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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

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ABSTRACT

The use of whey permeate as the fermentation substrate for the acetone:butanol:ethanol production of (solvents), usina 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 An inverse relationship between the lactose utilization production. 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/1.

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.

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To my parents, Eric and Betty

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ABBREVIATIONS

°C	degrees Celcius
CM	centimetre
g	gram
h	hour
1	litre
m	metre
mg	milligram
min	minute
ml	millilitre
ារា	millimetre
rpm	revolutions per minute
μl	microlitre
М	mojlar

OTHER ABBREVIATIONS

CA	acetone concentration (g/l)
CB	butanol concentration (g/l)
C _E	ethanol concentration (g/l)
CHAC	acetic acid concentration (g/l)
C _{HBu}	butyric acid concentration (g/l)
CL	lactose concentration (g/l)
C _{B,max}	maximal butanol concentration (g/l)
C _{so}	feed substrate concentration (g/l)
Cs	effluent substrate concentration (g/l)
C _{xs}	biomass in solid phase (g/l)
C _X	biomass in liquid phase (g/l)
$\Delta C_{\rm S}$ or ΔS	5 consumed substrate concentration (g/l)
d.w.	dry weight
D or Dt	dilution rate based on the total reactor volume (h^{-1})
(1-ε)	bead fraction of alginate beads in the reactor (l. alginate/l)
D _t /(1-ε)	normalized dilution rate (1/1. alginate h)
[¢] vi	volumetric flow rate in (l/h)
\$vo	volumetric flow rate out (l/h)
k	ratio of butanol/acetone (g/g)
Р	specific butanol production rate (g. butanol/l. alginate h)

Prod	volumetric fermenter productivity (g/l.h)
rs	substrate consumption rate (g/l.h)
r _{max}	maximal specific substrate consumption rate (g. substrate/
	1. alginate h)
Vt	total working volume (1)
Y	solvents yield based on substrate consumed $(g/g) =$
	$(C_{A} + C_{B} + C_{E})/\Delta S$
Ysb	butanol yield on substrate (g/g)
Yss	total solvents yield on substrate (g/g)
Y _{SX}	biomass yield factor on substrate (g d.w./g)
Umax	maximum growth rate (h ⁻¹)
>	greater than
<	less than
% w/v	percentage weight by volume

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