PRODUCTION OF 2,3-BUTANEDIOL FROM RENNET WHEY PERMEATE BY *KLEBSIELLA PNEUMONIAE* IMMOBILIZED IN ALGINATE GEL

A THESIS PRESENTED IN PARTIAL FULFILMENT OF
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ABSTRACT

The successful immobilization of cells of Klebsiella pneumoniae(NCIB 8017) in sodium alginate gels was demonstrated. A cell to alginate ratio of 1ml original cell culture to 1.25ml sodium alginate solution(20g/l) was found to be optimum for butanediol production from rennet whey permeate. Preliminary batch fermentation studies revealed that immobilized cells incubated in a non-agitated mode produced a higher concentration of 2,3-butanediol than those in an agitated mode. Smaller beads(1.8mm diameter) produced higher quantities of 2,3-butanediol than larger beads(5.5mm diameter), while bead storage at 4°C in either 0.1M Tris-HCl buffer or the gelating agent proved satisfactory although some activation was required to realise the full butanediol producing potential of the beads. Supplementation of 3g/l calcium chloride to the whey permeate was non-inhibitory to butanediol production and led to enhanced calcium alginate bead stability. Acclimatization of cells in high lactose concentration prior to cell immobilization did not result in enhanced butanediol production or lactose utilization. Product and substrate inhibition effects were not detected. In batch fermentation, a butanediol productivity of 0.11g/l.h was obtained. In continuous fermentation in a CSTR, the productivity was increased to 0.74g/l.h. Using packed columns operated in the vertical mode, similar productivities to those using the CSTR was attained. However, the columns suffered from an accumulation of carbon dioxide bubbles. This problem was overcome by placing a stainless steel mesh inside the column, and operating at an angle of 10° to the horizontal. Under these conditions, a butanediol productivity of 2.40g/l.h was achieved, representing an improvement over values reported in the literature.

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CHAPTER 1

INTRODUCTION

Whey is a by-product of cheese and casein manufacture. It contains approximately 5% lactose, 0.9% nitrogenous materials and small amounts of vitamins and minerals (Sandhu and Waraich, 1983). There are many types of whey produced by various manufacturing processes. In New Zealand, the estimated volume of whey produced and the volume processed for the 1982-1983 dairying season is presented in Table 1.1(Hobman, 1982). Whey permeate is the material remaining after ultrafiltration of whey to recover soluble proteins. The chemical co…pusition of whey permeate is shown in Table 1.2. The abundance of this dairy effluent, coupled with its high biochemical oxygen demand(ca. 33,000mg/1) means that it cannot be discharged directly into natural water systems, and thus pose a waste disposal problem to the dairy industry. In New Zealand, it is common practice to spray irrigate pasture with this material, which in turn helps to maintain soil and pasture fertility. In recent years, the dairy industry has recognised whey as a by-product rather than a waste product(Maddox and Archer, 1984). Processes such as the extraction of protein using ultrafiltration have become quite common in dairy factories. More recently, the realization that whey and its permeates are potentially useful substrates for microbial fermentation has generated interest in many temperate countries. A list of some microbial products that can be obtained via fermentation using various microorganisms is shown in Table 1.3.

The ever-increasing international demand for crude oil has resulted in fear and uncertainty as to the future supply of this dwindling natural resource . Thus, the idea of producing chemical feedstocks and liquid fuels, such as 2,3-butanediol, from naturally renewable resources such as whey, becomes an intriguing possibility for the future. The importance of 2,3-butanediol lies in its chemical properties. Its dehydration yields the industrial

Table 1.1 An estimate of the volume of whey produced and the volume processed for the 1982-1983 dairying season in New Zealand(Hobman, 1982)

	Base Product	
	Cheese	Casein
Quantity(tonnes)	120,000	57,000
Volume of whey produced (m^3)	912,000	1,480,000
Volume of whey used(m^3) for:		
Whey cheese	2,600	-
Lactose	373,000	-
Whey powder	100,000	108,000
Whey protein (extraction only)	-	293,000
Whey protein extraction plus lactose utilization	60,000	371,000
Miscellaneous	500	-
Total	536,100	772,000
Percentage of whey partially or totally processed	59	52
Percentage of whey not processed	41	48

Table 1.2 Chemical composition of whey permeates (Matthews, 1978)

	Sulphuric casein whey permeate	Lactic casein whey permeate	Rennet whey permeate
Total solids %	5.69	5.93	5.40
Ash %	0.78	0.71	0.44
Lactose %	4.26	4.21	4.77
Na g/kg	0.6	0.48	0.36
Ca g/kg	1.17	1.36	0.36
K g/kg	1.45	1.53	1.41
Cl g/kg	0.09	0.98	1.06
PO_4 g/kg	1.92	2.0	0.68
SO_4 g/kg	1.51	-	-
Lactate g/kg	-	0.64	-

Table 1.3 Some products obtained from whey by microbial fermentation

Organism	Product	Reference
Yeast	ethanol	Maddox & Archer (1984)
Yeast	ethanol (whey wine)	Kosikowski (1979)
Kluyveromyces ragi	ethanol	King & Zall (1983)
Kluyveromyces fragilis	single cell protein	Sandhu & Waraich (1983) Kosikowski(1979)
Wingea robertsii	single cell protein	Sandhu & Waraich (1983)
Fusarium moniliforme	gibberellic acid	Gohlwar *et al* (1984)
Clostridium acetobutylicum	butanol	Maddox & Archer (1984)
Methanogens	methane	-do-
Asperigillus niger	citric acid	-do-
Klebsiella pneumoniae	2,3-butanediol	Lee & Maddox (1984)

solvent methyl ethyl ketone. Further dehydration produces 1,3-butadiene which is the monomer of synthetic rubber. Dimerization of butadiene by the Diels-Alder reaction gives styrene, an important aromatic intermediate in the lucrative polymer industry.

The advantages of fermentation systems based on immobilized cell technology have resulted in enhanced productivities over those observed in traditional batch systems. The objective of the present work was to investigate the feasibility of producing 2,3-butanediol from rennet whey permeate based on the principles of continuous fermentation and immobilized cell technology.