NUTRIENT REQUIREMENTS FOR ACETONE-BUTANOL-ETHANOL PRODUCTION FROM WHEY PERMEATE BY *CLOSTRIDIUM ACETOBUTYLICUM* P262 IN A RANGE OF BIOREACTORS

by

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To my father who passed away in peace,
To my beloved mother, sister 'Lek' and brother 'Som'

ABSTRACT

The acetone-butanol-ethanol (ABE) fermentation process, using *Clostridium acetobutylicum* P262, was studied. Experiments were conducted in a traditional batch fermentation using freely-suspended cells in a defined medium with lactose as carbon source. Solvent production did occur under conditions of nitrogen-, phosphate- or iron-limitation. However, the optimum conditions were observed when all nutrients were present slightly in excess of growth requirements. A greater excess of nutrients caused the fermentation to be acidogenic rather than solventogenic.

Sulphuric acid casein whey permeate, without nutrient supplementation, proved to be a poor substrate for growth and solvent production by this organism. However, the addition of yeast extract (5 g/l) led to strong solventogenesis. The deficiency in the whey permeate was shown to be iron rather than assimilable nitrogen, phosphate or vitamins.

Experiments were also performed in a defined medium in continuous culture using freely-suspended cells in a CSTR. An inverse relationship was observed between the biomass concentration and the specific butanol productivity. It is suggested that this was due to the cell population not being homogeneous, and that a change in the nutrient *balance* led to a change in the relative proportions of acidogenic, solventogenic and inert cells (spores).

The addition of supplementary yeast extract during continuous solvent production from whey permeate using free cells in a CSTR showed that yeast extract supplementation of less than 1 g/l favoured acid production rather than solvent production. It is unlikely that high solvent productivities can

be attained with this substrate in a single stage continuous culture system with freely-suspended cells. A maximum solvent productivity of 0.05 g/l.h was observed in continuous culture compared to 0.06 g/l.h in batch culture.

A continuous fermentation process was investigated using cells immobilized by adsorption onto bonechar and operated in a packed bed reactor. Three nutrients (i.e. nitrogen, phosphate and iron) were selected for this investigation. Solvent production was favoured by high concentrations of these nutrients in the influent medium. It was not possible to restrict the supply of phosphate or iron in the reactor due to leaching of the bonechar. However, conditions where biomass growth was restricted by a restriction in the nitrogen supply were not conducive to solvent production.

This reactor was also operated using whey permeate as the substrate. The biomass build-up was controlled by minimizing the supplementary yeast extract concentration of the feed, but this had deleterious effects on solvent production. Hence, when producing solvent from whey permeate in this type of reactor a compromise must be made between strong solventogenesis and reactor longevity.

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ABBREVIATION

ABE	Acetone-Butanol-Ethanol
ATP	Adenosine 5′-tri-phosphate
CoA	Coenzyme A
°C	Degree Celcius
cm	Centimetre(s)
CSTR	Continuous Stirred Tank Reactor
D	Dilution rate(s)
g	Gram(s)
GPR	General Purpose Reagent
h	Hour(s)
l	Litre(s)
M	Molar
mg	Milligram(s)
min	Minute(s)
ml	Millitre(s)
mm	Millimetre(s)
NAD^+, NADH	Nicotinamide adenine dinucleotide, and its reduced form
nm	Nanometre
OD_{625}	Optical density at 625 nm
PBR	Packed Bed Reactor
rpm	Revolutions per minute
µl	Microlitre(s)
%(w/v)	Percentage weight by volume
%(v/v)	Percentage volume by volume