INTENSIFICATION OF THE ACETONE : BUTANOL : ETHANOL FERMENTATION USING WHEY PERMEATE AND *CLOSTRIDIUM ACETOBUTYLICUM* :

A Preliminary Study

A Thesis presented in partial

fulfilment of the requirements for the degree

of Doctor of Philosophy

in Biotechnology at Massey University

BRETT MILLS ENNIS

1987

ABSTRACT

The use of whey permeate as the fermentation substrate for the production of acetone:butanol:ethanol (solvents), using C. acetobutylicum P262 was studied. Initial experiments were conducted in a batch mode using sulphuric acid casein whey permeate medium, in an attempt to optimize the culture conditions for maximal extent of lactose utilization and solvents production. A high initial lactose concentration (65-75 g/l) in combination with a culture pH maintained in the region pH 5.4 to 5.6 were the most favourable conditions for solvent production. An inverse relationship between the lactose utilization rate and solvents yield was observed. Solvent productivities were only 60% however, of that achievable with this strain of organism on an industrial scale using a molasses medium, but comparable productivities were obtained using a semi-synthetic medium containing glucose. Hydrolysed-lactose sulphuric acid casein whey permeate medium was investigated as a medium for solvent production. Glucose and galactose were utilized simultaneously, although glucose was used preferentially. Only a small increase in solvents productivity was obtained compared with that obtained using non-hydrolysed permeate.

Experiments were performed in continuous culture using cheese whey permeate medium and alginate-immobilized cells. Significantly greater solvent productivities were obtained, compared with those achieved using free cells in batch culture. Fermentations were operated for over 650 hours with no detectable loss in fermentation performance. The extent of lactose utilization was low, however (less than 40%), and attempts to increase this by the use of pH regulation or a two-stage process were unsuccessful. This fermentation process was described as a biomass volume process (volumetric fraction of alginate beads in the reactor), where the lactose utilization and hence the solvents production, was defined by an inhibitory concentration of butanol, approximately 5 g/l.

An alternative continuous fermentation process using free cells and cheese whey permeate medium was investigated. External cell recycle using cross-flow microfiltration (CFM) membrane plant to continuously separate cells from the fermentation culture and recycle them back to the fermenter was utilized. Biomass was continuously removed from the fermenter in order to achieve a stable biomass concentration. Stable

solvents production was not achieved under the range of culture conditions investigated; culture degeneration was attributed to the complex interactive morphological cyclic behaviour of the organism. A tubular CFM unit which could be periodically backflushed to maintain the filtrate flux, was found to be the most suitable of those tested.

The integration of in-situ or in-line solvents recovery with batch culture using free cells, and continuous fermentation using cells immobilized by adsorption to bonechar, was investigated in order to remove toxic solvents and so increase the extent of lactose utilization and solvents productivity. A novel process using gas-stripping with an inert gas, and solvents recovery from the vapour phase by condensation using a cold trap, was described. An increase in lactose utilization and solvents productivity was achieved in both fermentation modes compared with control fermentations. The use of adsorbent resins and a molecular sieve for integrated fermentation solvents recovery was also demonstrated. However, the adsorption of medium components may mitigate against the usefulness of such a process option.

The batch refermentation of batch fermentation effluent treated by gas-stripping to remove solvents was investigated. However, solvent production was favoured only when lactose and nutrients were supplemented to concentrations similar to those present originally. Conversely, fermentation medium treated by gas-stripping to remove solvents could be readily refermented to produce solvents when an existing cell population was used, suggesting that this option of an integrated continuous fermentation-product recovery process may be promising for whey permeate solvent production.

To my parents, Eric and Betty

ACKNOWLEDGEMENTS

I wish to acknowledge and thank the following people:

Dr I S Maddox for his guidance and supervision. His encouragement, patience and enthusiasm for this project was greatly appreciated.

Drs A H J Paterson and T D Thomas for their supervision and interest in this project.

Professor R L Earle, Head of the Department of Biotechnology, for his interest in this project.

Dr P S Robertson, Director of the New Zealand Dairy Research Institute, for his permission to undertake this project and interest throughout.

Mr P G Hobman and Dr W J Harper of the New Zealand Dairy Research Institute and Dr K R Marshall of the New Zealand Dairy Board (formerly NZDRI) who were "champions" for my undertaking this project.

Professor D R Woods, of the University of Cape Town, South Africa for the gift of C. acetobutylicum P262 and Dr L E Pearce of the New Zealand Dairy Research Institute for importing this strain and making it available for use.

I gratefully acknowledge my employer, the New Zealand Dairy Research Institute for the receipt of a Post-Graduate Fellowship and a Staff Development Award.

Part of this project was undertaken in the Laboratory of Bioengineering, Department of Chemistry and Chemical Engineering, Delft University of Technology, Delft, The Netherlands. I am indebted to this Department for providing some financial assistance with my research costs and to the following people:

Professor Ir K Ch A M Luyben for his permission to work in this laboratory.

Dr G H Schoutens for arranging many aspects of my visit to Delft, and for her friendship and supervision.

Mr J P Lispet and Mr B Kerkdijk for their excellent laboratory support.

I wish to also thank the following people:

Mr J Alger and Mr B Collins of the Department of Biotechnology for their excellent and willing assistance with the many technical matters and laboratory equipment fabrication requirements that arose during this project.

Mr M Stevens, Mr M Yates, Mr D Couling, Dr N Qureshi, Miss C Marshall and Mrs A McCutcheon of the Department of Biotechnology for their excellent laboratory support.

Drs J Mawson and D Cleland of the Department of Biotechnology for their assistance with the cell-recycle mass balance determination and Minitab computer package respectively.

Mr R K Richardson of the New Zealand Dairy Research Institute for some gas chromatography analysis.

Mr D Hopcroft and Mr R Bennett of the Applied Biochemistry Division, Department of Scientific and Industrial Research, for the electron microscope photographs.

Noemi Gutierrez for her friendship.

Mrs S Enoka for her excellent typing of the publications arising from this project.

Mrs C McDonell for her excellent typing of this thesis.

Mrs A Hammer for kindly typesetting Figure captions.

My parents, for their constant support, love and encouragement.

My wife, Christine, for proof-reading, advice on the layout of the thesis, for accompanying me on many, many odd-hour visits to the laboratory, and for her patience, understanding, love and encouragement.

And finally, thanks to the 'Moody Blues', 'Genesis' and Radio Station 92.2XS FM for greatly easing the task of 'writing up'.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

Page

Page

LIST OF TABLES

Page

Page

ABBREVIATIONS

°C	degrees Celcius
cm	centimetre
g	gram
h	hour
l	litre
m	metre
mg	milligram
min	minute
ml	millilitre
mm	millimetre
rpm	revolutions per minute
µl	microlitre
M	molar

OTHER ABBREVIATIONS

C_A	acetone concentration (g/l)
C_B	butanol concentration (g/l)
C_E	ethanol concentration (g/l)
C_{HAc}	acetic acid concentration (g/l)
C_{HBu}	butyric acid concentration (g/l)
C_L	lactose concentration (g/l)
$C_{B,max}$	maximal butanol concentration (g/l)
C_{so}	feed substrate concentration (g/l)
C_s	effluent substrate concentration (g/l)
C_{xs}	biomass in solid phase (g/l)
C_x	biomass in liquid phase (g/l)
ΔC_s or ΔS	consumed substrate concentration (g/l)
d.w.	dry weight
D or D_t	dilution rate based on the total reactor volume (h^{-1})
$(1-\varepsilon)$	bead fraction of alginate beads in the reactor (l. alginate/l)
$D_t/(1-\varepsilon)$	normalized dilution rate (l/l. alginate h)
ϕ_{vi}	volumetric flow rate in (l/h)
ϕ_{vo}	volumetric flow rate out (l/h)
k	ratio of butanol/acetone (g/g)
P	specific butanol production rate (g. butanol/l. alginate h)

Prod	volumetric fermenter productivity (g/l.h)
r_s	substrate consumption rate (g/l.h)
r_{max}	maximal specific substrate consumption rate (g. substrate/ l. alginate h)
V_t	total working volume (l)
Y	solvents yield based on substrate consumed (g/g) = $(C_A + C_B + C_E)/\Delta S$
Y_{sb}	butanol yield on substrate (g/g)
Y_{ss}	total solvents yield on substrate (g/g)
Y_{sx}	biomass yield factor on substrate (g d.w./g)
μ_{max}	maximum growth rate (h^{-1})
>	greater than
<	less than
% w/v	percentage weight by volume