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**CITRIC ACID PRODUCTION USING *ASPERGILLUS*
NIGER BY SOLID SUBSTRATE FERMENTATION**

**A thesis presented in partial fulfilment of the requirement
for the degree of Doctor of Philosophy in Process
and Environmental Technology at
Massey University**

MINYUAN LU

1995

To My Mother

Abstract

The aim of this work was to investigate solid substrate fermentation for citric acid production using *Aspergillus niger*, in an attempt to provide systematic information and an understanding of the process. Initial experiments were performed to select an appropriate substrate and organism. Thus, kumara and a strain of *Aspergillus niger*, Yang No.2 were found favourable for citric acid production, while potato was a poor substrate due to its excessive nitrogen content. The fermentations were carried out under various conditions, i.e. inoculum size, moisture content and particle size of the substrate to optimize these parameters. Inoculum sizes between 10^4 and 10^6 spores/40 g kumara, moisture contents between 65 and 71% and particle sizes between 4 and 6 mm were optimal for citric acid production. It was found that the organism takes up nutrient by penetrating into the substrate, thus the fermentation had a direct relationship with the available surface area. The solid substrate was found to have the potential ability to overcome the adverse effect of high concentrations of metal ions. Addition of 150 mg/kg substrate of Fe^{2+} , 25 mg/kg substrate of Cu^{2+} , 75 mg/kg substrate of Zn^{2+} and 150 mg/kg substrate of Mn^{2+} had slightly stimulatory effects on citric acid production rather than inhibitory effects. Based on the optimized conditions, the kinetics of the solid substrate fermentation in flasks were studied. The maximum observed gravimetric rate, maximum observed specific rate and overall productivity of citric acid production were 1.5 g/kg.h, 122 mg/g.biomass.h and 0.48 g/kg.h, respectively.

To develop the solid substrate fermentation process, experiments were performed in different types of reactors, including a gas-solid fluidized bed, a gas-liquid-solid fluidized bed, a rotating drum and a packed bed. Except for the packed bed reactor, these systems were found to be unsuitable for the fermentation, due to harsh conditions of abrasion, friction, low moisture supply, or combinations of these factors. The fermentation in the packed bed reactor was optimized with respect to air flow rate, bed loading and particle size. Based on these optimized conditions, the kinetics were studied,

and it was determined that the fermentation allowed much higher rates of citric acid production than were observed in flasks, i.e. a maximum observed gravimetric rate of 1.9 g/kg.h and an overall productivity of 0.82 g/kg.h. In an attempt to understand mass and heat transfer in the solid substrate fermentation, experiments were conducted in a multi-layer packed bed reactor. However, because of the complexity of mass transfer in solid substrate fermentation, the understanding of this aspect in this process was rather limited. Nevertheless, the multi-layer packed bed reactor improved the mass transfer considerably compared with the single layer packed bed with the same bed loading, and allowed precise measurement of the gradients for gases, citric acid, biomass and starch. The results suggest that the multi-layer packed bed reactor is a suitable reactor for further investigations, and has the possibility of being used for large scale production of citric acid in solid substrate fermentation.

This systematic investigation of solid substrate fermentation for citric acid production, which is the first reported, provides detailed information and understanding of this fermentation technology.

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Nomenclature

ATM	atmospheric pressure, 1 kg/cm ²
bar	pressure, 1.02 kg/cm ²
BM	Biomass concentration, in g/kg initial wet substrate
CA	Citric acid concentration, in g/kg initial wet substrate
cm	Centimetre(s)
°C	Degree Celsius
g	Gram(s)
h	Hour(s)
kcal	1000 Caloric
kg	kilogram
kj	1000 joule
l	Litre(s)
M	Mole concentration
mg	Milligram(s)
min	Minute(s)
ml	Millitre(s)
mm	Millimetre(s)
NAD ⁺ , NADH	Nicotinamide adenine dinucleotide, and its reduced form
ppm	Parts per million
q	Specific rate, in g/g biomass.h
r	Rate of production or utilization, in g/kg.h
rpm	Revolutions per minute
RS	Resident starch concentration, in g/kg initial wet substrate
t	time, in hour or day
μ	Specific growth rate, in h ⁻¹
μl	Microlitre(s)
%(w/w)	Percentage weight by weight
%(w/v)	Percentage weight by volume
%(v/v)	Percentage volume by volume