

Thesis Title Citric Acid Production from Tapioca Starch
in Stirred Tank Bioreactor by *Aspergillus niger*

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ABSTRACT

Five strains of *Aspergillus niger*, C1, Ck, DSM 821, DSM 823, and DSM 872, were screened for the highest citric acid production on both bromocresol green (BCG) agar plates and tapioca starch agar plates. It was found that *Aspergillus niger* C1 gave the greatest differences in diameter between acid or clear zone and colony diameter on both types of plates. The differences were 3.8 ± 0.2 cm in BCG plates and 1.7 ± 0.2 cm in tapioca starch agar plates, after incubation at 37°C for 3 days and 4 days, respectively. Confirmatory studies were carried out in 500 ml baffle flasks containing 100 ml fermentation medium, and using the same 5 strains of *Aspergillus niger*. The cultures were incubated at 30°C. The highest citric acid concentration of 39.5 g/l at day 8 and the highest glucoamylase activity of 3.42 U/ml at day 2 were obtained from shake flask culture of *A. niger* C1 using 12% tapioca starch liquefied with α -amylase as a sole carbon source. Therefore, *A. niger* C1 was chosen as the appropriate strain to produce citric acid from tapioca starch. Various initial concentrations of liquefied tapioca starch and initial pH were studied. It was observed that 12% liquefied tapioca starch and an initial pH of

2.5 were suitable for the maximum citric acid production. The effect of type and concentration of nitrogen source on citric acid fermentation was also investigated. Citric acid reached a maximum amount of 60.0 g/l (representing 64.9% yield) at day 8 when 0.2% ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$ was used. Effect of pH on citric acid production was examined in a stirred tank bioreactor. A pH value at 2.5 maintained throughout the whole fermentation process gave better production of citric acid than maintenance at pH 4.0 for the first 2 days followed by a natural lowering to pH 2.5. Citric acid production was carried out by controlling the dissolved oxygen tension (DOT) at not less than 30% air saturation. The citric acid content increased to 80.2 g/l on day 10 (69.5% yield) compared to 76.0 g/l (68.2% yield) with uncontrolled DOT. Fed batch cultivation was subsequently examined. A growth-limiting substrate (nitrogen in the form of ammonium sulfate) was continuously fed to the fermentation medium from days 3 to 5 during the interval of citric acid production, prior to nitrogen exhaustion. The cultivation time for producing the highest citric acid (84.5 g/l) was reduced to 8 days and this led to an increase in the volumetric citric acid production rate to 440 mg/l h. This was 1.3 times higher than that for conventional batch fermentation. High performance liquid chromatography (HPLC) and UV-test kit analyses were employed to investigate the acid profile of fermentation filtrates. It was obviously shown that citric acid was mostly produced.