permeate contains all the original lactose and minerals, but the proteins have been removed by ultrafiltration. This ultrafiltration process may have contributed to the higher yields of citric acid obtained by Hossain et al. in their experiments. They also used a mutant strain of *A. niger*.

CONCLUSION

This study indicated that raw cheese whey can serve as a potential carbon and energy source for the production of mycelial biomass. Citric acid production of 0.32 g/L with 35.5% COD reduction of cheese whey were obtained.

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