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

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ABSTRACT

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 (μ = 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.

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ABBREVIATIONS

ABBREVIATIONS OF UNITS

^{o}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
ul	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

NADH	Reduced Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Reduced Nicotinamide Adenine Dinucleotide Phosphate
PO_4^{3-}	Phosphate
TCA	Tricarboxylic Acid
u	Specific Growth Rate