CITRIC ACID PRODUCTION BY IMMOBILIZED CELLS OF THE YEAST *CANDIDA GUILLIERMONDII*.

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ABSTRACT

The feasibility of using cells of *Candida guilliermondii* immobilized onto sawdust particles for production of citric acid was investigated.

C. guilliermondii IMK1 from a stock culture (Department of Process and Environmental Technology, Massey University, Palmerston North, New Zealand) was reisolated for further study including strain improvement work by induced mutation using UV light. A mutant strain DT2 was isolated which produced a citric acid concentration of 9.2 g/l (yield 25 % (w/w)) in shake flask culture, using a defined medium containing 36 (g/l) glucose, compared with 4.9 (g/l) citric acid produced (yield 14 % (w/w)) by the parent strain. Experiments in a laboratory scale batch fermenter, in which a higher concentration of citric acid (11.7 g/l) was achieved, proved that citric acid production using the mutant strain *C. guilliermondii* DT2, could be scaled up successfully from shake flask to a 2 l fermenter. This mutant was used throughout subsequent experiments.

Sawdust was selected, as the most appropriate support material to immobilize the mutant strain *C. guilliermondii* DT2 via the adsorption method.

Experiments using different concentrations of nitrogen nutrient in defined medium using cells of *C. guilliermondii* DT2 immobilized onto sawdust particles, in repeated batch shake flask culture, demonstrated a marked effect of the nitrogen concentration on citric acid production. Thus, an overall

productivity of 0.11 (g/l.h) was obtained using a defined medium containing 0.53 (g/l) ammonium chloride, compared to overall productivities of 0.04 (g/l.h) and 0.01 (g/l.h) using defined media containing 0.1 (g/l) and no ammonium chloride, respectively. No significant effect of nitrogen concentration on citric acid yield was observed in this investigation. In contrast, similar experiments, in repeated batch shake flask culture, for the effect of phosphate concentration on citric acid production showed no effect of phosphate on either the production rate or yield of citric acid.

In bubble column culture experiments, using cells of *C. guilliermondii* DT2 immobilized onto sawdust, the importance of pH control in citric acid production was demonstrated. In addition, it was demonstrated that the activity of immobilized cells which have lost the ability to produce citric acid can be revived by supplying medium containing sufficient concentrations of nitrogen and phosphate. Reduction of the nitrogen concentration in the medium from 0.53 (g/l) to 0.05 (g/l), provided that the reactor was well established, showed no significant influence on citric acid productivity, but significantly improved the citric acid yield. The highest productivity of around 0.21 - 0.24 (g/l.h) at a dilution rate of 0.21 h^{-1}, accompanied by a citric acid yield of about 10 - 11 % (w/w), was reached and maintained for more than 140 hours of stable operation.

Overall, it was concluded that cells of *C. guilliermondii* were succesfully immobilized onto sawdust particles, and the immobilized cell reactor produced

citric acid at a higher rate compared to a free cell system. In particular, a high rate of citric acid production in a bubble column reactor, operated in continuous mode, was achieved.

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TABLE OF CONTENTS PAGE

LIST OF FIGURES PAGE

LIST OF TABLES PAGE

CHAPTER 1

INTRODUCTION

Citric acid is produced commercially through a fermentation process, and selected strains of *Aspergillus niger* are usually applied in this process. However, several species of yeasts, especially from the genus *Candida*, have proved their potential as citric acid producers.

Yeasts have several advantages compared to fungal species, including ease of growth and ease of handling in a fermenter since they do not block ports or grow on probes, and they grow as a homogeneous suspension rather than as pellets or large aggregates. Moreover, yeasts can be grown on various kinds of carbon source including n-alkanes and sugars. Due to their ease of assimilation and lower formation of the by-product, isocitric acid, sugars , particularly glucose, are auspicious carbon sources for the production of citric acid (Rohr *et al*, 1983; McKay *et al*, 1990).

Recently, with respect to the optimization of the process, mutation of strains (Furukawa *et al*, 1977, 1982; McKay *et al*, 1990; Gutierrez and Maddox, 1993) and immobilization of cells (Maddox and Kingston, 1983; Kautola *et al*, 1991; Rymowicz *et al*, 1993; Forster *et al*, 1994) have become of central interest to workers to improve reactor productivity. Hence, it seemed appropriate to investigate the use of immobilized cells of *C. guilliermondii* for

the production of citric acid.

The present work aimed to examine the performance of immobilized cells of *C. guilliermondii* for citric acid production from glucose in repeated batch shake-flask culture and in a continuous bubble column reactor. Attempts to accelerate the process by means of manipulation of limiting nutrient concentrations, i.e. nitrogen and phosphate, were also examined in this work.