SUBMERGED CITRIC ACID FERMENTATION OF WHEY PERMEATE BY *Aspergillus niger*

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ERRATUM

In the text and figures, for isocitric dehydrogenase read isocitrate dehydrogenase. On page 11, for pyruvic kinase read pyruvate kinase.

PAGE 133

Citric acid yield in whey permeate has been calculated on the basis of total lactose utilized. Separate shake-flask experiments using lactic acid as a carbon source in the medium of Kristiansen and Charley (1981) produced no citric acid, however, sparse growth did occur. Thus, the quantity of lactic acid present in the whey permeate (5.2 g/l) being metabolized by the culture, will make a small contribution to the biomass produced, but is ignored in the calculations of citric acid yield in this thesis.

PAGE 194, 200 AND 204

In this thesis the terms "inhibition" and "stimulation" of 2-oxoglutarate dehydrogenase and pyruvate carboxylase respectively are taken to mean changes in the specific activity of these enzymes. It is acknowledged that this is an incorrect use of these words, which refer strictly to rates of reaction of enzymic reactions.

Figure 6.33 contains a mis-plot. Figures 6.32 and 6.33 have been replotted, including points corresponding to zero inhibitor concentration.

NOTE: (a) competitive inhibition is shown in Figure 6.32 (page 174), (b) Figure 6.33 (page 174) shows competitive inhibition and allows the estimation of an approximate value of Ki, (c) the use of Figure 6.33 to estimate Ki is not strictly correct, since the data are obtained from batch culture; steady state chemostat data are required to analyse the inhibition in this way.

Figure 6.33 has been plotted with volumetric rate data. This is possible in this case, since the dry weight of mycelium at day 6 was the same at all inhibitor concentrations. Thus specific and volumetric rates are equivalent and the value of Ki obtained will be the same. However, under most circumstances, the dry weight might be expected to vary with inhibitor concentration and thus specific rate data should be used to determine Ki.

ABSTRACT

The feasibility of using lactic casein whey permeate as an alternative source of raw material for the production of citric acid by *Aspergillus niger* was studied.

A. niger (10 strains) and *A. carbonarius* (1 strain) were screened for their ability to produce citric acid from lactic casein whey permeate in shake-flask culture. Of the organisms tested, *A. niger* IMI 41874 produced the highest citric acid concentration of 5.0 g/l, representing a yield of 13.5% (w/w) based on lactose utilized. When the permeate was supplemented with additional lactose (final concentration 140 g/l), a concentration of 8.2 g/l was obtained, representing a yield of 15.5% (w/w). This organism was selected for further study including strain improvement work by induced mutation using UV light. A mutant strain (MH 15-15) was isolated which produced a citric acid concentration of 10.2 g/l in lactose-supplemented whey permeate. Using a sucrose-based synthetic medium a concentration of 52.8 g/l (yield 48% (w/w)) was observed, compared with 34.0 g/l (yield 33% (w/w)) produced by the parent strain. This mutant was used throughout subsequent experiments.

In fermenter culture experiments using lactose-supplemented whey permeate a citric acid concentration of 14.8 g/l was obtained. When extra nitrogen was fed to the culture after the onset of citric acid production, a concentration of 19.5 g/l was observed. Experiments with decationized whey permeate, supplemented with various amounts of different trace elements, proved unsuccessful in respect of improved citric acid production when compared with untreated whey permeate.

Experiments with different sugar sources using a synthetic medium demonstrated a marked effect of the sugar source on citric acid production. Thus, concentrations of 52.8 g/l, 31.0 g/l, 23.0 g/l, 5.0 g/l and 0 g/l were obtained from sucrose, glucose, fructose, lactose and galactose respectively. Good mycelial growth was observed with all the sugars. Similar experiments in fermenter culture showed the same trend of results, but in contrast to the experiments using whey permeate, citric

acid production was lower than in shake-flask culture. The activities of some TCA-cycle enzymes in mycelial cell-free extracts were investigated during fermenter culture experiments using the different sugar sources in synthetic medium and whey permeate. The initial activities of aconitase and both NAD- and NADP-linked isocitric dehydrogenase showed a strong relationship with subsequent citric acid accumulation. During citric acid accumulation the activities of these enzymes decreased significantly compared with those found during growth phase, but did not completely disappear. 2-oxoglutarate dehydrogenase disappeared completely when citric acid production was high but activity was maintained when production was low. The activity of pyruvate carboxylase increase considerably during citric acid production but little activity was detected when citric acid was not produced. It was concluded that accumulation of citric acid is not a consequence of the complete disappearance of the activity of aconitase or isocitric dehydrogenase (both NAD- and NADP-linked), but rather the accumulation is caused by the repression of 2-oxoglutarate dehydrogenase causing a block in the TCA-cycle, and the concomitant increase in pyruvate carboxylase activity. It was hypothesized that glucose and fructose cause repression but galactose does not.

Experiments using various combinations of glucose and galactose as sugar source demonstrated that galactose caused competitive inhibition of citric acid production from glucose. The inhibition showed a strong relationship with the levels of activity of 2-oxoglutarate dehydrogenase and pyruvate carboxylase.

The effect of methanol on citric acid production from lactose, glucose, galactose and whey permeate was investigated. In shake-flask culture, 1% (v/v) methanol caused increased production and yields of citric acid from both glucose and lactose. Citric acid production from galactose was also observed (12.5 g/l). In fermenter culture, using whey permeate, the presence of 3% (v/v) methanol gave a 69% increase in citric acid production (25.0 g/l compared with 14.8 g/l in the absence of methanol). The presence of methanol showed a general inhibitory effect on the various TCA-cycle enzymes studied,

in particular 2-oxoglutarate dehydrogenase.

Overall, it was concluded that the main obstacle to the improved production of citric acid from whey permeate is the nature of the sugar source rather than the other components of the substrate. In particular, the galactose moiety of lactose is not a favourable sugar source.

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ABBREVIATIONS

ABBREVIATIONS OF UNITS

^{o}C	degrees Celsius
cm	centimetre
d	day
g	gram
h	hour
l	litre
m	metre
mg	milligram
min	minute
mL	millilitre
mm	millimetre
mM	millimole
nm	nanometer
rpm	revolutions per minute
µg	microgram
µL	microlitre

OTHER ABBREVIATIONS

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
cAMP	cyclic AMP
DNA	Deoxyribonucleic acid

DOT	Dissolved Oxygen Tension
DW	Dry Weight
EDTA	Ethylenediaminetetraacetic acid
HPLC	High Performance Liquid Chromatography
ID	Internal Diameter
IMI	Commonwealth Mycological Institute
mRNA	messenger RNA
N	Nitrogen
NAD	Nicotinamide Adenine Dinucleotide
NADH	Reduced Nicotinamide Adenine Dinuclotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Reduced Nicotinamide Adenine Dinucleotide Phosphate
RNA	Ribonucleic Acid
TCA	Tricarboxylic Acid
UV	Ultra-violet

PREFACE

The Departments of Food Technology and Biotechnology of Massey University have a continuing interest in developing indigenous raw materials for use as foods or pharmaceuticals, thus making New Zealand more self-sufficient and extending the range of exports. It was apparent that large quantities of lactic casein whey were being used for spray irrigation and a potentially valuable fermentation feedstock (lactose) was being wasted. A number of chemicals which could be made from lactose are imported into New Zealand and citric acid was chosen for study on the basis of its continuing need in the food industry as an acidulant. This thesis is the result of that study.

Except where stated specifically in the text or Acknowledgements, all experimental work and chemical and physical analyses were carried out by the candidate personally.

Where the published work of others has been used, it is acknowledged in the text by the quotation of the authors' names and the date of publication. All cited work is listed in (alphabetical) order of the first author's names in the Reference section.