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A STUDY OF CITRIC ACID PRODUCTION BY
SUBMERGED AEROBIC FERMENTATION
USING THE FUNGUS
*Aspergillus niger*

A thesis presented in partial
fulfilment of the requirements for the degree
of Doctor of Philosophy
in Biotechnology at Massey University

MARK WILLIAM DAWSON
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The aim of this work was to obtain detailed information on the production of citric acid during submerged fermentation by Aspergillus niger, in an attempt to understand and optimize the process. Initial experiments were performed to determine the effect of interruptions to aeration on citric acid production. Unless the value of the Dissolved Oxygen Tension (DOT) of the culture fell below the DOT_{crit} (20% of saturation), no gross effect was observed. When the DOT value fell to zero, citric acid production ceased. Production however, recovered after recommencement of aeration, albeit after a delay.

Experiments were performed in batch fermentation using various non-carbohydrate medium components as the growth-limiting nutrient. Nitrogen-, phosphate- or sulphate-limited cultures resulted in strong citric acid production. The most significant observation during these fermentations was that the maximum citric acid production rate occurred prior to the exhaustion of the limiting nutrient, i.e. when the organism was at a positive growth rate.

Chemostat experiments were performed in order to determine the effect of the growth rate and the culture DOT on citric acid production. Maximum citric acid production rates and yields were achieved at low growth rate (\( u = 0.017 \) h\(^{-1} \)) and high DOT (90% of saturation) values. The specific citric acid production rate was twice the maximum observed in batch fermentation, and the product yield was increased.
by 23%.

The information regarding growth rate and DOT gained from the chemostat experiments was applied to a continuous fed-batch fermentation using nitrogen as the growth-limiting nutrient. The overall fermenter productivity attained was double that of the batch fermentation, resulting in a halving of the fermentation period. This is the first reported use of the continuous fed-batch technique for citric acid production.

In all three fermentation modes (batch, chemostat and fed-batch), nitrogen limitation was superior to phosphate limitation in terms of citric acid production rates and yields. A double nitrogen/phosphate limitation gave results almost identical to a nitrogen limitation. The evidence suggests that the nitrogen nutrient exerts a form of catabolite repression on citric acid accumulation.
ACKNOWLEDGEMENTS

The author wishes to acknowledge the following people:

Drs I.S. Maddox, J.D. Brooks and R.C. Lawrence for guidance and supervision. Their availability and willingness to discuss problems when they arose, was greatly appreciated.

Professor R.L. Earle, Head of the Department of Biotechnology for his encouragement throughout the project.

Mr I.K. Gray and his staff of the New Zealand Dairy Research Institute for trace element analysis.

Dr R.D. Reeves of the Department of Chemistry, Massey University, for sulphate analysis.

Mr J. Alger and Mr B. Collins of the Department of Biotechnology for their assistance with the many technical problems which arose with the equipment.

Dr I.F. Boag of the Department of Production Technology for his assistance with computer analysis of data and developing the program for the construction of 3-D response surfaces.

Mr M. Stevens, Mr M. Lubbers, Miss A. Moran and Mrs A. McCutcheon of the Department of Biotechnology for their excellent laboratory support.

The New Zealand Dairy Research Institute, Palmerston North for financial support of myself and this project.
My parents, for their constant support and encouragement throughout this project.

My wife Deborah, for typing, proof-reading and advising on layout of the thesis, and for her patience, love and encouragement.
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ABBREVIATIONS

ABBREVIATIONS OF UNITS

°C  degrees Celcius

d  day

g  gram

h  hour

l  litre

m  meter

mg  milligram

min  minute

ml  millilitre

mm  millimeter

mM  millimole

nm  nanometer

rpm  revolutions per minute

µl  microlitre

vvm  volume per volume per minute

OTHER ABBREVIATIONS

AMP  Adenosine monophosphate

ATP  Adenosine triphosphate

D  Dilution rate

DOT  Dissolved Oxygen Tension

DW  Dry Weight

EDTA  Ethylenediaminetetraacetic acid

HPLC  High Performance Liquid Chromatography

ID  Internal Diameter

N  Nitrogen

NAD  Nicotinamide Adenine Dinucleotide
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