APPLICATION OF ALTERNATIVE FERMENTER DESIGN

IN

WHEY-ETHANOL PRODUCTION

(A Preliminary study)

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To

Mt. Egmont, Mt. Ngauruhoe, Mt. Tongariro and

Mt. Ruapehu

ABSTRACT

The performance of a crossflow-microfiltration recycle reactor for whey-ethanol production was studied. Experiments using the yeast strain *Kluyveromyces marxianus* Y-113, an industrial whey-ethanol strain, and reconstituted acid whey permeate powder were carried out. Unsteady state experiments (i.e. with 100% cell recycle) were conducted at 46-137 g/l feed lactose concentration and dilution rates of 0.44-1.3 hr^{-1}. These experiments were used to estimate the maximum specific growth rate (μ_m), biomass substrate yield coefficient (Y_{xs}), product substrate yield coefficient (Y_{ps}). A mathematical model for biomass, lactose and ethanol concentration prediction was also developed. The model was based on Monid kinetics incorporating the concepts of a significant biomass volume fraction and single product inhibition. Two unsteady state experiments were conducted at 53.4-55.7 g/l lactose and dilution rate of 0.88-0.95 hr^{-1} to check fermentation model accuracy. Two steady state runs at 64-110 g/l lactose, dilution rates of 0.34-0.43 hr^{-1} were established for comparison with the unsteady state runs and to observe the effect of operation under stable conditions with the cell concentration regulated at 10 g/l..

Productivity increases of up to 13 times over the commercial batch fermentation process using the same organism was obtained. The highest productivity obtained was 13.7 g/l.hr. when the biomass was allowed to accumulate to 29.6 g/l, but lactose utilization (46%) and ethanol concentration (10.5 g/l) were low. In general, lower values of substrate utilization and ethanol concentration were noted at high dilution rates. At high feed lactose concentrations, lower lactose utilization was obtained. It was also noted that the growth rate was not significantly affected by substrate concentration and dilution rate. The product substrate coefficient (Y_{ps}) was affected by dilution rate but independent of lactose concentration. Increasing dilution rate also decreased the biomass yield coefficient (Y_{xs}) and the product substrate yield coefficient (Y_{ps}). Further experiments are needed to better understand the effects of these parameters on yield coefficients. Steady state runs showed close agreement to the corresponding unsteady state experiments.

Major problem of the fermenter operation was insufficient membrane flux which resulted in short fermentation runs at some condition. To solve this

problem, a dual membrane configuration coupled with a permeate back flushing mechanism should be introduced.

The mathematical model developed was adequate, but not optimal, an uncertainties of ± 30% and ± 20% in prediction of lactose and biomass concentrations were noted. While this was acceptable in the context of preliminary economic analysis and process optimization, to further improve the model accuracy, a relationship between the various yield coefficients and operating conditions has to be determined. Better estimation of the maximum specific growth rate (μ_m) and incorporating a function to describe the variation of specific growth rate (μ) with biomass and ethanol concentrations is needed. More accurate estimation of the biomass substrate yield coefficient (Y_{xs}) is also necessary for further model refinement.

In conclusion, the crossflow-mircofiltration recycle fermenter has demonstrated potential application in whey-ethanol production with much improved productivity over current commercial and batch systems. Further studies are needed to determine its performance as compared to other intensive fermenter designs. The mathematical model developed also provides sufficient accuracy for preliminary process economic analysis and for process optimization study.

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CHAPTER 1

INTRODUCTION

Rising environmental concern has raised interest in the utilization of whey, whey a by-product from cheese and casein manufacture whey is a solution comprising approximately 5.7-6.7% solids by weight of which the major components are protein, lactose and minerals (Sanderson & Reed 1986). The proteins can be recovered by heat treatment and centrifugation thus producing lactalbumin, or by ultrafiltration which produces whey protein concentrate (Marshall 1982). Because of the small percentage of protein present (8.9% of total solids of sweet whey, Sanderson & Reed 1986), a large volume of deproteinated whey remains for disposal. This is illustrated by the ultrafiltration process, where only about one sixth of the volume of whey is used to make the whey protein concentrate (Barry 1982). Processing of the remaining large volume of lactose-rich deproteinated whey has proved to be a considerable problem. This stream cannot be discharged directly into the environment in an uncontrolled fashion as it has a very high biological oxygen demand (typically 34,000 mg/l, Titchener 1982) and would be detrimental to the ecosystem.

Deproteinated whey can be utilized as a substrate by microorganisms, for example by lactose-utilizing yeasts to produce ethanol. In New Zealand, apart from ethanol, other fermentation products which have also been produced or considered for production include biomass, lactic acid, propionic acid, citric acid, acetone-butanol-ethanol and microbial oils as cocoa butter substitute. Despite these many possible products, production of whey-ethanol has developed as the only important commercial fermentation process for whey utilization.

Ethanol is an important industrial solvent and can also be used as a substitute fuel in internal combustion engines or in petroleum blends (as octane boosters or to prevent phase separation in such blends, Coombs 1984). Potable grade ethanol is used for spirit manufacture and wine fortification. The production of fermentation ethanol can be divided into two stages. The first is a biological conversion stage in which the yeast utilizes the sugar and produces ethanol. This product is then recovered and/or further purified by distillation.

Brazil and the USA are two of the major ethanol producers. Brazil produces ethanol from sugar cane largely to fuel its ethanol-powered vehicles while the USA employs maize-based fermentation plants to produce ethanol for which gasohol (a blend of gasoline and alcohol) is a major end-use. Owing to the current low oil prices, using ethanol as a fuel substitute is uneconomic and subsidies are required to sustain these plants in operation (Paul 1979).

In New Zealand, other factors have made whey-ethanol a commercially viable option. The production of whey-ethanol further diversifies the variety of dairy derived products being produced in New Zealand and the significant reduction in whey effluent load in whey-ethanol production also contributes favourably to the process economy.

In order to increase the competitive edge over other ethanol producers, technologies which could improve the process economy should be introduced. This has not yet happened as far as New Zealand is concerned. There are four whey distilleries operating which supply the entire local market and limited export markets for both potable and industrial ethanol. Three of the four distilleries employ the relatively unsophisticated batch fermentation with only the Tirau Distillery of New Zealand Co-operative Dairy Company using a continuous cascade fermentation with limited success (Howell 1981, Mawson 1990).

To overcome the conservative nature of the distillery industry and its reluntance in investing in modern technologies, further study is required into the use of new technologies in the areas of fermenter design or product recovery which can increase the productivity or reduce energy costs thus improving the profit margin.

The primary objective of this study was to investigate the application of new technologies, particularly new reactor designs for intensive ethanol production. The secondary objective was the development of a mathematical model for fermentation process simulation.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

A large volume of literature is available on the subject of ethanol production by fermentation e.g. see Paul (1979), Kosaric *et al* (1983), NEAC (1980), Slapack *et al* (1987), Cheremisinoff (1980) and Maiorella *et al* (1981). Topics covered range from the social and political importance of fuel ethanol to technical details of different ethanol production systems. No attempt will be made here to give a detailed review of all this literature. Instead, this review addresses whey production and utilisation, and provides an overview of the current fermentation technologies and their potential application to whey ethanol production.

2.2 Whey production and utilization

2.2.1 Origins of whey and deproteinated whey

Whey is the fluid obtained by separating the coagulum from milk, cream or skim milk and is a by-product from cheese, rennet casein or acid casein manufacture as shown in Figure 2.1 (Webb & Whittier 1970). There are essentially two types of whey produced depending on the different manufacturing processes. Sweet whey has a pH value greater than 5.5 and is derived from the manufacture of cheese or rennet casein. Manufacture of cottage cheese, lactic casein or mineral acid casein produces acid whey with a pH value less than 5 (Short 1978). Whey can be further processed to recover the protein through production of whey protein concentrate (WPC) or lactalbumin. Figure 2.2 shows a processing plant for the production of whey protein concentrate from sweet whey by ultrafiltration. The by-product of such a process is whey permeate. Diagrams of typical lactalbumin and lactose production processes is shown in Figure 2.3. Both whey permeate and the deproteinated lactalbumin serum are suitable for whey ethanol production.

Figure 2.1 Milk utilisation (Webb & Whittier 1970)

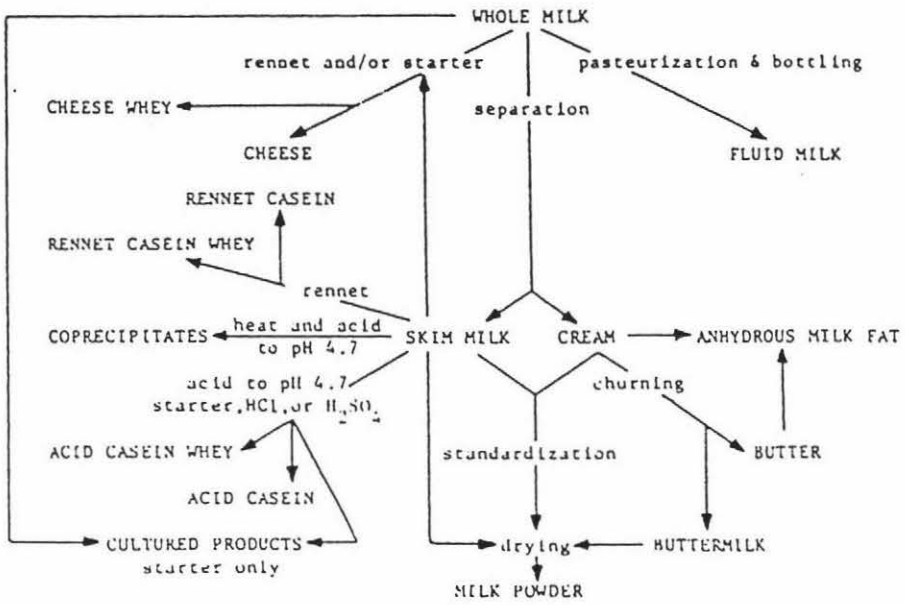


Figure 2.2 Processing plant for whey protein concentrate production from sweet whey (APV 1988)

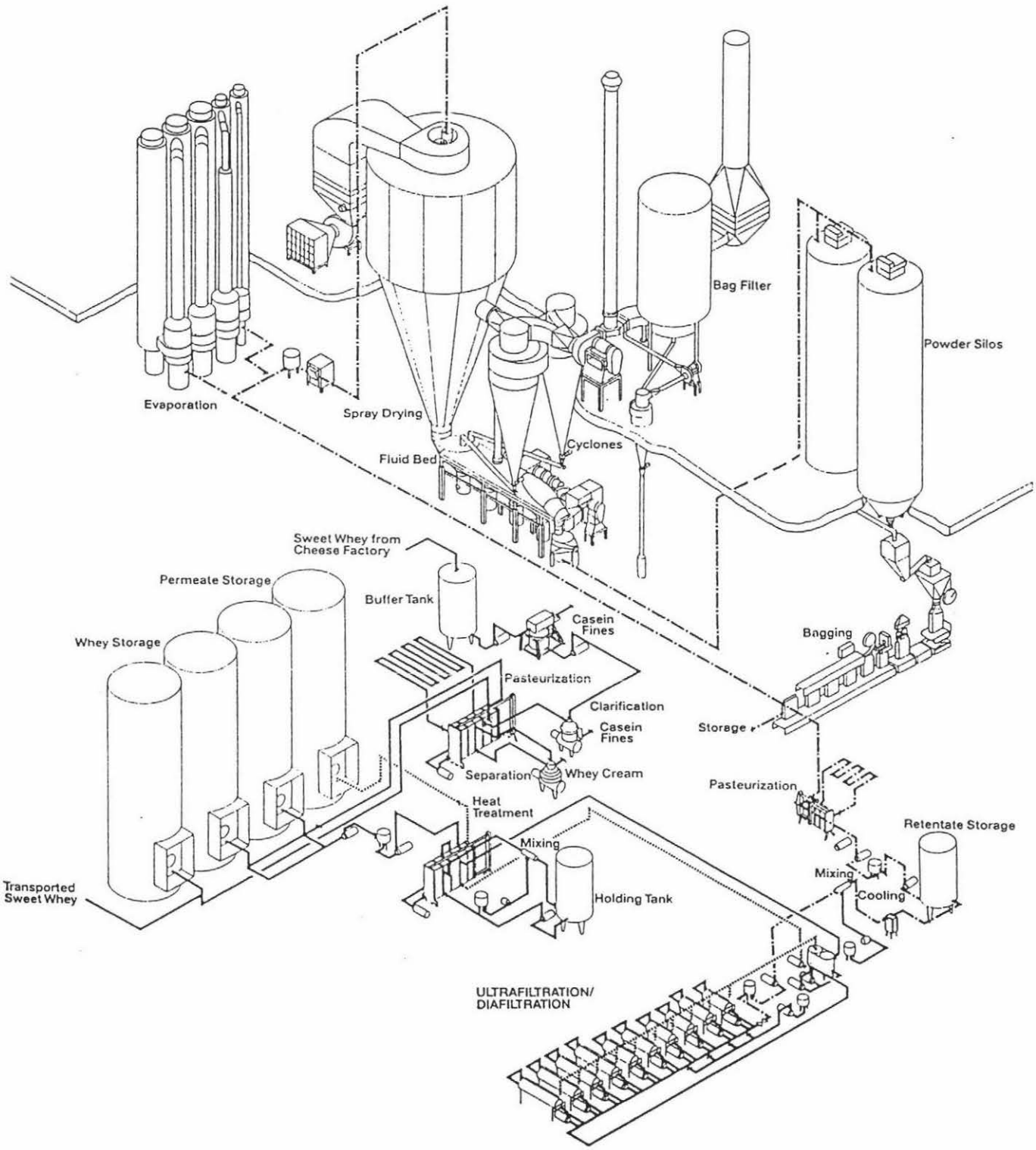
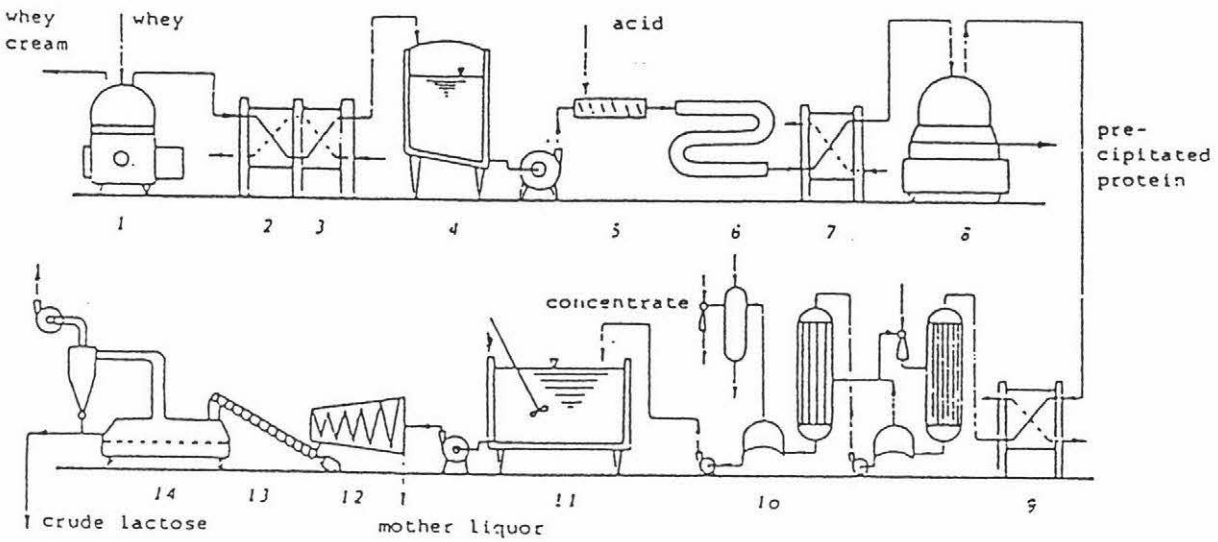


Figure 2.3 A schematic diagram of a lactalbumin and lactose production process (Kessler 1981)



1 separator for the separation of fat
2 preheater
3 heater
4 holding tank
5 tubular mixer
6 tubular holder
7 cooler
8 self-cleaning separator
9 preheater
10 vacuum evaporator
11 crystallizing vat
12 centrifuge
13 screw conveyor
14 hot air drier

2.2.2 Composition of whey

Table 2.1 summarizes the composition of wheys produced in New Zealand. The major components are proteins, lactose and minerals. The protein fraction comprises about 90% α-lactalbumim and ß-lactoglobulin which have a high nutritional value due to their favourable amino acid composition, and especially their high lysine content (Smith 1979, Ewen 1980). The major mineral constituents of whey are calcium, sodium, potassium, magnesium, chloride and phosphate. Part of the phosphate and calcium of milk are retained in the cheese and casein produced, but the other minerals are present in whey in the same quantity as in whole milk (Ewen 1980).

Table 2.1 Typical composition of wheys produced in New Zealand (g/kg) (Short & Doughty 1977)

	Cheese whey	Casein whey	
		lactic	sulphuric
Total solids	67	64	59
Lactose	50	44	47
Protein	5.7	5.7	5.3
Non protein nitrogen	0.5	0.5	0.3
Fat	0.3	0.2	0.4
Ash	5.3	5.8	6.7

2.2.3 Whey production

Each tonne of cheese and casein manufactured produce approximately 7.6 and 25 tonnes of whey, respectively (Boontanjai 1983). Large volume are therefore produced, for example approximately 2.5 million tonnes of whey (based on cheese and casein production) were produced in New Zealand in 1989 (New Zealand Dairy Board 1989). Table 2.2 summarizes New Zealand's annual cheese and casein production from 1984 to 1989. By way of comparison, Australia produced 1.3 million tonnes of whey in 1987 (estimation based on cheese production) (EEC Dairy Facts & Figures 1988).

Table 2.2 Summary of the cheese and casein production (tonne)of New Zealand from 1984-1989 (New Zealand Dairy Board 1989)

product	84/85	85/86	86/87	87/88	88/89	5 yr. ave.
cheese	117679	127269	113250	128414	124200	122162
acid casein caseinates rennet casein	(64168)	(75382)	(61840)	(65759)	38000* 14700* 10000*	(65970)
lactose	11500	13760	14410	17655	17853	15036
whey powder	11444	12475	9500	14281	13000	12140

source : NZDB Annual Report 1989 Livestock Improvement report 86/87

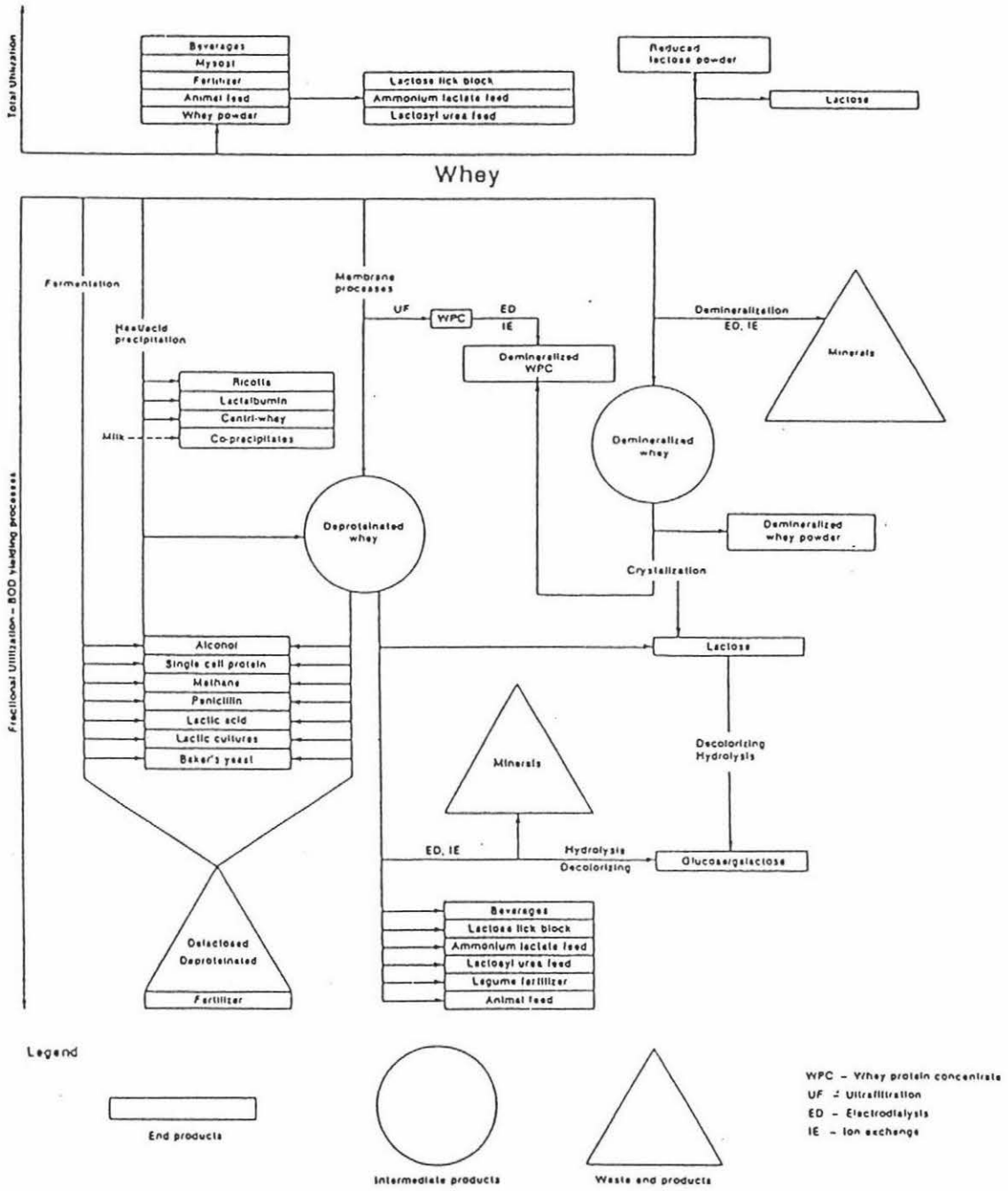
key : * NZDRI () Quantity as total casein products

2.2.4 Whey utilization

Figure 2.4 outlines various whey utilization processes. In New Zealand, approximately 40% of the available whey solids from the dairy industry are processed into over 50 individual whey products including whey protein concentrates, whey powders, lactalbumin, ethyl alcohol, lactose and whey cheese. The majority of these products are sold into the food ingredient trade (New Zealand Dairy Board 1985). In the USA, in 1981, about 50% of the liquid whey produced was utilized within the dairy industry, generally as a substitute in products in which non-fat dry milk is utilized (Janssens *et al* 1983). There are also a wide range of whey-based alcoholic drinks produced, for example whey champagne, whey kwas and whevit (Holsinger *et al* 1974).

Whey can also be fed to animals. In the United Kingdom, 40% of the whey produced was used as pig feed in 1973 (Coton 1976), while in 1979, in the European Economic Community, approximately 34% of the whey produced was fed to animals (mostly pigs) (Irvine & Hill 1985). In Switzerland, 92% of the whey production is used for pig feeding (Coton 1980). However, this is not widely employed in New Zealand due to the relatively small scale pig industry and large scale of dairy industry.

Figure 2.4 Summary of processes for whey utilization (Irvine & Hill 1985)



2.2.5 Whey disposal

General methods of whey disposal include spray irrigation and discharge into natural waterways or municipal sewers. Because of the high plant capital cost required to treat the high biological oxygen demand of whey, very few factories have effluent treatment plants for whey disposal (Oborn 1968). In 1981 in the United States, approximately 50% of the liquid whey produced was disposed of (Janssens *et al* 1983). In New Zealand, about 20% of the effluent from all the cheese, butter and milk powder factories are disposed of by spray irrigation. Some costs are recovered by charging for the fertilizer value of this irrigated whey (Radford *et al* 1986).

2.3 Ethanl fermentation by *Kluyveromyces* strains

2.3.1 Introduction

The productivity of a whey-ethanol production plant is affected by the yeast strain and fermentation conditions as well as the fermenter design. This section provides a brief introduction to the effect of various process parameters on yeast growth and ethanol production.

2.3.2 Morphology and metabolism of the *Kluyveromyces* species

Kluyveromyces species are commonly used in whey-ethanol production because of their ability to ferment lactose. *Kluyveromyces* species are unicellular, heterotropic and resembles higher plants and animals in the anatomical complexity of their cells. To be able to utilise lactose, the yeast transports the sugar across the plasmalemma by a carrier which is induced by growth on lactose. Lactose molecules are then hydrolysed intracellularly by β-galactosidase into glucose and galactose. Glucose follows the Emden-Myerhoff glycolytic pathway and is broken down to pyruvate. The galactose is converted to D-glucose-1-phosphate before entering the Emden-Myerhoff pathway and also being converted to pyruvate.

Under aerobic conditions, pyruvate produced by glycolysis is further metabolised in the tricarboxylic acid cycle and thence by oxidative phosphorylation. Under anaerobic conditions, fermentation occurs in which the pyruvate is converted to ethanol and carbon dioxide. Less ATP is produced,

with much chemical energy remaining bound in the ethanol molecule.

The species *Kluyveromyces marxianus* appears most widely used for ethanol production. This species was classified as *Saccharomyces fragilis* and *S. marxianus* in the 1970s. However, in the 1980s, these 3 species were collectively named as *K. marxianus* (Priest & Campbell 1987). In the following discussion, the name used in the original reference will be retained.

2.3.3 Effect of lactose and ethanol concentration

High lacotse and ethanol concentrations have been shown to have a major influence on the fermentation, affecting yields coefficients, growth rate and ethanol productivity. The effect of lactose and ethanol are obviously linked, as high ethanol concentrations should result from a high initial lactose concentration.

Lactose inhibition of ethanol production has been reported for various yeast strains and the data are summarized in Table 2.3. The inhibition effect is also reported for *Kluyveromyces marxianus* Y-113. Ethanol yield decreases from 0.44 at 50 g/l lactose to 0.38 at 150 g/l lactose (Mawson & Taylor 1988). The inhibitory effect is also strain dependent. For example,.for *K.fragilis* CBS-397, the ethanol yield decreased by 8.2% when lactose concentration increased from 50 to 200 g/l, while a 55.6% decrease in ethanol yeild is recorded for *K. marxianus* NRRL Y-1120.

Some yeasts show decreasing lactose utilization activity only above about 150 g/l lactose. For example, Janssens *et al* (1983) noted the fermentation time for complete lactose utilization increased from 15 to 36 hours at 50 g/l and 150 g/l lactose respectively, but at 200 g/l lactose, greater than 90 hours was required.

Table 2.3 Summary of ethanol yield data

Yeast strain	Lactose concentration (g/l)	Ethanol yield (g/g)	reference
K. marxianus NRRL Y-1120	50-200	0.72-0.32	(1)
K. fragilis NRRL Y-1109	50-150	0.49-0.45	(2)
K. fragilis CBS-397	50-200	0.49-0.45	(3)
K. fragilis CBS-5795	50-250	0.48-0.06	(4)
K. fragilis CBS-397	5%-25% solid	0.48-0.37	(5)
K. marxianus Y-113	50-150	0.44-0.38	(6)

Key : (1) Bothast *et al* 1986 (2) Burgess & Kelly 1979
(3) Janssens *et al* 1983 (4) Moulin & Galzy 1981
(5) Sanderson & Reed 1986 (6) Mawson & Taylor 1989

An overall decrease in specific activity with increasing lactose concentration has also been observed for 73 strains of *Kluyveromyces marxianus* (Bothast *et al* 1986). For 10 *K. marxiamus* strains with superior ethanol production rates, the specific productivity decreased by over 50% as the lactose level increased from 50 to 200 g/l (Bothast *et al* 1986).

High ethanol concentrations also depress the ethanol productivity (Zertuche & Zall 1985) and in some cases a threshold concentration, above which ethanol production ceases, has been observed. Studies have been carried out to establish the threshold ethanol concentration for several strains (Table 2.4).

Table 2.4 Summary of the threshold ethanol concentration

Yeast	Threshold ethanol concentration g/l	Reference
K. fragilis NRRL Y-665	45	(1)
K. fragilis CBS-5795	65.5	(2)
K. marxianus Y-113	40	(3)

Key : (1) Vienne & von Stockar (1985) (2) Ruggeri *et al* (1988)
(3) Grubb (1990)

Increasing ethanol concentration has also been observed to reduce the biomass yield in some cases (Vienne & von Stockar 1985, Ruggeri *et al* 1988).

2.3.4 Media supplementation (yeast extract, ergosterol, lipid)

Several studies on *Saccharomyces fragilis* (*K. marxianus*) have demonstrated an improvement in ethanol production with yeast extract supplementation. For example, the ethanol concentration increased from 24.3 g/l to 31.6 g/l for 0.3 and 0.7% yeast extract addition (Chen & Zall 1982). Vienne & von Stockar (1983) suggested that early cessation of growth and low biomass yield could be overcome by supplementing the medium (50 g/l lactose) with a minimum of 0.375% yeast extract. Ethanol production is also affected by ergosterol supplementation (Chen & Zall 1982). Ethanol increased by 1.3 fold with 10 mg/l ergosterol and more cell mass was also obtained with ergosterol supplement (Table 2.5). Shorter fermentation times resulted (Janssens *et al* 1983).

Table 2.5 Summary on effect of ergosterol supplementation on biomass (data extracted from Chen & Zall 1982)

Ergosterol concentration mg/l	Biomass g/l	Ethanol concentration g/l
0	1.75	28.73
10	2.7	36.75
20	2.7	38.1

N.B. data are after 48 hours of fermentation

A pronounced effect on biomass growth rate, biomass concentration was observed with addition of lipid. At 2 % v/v ethanol, a 33.3% increase in maximum specific growth rate (μ_m) to 0.2 hr^{-1} and a near doubling of the maximum biomass concentration was reported (Janssens *et al* 1983).

2.3.5 Effect of inoculum size and temperature

Increased fermentation rate with larger inoculum size was reported by Rajagopalan & Kosikowski (1982). The ethanol concentration increased from 1 to 12.5 % v/v when larger inoculum size was used (1 to 10 g wet wt./100ml). Fermentation temperature was shown to affect yeast performance (Burgess &

Kelly 1979). Complete yeast utilization was obtained in 24 hours at 35°C whereas at 25 and 30°C, 27 hours was necessary. Overall, the literature suggests an optimum temperature of about 35°C, although this is strain specific as would be expected.

2.4 Industrial and pilot scale plant operations for whey ethanol production

Whey ethanol production has been studied since the 1940s. However, it was only in the late 1970s that the first commercial whey ethanol plant was built at Carbery, Ireland. At present, there are at least nine whey ethanol production plants and one pilot scale plant in operation. Table 2.6 summarizes the mode of fermentation employed by these known commercial or semi-commercial scale distilleries and their respective capacity.

Table 2.6 Summary of the fermentation methods employed by nine commercial size distilleries and one pilot scale plant

Distillery	Country	Mode of fermentation	Capacity	Reference
Reporoa	New Zealand	batch	12,000 l/day 96% alcohol	(1)
Edgecumbe	New Zealand	batch	2 million l/annum	(2)
Temuka	New Zealand	batch	NA	(3)
Tirau	New Zealand	continuous cascade	32,000 l/day 96% ethanol	(1)
Carbery	Ireland	batch	164,000 l working capacity	(4)
Juneau	USA	batch	NA	(5)
Corona	USA	batch	18.93 million l/annum	(6)
Melrose	USA	NA	NA	(5)
Tulare	USA	same as Melrose	3.785 million l/annum	(6)
Dansk Gaering Industri	Denmark	continuous 2 stage	pilot scale	(7), (8)

Key : (1) Howell 1981 (2) Mawson 1988
(3) Gooding 1982 (4) Barry 1982
(5) Sanderson & Reed 1986 (6) Lyons 1986
(7) Fergusson 1980 (8) Reesen & Strube 1978
NA not available

Four of the known commercial scale operations are situated in New Zealand at Reporoa (Howell 1981), Edgecumbe (Mawson 1988), Temuka (Gooding 1982) and Tirau (Howell 1981).

The Reporoa plant was designed to produce 12,000 litres per day of 96% or anhydrous alcohol. This replaced all of the industrial alcohol imported into New Zealand. Mawson (1990) reported the Edgecumbe plant has achieved a yield of 0.46 g ethanol/g lactose utilized (85.5% theoretical maximum) based on 4 % w/w lactose in whey. The overall ethanol productivity of the plant was approximately 1.1 g/l.hr. The annual ethanol production of the plant was 2 million litres (Mawson 1988).

The plant at Tirau has attempted continuous cascade fermentation. This plant employs two sets of three fermenters in series and processes a maximum of 1500 m^3 of deproteinated whey per day to produce 32,000 litres of 96 % v/v ethanol (Howell 1981).

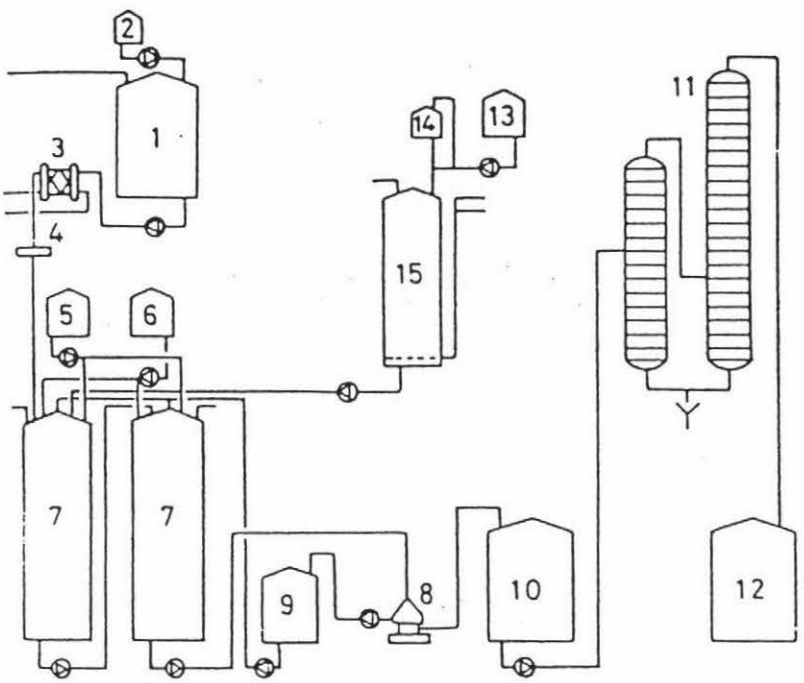
The Carbery plant in Ireland, consists of six fermenters of 164,000 litres working capacity for batch fermentation, yeast propagation and yeast storage. A centrifugation system for separating and concentrating yeast is used. An average fermentation efficiency of 85% of the theoretical figure has been reported. For whey permeate containing 47 g/l lactose, an alcohol content of 2.59% v/v has been achieved (Barry 1982).

There are also four known commercial whey ethanol distilleries in the USA. In Juneau, the Universal Foods Corporation produces beverage grade 192 proof ethanol in Amber Laboratory as a by-product of yeast production (Sanderson & Reed 1986, Lyons 1986). Express Dairy at Corona, California, USA employs technology similar to the Carbery plant. The plant has the capacity of approximately 18.93 million litres per annum and was commissioned during 1986 (Lyons 1986). Kraft in Melrose, USA, operates a plant for production of 100% fuel grade ethanol (Sanderson & Reed 1986). The Dairyman's Association in Tulare, USA uses a process from Kraft with production capacity of approximately 3.785 million litres of alcohol per annum (Lyons 1986).

In Denmark, a pilot-plant scale investigation was carried out by the Dansk Gaering-Industri (Fergusson 1980, Reesen & Strube 1978). Figure 2.5

shows a flow sheet of the ethanol plant. Yeast cultivation was done in a two-stage batch propagating process. After separation by centrifugation, the first continuous fermenter containing the yeast was filled with whey permeate. The second continuous fermenter was started when the predetermined fermentation conditions of the first fermenter was achieved. Part of the yeast from the separator was recycled back to the fermenters while the yeast free wort was pumped to the distillation plant.

Figure 2.5 Flow sheet of the whey ethanol plant by Dansk Daering Industri (Fergusson 1980)

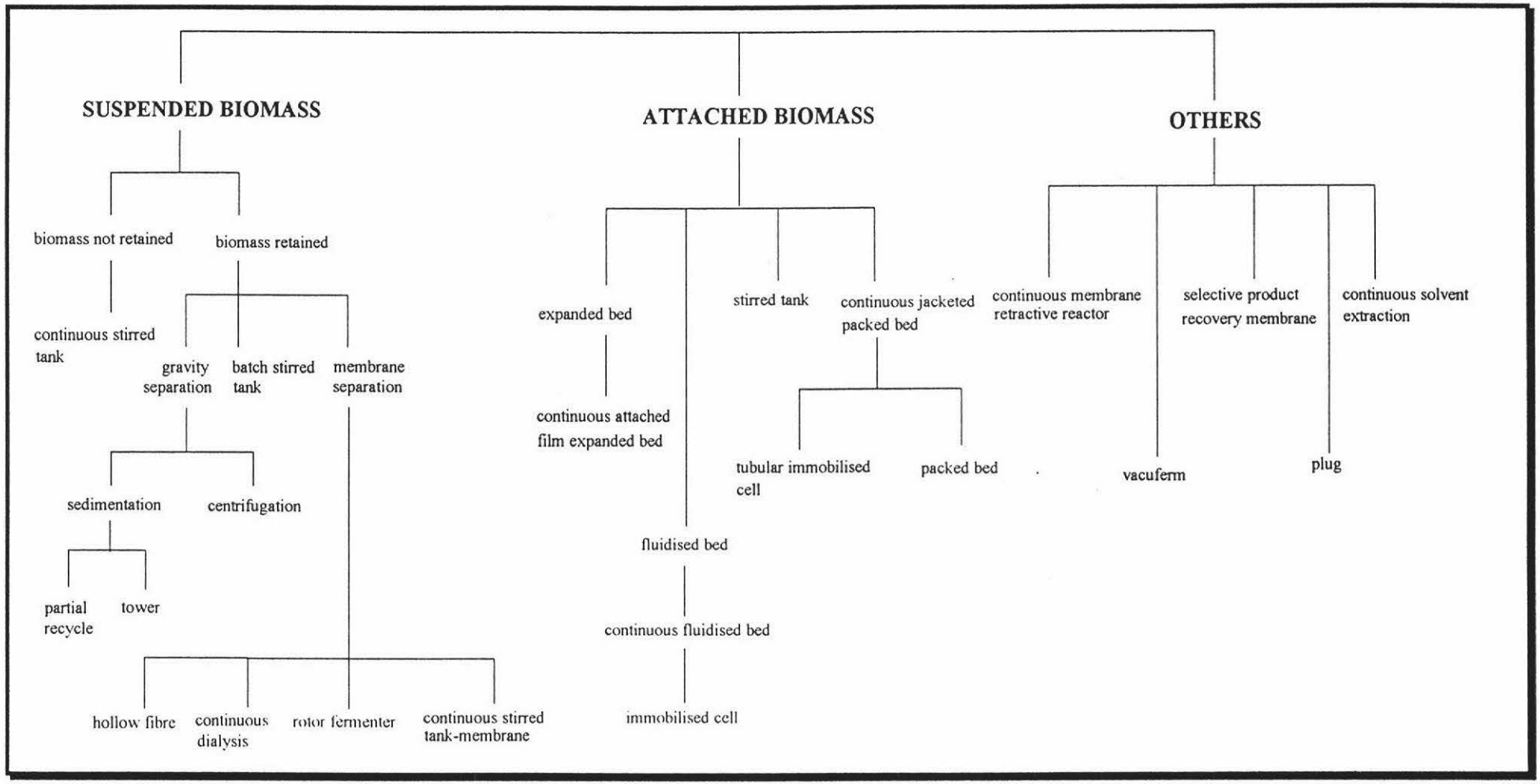


Key : (1) storage tank for whey permeate (2) acid container
(3) plate heat exchanger (4) control unit (5) tank for antifoam
(6) tank for chemicals (7) fermenting vat (8) separator
(9) storage tank for yeast cream (10) buffer tank (11) distillation plant
(12) storage tank for alcohol (13) substrate reservoir (14) propagation plant
(15) propagation plant

2.5 Overview of alternative whey-ethanol fermentation systems

There are over 20 different reactor designs reported for fermentation ethanol production and which could be adapted for whey ethanol production. Maiorella *et al* (1981) provided a very comprehensive summary of these different fermentation configurations. Developments in bioreactors for ethanol production were also discussed by Shama (1988). For easier analysis individual systems are grouped under three classes, namely suspended biomass, attached biomass and others, as shown in Figure 2.6.

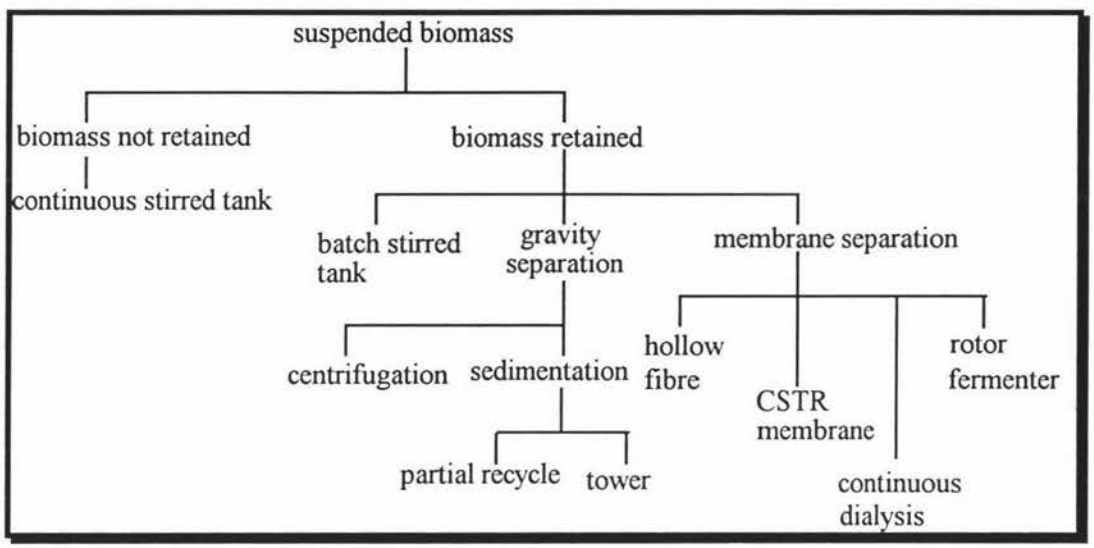
Figure 2.6 A fermenter classification chart



2.5.1 Suspended biomass

Figure 2.7 shows a classification chart for suspended biomass fermenters. This class is subdivided into retained biomass and biomass not retained catagories.

Figure 2.7 Classification chart of suspended biomass fermenters

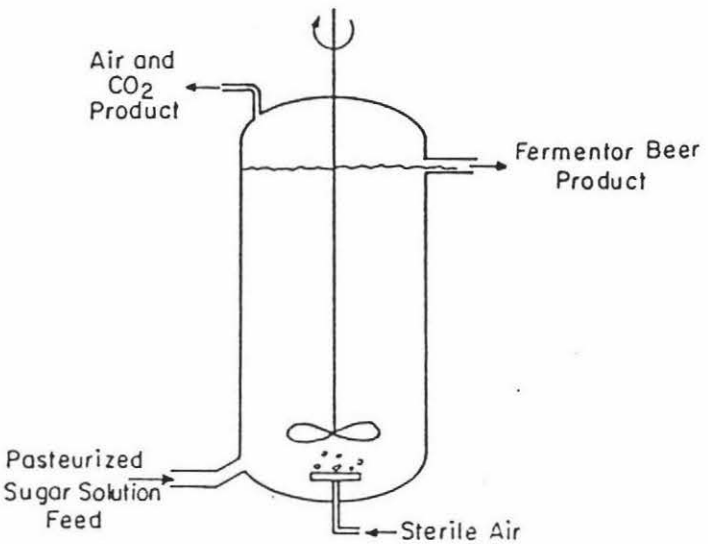


2.5.1.1 Biomass not retained

2.5.1.1.1 Continuous stirred tank

A schematic diagram of a single stage continuous stirred tank fermenter (CSTR), the sole reactor type in this category, is given in Figure 2.8. Substrate is continuously fed into the fermenter and a product stream containing the cell mass and ethanol produced flows out continuously at the same rate as the feed is added. Dilution rate is controlled to maintain the desired cell concentration in the fermenter.

Figure 2.8 Schematic diagram of a single stage continuous stirred tank fermenter (Maiorella *et al* 1981)



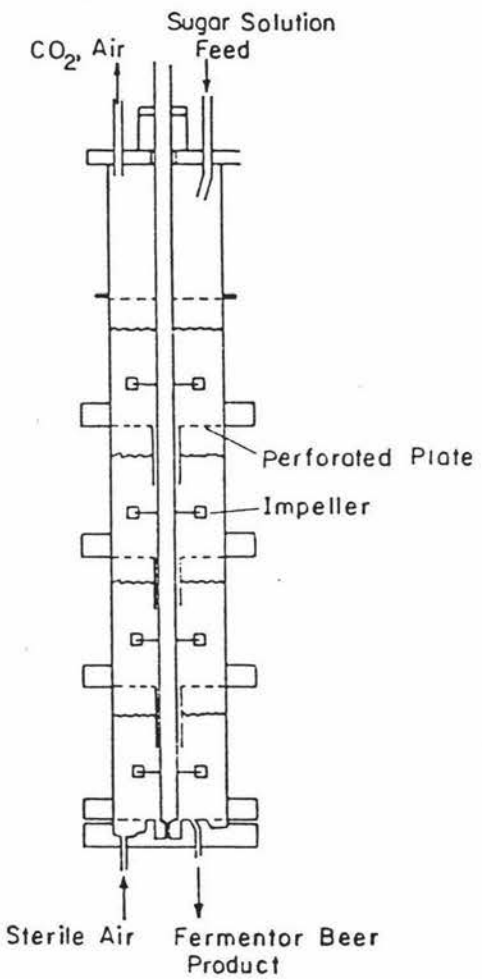
Maiorella *et al* (1981) reported that 3 times the average batch productivity could be obtained for ethanol production from glucose in a CSTR. The process could be conducted at a single optimum condition. A reduced downtime resulting from the longer production cycle and higher product quality consistency could also be expected. The fermenter also has a steady demand on utilities. The equipment is mechanically simple when compared to recycle systems and this simplifies process control and automation.

The risk of contamination is an important concern. Contamination will not be confined to the fermenter. The process must be stopped for an extended period of time for cleaning and sterilization. A temporary whey storage facility is necessary, or some other form of disposal must be employed, e.g. irrigation.

A schematic diagram of a multistage fermenter is given in Figure 2.9. Partial substrate utilization occurs in the first stage, and the outflow is then fed into the second stage where further fermentation occurs. Cells at the earlier stages experience a lower ethanol concentration, and thus higher productivities can be obtained (Maiorella *et al* 1981). Similar to a single stage continuous stirred tank fermenter, greater product output, higher product quality consistency and steady utility demand are the main advantages of this system.

A multistage fermenter system can be mechanically complicated and the capital cost is high. In the event of contamination, a longer downtime is expected. This fermenter has not been well accepted, although in New Zealand, the New Zealand Cooperative Dairy Company at Tirau operates a continuous cascade fermenter for whey-ethanol production. The two major breweries in New Zealand have also operated two-stage continuous plants.

Figure 2.9 Schematic diagram of a multistage continuous fermenter (Maiorella *et al* 1981)



2.5.1.2 Biomass retained

2.5.1.2.1 Batch stirred tank

Much research has been carried out on batch fermentation due to its simplicity, ease of construction and the proven commercial applications. The Edgecumbe plant uses *Kluyveromyces marixanus* Y-113 and achieved 0.46 g/g ethanol yield with 4 % w/w lactose feed. An ethanol productivity of 1.1 g/l/hr was reported (Mawson 1988). The Carbery and Corona plants also use batch fermentation (Barry 1982, Lyons 1986).

Overall, high ethanol concentration and complete lactose utilization have been demonstrated on both synthetic medium and whey permeate (Table 2.7). But a low ethanol productivity is generally reported (0.6-3.5 g/l/hr). As discussed in section 2.3.3, high lactose and ethanol concentrations both inhibit the ethanol yield. Exposure of biomass to increasingly high ethanol concentration therefore contributes to the low productivity. The other major factor is the low initial biomass concentration employed in these batch systems. Other disadvantages of batch fermenters include longer downtime due to short production cycles, batch to batch product quality or yield variation, and high labour costs.

Table 2.7 Summary of batch fermentation data

Yeast	Medium	Lactose conc. g/l	Substrate utilization	Ethanol conc. g/l	Productivity g/l/hr	Reference
K. fragilis	synthetic lactose	50-200	85-100	23-85	2-3.5	(1)
NA	whey permeate	47	100	25.9	NA	(2), (3), (4)
NA	whey permeate	45-50	NA	22.1	NA	(5)
NA	deproteinized whey	41.4	98	18.6	1.1	(6)
K. fragilis NRRL Y-2415	demineralized concentrated acid whey	240	100	108.8	3.2	(7)
S. fragilis	reconstituted cottage cheese acid whey	100.1	76	30	0.6	(8)
K. marxianus NCYC-179	whey permeate	98	NA	42.6	NA	(9)
K. fragilis NRRL 1109	ultrafiltration permeate	80-160	NA	23-37.2	NA	(10)

Key : *K. fragilis* - *Kluyveromyce fragilis* *K. marxianus* - *Kluyveromyces marxianus*
S. fragilis - *Saccharomyces fragilis* NA - not available
(1) - Mehaia & Cheryan 1984 (2) - Cheryan & Mehaia 1983
(3) - Cheryan & Mehaia 1985 (4) - Barry 1982
(5) - Sandbach 1981 (6) - Tichener 1982
(7) - Rajagopalan & Kosikowski 1982 (8) - Chen & Zall 1982

2.5.1.2.2 Gravity settling

Systems where the biomass is not retained suffer from biomass escaping in the product stream. Cell concentration is low because of the low yield and hence a low productivity is obtained. In gravity settling designs, cells are retained in the fermenter by natural or forced gravity settling and a high productivity can be achieved. Examples of natural settling fermenters are partial recycle and tower designs. Figure 2.10 shows a diagram of the partial

recycle fermenter. In the partial recycle fermenter, cell separation relies on the region between the pipe and sleeve escaping agitation. This allows the yeast to separate from the product stream rising to the outflow nozzle.

The tower fermenter consists of a vertical cylindrical tower. Substrate is fed to the bottom of the fermenter and progressively rises to the top where a product stream of constant flowrate is removed. A diagram of the APV tower fermenter is given in Figure 2.11.

Figure 2.10 Schematic diagram of a partial recycle fermenter (Maiorella *et al* 1981)

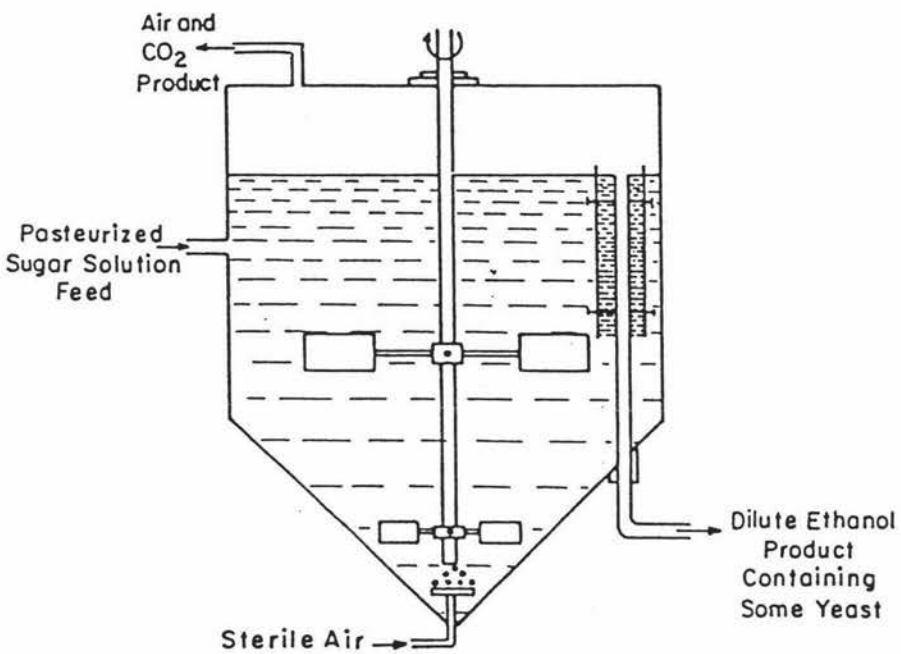
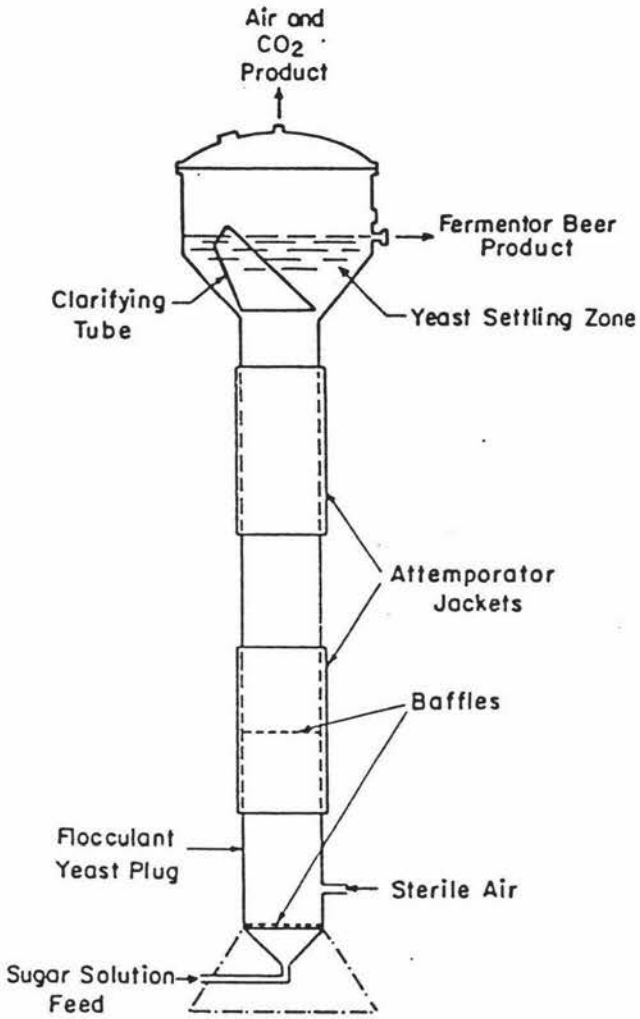


Figure 2.11 Schematic diagram of a APV tower fermenter (Maiorella *et al* 1981)

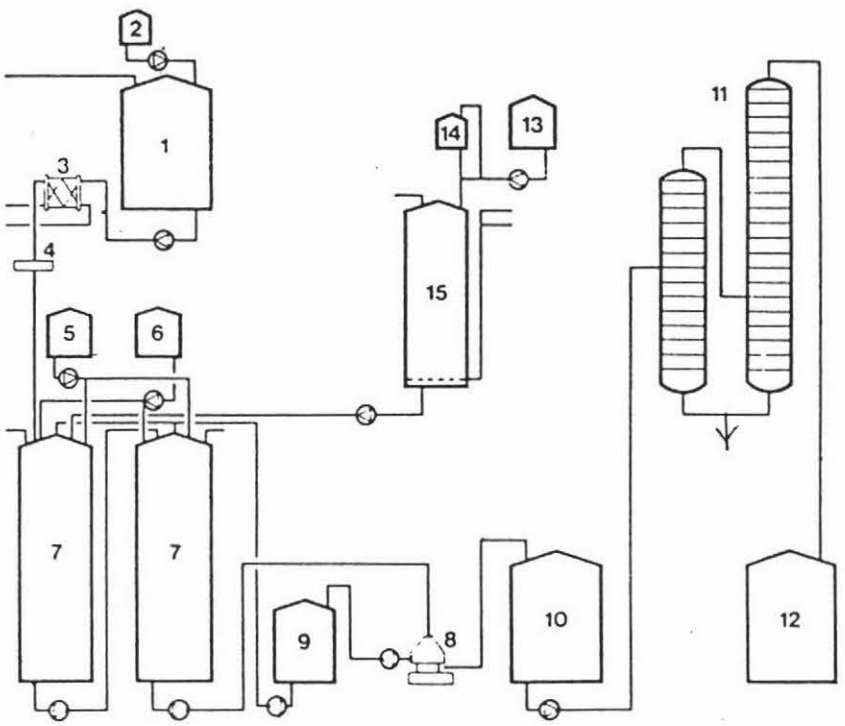


These natural gravity settling systems are mechanically simple and have a relatively low energy requirement. Capital cost is low and high productivity could be obtained (Maiorella *et al* 1981). As the driving force for natural settling is low, a large settler is required for commercial application. A long start-up time is also needed for the tower fermenter (Maiorella *et al* 1981). Improved prospects for these fermenters lies with techniques such as genetic engineering to improve the flocculence of the yeast which appears less marked in *Kluyveromyces* strains. There have been no reports on their commercial application for whey ethanol production.

Similar to natural gravity settling fermenters, forced settling fermenters uses gravitational force for cell separation. However, mechanical devices such as a centrifuge are used to raise the driving force. Figure 2.12 shows a schematic drawing of a continuous stirred tank-centrifuge fermenter. Maiorella

et al (1981) claimed a marked improvement in volumetric productivity can be obtained and this was shown by Singh *et al* (1983), who reported a productivity of 5.7 g/l/hr with 160 g/l lactose. Near complete lactose utilization (98%) was also demonstrated and a high ethanol concentration (71 g/l) was achieved.

Figure 2.12 Schematic drawing of a continuous stirred tank-centrifuge fermenter (Reesen & Strube 1978)



Key : (1) - Storage tank for whey permeate (2) - Acid container
(3) - plate heat exchanger (4) - Control unit
(5) - Tank for antiform (6) - Tank for chemicals
(7) - Fermenting vat (8) - Separator
(9) - Storage tank for yeast cream (10) - Buffer tank
(11) - Distillation plant (12) - Storage tank for alcohol
(13) - Substrate reservoir (14), (15) - Propagation plant

The introduction of mechanical separator does add to the complexity of the process. This is specially true for an industrial scale continuous centrifuge for which the capital and operating costs are high. More supervision of fermenter operation is required and increases in utility demand, and maintenance cost are expected. Contamination is another potential problem as it is difficult to sterilize an industrial size continuous centrifuge. Recycle of contaminated yeast may lead to reduced yeild and productivity. Cell damage may also occur during centrifugation. A semi-production scale of continuous

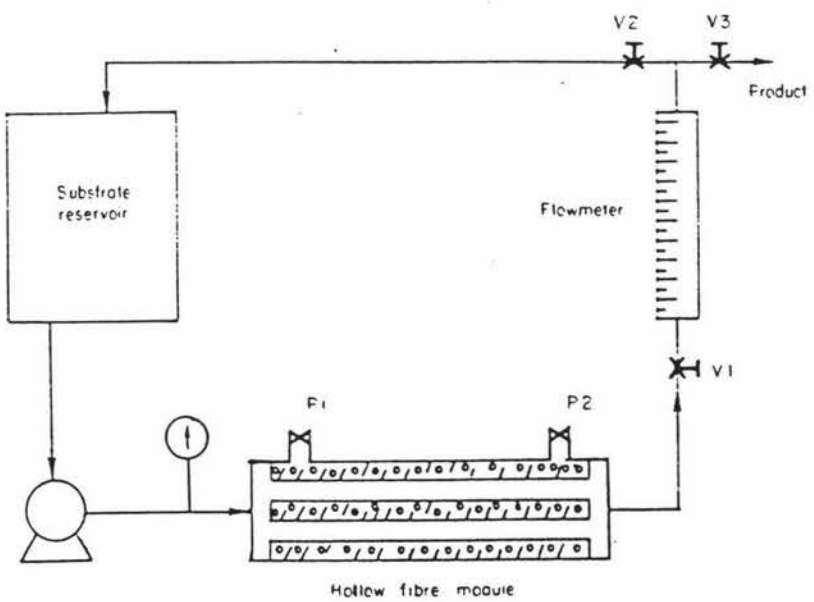
fermenter with centrifuge was developed at Dansk Gaerings-Industri (Fergusson 1980, Reesen & Strube 1978).

2.5.1.2.3 Membrane separation

In general, there are two main catagories of membrane fermenter. The membrane filter could be operated as a stand-alone fermenter (hollow-fibre fermenter) or it can be coupled to a continuous stirred tank fermenter (membrane recycle bioreactor - MRB). The role of the membrane in both designs is for cell separation, with biomass retained in the fermenter to improve the fermenter productivity.

Some examples of membrane fermenters are hollow fibre fermenter (Mehaia & Cheryan 1984), continuous dialysis, and the rotor fermenter designs (Maiorella *et al* 1981). A diagram of a hollow fibre fermenter is given in Figure 2.13. Yeast cells were pumped into the shell side of the filter and the substrate was fed into the tube side continuously. Mehaia & Cheryan (1984) reported volumetric productivities of 60-135 g/l/hr with 50-150 g/l lactose synthetic medium. Much lower productivities were obtained (2-8 g/l/hr) for batch fermentation studies (Mehaia & Cheryan 1984). The author predicted with a 150 g/l lactose feed, productivity greater than 100 g/l/hr could be achieved at 99% lactose utilization. High ethanol concentrations (62.5-76.9 g/l) could also be maintained.

Figure 2.13 Schematic diagram of hollow fibre fermenter
(Mehaia & Cheryan 1984)



High productivity is also obtained with whey permeate studies. At near complete utilization of a 45 g/l lactose feed, the volumetric productivity was 30 g/l/hr and the ethanol concentration was 22 g/l (Mehaia & Cheryan 1984, Cheryan & Mehaia 1985). The productivity is 8 times that of a batch fermenter although at the expense of ethanol concentration.

Figure 2.14 and 2.15 show schematic diagrams of a dialysis fermenter and a rotor fermenter. A dialysis membrane is primarily used for cell containment and especially mammalian cells. Substrate diffuses through the membrane into the cell compartment. Fermentation takes place and ethanol or other products diffuses back into the substrate compartment. High productivity could be achieved (Maiorella *et al* 1981) but there has been no commercial application of the fermenter in alcohol fermentation. For the rotor fermenter, the stationary membrane is replaced by a rotating membrane cylinder. A centrifugal force is developed and large molecules are thrown back into the annular zone. The fermenter could potentially achieve high productivity. However, it is mechanically complicated and considerable skilled labour time is needed for periodic membrane replacement (Maiorella *et al* 1981).

Overall, large improvements in productivity can be achieved with such membrane fermenters and complete lactose utilization could be attained. However, membrane plugging remains a problem, a buffer tank for temporary storage of whey is needed and capital investment is further increased by the high cost of the membrane unit.

Figure 2.14 Schematic diagram of a dialysis fermenter (Maiorella *et al* 1981)

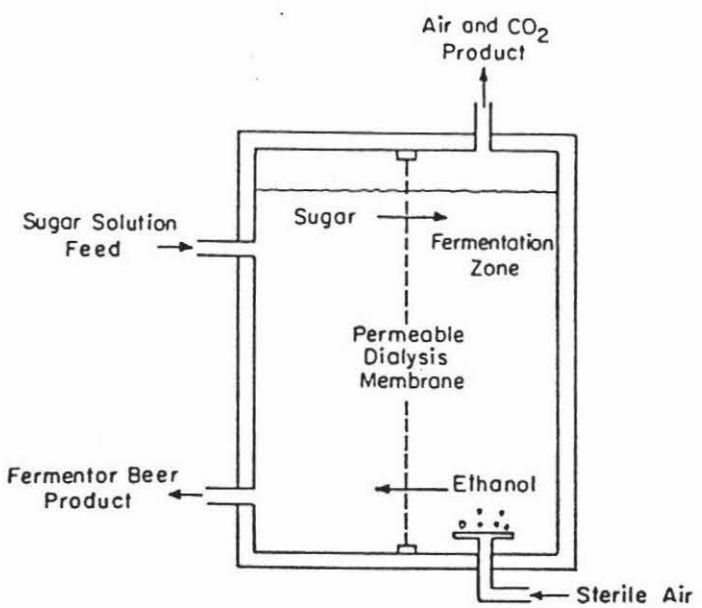
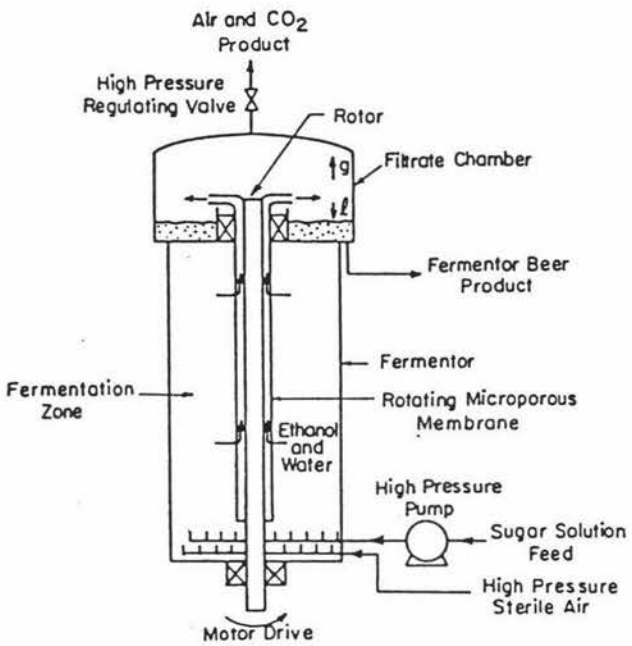


Figure 2.15 Schematic diagram of a rotor fermenter (Maiorella *et al* 1981)



The continuous stirred tank-membrane fermenter (or MRB) has received much attention in its application for whey-ethanol production. Studies of a CSTR-hollow fibre fermenter showed 13 g/l/hr productivity and an ethanol concentration of 25 g/l could be obtained with a 50 g/l lactose substrate. At 150 g/l lactose, the productivity was maintained at a high value (73 g/l/hr) with a product concentration of 72 g/l. Complete lactose conversion was achieved for both studies (Cheryan & Mehaia 1983). A higher productivity was also attained (118 g/l/hr) but at the expense of lactose utilization (80%) and ethanol concentration (19.7 g/l) when operating at very high dilution rate. In contrast, only a relatively low productivity was reported for a CSTR-UF fermenter (Janssens *et al* 1984). For 100-120 g/l lactose substrate, the volumetric productivity was 2.6-7.1 g/l/hr. The lactose utilization was between 86.2 and 100%, and the ethanol concentration was in the range of 36.7-58 g/l. A low productivity was also reported by Chen & Zall (1982) using CSTR-UF fermenter. At 100 g/l lactose, a productivity of 3.4-7.4 g/l/hr and ethanol concentration 29-37 g/l was recorded.

Overall, the high cost of the membrane increases the capital cost and membrane fouling limits both the length of production cycle and increases the complexity of the control of the operation. This latter problem could be

improved with backflushing or the use of a dual membrane system, where one membrane is in operation and the other being cleaned.

2.5.1.2.3.1 Brief description of microfiltration membrane

Microfiltration separation has a cut off size of 300 - 140000 A (Smith & Gregario 1970). Solution components are separated based on molecular size : under an applied pressure differences across the membrane, solvent and small solute species pass through the membrane and are collected as permeate, while high molecular weight species are retained by the membrane and recovered as a concentrated retentate (Henry 1985).

Asymmetric membranes have a thin layer of defined pore size above a more open layer and provide a low friction resistant to flow. Ceramic material has greater chemical resistant and mechanical strength. Sterilization by steam or chemical is possible with ceramic (Matsumoto *et al* 1988). The tubular design is selected as in the presence of membrane failure, module capacity is less affected due to the lower packing density.

The cross-flow mode of operation is particularly suitable for higher fouling tendency operation. The bulk of the fluid travels parallel with membrane surface and only a relatively small part of this passes through the filter. The concentrate is continuously swept away, providing a relatively unchanged surface concentration. Other advantages reported for crossflow microfiltration (Hanisch 1986) are :-

(1) filter performance is not a strong function of cell size

(2) flux is independent on a density differences between cells and suspending media

(3) filter aids or flocculating agents are not required

(4) accumulation of cells on the filter medium is minimized such that high fluxes can be maintained over long periods

2.5.2 Attached biomass

Yeast cells could be immobilized by bonding onto carriers such as activated carbon or by entrapment in gels. Some examples of gel entrapment materials are cellulose acetate, polyacrylamide, K-carrageenan and alginate. The fermenter biomass concentration is increased and higher productivity is expected. Table 2.8 summarizes fermentation data of various attached biomass fermenters.

Low productivity but high ethanol concentration was reported for a batch immobilized cell fermenter (Linko *et al* 1984). The fermenter can be designed to minimize effluent biomass, and high substrate conversion and increased product concentration are other advantages. However as for the batch stirred tank fermenter, the batch-immobilized design has inconsistent product quality. Higher operating cost will also arise due to the short production cycle.

Table 2.8 Summary of fermentation data of various attached biomass fermenter

Fermenter	Yeast	Medium	Lactose conc. g/l	Ethanol conc. g/l	Product-ivity g/l/hr	Lactose utiliz-ation %	Ref.
Batch immobilized alginate gel	*K.fragilis*	reconstituted whey permeate	50-200	24-53	0.5-1.5	NA	(1)
Continuous fluidized bed	*K.fragilis*	reconstituted sweet whey powder	100	4-22	6.2-13.3	14-50	(2)
	K.fragilis	deproteinized demineralized whey lactose	150-250	6.6-49.5	7.5-17.2	NA	(6)
	S.fragilis	reconstituted cottage cheese acid whey	100	5-14	3.6-7.0	12-26	(3)
continuous packed bed	*K.fragilis*	reconstituted whey permeate	50-100	15-28	NA	NA	(1)
	K.fragilis	demineralized whey powder	50-100	20-45	5.13-5.42	NA	(4)
	K.fragilis	cheese whey permeate	45	78.9	1.1	NA	(5)

Key : *K.fragilis* - *Kluyveromyces fragilis* *S.fragilis* - *Saccharomyces fragilis*
NA - not available
(1) - Linko *et al* (1984) (2) - King & Zall (1983)
(3) - Chen & Zall (1982) (4) - Linko *et al* (1981)
(5) - Hahn-Hagerdal (1985) (6) - Gianetto *et al* (1986)

Studies were carried out on various continuous immobilized cell fermenters (King & Zall 1983, Chen & Zall 1982, Linko *et al* 1981, Linko *et al* 1984, Hahn-Hagerdal 1985, Gianetto *et al* 1986). In general, cells were immobilized onto a carrier in these studies and the carrier was retained in the fermenter by a physical barrier (e.g. wire mesh). A continuous flow of medium was pumped to the bottom of the fermenter. For lactose concentration of 45-250 g/l, ethanol concentrations of 4-78.9 g/l and productivities 1.1-17.2 g/l/hr were obtained. High productivity could be achieved but with a lower ethanol concentration (e.g. Gianetto *et al* 1986 : productivity 17.2 g/l/hr, ethaol

concentration 18 g/l) when operating at high dilution rates.

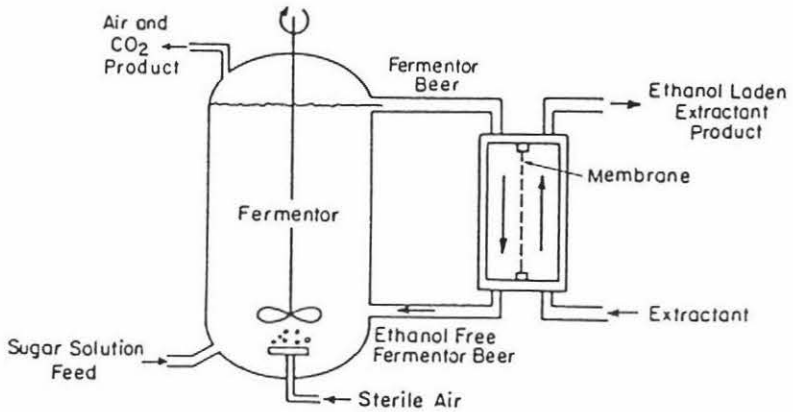
More consistent product quality is obtained with the continuous fermenter. Better process control and lower biocatalyst cost are some other advantages. The production cycle is, however, limited by the need for periodic biomass regeneration. This is necessary to ensure that the biomass is not dominated by other undesirable mutant strains. There is some bead disruption by the fluid flow so very small carriers with good mechanical properties are necessary. This will also improve the catalyst efficiency by minimizing mass transfer limitation. Klein & Kressdorf (1983) suggested fluidized bed fermenter with good back-mixing in the front stage combined with plug flow characteristic in the last stage be used for fermentations suffering from product inhibitory.

2.5.3 Other configurations

Attempts have been made to improve ethanol fermentation performance with some novel reactor designs. These include the continuous membrane extractive process, vacuferm reactor, selective product recovery membrane reactor, continuous solvent extractive fermenter, flashferm configuration and the plug reactor.

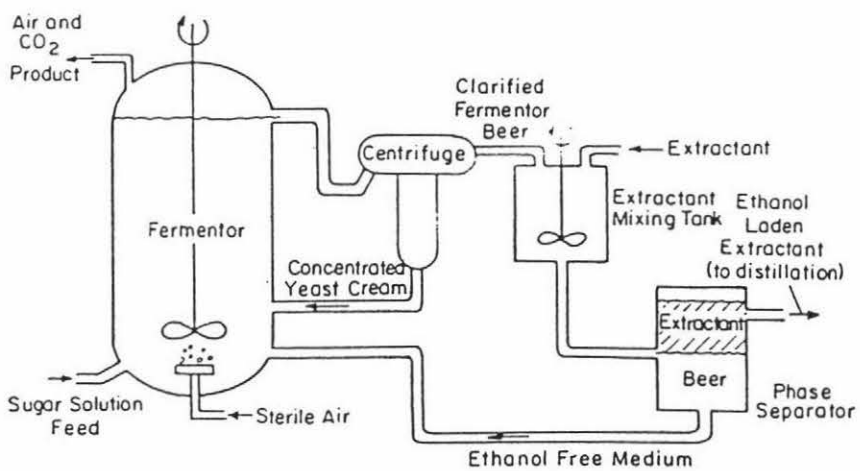
Figure 2.16 shows a schematic diagram of the continuous membrane extractive fermenter. Ethanol diffuses across the membrane into the extractant. This produces an ethanol rich product stream. Energy cost in distillation is reduced. The process is simple with little added equipment. Improvement on membrane design is needed to reduce membrane fouling. The design is not applicable to potable grade alcohol production due to contamination by the extractant.

Figure 2.16 A schematic diagram of continuous membrane extractive fermenter (Maiorella *et al* 1981)



A diagram of the continuous solvent extractive fermenter is given in Figure 2.17. Liquid extractant, immiscible with the beer (clarified fermenter content), absorbs ethanol and thus an ethanol rich stream is produced. However, the fermenter is mechanically complex, a high risk of contamination is expected and there is contamination by the extractant on the product ethanol.

Figure 2.17 Schematic drawing of continuous solvent extractive fermenter (Maiorella *et al* 1981)

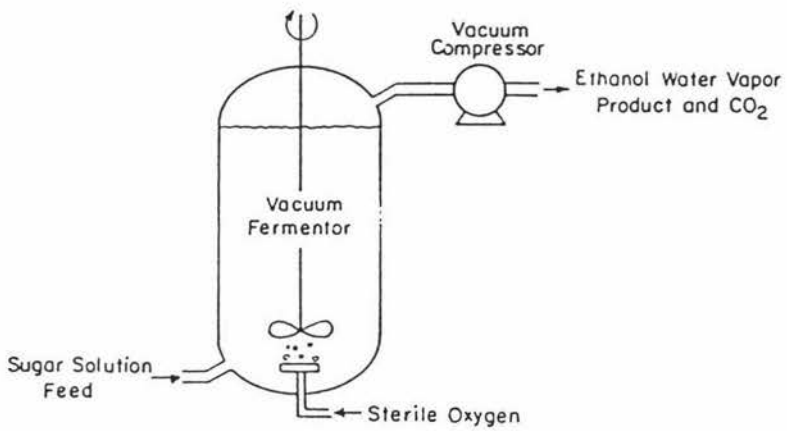


A pervaporation fermenter operates in similar principle as the membrane extractive fermenter. No extractant is used. Instead, the membrane performs the

product separation and facilitates the ethanol diffusion through the membrane. Large membrane area is required for ethanol diffusion. Membrane fouling is also a disadvantage.

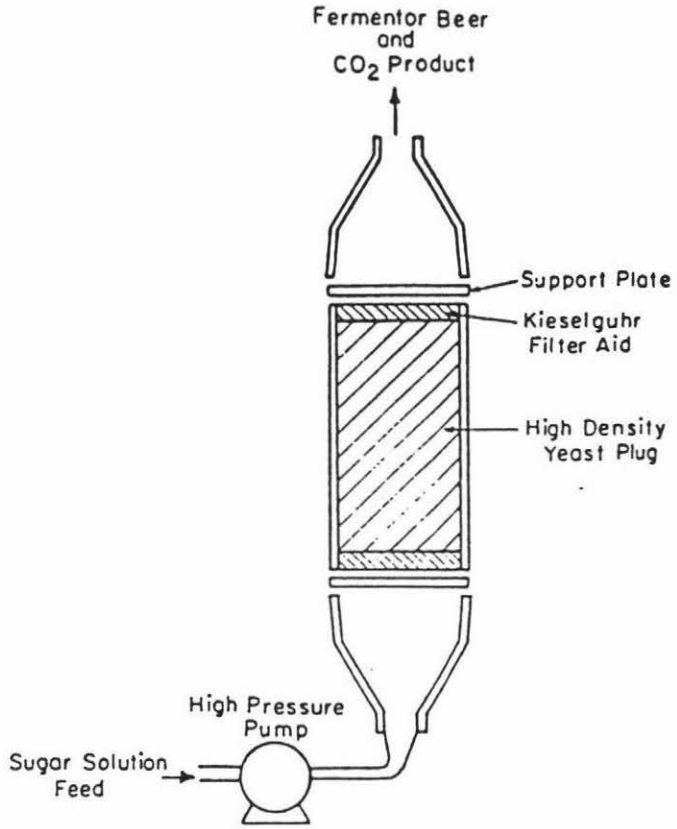
Figure 2.18 shows a diagram of the vacuferm fermenter. The fermentation is conducted under vacuum so the boiling point is reduced and the ethanol boils away. The vapour is collected for further purification. A very large compressor is necessary to operate at the unusually low pressure and capital investment will be high (Maiorella *et al* 1981).

Figure 2.18 Schematic diagram of a vacuferm fermenter (Maiorella *et al* 1981)



A diagram of the plug fermenter is given in Figure 2.19. Kieselguhr filter aid laid over a porous grit support is used to retain the yeast plug. Kieselguhr is also mixed into the yeast plug to prevent dense packing of yeast. A well designed flow distribution system is needed to prevent channelling and methods to prevent plugging such as controlled feed substrate concentration are necessary. Regular shut down is required for yeast regeneration (Maiorella *et al* 1981). The need for high power feed pump is another disadvantage.

Figure 2.19 Schematic diagram of a plug fermenter (Maiorella *et al* 1981)



Maiorella *et al* (1981) reported high productivities for the above fermenters on glucose-ethanol production. There are no reports on their experimental or commercial application in whey-ethanol production.

2.6 Discussion

Overall, more than 14 different fermenter designs have been reviewed with the aim of identifying fermenter systems with improved performance and potential for application to industrial whey-ethanol production. An ideal whey-ethanol fermenter has high productivity, a high product stream ethanol concentration and low effluent lactose level. A batch fermenter fulfills the high ethanol and lactose utilization requirements but with low productivity. The fermenter is mechanically simple and has been well established for commercial scale whey-ethanol production in New Zealand (section 2.4). A summary of performance data on whey permeate of various fermenters is given in Table 2.9. Direct comparison of performance data is impossible as the studies are conducted with different yeast strains, inoculum size and substrate lactose level. Nevertheless, some interesting conclusions can be drawn.

In general, all continuous fermenters showed higher volumetric productivities than batch configurations. The hollow fibre, continuous stirred tank (CSTR)-hollow fibre or CSTR-ultrafiltration fermenters exhibit highest productivities of all the fermenter studied. Relatively high productivity as compared to batch fermenter is still achieved at near complete lactose utilization (e.g. at >99% lactose utilization, productivity is 30 g/l/hr for the hollow fibre, Mehaia & Cheryan 1984). Table 2.10 summarizes the fermentation data on batch, hollow fibre and CSTR-hollow fibre studies on *K. fragilis* NRRL Y-2415. Synthetic medium was used for all the studies.

Table 2.9 Summary of performance data of various fermenters

Model of operation	Lactose conc. g/l	Ethanol conc. g/l	Lactose utilisation %	Productivity g/l/hr	Comment	Ref.
Batch stirred tank	41.4-240	18.6-108.8	76-100	0.6-3.2	NA	(1)-(8)
Continuous stirred tank	NA	NA	NA	3x of batch fermenter	on ethanol from glucose	(9)
Partial recycle	NA	NA	NA	high	on ethanol from glucose	(9)
Tower fermenter	NA	NA	NA	high	on ethanol from glucose	(9)
CSTR-centrifuge	160	71	98	5.7	NA	(10)
Hollow fibre	45	12-22	55->99	30-34	NA	(1)
Continuous dialysis	NA	NA	NA	NA	NA	(9)
Rotor fermenter	NA	NA	NA	high	on ethanol from glucose	(9)
CSTR-hollow fibre	50-150	9.3-40	34-63	24-240	on synthetic medium	(2)
CSTR-UF	45	19	97	80.6	NA	(3)
CSTR-UF	100-120	36.7-58	86.2-100	2.6-7.1	on reconstituted spray dried deproteinized whey powder with lipid supplement	(11)
Batch immobilized	50-200	24-53	NA	0.5-1.5	NA	(12)
Continuous fluidized and packed bed	45-250	4-78.9	NA	1.1-17.2	NA	(8), (12)-(16)
Continuous membrane extractive fermenter	NA	NA	NA	high	on ethanol from glucose	(9)
Continuous solvent extractive fermenter	NA	NA	NA	high	on ethanol from glucose	(9)
Vacuferm	NA	NA	NA	high	on ethanol from glucose	(9)
Plug fermenter	NA	NA	NA	72x of batch stirred tank	on ethanol from glucose	(9)

Key : NA - not available

(1) - Mehaia & Cheryan 1984
(2) - Cheryan & Mehaia 1983
(3) - Cheryan & Mehaia 1985
(4) - Barry 1982
(5) - Sandbach 1981
(6) - Titchener 1982
(7) - Rajagopalan & Kosikowski 1982
(8) - Chen & Zall 1982
(9) - Maiorella *et al* 1981
(10) - Singh *et al* 1983
(11) - Janssens *et al* 1984
(12) - Linko *et al* 1984
(13) - King & Zall 1983
(14) - Linko *et al* 1981
(15) - Hahn-Hagerdal 1985
(16) - Gianetto *et al* 1986

Table 2.10 Fermentation data on batch, continuous hollow, CSTR-hollow fibre studies (K. fragilis NRRL Y-2415, synthetic medium)

Mode of ferment-ation	Inoculum (dry wt. g/l)	Substrate lactose conc. (g/l)	Dilution rate (hr^{-1})	Lactose utilizat-ion %	Ethanol Conc. (g/l)	Volumetric productivity (g/l/hr)	Ref.
Batch	3.8	50	NA	66	15	3.9	(1), (2)
Continu ous-hollow fibre	82	50	4	66	15	60	(2)
CSTR-hollow fibre	3.8	50	1.5	63	16	24	(1)
Batch	2.4	150	NA	63	50	5	(2)
Continu ous-hollow fibre	82	150	6	63	22.5	135	(2)
CSTR-hollow fibre	90	150	6	60	41	240	(1)

Key : (1) - Cheryan & Mehaia 1983 (2) - Mehaia & Cheryan 1984

At 50 g/l lactose, six fold increase in productivity is achieved using a CSTR-hollow fibre fermenter over the batch design. A similar ethanol

concentration was recorded. The CSTR-hollow fibre fermenter also demonstrated a higher volumetric productivity over hollow fibre fermenter. At 150 g/l lactose, an 78% improvement in productivity is recorded over the hollow fibre unit and a higher ethanol concentration was also obtained (Table 2.10). Unequivocable comparisons would not be drawn between the CSTR-hollow fibre and the hollow fibre fermenter at 50 g/l lactose, as the higher inoculum size used in the hollow fibre study would result in much higher productivity value. This also applies to the batch fermenter at 150 g/l, where the inoculum size was 3.8 g/l dry weight. The above studies suggested CSTR-hollow fibre fermenter as capable of providing the highest productivity of the three configurations. Comparable ethanol concentrations could also be reached with similar lactose utilization.

In terms of industrial application, the batch and the continuous stirred tank fermenter have already been applied in the whey-ethanol industry (section 2.4). Partial recycle and the tower fermenters are unsuitable due to the low driving force for natural settling. Very large settlers are required for commercial application and a long initial start-up time is required by the tower fermenter. In a case of contamination, the system would have to be shut down for an extended period of time. So, very large whey permeate storage is necessary. Successful semi-production scale trials on a CSTR-centrifuge fermenter were reported by Reesen & Strube (1978), Fergusson (1980) and Singh *et al* (1983). The design could therefore be employed in industrial scale. However, the economics of this fermenter is affected by the high capital and operating cost of a continuous centrifuge, and the risk of contamination is another potential problem.

The hollow fibre membrane fermenter is not suitable for industrial whey-ethanol production as a very large membrane area is necessary. Capital investment is high due to the high cost of membrane. In contrast, CSTR-membrane design has commercial application potential. Less membrane area is needed as the fermentation takes place in the CSTR and the role of the membrane is for cell recycling. The design is mechanically simpler than the CSTR-centrifuge configuration and scaling up is also easier as addition membrane area can be added by using additional modules.

Industrial application of immobilized cell fermenters appears impractical principally as it is difficult to perform cell immobilization on a commercial

scale. Bead disruption at the bottom of the fermenter is also expected. Continuous solvent extractive fermenter could not be used for potable grade ethanol production due to possible solvent contamination. The large membrane surface area required for the continuous membrane extractive fermenter (Maiorella *et al* 1981) limits their application. Future development of high flux membranes with improvement in anti-fouling properties are necessary for industrial use. The vacuferm fermenter requires a large compressor working at very low pressure. Higher capital cost and difficulties in compressor control are problems for its application. The risk of contamination is also higher. Further study is needed to determine the benefit and operation difficulties of this design. The plug fermenter is relatively simple in operation and its mechanical simplicity makes it potentially suitable for commercial use, but further evaluation is required.

Overall, the CSTR-centrifuge, CSTR-membrane, continuous membrane extractive fermenter, and plug fermenter have potential application in the whey-ethanol production industry. Further studies are necessary to determine their fermentation performances and operating characteristics. A CSTR-microfiltration membrane fermenter was selected for the current study for its much improved productivity, comparable ethanol concentration and potential commercial application. It is relatively easy to scale-up and more suitable for laboratory-scale study. There are also no studies reported on its performance with an industrial yeast strain used in New Zealand or with acid casein whey as substrate.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Microbiological Media

The compositions of media used for culture maintenance and inoculum preparation are given in Tables 3.1 and 3.2. Lactose was obtained from the Lactose Company Ltd (Kapuni, New Zealand) and Bacto yeast extract was obtained from Difco (Detroit, USA). Bacteriological peptone and agar powder were obtained from Oxoid Ltd (Hampshire, England) and Davis Gelatine (NZ) Ltd (Christchurch, New Zealand) respectively. Table 3.3 shows the composition of the sulphuric acid whey permeate powder used in all studies. This was supplied by the New Zealand Dairy Research Institute (Palmerston North, New Zealand). This was reconstituted in distilled water to give lactose concentrations in the range 40-150 g/l.

3.1.2 Chemicals

All chemicals used were of AR grade (analytical reagent) unless otherwise specified.

3.1.3 Gases and other materials

Oxygen-free nitrogen gas was supplied by New Zealand Industrial Gases Ltd (Palmerston North, New Zealand). Pyroneg detergent was supplied by Diversey-Wallace Ltd (Papatoetoe, New Zealand). "Space Maid" granular chlorine (65% calcium hypochlorite) was obtained from Scientific Supplies Ltd (Auckland, New Zealand).

Table 3.1 Composition of slant agar for culture maintenance

Component	Concentration (g/l)
lactose	50
bactopeptone	5
yeast extract	3
agar	20

Table 3.2 Composition of broth for inoculum preparation

Component	Concentration (g/l)
lactose	50
bactopeptone	5
yeast extract	3

Table 3.3 Composition of the deproteinated whey permeate powder for recycle fermentation trials

Component	Concentration
moisture	2.51 % w/w
total nitrogen	0.70 % w/w
non-protein nitrogen	0.51 % w/w
ash	13.1 % w/w
lactose	80.3 % w/w
Ca^{2-}	429 mM/kg
PO_4^{3-}	361 mM/kg
SO_4^{2-}	278 mM/kg
fat	NA*

NA* - not available

3.1.4 Organism

The yeast *Kluyveromyces marxianus* Y-113 was used for all fermentation experiments. The yeast culture was originally supplied by the

New Zealand Distillery Company Ltd (Edgecumbe, New Zealand) and has been maintained in the culture collection of the Biotechnology Department, Massey University (Palmerston North, New Zealand). The yeast was selected because of its proven commercial use.

3.1.5 Recycle fermenter

A schematic diagram of the crossflow-microfiltration recycle fermenter (CMRF) is shown in Figure 3.1. Figure 3.2 is a photograph of the fermentation system. The fermenter used was a Multigen F2000 Benchtop culture apparatus from New Brunswick Scientific Company Inc. (New Jersey, USA) equipped with a 2-L pyrex glass vessel (4). The working volume of the fermenter was 750 ml and the fermenter vessel was fitted with a polyethylene top plate with ports for probe insertion. The fermenter contents were agitated at 500 rpm during the aerobic stage and at 200 rpm in the anaerobic fermentation stage using an indirect magnetic coupling driven 4-blade impeller (3). During the aerobic stage, air was introduced into the vessel via a sparger to promote oxygen transfer. Silicon or Tygon tubing were used for liquid transfer except for the recycle pump suction stream which used PVC tube. The rigidity of the pvc tube provided extra support to prevent tube collapsing due to the pump suction pressure.

A Ceraflo Asymmetric Ceramic Microfilter (10) supplied by the Norton Company (Worchester, USA) was used as the cell separator. Figure 3.3 is a photograph of the disassembled filter. The unit consisted of 28 sintered alumina tubes of internal diameter 2.8 mm with an rated surface pore size of 0.45 μm. The total filter area was 0.1 m^2 and the pressure rating of the unit was 1550 kPa. The dead volume of the filter including holdup in the pipe lines was 250 ml. The recycle pump was a Hoover A.C. motor (7) (type 7407AF18341) rotating at 1425 rpm with a rotary vane type Jabsco pump head (ITT Fluid Handling Ltd., England) delivering a flowrate of 468 l/hr.. The fermenter contents were constantly pumped through the membrane, product stream was removed, and yeast cells were recycled back to the fermenter. The transmembrane flux of the microfilter was controlled by a solenoid valve (Delta, model DV2 124A2, Fluorocarbon, Anaheim, USA) which in turn was controlled by a custom-made level controller. The valve was shut off when the liquid level of the permeate vessel (11) was above or at the target level, and was opened when the liquid level was below the set level.

The fermentation temperature was controlled by an electrical heating element, a cooling finger and a recycle stream cooler (8). The heating element was automatically controlled by the fermenter temperature sensor which was set to 30 ± 1°C. Chilled water supply to the cooling finger and the recycle stream cooler was from a Grant type SU5 portable refrigeration unit, Grant Instruments Ltd (Cambridge, England). The chilled water flow was maintained by a Heidolph type T50 heater/circulator obtained from Grant Instruments Ltd (Cambridge, England).

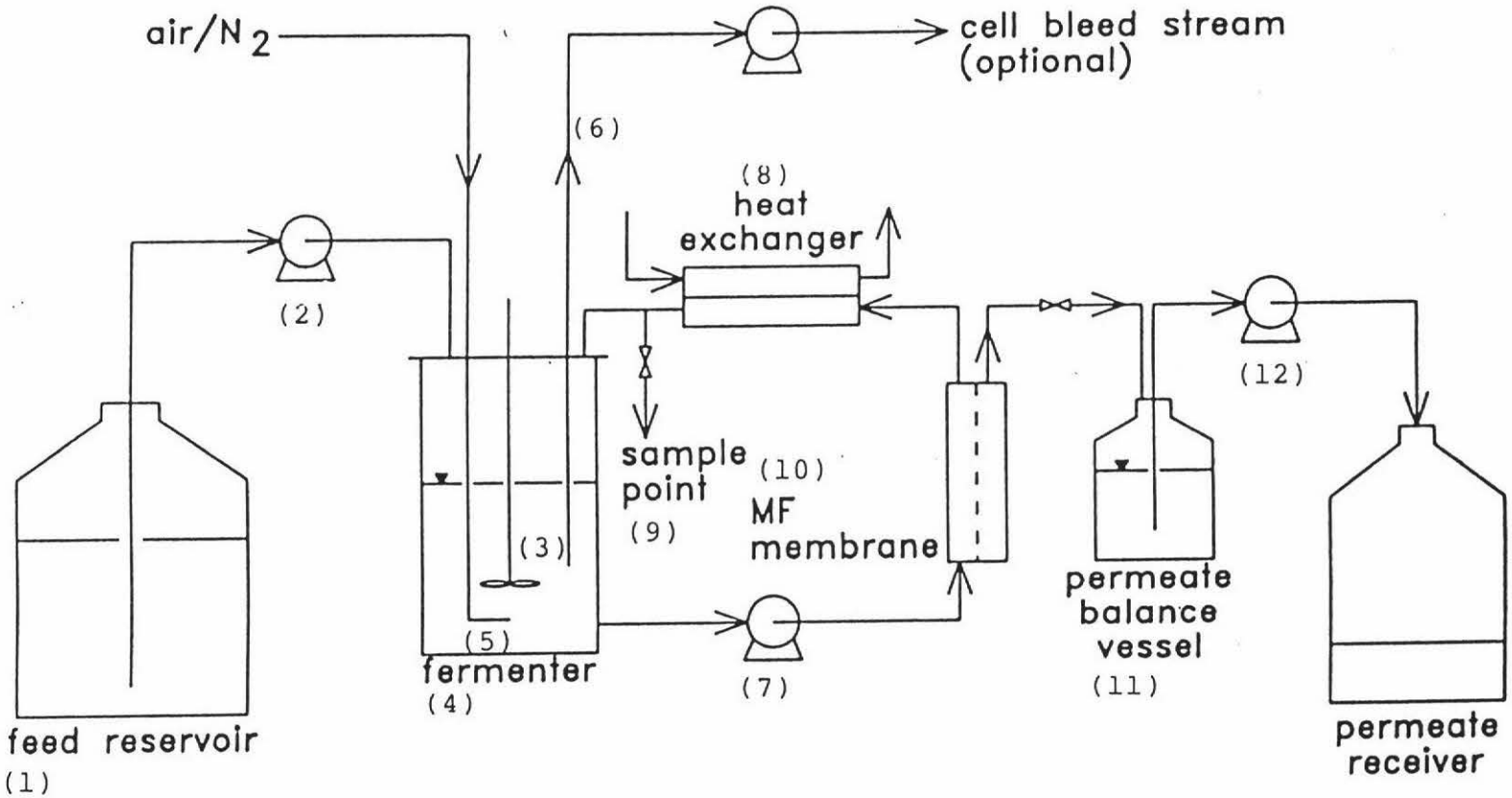
Solid-state controllers made in the Biotechnology Department were used for liquid level control. One controller coupled with the feed permeate pumps maintained the desired fermenter level. The feed pump (2) was a piston pump (micropump series II, F.A. Hughes, England) fitted with a long stroke mechanism and a size 4 pump head. The maximum pressure of the pump was 1723.7 kPa and the nominal stroke frequency was 100/min.. The permeate pump was piston type (12) (series II, Metering Pumps Ltd, England) also with a maximum pressure rating of 1723.7 kPa. The pump was also equipped with a long stroke mechanism and a size 4 pump head operated with a nominal stroke rate of 100/min..

The feed pump (2) was switched on and the permeate pump (12) turned off when the fermenter level dropped below the target level. The reverse control action was implemented when the fermenter liquid level was above the set point. There was also a third controller acting as a safety switch. This controller was used to turn off all the pumps when the permeate vessel liquid level fell below a preset level. This provided a safeguard against pump burn-out in the event of no fluid flow to the pump due to pump failure or blockage.

A bleed stream (6) was used for all steady-state experiments to control the fermenter biomass concentration. A Masterflex peristaltic pump (Cole-Palmer Instrument Co., Chicago, USA) with a 7013 pump head was used for this purpose. The pump was controlled by a solid-state Masterflex controller and was manually adjusted to maintain the desired biomass concentration in the fermenter.

A port (9) situated between the recycle pump exit and the microfilter inlet was provided for sampling. The positive pressure of the pump safeguarded the fermenter against contamination during sampling.

Figure 3.1 Schematic diagram of the recycle fermentation system



Key

(1) - feed vessel (20 L)
(2) - feed pump
(3) - impeller
(4) - fermenter vessel
(5) - air inlet
(6) - bleed stream
(7) - recycle pump
(8) - recycle stream cooler
(9) - sample stream
(10) - ceramic microfilter
(11) - intermediate permeate vessel (1 L conical flask)
(12) - permeate pump

Figure 3.2 A photograph of the recycle fermentation system

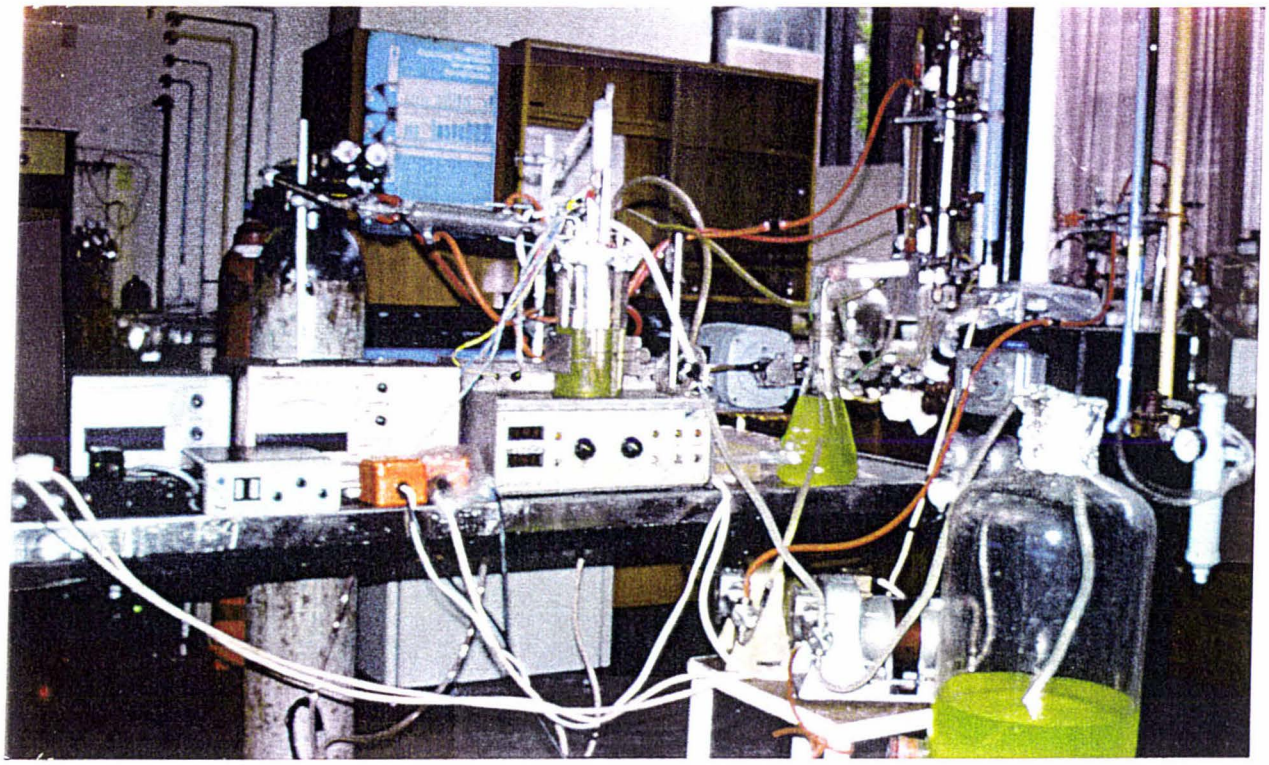
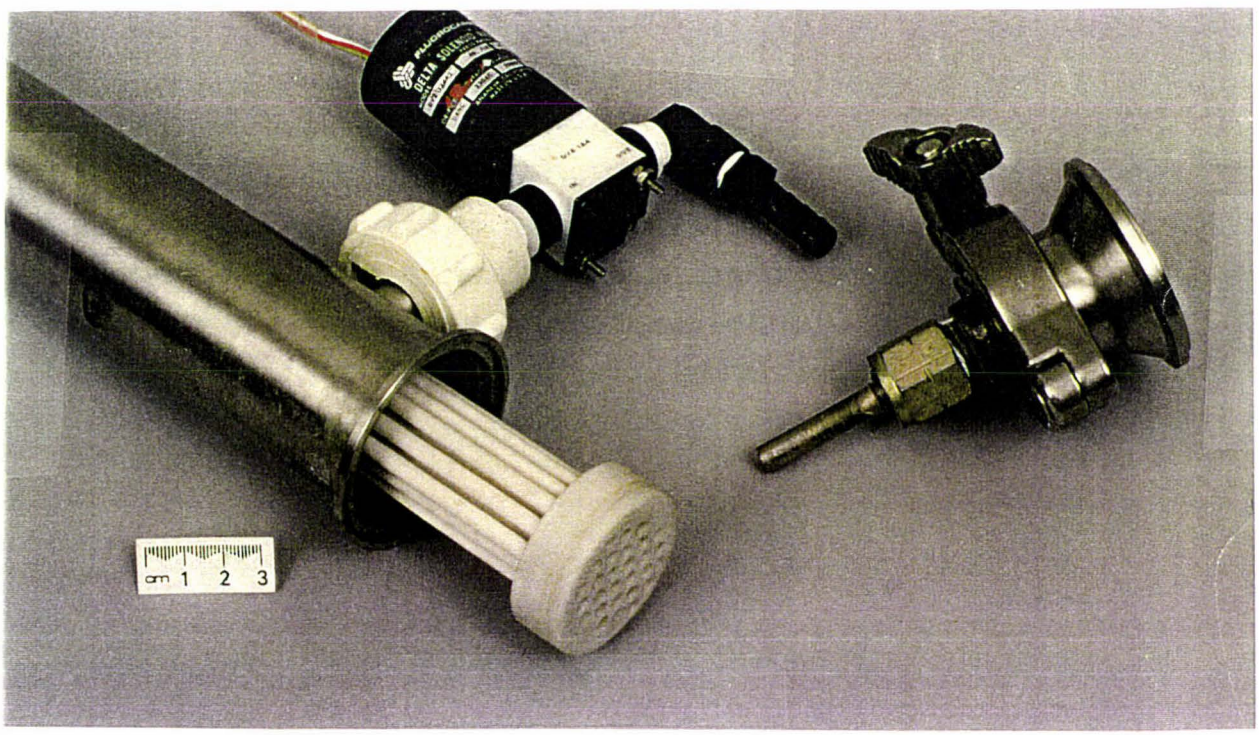


Figure 3.3 Photograph of the Ceraflo Asymmetric Ceramic Microfilter



3.2 Methods

3.2.1 Inoculum preparation

K. marxianus Y-113 was transferred by loop from the stock culture to agar slants of the maintenance medium (Table 3.1). After incubation at 30°C for 24 hours, the agar slope was washed with 1 ml sterile distilled water and the suspension obtained was then used to inoculate 100 ml of culture broth in a 200 ml conical flask. The broth was incubated for 24 hours at 30°C on a shaker (Gyrotory Shaker model G10, New Brunswick Science Company, New Jersey, USA) operated at 200 rpm and was used as the inoculum for the recycle fermentations.

3.2.2 Medium preparation

Media for culture maintenance and inoculum preparation were prepared by dissolving the components (Table 3.1, 3.2) in 1 L of distilled water. The media were then sterilized by autoclaving at 121°C, 20 min..

Reconstituted whey permeate used for all recycle fermentation trials was prepared by dissolving the required amount of whey powder in distilled water. The total amount of whey powder requirement was calculated based on the lactose concentration of the whey powder. The media was not sterilized.

3.2.3 Fermenter cleaning

The fermenter, all transfer tubing, the feed and permeate pump heads were cleaned at the end of each run by soaking in hot Pyroneg solution followed by a hot water rinse. The recycle pump head and the Ceraflo filter were cleaned by recirculating cleaning solutions through the pump head and the filter using the recycle pump. The cleaning scheme was as following :-

1) flush with tap water at room temperature, 10 min.

2) circulate with hypochlorite solution (2 g/l) at room temperature, 5 min.

3) flush with tap water at room temperature, 10 min.

4) circulate with sodium hydroxide solution (10 g/l) at 50-55°C, 10 min.

5) flush with hot water at 50-55°C, 10 min.

6) circulate with hot nitric acid solution (0.3 % v/v) at 40-45°C, 10 min.

7) flush with tap water at room temperature, 10 min.

3.2.4 Fermenter sterilization

The fermenter vessel was disconnected from the recycle pump and the microfiltration unit. All open-ended tubes were covered with aluminium foil and the fermenter was autoclaved at 121°C, 20 minutes. The recycle pump head and the Ceraflo filter together with all attached tubing were sanitized by free-steaming for 10 minutes. Immediately after autoclaving, the fermenter was reconnected to the recycle pump and the filter.

3.2.5 Gas sterilization

Both the air and the nitrogen gas were sterilized by passing through a sterile cotton wool air filter.

3.2.6 Continuous fermentation

A 10 % v/v inoculum was used for all experiments. The fermentation temperature was controlled at 30°C. At startup, the fermenter was manually filled with the inoculum and media to the 1 L level. The fermenter was then run in batch mode with the stirrer speed set at 500 rpm and an air supply of 1 L per minute (~1 vvm) until the biomass concentration reached approximately 6 g dry wt./l. The recycle, feed and permeate pumps were then switched on and the air supply was replaced with nitrogen supplied from gas cylinder at a flowrate of approximately 100 ml/min. to initiate the anaerobic stage. The stirrer speed was set at 200 rpm.

Based on the modified factorial experimental plan (section 3.2.11), the appropriate dilution rates and substrate concentrations were used. Fermenter temperature, pH and stirrer speed were recorded every 1-2 hours. Samples of approximately 20 ml volume were removed from the fermenter through the

sample port at 1 or 2 hour intervals. The sample absorbance at 620 nm and the ethanol concentration were determined immediately. Samples for lactose concentration analysis were batched and stored at -40°C for later analysis.

3.2.7 pH measurement

Samples were removed from the fermenter and the pH measured with an Orion Research model 701A/digital ionalyzer (Orion Research Incorporated, USA). The pH probe was calibrated with commercial pH 4 and 7 buffers prior to use.

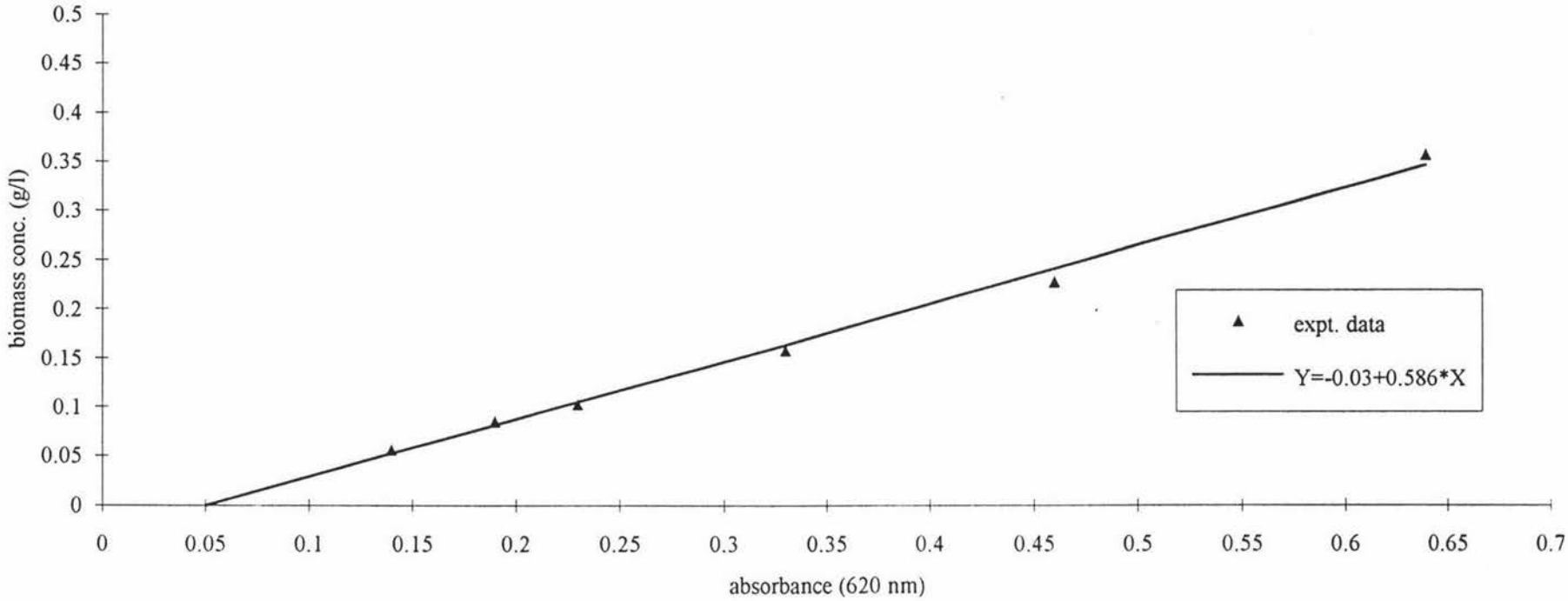
3.2.8 Biomass dry weight measurement

Biomass concentration was determined indirectly from the absorbance at 620 nm. A standard dry weight-absorbance curve was prepared and is shown in Figure 3.4. Raw data of the standard curve are shown in Table A1.1 in the appendix together with details of the statistical analysis. For the dry weight determination, 100 ml samples were placed in aluminium moisture dishes and dried overnight in a 110°C oven. The samples for absorbance determination were first centrifuged at 5000 rpm, 4°C for 4 min. using a Sorvall centrifuge (Sorvall Instruments, DuPont RC5C, SS-34 fixed angle rotor). The pellet was resuspended in distilled water. The sample was then diluted 100 times with distilled water before measurement at 620 nm using a ultraviolet spectrophotometer (model CE 202 with tungsten lamp, Cecil Instruments Ltd., Cambridge, England). The relationship between dry weight and absorbance was calculated at 95% confident level as :-

dry weight = -0.03 + 0.586*absorbance ($\pm$ 0.05* dilution factor)

During fermentation runs, a 20 ml sample was centrifuged for 10 min. at 5000 rpm, 4°C using the Sorvall centrifuge. The supernatant was decanted and retained for ethanol and lactose analyse. The pellet was resuspended in distilled water and appropriate dilutions made to bring the absorbance reading (620 nm) to the 0.1-0.6 range. The biomass concentration was then calculated using the above equation taking into account any dilutions made.

Figure 3.4 Standard curve of biomass concentration (dry wt. g/l) vs absorbance(620nm)



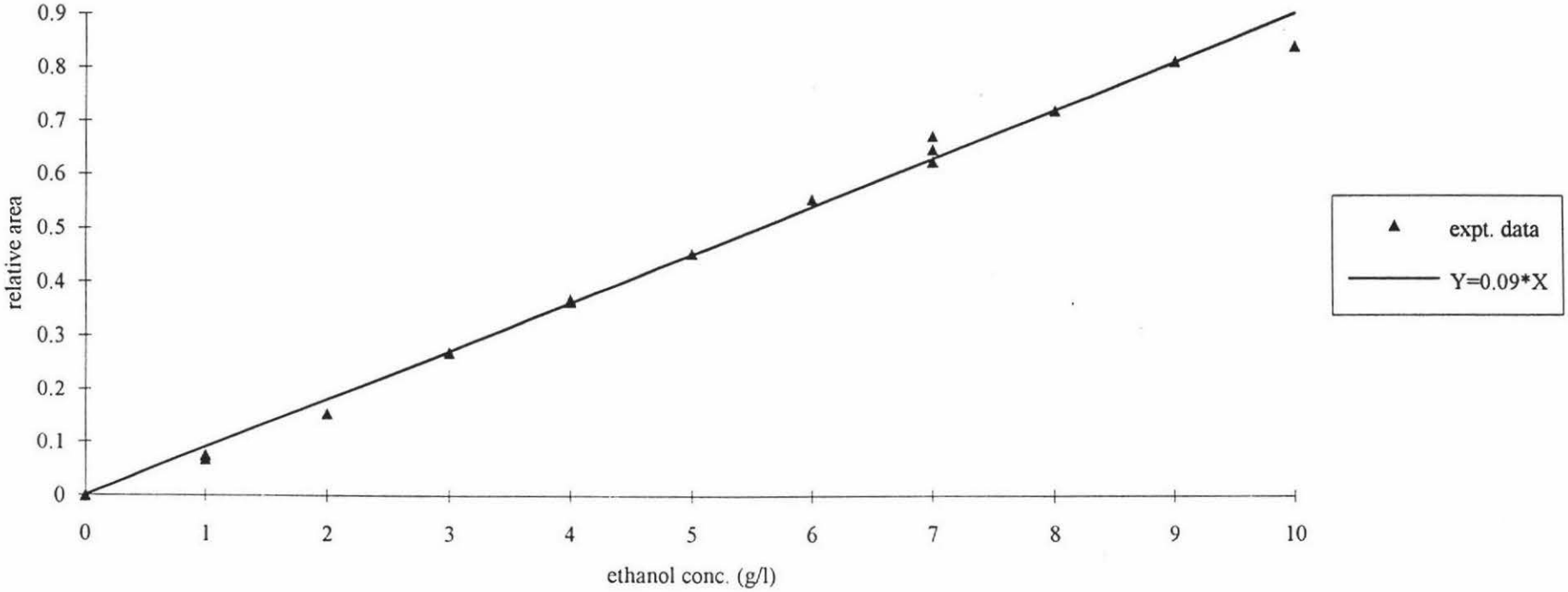
3.2.9 Ethanol concentration determination

Ethanol concentration was determined from relative peak area of the GC spectrum. A standard curve was prepared using AR grade ethanol (95 % w/v) containing the isopropanol (99 % w/v) internal standard. Raw data for the standard curve are shown in Table A1.2 in the Appendix together with the statistical analysis. Figure 3.5 shows a diagram of the standard curve obtained.

Samples for ethanol analysis were first diluted with distilled water to bring the ethanol concentration to the 1-10 g/l range. A Shimadzu GC (Shimadzu Corporation, Kyoto, Japan, Model GC- 8APF) was used with a flame ionization detector. A 1 m x 0.15 cm column containing Porapak Q was used at a carrier gas (nitrogen) flowrate of 60 ml/min. and a column temperature of 180°C. The detector and injector temperatures were 220°C. A sample volume of 2 μl was used and isopropyl alcohol (10 g/l) was added as the internal standard. The sample ethanol concentration was quantified by peak area measurement using a Varian Chromatography data system 111C (Varian Incorporate, California, USA). The peak spectrum was also recorded using a Sekonic SS250F recorder (Japan). At 95% confident level, the equation relating ethanol concentration and relative peak area was :-

ethanol concentration = relative peak area/0.09 (± 1.02)

Figure 3.5 Standard curve of relative area vs ethanol conc. (g/l)



3.2.10 Determination of lactose concentration

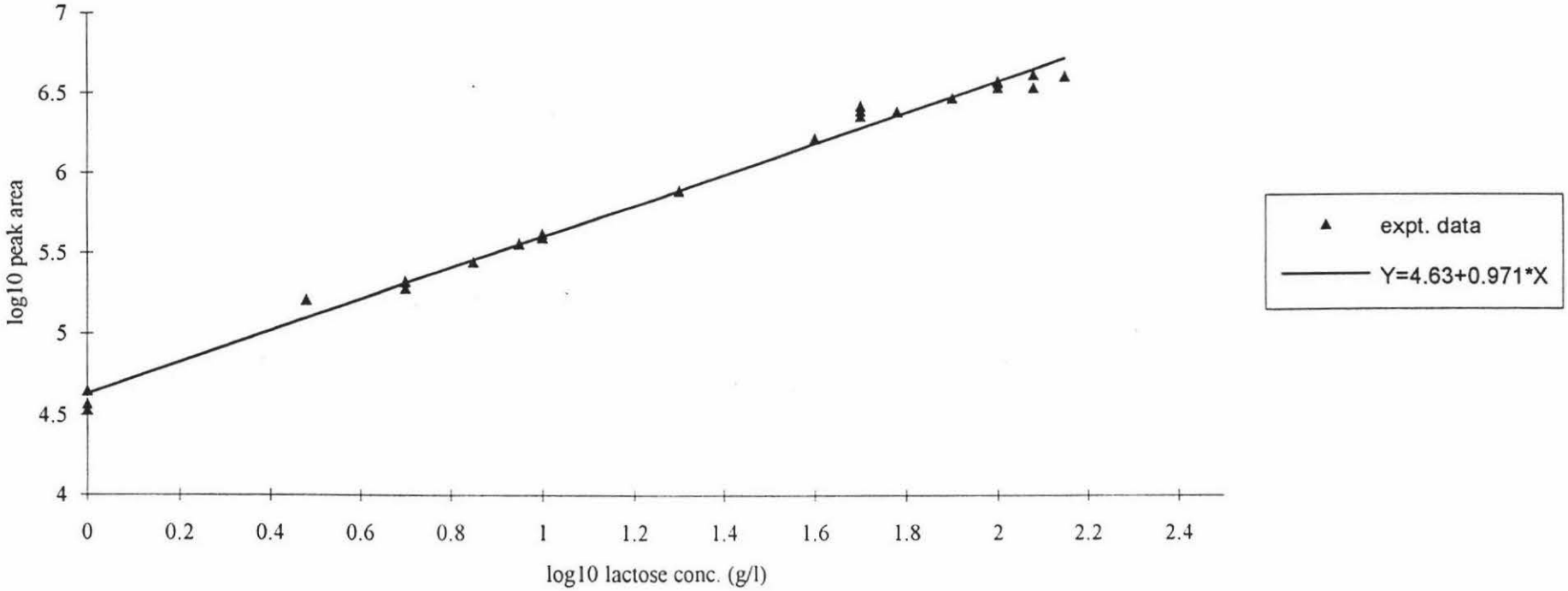
Lactose concentration was determined by high performance liquid chromatography (HPLC). Figure 3.6 shows the standard curve relating peak area and lactose concentration. Raw data of the standard curve and the statistical analysis are shown in Table A1.3 in the Appendix. At the 95% confident level, the equation relating lactose concentration to peak area was :-

$$\log_{10}\text{lactose concentration} = (\log_{10}\text{peak area} - 4.63)/0.971\ (\pm 0.13)$$

The standard curve was prepared using AR grade lactose monohydrate dissolved in distilled water. For all analysis, a 50 μl injection volume was used. For high lactose concentration trials (section 3.2.11), the samples for lactose determination were first diluted 10 times with distilled water prior to analysis to bring the lactose concentration to the range 1-14 g/l. The sample lactose concentration was calculated by peak area measurement using a Waters 740 data module. A liquid chromatograph (Model ALC/GPC 244, Waters Associates Inc., Milford, Massachusetts, USA) was used together with a solvent delivery system (Model 6000A) and septumless injector (Model U6K).

A Sugar-Pak 1 Carbohydrate column (30 cm x 0.65 ID) supplied by Waters Associates was used for the analysis. The detector was a differential refractometer (Model R401). The response was recorded on a CR600 twin-pen, flat-bed chart recorder (J.J. Lloyd Instruments Limited, Southhampton, England). A filtered (0.45 μm filter, Millipore Corporation, Massachusetts, USA) and degassed calcium acetate solution (20 mg/l) prepared using distilled water was used as the mobile phase. The column was operated at 90°C and at a solvent flowrate of 0.5 ml/min.

Figure 3.6 Standard curve of log10 peak area vs log10 lactose conc. (g/l)



3.2.11 Experimental plan

An experimental plan was designed to fulfill the following objectives :-

1) To investigate the application of new technologies
2) To develop a mathematical model for fermentation process simulation

Evaluation of this new technology required the performance data of the CMRF to be compared with other reported designs. Table 2.9 (section 2.6) shows a summary of fermentation data for various fermenters. Most of the studies were performed at 40-150 g/l lactose, although also some studies were carried out at >150 g/l lactose. However, preliminary studies (not reported) showed that the whey powder became very difficult to completely dissolve at above 150 g/l lactose. Lactose concentrations at 40-150 g/l were therefore chosen for the study. For the dilution rate, preliminary studies (not reported) demonstrated that dilution rate higher than 1.5 hr^{-1} resulted in filter blockage in a relatively short period (<12 hr.). Therefore a dilution rate of 1.3 hr^{-1} was selected as the high dilution rate; the low dilution rate was arbitrarily set at 0.4 hr^{-1}.

The experimental plan also supported the development of a mathematical model for preliminary financial study. Factorial experiments with 2 levels enables a linear model to be constructed. This model will be useful for simulating fermenter performance for operating conditions within the high and low level conditions studied. The underlying assumption that it is a linear relationship between the parameters studied and the effects measured is justifiable in the context of a preliminary study.

A modified 2x2 factorial experimental plan was used. Table 3.4 shows a standard factorial plan for two effects. The modified plan is shown in Table 3.5. This comprised of six unsteady-state experiments and two steady-state runs at selected dilution rates and lactose concentrations. Unsteady-state experiments were conducted without bleeding of biomass, i.e. the yeast concentration was allowed to increase during the course of the experiment. For steady-state experiments, the biomass concentration was controlled at approximately 10 g/l by the use of a bleed stream. Four of the six unsteady-state experiments were designed to estimate the maximum specific growth rate

(μ_m), biomass yield coefficient (Y_{xs}) and the product biomass yield coefficient (Y_{px}). The remaining two unsteady-state runs were used to check the accuracy of the fermentation model. The design used a low substrate concentration (approximately 50 g/l) and a mid-level dilution rate (approximately 0.9 hr^{-1}) for these two validation runs. The former condition was selected because the lactose level is similar to the natural whey permeate lactose concentration (approximately 50 g/l) which is utilized by the whey-ethanol distilleries. The mid-level dilution rate was chosen as it is necessary to check the fermentation model at conditions other than those used to estimate the fermentation model parameters. The two steady-state trials were established for comparison with the unsteady-state runs and to observe the effect of operation under stable conditions.

As the behaviour of the yeast in the CMRF was unknown, the runs were not randomized. Instead, the runs with the most extreme conditions (e.g. high dilution rate) were carried out first. This served the purpose of guarding against system failure at a later stage.

Table 3.4 A standard 2X2 factorial experimental plan

Run	Lactose conc.	Dilution rate
1	+	+
2	-	+
3	+	-
4	-	-

Key :- +,- - coded level = (x-mid.pt.)/(1/2*range)

Table 3.5 A modified 2X2 factorial experimental plan

Run	Lactose conc. (g/l)	Lactose conc. (coded level)	Dilution rate (hr^{-1})	Dilution rate (coded level)	Steady state
1	40	-	1.3	+	N
2	150	+	0.4	-	N
3	40	-	0.4	-	N
4	150	+	1.3	+	N
5	40	-	0.4	-	Y
6	150	+	0.4	-	Y
7	40	-	0.9	0	N
8	50	-	0.9	0	N

Key :- +,-,0 - coded level = (x-mid.pt.)/(1/2*range) (+ high , - low , 0 mid. pt.)

Y,N - with or without steady state

3.2.12 Statistical analysis

Non-linear regression was performed using the computer package BMDP (California, USA) available on the Prime 750 mainframe computer. Minitab (Minitab Inc., USA) was used to perform linear regression analysis on the Unix network with a Philips IBM- compatible 386 based computer.

CHAPTER 4

FERMENTATIONS : RESULTS AND DISCUSSION

4.1 Continuous fermentation results

4.1.1 Introduction

Four unsteady state runs (1-4) and two steady state runs (5-6) were carried out. Lactose concentrations of 46-137 g/l and dilution rates at 0.34-1.3 hr^{-1} were studied as summarized in Table 4.1. The data shown are experimental data and show slight deviation from the nominal value as described in section 3.2.11.

Table 4.1 Summary of the operating conditions for run 1-6

Run	Lactose conc. (g/l)	Dilution rate (hr^{-1})	Mode of operation
1	46	1.3	unsteady state
2	132	0.47	unsteady state
3	53	0.44	unsteady state
4	137	1.15	unsteady state
5	64	0.34	steady state
6	110	0.43	steady state

All runs were controlled at 30°C and used a 10% inoculum. The unsteady state runs were operated with 100% cell recycle. The steady state trials were operated with a cell bleed stream to maintain a constant biomass concentration of 10 g/l dry weight. All fermentations were operated until the membrane flux was insufficient to maintain the dilution rate or a steady state condition (with reference to biomass concentration) was achieved

Samples were taken every one or two hours for analysis of biomass, lactose and ethanol concentrations; pH and temperature were also recorded. The data were then fitted with a logistic equation in the form :-

$$Y = p_1/(1+e^{p_2+p_3*t})$$

where Y = dependent variable

t = time

p_1, p_2, p_3 = constant terms

A sample print out of the non-linear regression analysis is shown in the Appendix. The specific growth rate (μ), biomass yield coefficient (Y_{xs}), product biomass yield coefficient (Y_{px}) and product substrate yield coefficient (Y_{ps}) were then calculated based on the fitted parameters. Details of the calculation procedure is available in the Appendix.

4.1.2 Unsteady state runs (1-4)

The biomass, lactose and ethanol concentration time profiles are shown in Figures 4.1-4.4. All the runs showed a similar trend of decreasing substrate concentration with time, while biomass and ethanol concentration increased. A transient period was also observed for runs 1-3 where the lactose concentration increased for 2-4 hours before following a decreasing trend. The highest ethanol concentration obtained was 21.6 g/l for run 2 at t = 44 hours. The maximum ethanol productivity was 13.7 g/l.hr. (run 1, t = 12 hours) and near complete lactose utilization (91.6%) was achieved with run 3 at t = 23 hours (Table 4.2).

As the final biomass concentration achieved in each unsteady state run differed (X_f, Table 4.2), the performance of runs were also compared at a constant biomass concentration of 10 g/l i.e. the value later used for steady state operation. This data is shown in Table 4.3.

The inability to maintain sufficient membrane flux over long period was a major problem and often resulted in short fermentation runs. Blockage of the air filter also resulted in some loss of fermenter contents in run 1. The fermentation run was repeated. Scattering of the lactose concentration data was also noticed and needs to be addressed (e.g. Figure 4.4).

Figure 4.1 Plot of biomass, lactose, ethanol concentration (g/l) vs time (hr.) for run 1

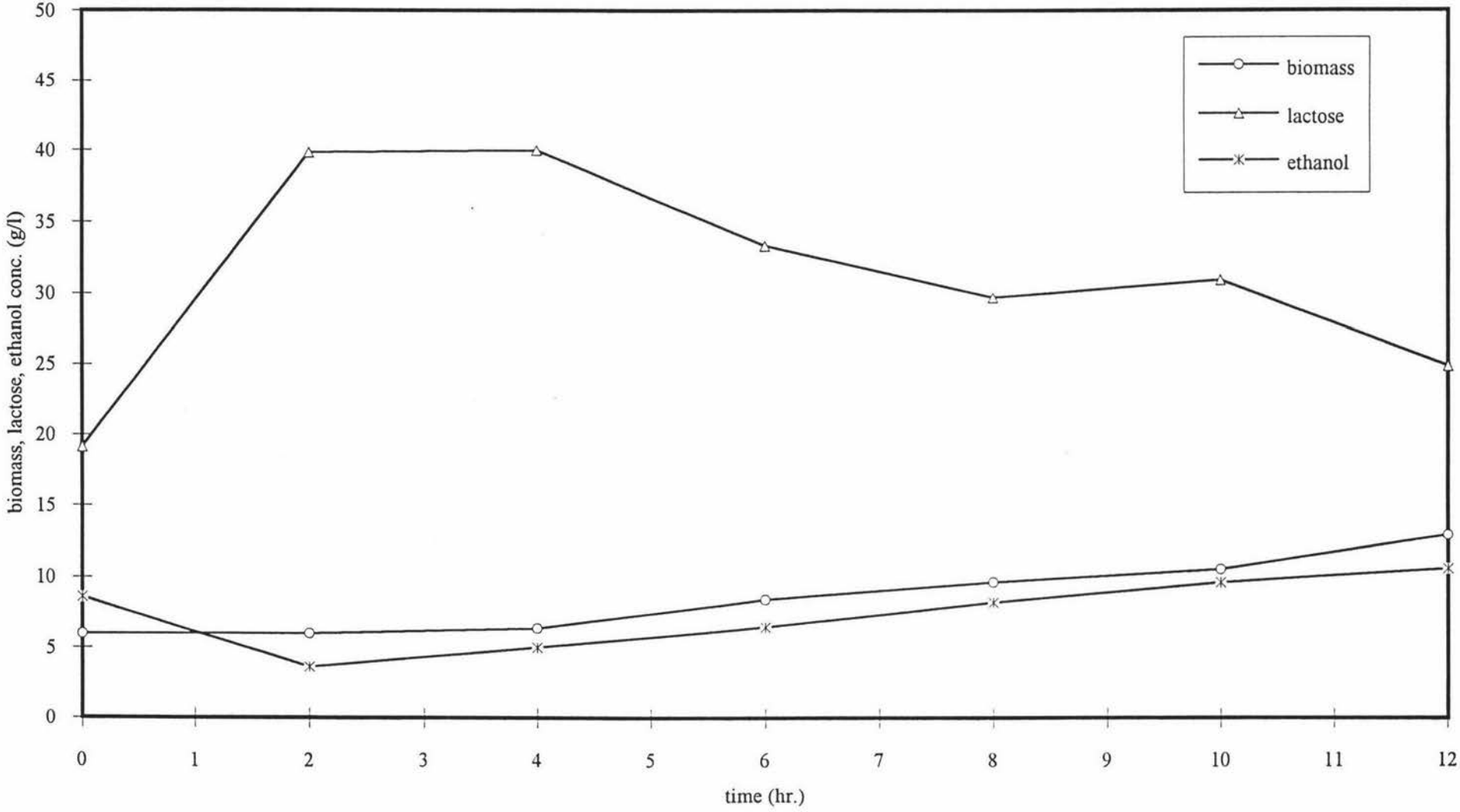


Figure 4.2 Plot of biomass, lactose, ethanol concentration (g/l) vs time (hr.) for run 2

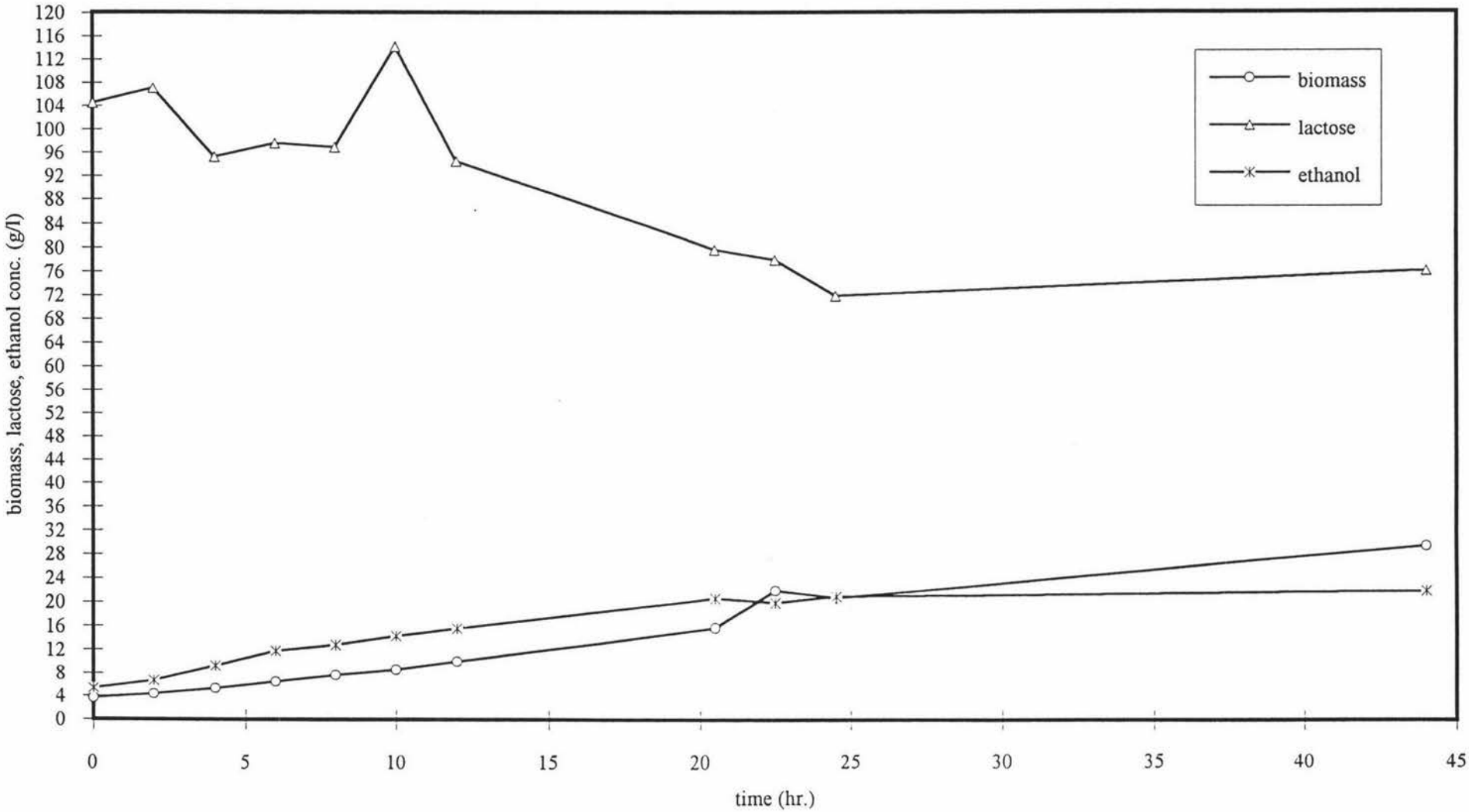


Figure 4.3 Plot of biomass, lactose, ethanol concentration (g/l) vs time (hr.) for run 3

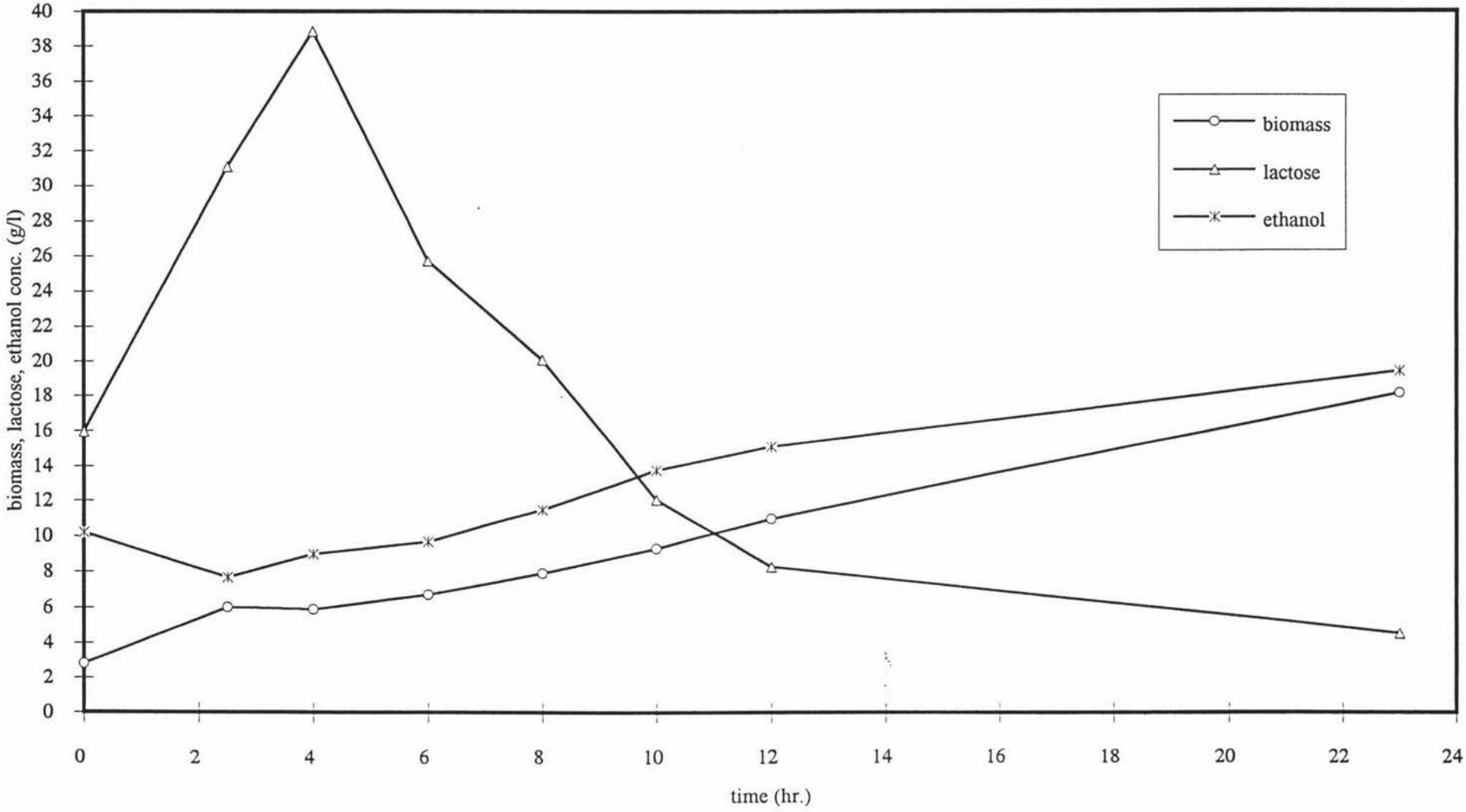


Figure 4.4 Plot of biomass, lactose, ethanol concentration (g/l) vs time (hr.) for run 4

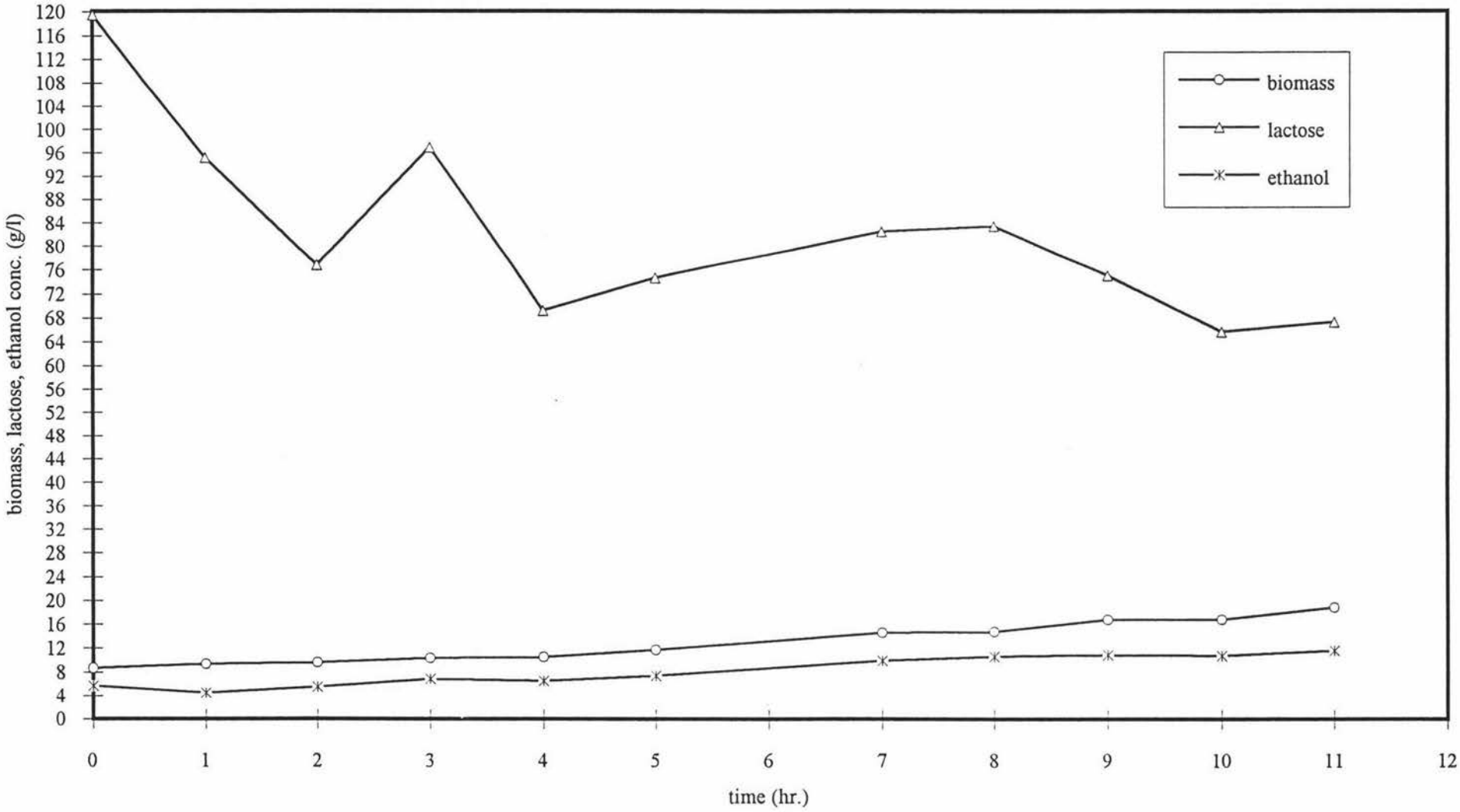


Table 4.2 Summary of fermentation performance parameters (unsteady state run 1-4, at end of fermentation run)

Run	S_0 (g/l)	D (hr^{-1})	X_f (dry wt. g/l)	E_f (g/l)	Y_{px}	Y_{xs}	Y_{ps}	Fermentation efficiency (%)	Lactose utilization (%)	Ethanol productivity (g/l.hr.)	μ (hr^{-1})
1	46	1.3	12.9	10.5	15.6	0.036	0.56	104	46	13.7	0.074
2	132	0.47	29.2	21.6	68.8	0.005	0.35	64	43	10.1	0.005
3	53	0.44	18.2	19.5	15.9	0.002	0.39	72	92	8.6	0.03
4	137	1.15	18.8	11.5	11.5	0.016	0.18	38	51	13.3	0.065

Key : S_0 - feed lactose concentration
D - dilution rate
X_f - final biomass concentration
E_f - final ethanol concentration
Y_{px} - product biomass yield coefficient
Y_{xs} - biomass substrate yield coefficient
Y_{ps} - product substrate yield coefficient
μ - specific growth rate

Table 4.3 Summary of fermentation parameters for unsteady state run (runs 1-4) at 10 g/l biomass

Run	S_0 (g/l)	D (hr^{-1})	X (g/l)	E (g/l)	Y_{px}	Y_{xs}	Y_{ps}	Fermentation efficiency (%)	Lactose utilization (%)	Ethanol productivity (g/l.hr.)	μ (hr^{-1})
1	46	1.3	9.6	8.2	16.37	0.039	0.65	120	35	10.6	0.075
2	132	0.47	10.1	15.6	9.16	0.051	0.47	86	28	7.3	0.078
3	53	0.44	9.3	13.8	9.56	0.045	0.43	80	77	6.1	0.074
4	137	1.15	10.3	6.9	9.91	0.013	0.13	23	29	7.9	0.081

Key : S_0 - feed lactose concentration

D - dilution rate

X - biomass concentration

E - ethanol concentration at 10 g/l biomass

Y_{px} - product biomass yield coefficient

Y_{xs} - biomass substrate yield coefficient

Y_{ps} - product substrate yield coefficient

μ - specific growth rate

4.1.3 Steady state runs (5-6)

Concentration plots of biomass, lactose and ethanol concentration showed similar trends (Figure 4.5-4.6). There was an initial transient period where biomass and lactose concentrations increased and ethanol concentration decreased with time. This was followed by an unsteady state period where biomass accumulated to 10 g/l. The lactose concentration decreased and ethanol concentration increased during this phase, and all the concentrations then stabilised. The steady state periods were estimated to be at t = 30.5-38.5 hours for run 5 and t = 11-15 hours for run 6. The highest steady state ethanol concentration (17.6 g/l) and lactose utilization (80.7%) were achieved with run 5 (Table 4.4).

Figure 4.5 Plot of biomass, lactose, ethanol concentration (g/l) vs time (hr.) for run 5

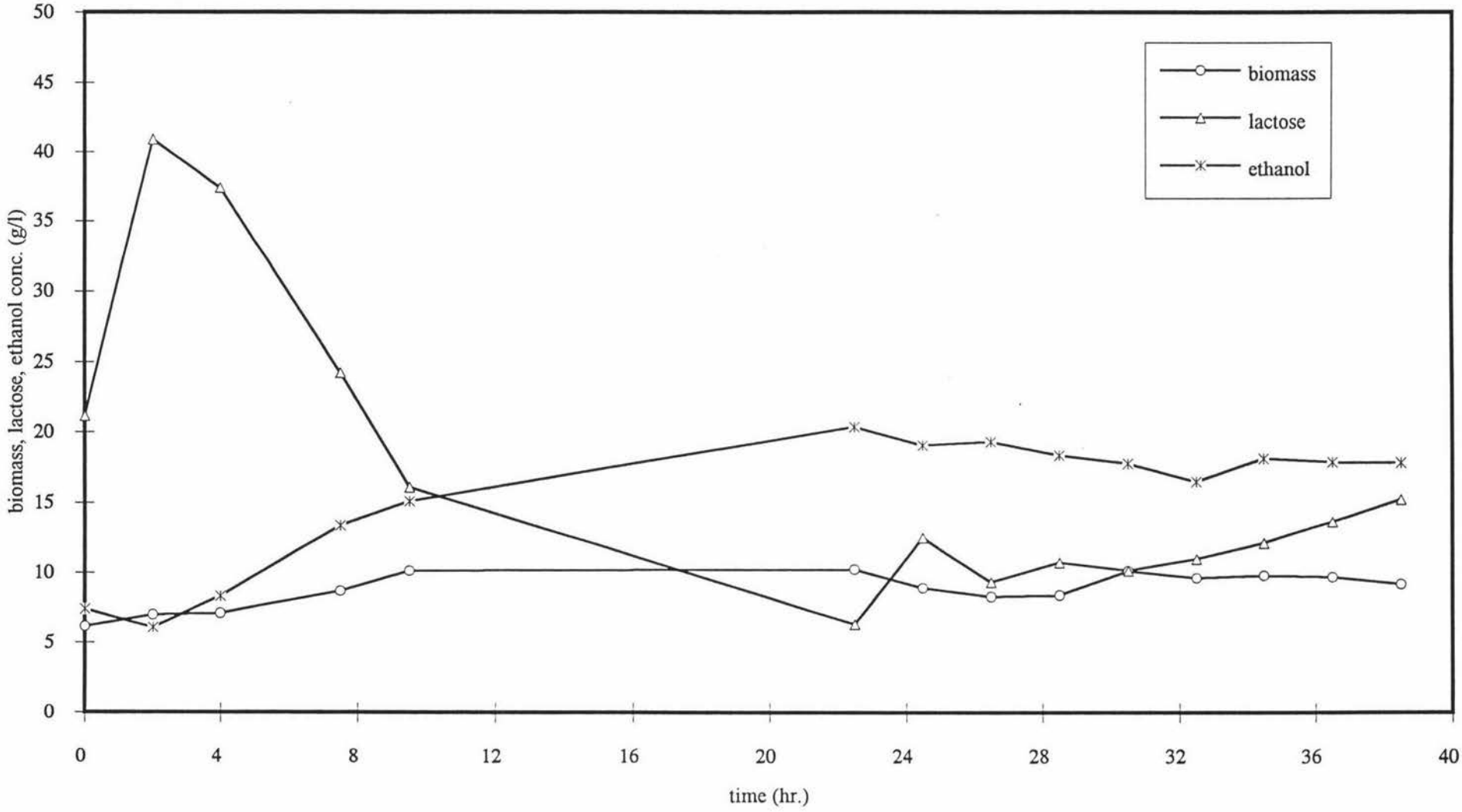


Figure 4.6 Plot of biomass, lactose, ethanol concentration (g/l) vs time (hr.) for run 6

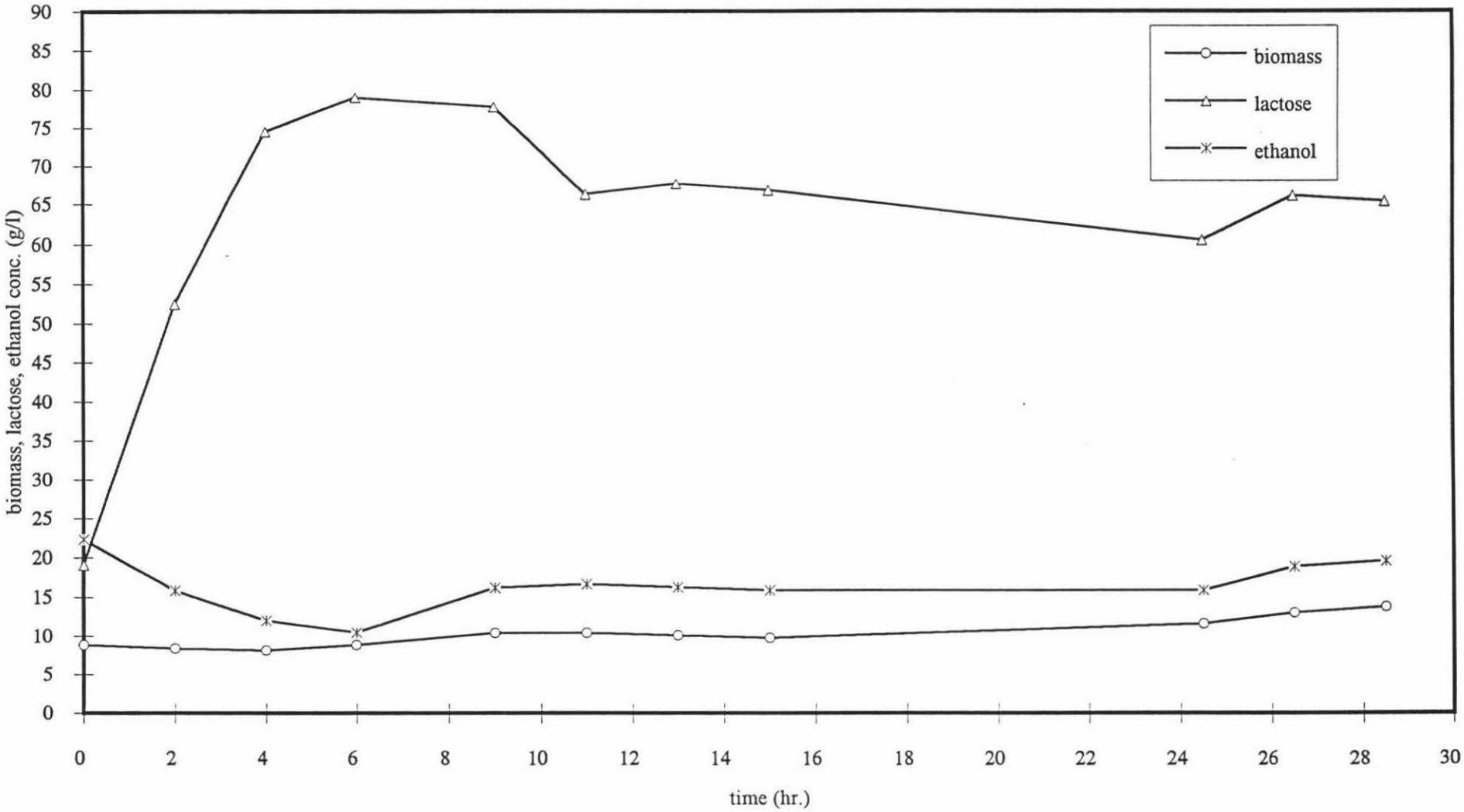


Table 4.4 Summary of fermentation performance parameters (steady state run 5-6)

Run	S_0	D (hr^{-1})	S_{ss} (g/l)	X_{ss} (g/l)	E_{ss} (g/l)	Y_{px}	Y_{xs}	Y_{ps}	Fermentation efficiency (%)	Lactose utilization (%)	Ethanol productivity (g/l.hr.)
5	64	0.34	12.4	9.7	17.6	1.82	0.188	0.34	63.6	81	6.0
6	110	0.43	66.9	12.1	16.3	1.61	0.235	0.38	70.3	39	7.0

Key : S_o - feed lactose concentration

D - dilution rate

S_{ss} - steady state lactose concentration

X_{ss} - steady state biomass concentration

E_{ss} - steady state ethanol concentration

Y_{px} - product biomass yield coefficient

Y_{xs} - biomass substrate yield coefficient

Y_{ps} - product substrate yield coefficient

4.2 Discussion

The performance of the CMRF (crossflow-microfiltration recycle fermenter) was investigated at a range of inlet lactose concentrations and dilution rates under both steady state and unsteady state conditions. Of particular interest were to compare the performance of the reactor with both batch and other intensive continuous fermentation systems, to compare the performance of acid whey permeate with other whey streams, and to note any operational problems.

The CMRF demonstrated improved ethanol productivity over batch fermenters. At a dilution rate of 0.44 hr^{-1} and feed lactose concentration of 53 g/l, a productivity of 8.6 g/l.hr. was achieved (Table 4.2). This is 9.5 times of the value obtained with batch fermentation using the same yeast strain at 50 g/l lactose in media supplemented with yeast extract (Mawson & Taylor 1989). At a dilution rate of 1.3 hr^{-1} and feed lactose concentration of 46 g/l, the CMRF achieved a productivity of 13.7 g/l.hr. (Table 4.2). As mentioned in section 2.3.4, improvement in ethanol production has been observed with yeast extract supplementation. Early cessation of growth and low biomass yield could be overcome by yeast extract supplementation and production were increased (section 2.3.4). The ethanol productivity with the CMRF could therefore be much higher with yeast extract supplemented medium.

The performance of the fermenter during unsteady state operation is summarized in Tables 4.2 and 4.3. From Table 4.2, at the highest productivity level (run 1, 13.7 g/l.hr.), low lactose utilization (45.9%) and ethanol concentration (10.5 g/l) were obtained. This is similar to, but less favourable than, the hollow fibre and CSTR-hollow fibre fermenters (15-16 g/l ethanol, 63-66% lactose utilization, Table 2.10). While this may indicate the CMRF is less favourable, the lower performance could be due to yeast strain differences. Fermentation studies with the hollow fibre and the CSTR-hollow fibre fermenter using *K. marxianus* Y-113 is necessary to confirm how the configuration effects fermentation performance.

When the runs were compared at the biomass concentration of 10 g/l (Table 4.3), the following trends were noted : decreasing the lactose concentration (runs 2 and 3) increased the lactose utilization by 180% but at the expense of a small decrease in ethanol productivity and ethanol concentration.

Raising the dilution rate at the higher feed lactose concentration (run 2 and 4) did not significantly alter lactose utilization or ethanol productivity but gave a much lower ethanol concentration in the product stream. At the lower lactose concentration, increasing dilution rate decreased both lactose utilization and ethanol concentration, but overall productivity was significantly increased. Unacceptably high fermentation efficiency value (104%) was recorded for run 1. This could be due to errors in lactose and ethanol concentration measurement. Both the lactose utilization and the product concentration play an important role in the whey ethanol economy : lower lactose utilization would increase waste treatment costs, while much more energy is required to recover the ethanol from a diluted product stream. A model accurately predicting the lactose utilization and ethanol concentration would be needed for process optimization. Such a model study is discussed further in Section 5.1.

The estimated specific growth rate were similar for all four runs at 10 g/l biomass (0.074-0.081 hr^{-1}). Thus growth rate was not significantly affected by the substrate concentration and dilution rate under the conditions tested. The product substrate yield coefficient is independent of the lactose concentration at the lower level (runs 2 and 3) but is affected significantly by the dilution rate (runs 2 and 4, 1 and 3). This particularly contradictory to reports of decreasing product yield with increasing lactose level in batch fermentation studies (Mawson and Taylor 1989) and indicates the more complex behaviour of cells in continuous recycle systems. Increasing the dilution rate decreased the biomass yield coefficient (runs 2 and 4, 1 and 3), which in turn caused the drop in product substrate yield coefficient. Further runs at different substrate level and dilution rates are needed to provide better understanding on the effects of these parameters on yield coefficients, as the latter is an important predictor for the fermenter model.

Scattering of the lactose concentration data was recorded (e.g. Figure 4.4). Its effect on the lactose utilization was examined by comparing the predicted lactose utilization using the predicted (simulated) lactose concentration (Table 4.5). At a biomass level of 10 g/l, a much lower lactose utilization was predicted with the logistic equation for run 1, while a higher lactose utilization was predicted for run 4 (Table 4.5). The predicted lactose utilization for runs 2-3 were relatively close to the experimental value. The experimental and predicted ethanol concentrations show good agreement for all the runs.

Table 4.5 Summary of predicted and experimental fermentation parameters (unsteady state, at 10 g/l biomass)

Run	X (g/l)	S_{te} (g/l)	S_{tp} (g/l)	E_{te} (g/l)	E_{tp} (g/l)	Productivity$_e$ (g/l.hr.)	Productivity$_p$ (g/l.hr.)	Lactose utilization$_e$ (%)	Lactose utilization$_p$ (%)
1	9.6	29.7	31.3	8.2	8.2	10.6	10.7	89	32
2	10.1	94.6	92.5	15.6	16.0	7.3	7.5	28	30
3	9.3	12.1	12.1	13.8	13.3	6.1	5.9	77	77
4	10.3	97.0	80.9	6.9	6.3	7.9	7.2	29	41

Key : X - biomass

S_{te} - lactose conc. (experimental value)

S_{tp} - lactose conc. (predicted value)

E_{te} - ethanol conc. (experimental value)

E_{tp} - ethanol conc. (predicted value)

Productivity$_e$ - ethanol productivity (experimental value)

Productivity$_p$ - ethanol productivity (predicted value)

Lactose utilization$_e$ - lactose utilization (experimental value)

Lactose utilization$_p$ - lactose utilization (predicted value)

Further experiments should be carried out repeating the conditions of runs 1 and 4 for better estimation of the lactose utilization. However prior to this, the accuracy of various lactose assay methods such as colorimetric, enzymatic and HPLC analysis should be examined for selection of a more robust method.

Insufficient membrane flux and air filter blockage were some of the difficulties noted in the fermenter operation. Incorporation of a back-flushing mechanism with a dual membrane system is one possibility to improve on the fermenter operation. With backflushing, a longer fermentation operation is possible by periodic removal of the fouling layer by passing permeate back through the filter membrane. A dual membrane system would allow one membrane to be cleaned while the other is in operation. Such changes should permit prolonged operation, allowing the long-term performance of the CMRF to be evaluated. Larger head space and better foam control could lower the risk of filter blockage by foaming. This design should be adopted to improve on the fermentation operation.

The steady state runs show close agreement to the respective unsteady state experiments when compared at a biomass concentration of 10 g/l (Table 4.6). The productivity for runs 3 and 5 varied by only 3%, and runs 2 and 6 also demonstrated similar volumetric productivities with a 5% difference between the two values. Higher ethanol concentration and lactose utilization was observed for the steady state experiment. The fermenter could, therefore, be operated in steady state mode without significant loss in performance. The steady state runs also reconfirmed the characteristic increase in lactose utilization with decreasing lactose concentration.

Table 4.6 Summary of fermentation parameters
(unsteady state run 2, 3 and steady state run 5, 6)

Run	S_0 (g/l)	D (hr^{-1})	Ethanol (g/l)	Productivity (g/l.hr)	Lactose utilization (%)	Fermentation efficiency (%)
5	64	0.34	17.6	5.9	81	44
3	53	0.44	13.8	6.1	77	80
6	110	0.43	16.3	7.0	39	73
2	132	0.47	15.6	7.3	28	86

Key : S_0 - feed substrate conc. D - dilution rate

4.3 Conclusions and recommendations

Superior performance as measured by ethanol productivity was obtained with the CMRF (crossflow-microfiltration recycle fermenter) over the batch configuration. The operating parameters (dilution rate, lactose concentration) had considerable influence over the performance of the yeast strain. The effects of dilution rate on the biomass yield coefficient and the product substrate yeild coefficient were demonstrated. Adequate performance in terms of high lactose utilization and high productivity was achieved at a dilution rate of 0.44 hr^{-1} for an initial lactose concentration of 53 g/l. Under these conditions the productivity was 8.6 g/l.hr. Overall, a model should be developed to evaluate the effects of different operating conditions and for process optimization, and this is addressed in the following chapter. Further study on effects of various parameters on yield coefficients is also necessary.

Similar productivity values were obtained with the steady state and unsteady state experiments when compared at 10 g/l biomass. It is concluded that the fermenter could be operated in the steady state mode without significant loss in performance.

It is concluded that the CMRF has potential application in whey-ethanol fermentation for its much improved productivity over batch fermenter. The major problem with the configuration is insufficient membrane flux. Backflushing combined with a dual membrane design is a potential solution to this problem.

CHAPTER 5

PROCESS MODELLING

5.1 Introduction

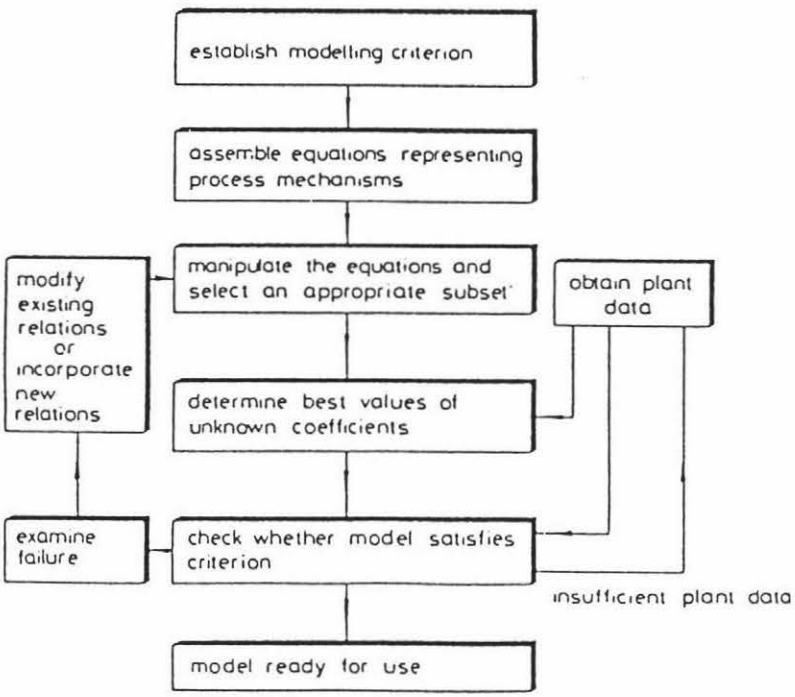
Process modelling is one of the most important tools in process design. With the help of high-speed large-memory computers, the response of a process to different forcing functions can easily be obtained. Selection of optimum operating conditions such as the dilution rate or feed lactose concentration which affect the process economy can then be carried out. Controllability and the stability of the process can also be assessed rapidly and with minimum cost by minimizing the number of experiments. Figure 5.1 shows a flow diagram of the general approach to modelling. This is a recursive operation in which the developed model has to be repeatedly tested and refined until the required model accuracy is reached.

Modelling a fermentation process is especially difficult due to the complexity of biological systems. There are a large number of variables which could affect the fermentation process as noted in section 2.3. Parameters such as temperature, pH, dissolved oxygen content, substrate concentration, size and the condition of the inoculum are just a few of the likely important variables.

In this chapter, a reactor model to describe the concentration time profiles (biomass, lactose and ethanol) of the CMRF (crossflow-microfiltration recycle fermenter) is developed. A modified Monod model was selected as the basis for the fermentation model. This is an unstructured, deterministic model and does not take into account the change in biomass composition with environmental changes (Bailey & Ollis 1986). Such models, however, have gained wide acceptance for their simplicity and good fit to fermentation data under balanced growth condition (Bailey and Ollis 1986).

The objective of the study was to develop a model to aid in providing answers to "what if" questions for future process optimization and economic analysis.

Figure 5.1 A flow diagram of modelling procedures (Nicholson 1980)



5.2 Model formulation

5.2.1 Abbreviations and nomenclature

V_r fermenter working volume (l)
F feed stream flowrate (l/hr.)
C_x fermenter biomass concentration (g/l)
C_{x0} feed stream biomass concentration (g/l)
C_{xw} permeate stream biomass concentration (g/l)
C_{xr} recycle stream biomass concentration (g/l)
C_{xb} bleed stream biomass concentration (g/l)
C_{p0} feed stream abiotic phase product concentration (g/l)
C_p abiotic phase product concentration (g/l)
C_{pr} recycle stream abiotic phase product concentration (g/l)
C_{pw} permeate stream abiotic phase product concentration (g/l)
C_{pb} bleed stream abiotic phase product concentration (g/l)
C_{pm} maximum abiotic phase product concentration (g/l)
μ_m maximum specific growth rate (hr^{-1})
C_s abiotic phase limiting substrate concentration (g/l)
C_{s0} feed stream abiotic phase substrate concentration (g/l)
C_{sr} recycle stream abiotic phase substrate concentration (g/l)
C_{sw} permeate stream abiotic phase substrate concentration (g/l)
C_{sb} bleed stream abiotic phase substrate concentration (g/l)
B bleed stream flowrate (l/hr.)
t time (hr.)
K_s Monod half saturation constant (g/l)
Y_{xs} biomass substrate yield coefficient (g biomass dry wt. produced/g limiting substrate used)
Y_{px} product biomass yield coefficient (g product synthesized/g biomass dry wt. produced)

5.2.2 Assumptions

The following assumptions apply :

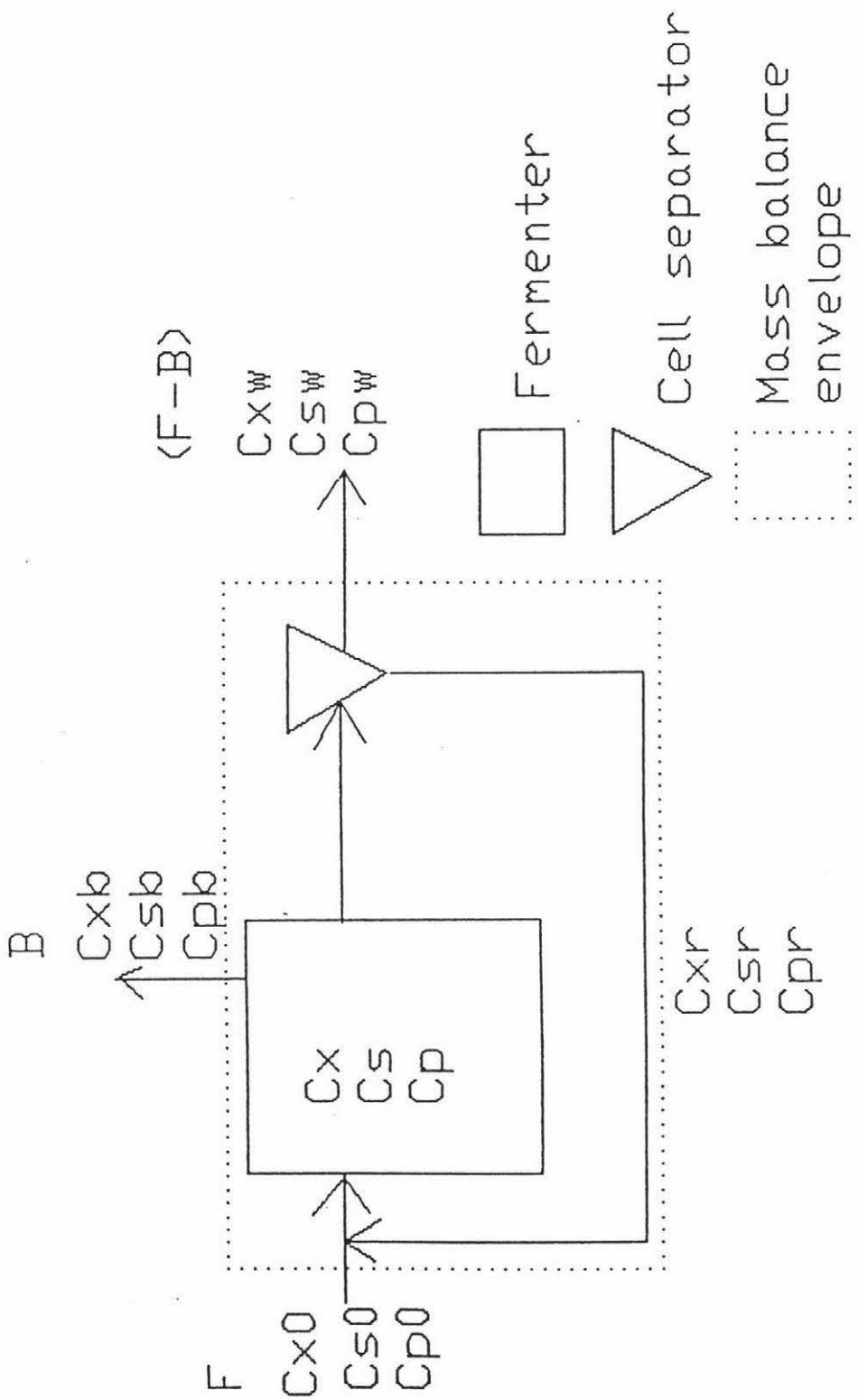
1) Negligible wall growth
2) Balanced growth (growth is balanced over a time interval if every extensive properties of the system increase by the same factor during that interval)
3) Single substrate limitation (lactose)
4) Single product inhibition (ethanol)
5) Biomass volume fraction significant
6) Negligible residence time in pipelines or cell separator
7) No leakage in the system

The effect of wall growth to the model accuracy is minimal as the total available surface area is relatively small. Balanced growth is the basic assumption for Monod's equation to apply. Single substrate limitation and product inhibition were assumed. These are justified in the context of developing a model for preliminary process optimization and economic analysis. A simple model is preferred and this will serve as the base for future development of a more sophisticated model. The effect of biomass volume fraction is specially introduced into the model as the aim of a recycle fermenter is to increase the productivity by increasing the biomass concentration. Residence time of the fermenter content in the pipelines and cell separator is short due to their relatively small volume and the high flowrate. No leakage in the system was ensured by checking periodically over the course of the fermentation.

5.2.3 Verbal-balance

A schematic diagram of the system is shown in Figure 5.2 together with the mass balance envelope.

Figure 5.2 Schematic diagram of the fermenter system



5.2.3.1 Biomass balance

rate of change of biomass = rate of biomass input in feed stream - rate of biomass outflow in product stream - rate of biomass outflow in bleed stream + rate of biomass production

5.2.3.2 Substrate balance

rate of change of substrate = rate of substrate input in feed stream - rate of substrate outflow in product stream - rate of substrate outflow in bleed stream - rate of substrate utilization

5.2.3.3 Product balance

rate of change of product = rate of product input in feed stream - rate of product outflow in product stream - rate of product outflow in bleed stream + rate of product production

5.2.4 Mathematical formulation

5.2.4.1 Biomass balance

(1) $d(C_x V_r)/dt = FC_{x0} - (F-B)C_{xw} - BC_x + \mu_m V_r C_x C_s/(K_s+C_s)[(1 - C_p/C_{pm})]$

5.2.4.2 Substrate balance

(2) $d(C_s V_r)/dt = FC_{s0} - (F-B)C_{sw} - BC_s - (\mu_m V_r C_x C_s/(K_s+C_s))/Y_{xs}[(1-C_p/C_{pm})]$

5.2.4.3 Product balance

(3) $d(C_p V_r)/dt = FC_{p0} - (F-B)C_{pw} - BC_p + (\mu_m V_r C_x C_s/(K_s+ C_s))Y_{px}[(1 -C_p/C_{pm})]$

5.2.5 Model solutions

5.2.5.1 Static (steady-state) analytical solutions

5.2.5.1.1 Solution for biomass concentration

At steady state, $d(C_xV_r)/dt = 0$, $C_{xw} = 0$ for 100% cell recycle, $C_{x0} = 0$ if no biomass in feed stream :-

(4) $(1 - C_p/C_{pm})\mu_m C_s C_x/(K_s + C_s) = BC_x/V_r$

(5) $\mu_m = BC_x(K_s + C_s)/(V_r C_s C_x(1 - C_p/C_{pm}))$

assume $C_s >> K_s$ (Bailey & Ollis (1986)) :-

(6) $\mu_m = BC_x/(V_r C_x(1 - C_p/C_{pm}))$

(7) $\mu_m = B/(V_r(1 - C_p/C_{pm}))$

5.2.5.1.2 Solution for substrate concentration

At steady state, $d(C_sV_r)/dt = 0$, $C_{sw} = C_s$:-

(8) $0 = FC_{s0} - FC_{sw} + BC_{sw} - BC_s - ((1 - C_p/C_{pm})\mu_m C_s C_x V_r/(K_s + C_s))/Y_{xs}$

(9) $Y_{xs} = ((1 - C_p/C_{pm})\mu_m C_s C_x V_r/(K_s + C_s))/(F(C_{s0} - C_{sw}))$

assume $C_s >> K_s$:-

(10) $Y_{xs} = ((1 - C_p/C_{pm})\mu_m C_x V_r)/(F(C_{s0} - C_{sw}))$

5.2.5.1.3 Solution for product concentration

At steady state, $d(C_pV_r)/dt = 0$, $C_{pw} = C_p$:-

(11) $0 = FC_{p0}/V_r - (F - B/V_r)C_{pw} - BC_p/V_r + ((1 - C_p/C_{pm})\mu_m C_s C_x V_r/(K_s + C_s))Y_{px}/V_r$

assume $C_s >> K_s$, no product in feed stream $C_{p0} = 0$:-

(12) $0 = ((1 - C_p/C_{pm})\mu_m C_x V_r/V_r)Y_{px} - (F - B)C_{pw}/V_r - BC_p/V_r$

(13) $Y_{px} = FC_{pw}/(\mu_m V_r C_x(1 - C_p/C_{pm}))$

5.2.5.2. Dynamic (unsteady-state) numerical solutions

Numerical methods for solving these differential equations are required. A Runge-Kutta fourth order method was chosen as the integrator. A Pascal program was written to implement this numerical integration (appendix A4.1).

5.3 Experimental

All the simulations were done using a Philips 386 IBM-compatible computer. The simulation program was written in Turbo Pascal V 3.01 (Borland International Inc., USA).

Two series of simulation trials predicting the biomass, lactose and ethanol concentration time profiles were carried out. Series one examined the fit of the model for two unsteady-state fermentation trials; runs 7 and 8. Linear regressions were performed over the end point fermentation parameters for runs 1-4 (appendix A3.3). The estimated biomass substrate yield coefficient (Y_{xs} = 0.028) and product biomass yield coefficient (Y_{px} = 13.7) were used as the predictors. The maximum specific growth rate was estimated to be 0.105 hr^{-1}. Table 5.1 summarizes the simulation conditions.

Table 5.1 Summary of simulation conditions for unsteady state runs 7-8 (simulation series one)

Run	S_0 (g/l)	D (hr^{-1})	Y_{xs}	Y_{px}	C_{pm} (g/l)	μ_m (hr^{-1})	K_s (g/l)
7	55.7	0.88	0.028	13.7	40	0.105	0.0001
8	53.4	0.95	0.028	13.7	40	0.105	0.0001

Key :- S_0 - feed substrate concentration D - dilution rate

Y_{xs} - biomass substrate yield coefficient Y_{px} - product biomass yield coefficient

C_{pm} - maximum abiotic phase product concentration

μ_m - maximum specific growth rate K_s - Monod half saturation constant

Experimental data at the end of the transient stage (about t = 2 hours) were used as the initial conditions for the simulations. This was done to reduce the error arising from incorporating the transient stage. The predicted biomass, lactose and the ethanol concentration were recorded and graphed together with the experimental data for comparison.

A second set of simulations was carried out on the steady-state experiments runs 5 and 6 (section 4.1.2). This was to test the accuracy of the model in describing the steady-state concentrations. The estimated fermentation parameters in series one and the analytical solutions (section 5.2.5) were employed (Table 5.2). Analytical solutions were calculated by substituting the experimental (steady state) data of runs 5 and 6 into equations (7), (10), (13) (section 5.2.5) and solve for the equations.

Table 5.2 Summary of simulation conditions (simulation series two)

Run	μ_{mana} (hr^{-1})	μ_{mpred} (hr^{-1})	Y_{xsana}	Y_{xspred}	Y_{pxana}	Y_{pxpred}
5	0.059	0.105	0.0184	0.028	18.38	13.7
6	0.106	0.105	0.0345	0.028	11.3	13.7

Key :- μ_{mana} - maximum specific growth rate (analytical solution)

μ_{mpred} - maximum specific growth rate (predictor used in series one)

Y_{xsana} - biomass substrate yield coefficient (analytical solution)

Y_{xspred} - biomass substrate yield coefficient (predictor used in series one)

Y_{pxana} - product biomass yield coefficient (analytical solution)

Y_{pxpred} - product biomass yield coefficient (predictor used in series one)

Both the concentration-time profiles and the steady-state concentration values were recorded. Concentration-time profiles were plotted along with the experimental data.

The sensitivity of the predicted biomass, lactose and ethanol concentrations to the model parameters selected was then studied. Run 7 was arbitrarily selected for the sensitivity test. Table 5.3 shows the factorial experimental plan for this study together with the fermentation parameters and the initial conditions. The various model parameters were maximum specific growth rate (μ_m), biomass yield coefficient (Y_{xs}), product biomass yield coefficient (Y_{px}), Monod half saturation constant (K_s) and the maximum

product concentration (Y_{pm}). These were varied by ± 50% as the high and low level and coded in the following manner :-

$$X = (x - \text{mid. pt.})/(1/2*\text{range})$$

where X - the coded level of the fermentation parameter

x - the uncoded parameter value

The midpoint values were 0.105 hr^{-1}, 0.0278 g/g, 13.7 g/g, 0.0001 g/l and 40 g/l for μ_m, Y_{xs}, Y_{px}, K_s, Y_{pm} respectively. The predicted biomass, lactose and ethanol concentrations at 12 hours were analyzed with linear regression and the sensitivity factors calculated. Details of the sensitivity analysis is shown in the Appendix (A3.4).

Table 5.3 Summary of factorial experimental plan (2 level, 5 factors) and simulation results on run 7 (t = 12 hr.) for sensitivity analysis

Simimulation	μ_m (hr^{-1})	Y_{xs}	Y_{px}	K_s	Y_{pm}	Biomass (g/l)	Lactose (g/l)	Ethanol (g/l)
1	1	1	1	1	1	18.3	19.5	31.1
2	-1	1	1	1	1	11.0	43.3	10.6
3	1	-1	1	1	1	17.0	0	27.2
4	-1	-1	1	1	1	11.0	18.5	10.6
5	1	1	-1	1	1	24.0	0	16.8
6	-1	1	-1	1	1	11.5	41.2	4.1
7	1	-1	-1	1	1	21.4	0	15.0
8	-1	-1	-1	1	1	11.5	12.2	4.1
9	1	1	1	-1	1	18.3	19.5	31.1
10	-1	1	1	-1	1	11.0	43.3	10.6
11	1	-1	1	-1	1	17.0	0	27.2
12	-1	-1	1	-1	1	11.0	18.5	10.6
13	1	1	-1	-1	1	24.0	0	16.8
14	-1	1	-1	-1	1	11.5	41.2	4.1
15	1	-1	-1	-1	1	21.4	0	15.0
16	-1	-1	-1	-1	1	11.5	12.2	4.1
17	1	1	1	1	-1	12.7	39.5	13.9
18	-1	1	1	1	-1	9.9	47.0	7.5
19	1	-1	1	1	-1	12.7	7.0	13.9
20	-1	-1	1	1	-1	9.9	29.6	7.5
21	1	1	-1	1	-1	18.0	19.8	10.3
22	-1	1	-1	1	-1	10.9	43.4	3.5
23	1	-1	-1	1	-1	16.7	0	9.0
24	-1	-1	-1	1	-1	10.9	18.9	3.5
25	1	1	1	-1	-1	12.7	39.5	13.9
26	-1	1	1	-1	-1	9.9	47.0	7.5
27	1	-1	1	-1	-1	12.7	7.0	13.9
28	-1	-1	1	-1	-1	9.9	29.6	7.5
29	1	1	-1	-1	-1	18.0	19.8	10.3
30	-1	1	-1	-1	-1	10.9	43.4	3.5
31	1	-1	-1	-1	-1	16.7	0	9.0
32	-1	-1	-1	-1	-1	10.9	18.9	3.5

33	1	0	0	0	0	18.2	1.5	20.7
34	-1	0	0	0	0	11.0	37.2	7.1
35	0	1	0	0	0	14.9	30.1	14.7
36	0	-1	0	0	0	14.3	0	12.7
37	0	0	1	0	0	13.7	24.4	17.9
38	0	0	-1	0	0	16.6	5.2	9.6
39	0	0	0	1	0	14.9	17.2	14.7
40	0	0	0	-1	0	14.9	17.2	14.7
41	0	0	0	0	1	16.0	9.9	17.5
42	0	0	0	0	-1	12.8	29.3	10.1

Key :- μ_m (1) - 0.1575 μ_m (-1) - 0.0525

Y_{xs} (1) - 0.0417 Y_{xs} (-1) - 0.0139

Y_{px} (1) - 20.55 Y_{px} (-1) - 6.85

K_s (1) - 0.00015 K_s (-1) - 0.00005

Y_{pm} (1) - 50 Y_{pm} (-1) - 30

biomass - predicted biomass concentration at t = 12 hr.

lactose - predicted lactose concentration at t = 12 hr.

ethanol - predicted ethanol concentration at t = 12 hr.

Initial condition :-

initial biomass concentration (7 g/l)

initial feed stream abiotic-phase substrate concentration (55.74 g/l)

initial fermenter abiotic-phase substrate concentration (44.26 g/l)

initial feed stream abiotic-phase product concentration (0 g/l)

initial fermenter abiotic-phase product concentration (5.96 g/l)

5.4 Results

5.4.1 Simulation series one

Concentration time profiles for the predicted and experimental biomass, lactose and ethanol concentrations of Run 7 are shown in Figure 5.3, 5.4 and 5.5. The time scale of the predicted curve is shifted by 2 hours (i.e. t = 0 on the predicted curve will be plotted as t = 2 hour). This is to compensate for using t = 2 hour experimental data as the initial condition for the simulation. Similar trends are observed for the predicted and experimental curves. Biomass and ethanol concentrations increase with time while the lactose concentration decreases. However, the predicted lactose concentration is consistently lower than the experimental value and over-estimation of the ethanol concentration at t = 2 to 9 hours was recorded. Table 5.4 is a summary of the data at 9 hours.

Table 5.4 Summary of biomass, lactose, ethanol concentration (run 7, t = 9 hr.)

Data type	Biomass conc. (g/l)	Lactose conc. (g/l)	Ethanol conc. (g/l)
predicted (t = 7 hr.)	12.1	22.3	12.7
experimental (t = 9 hr.)	10.8	31.7	12.4
% error	12.2	29.7	2.6

Key :- % error = (predicted value - experimental value)/experimental value*100

Figure 5.3 Plot of experimental and predicted biomass conc. (g/l) vs time (hr.) (run 7)

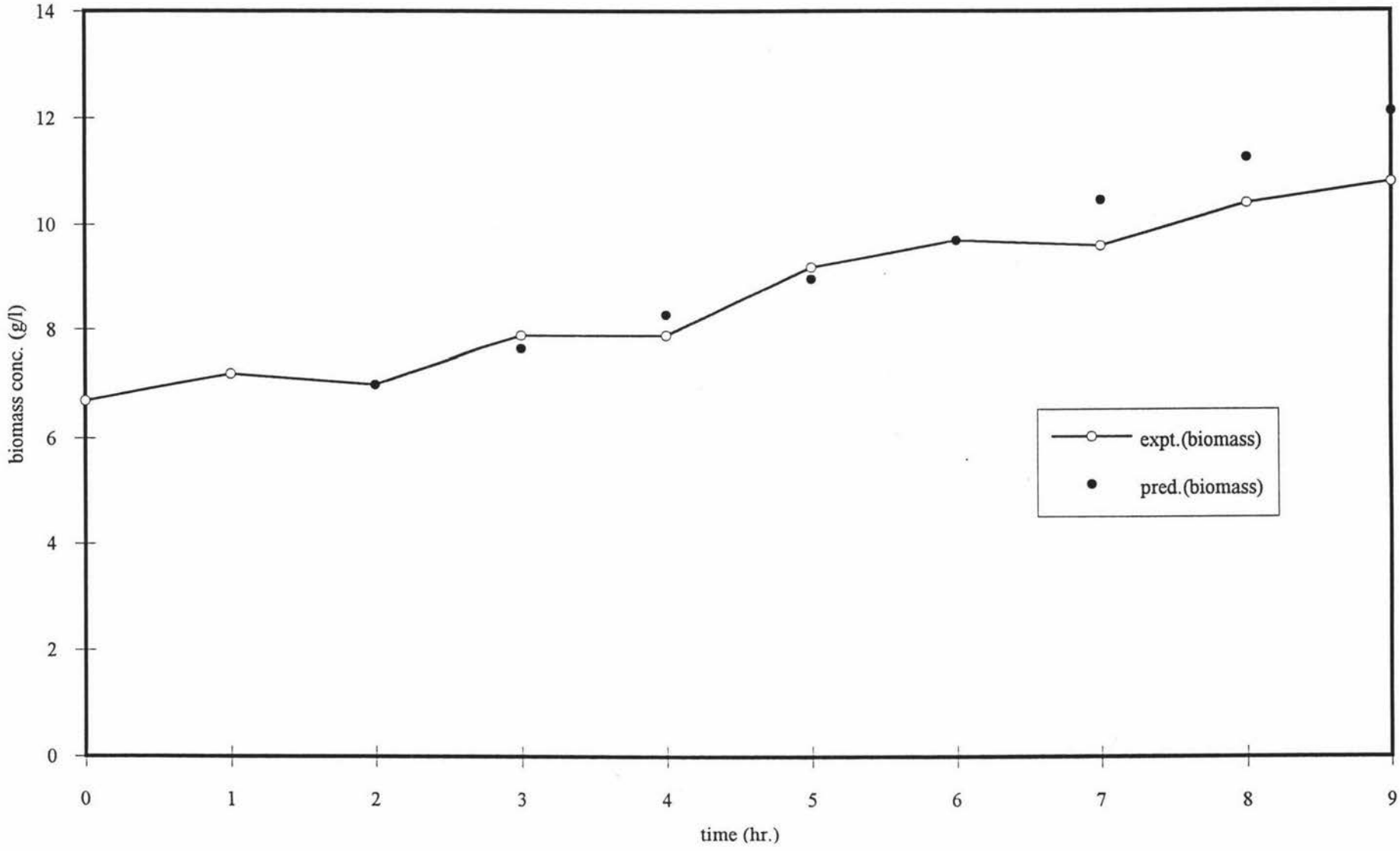


Figure 5.4 Plot of experimental and predicted lactose conc. (g/l) vs time (hr.) (run 7)

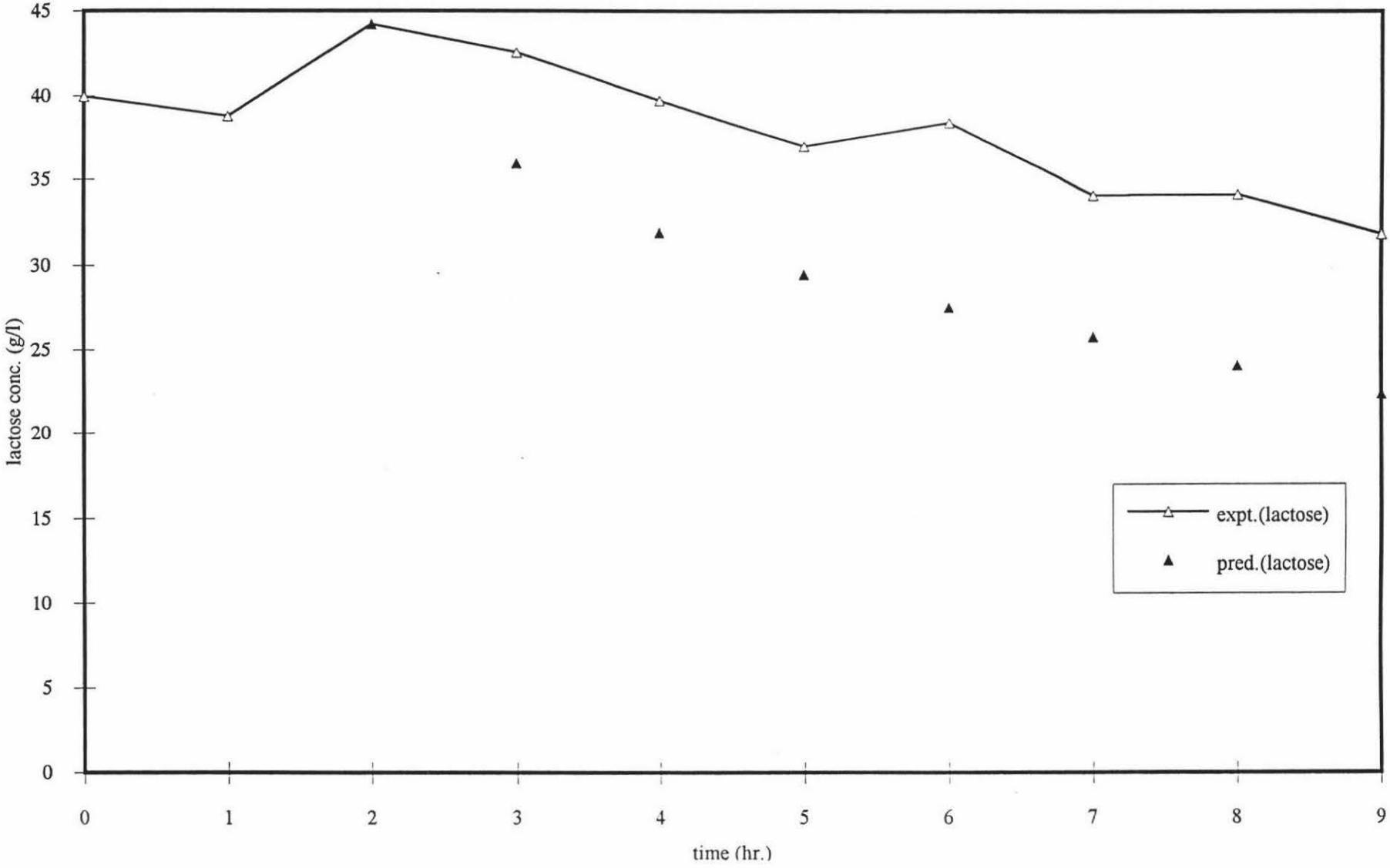


Figure 5.5 Plot of experimental and predicted ethanol conc. (g/l) vs time (hr.) (run 7)

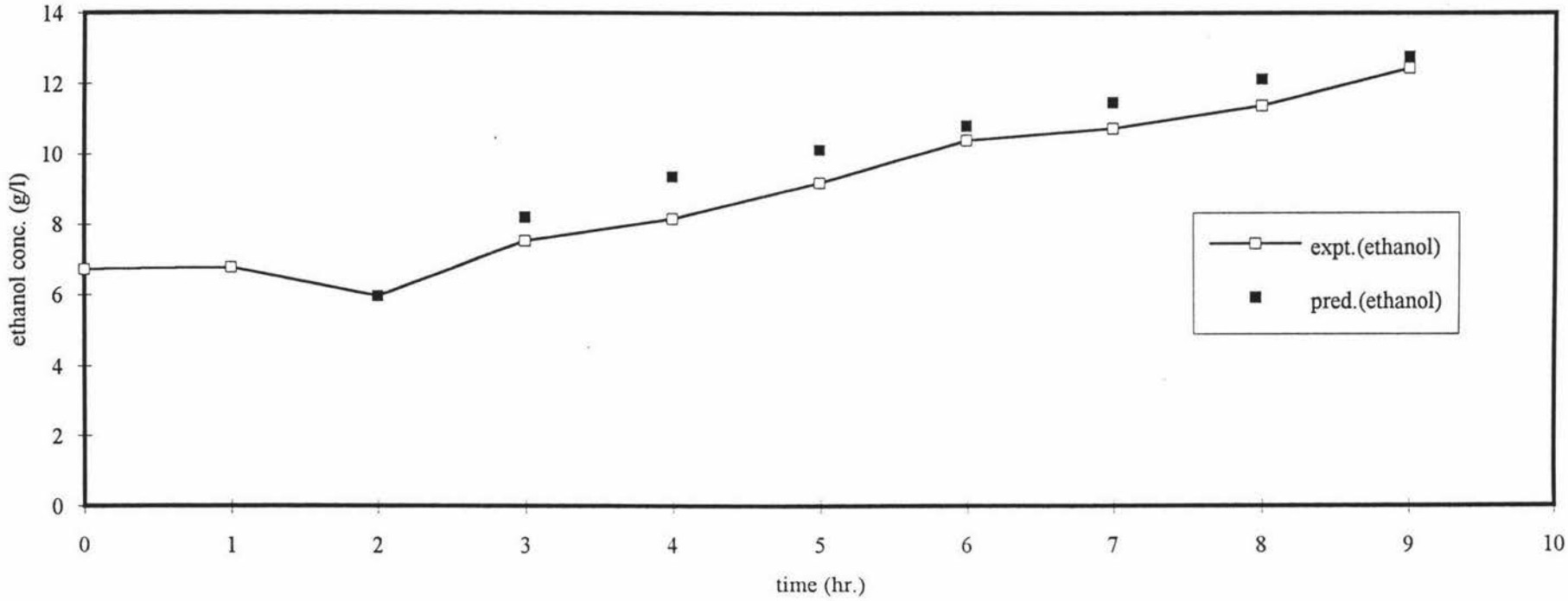


Figure 5.6, 5.7, 5.8 show the concentration time profiles of the predicted and experimental biomass, lactose and ethanol concentrations for run 8. The time scale of the predicted curve is again shifted by 2 hours (i.e. t = 0 on the predicted curve will be plotted as t = 2 hour). The predicted and experimental concentration time profiles show similar trends. The predicted biomass profile shows a steeper gradient than observed experimentally and over-estimates the data at t = 5 to 9 hours. The predicted lactose concentration is consistently lower than the experimental value. The ethanol profile shows a positive slope with over-prediction at t = 2 to 7 hours and under prediction at t = 8 to 9 hours. Table 5.5 shows a summary of the t = 9 hour data.

Table 5.5 Summary of biomass, lactose, ethanol concentration (run 8, t = 9 hr.)

Data type	Biomass conc. (g/l)	Lactose conc. (g/l)	Ethanol conc. (g/l)
predicted (t = 7 hr.)	11.1	23.4	11.43
experimental (t = 9 hr.)	8.9	26.38	14.23
% error	25.0	11.3	19.7

Key :- % error = (predicted value - experimental value)/experimental value*100

Figure 5.6 Plot of experimental and predicted biomass conc. (g/l) vs time (hr.) (run 8)

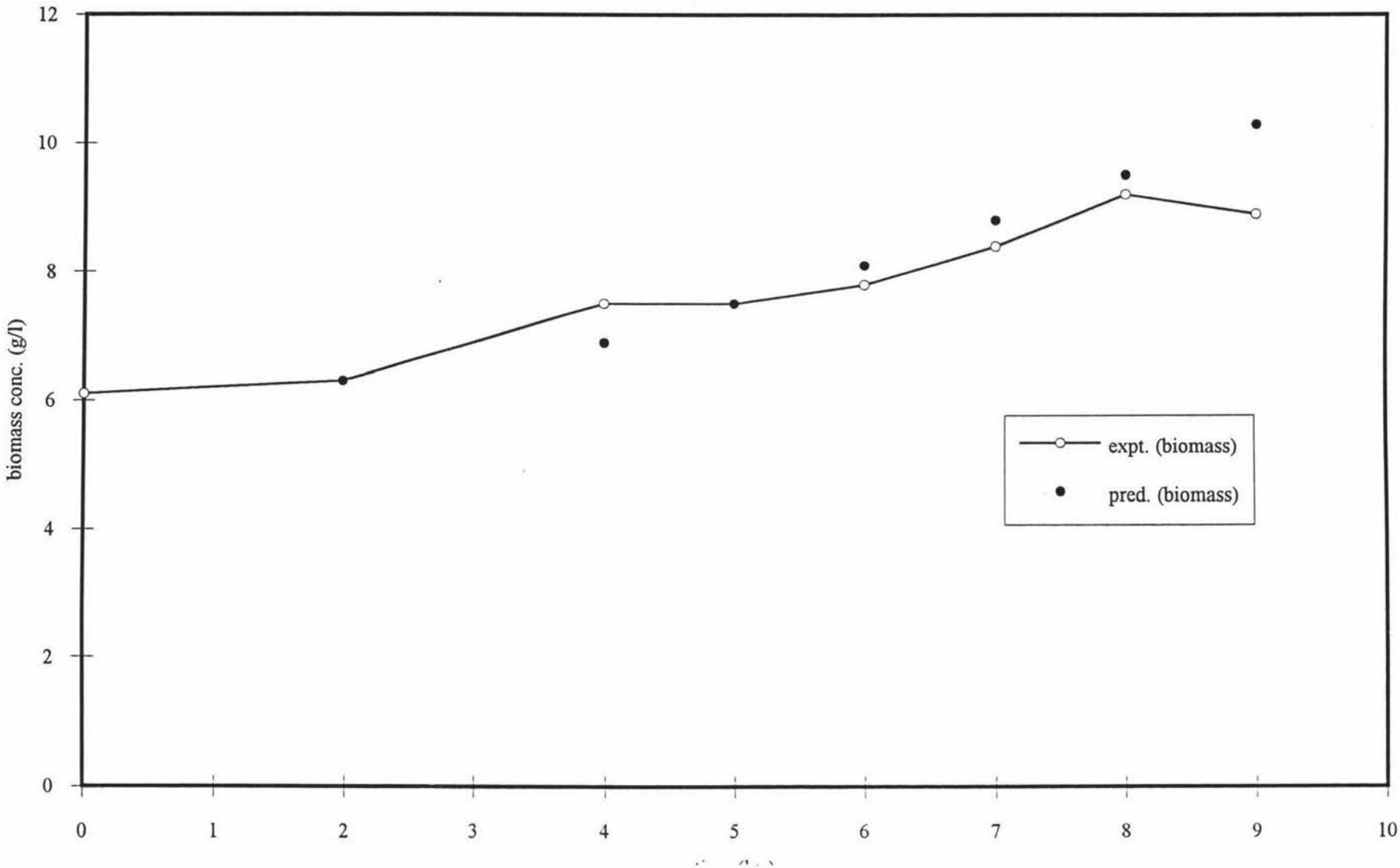


Figure 5.7 Plot of experimental and predicted lactose conc. (g/l) vs time (hr.) (run 8)

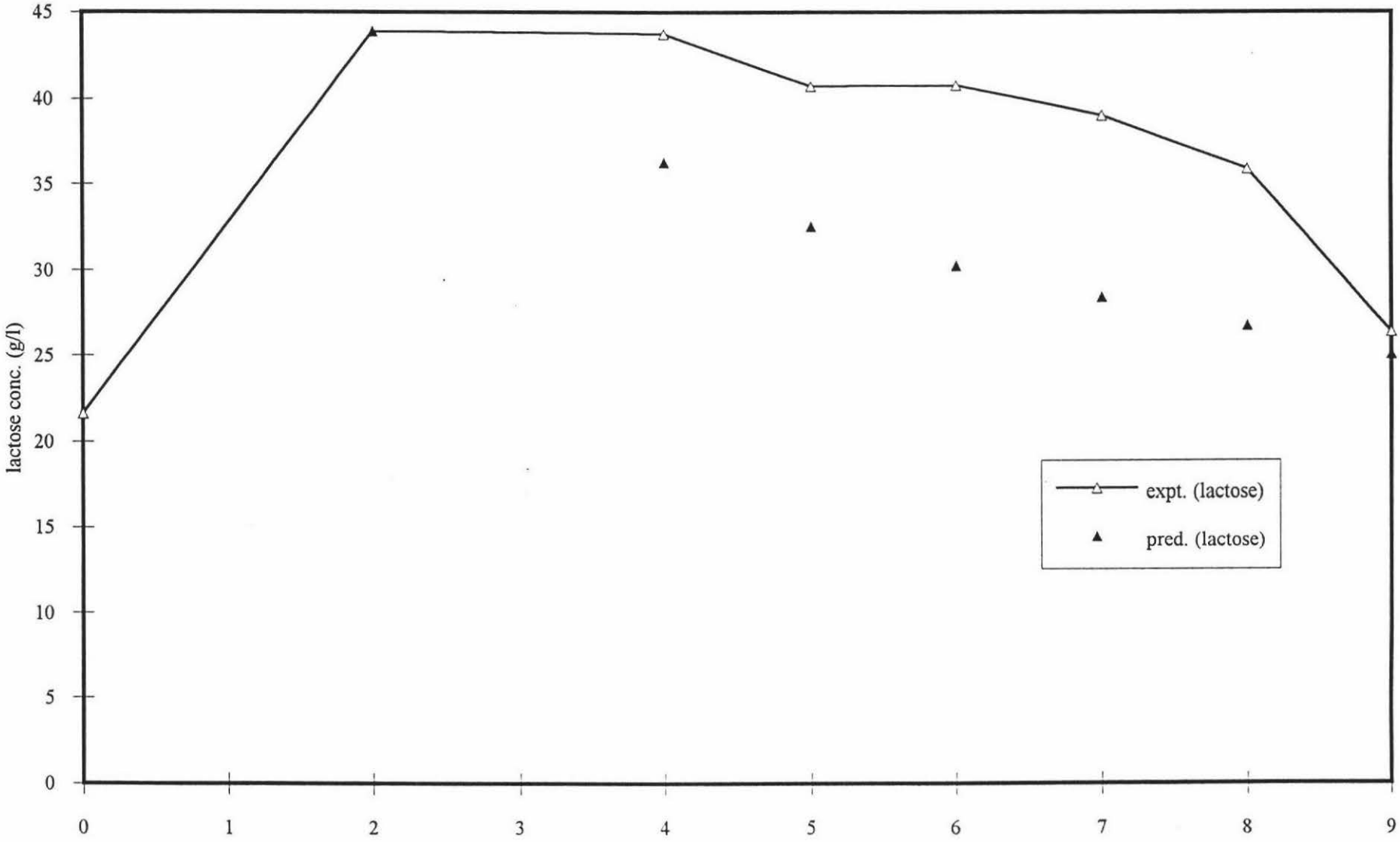
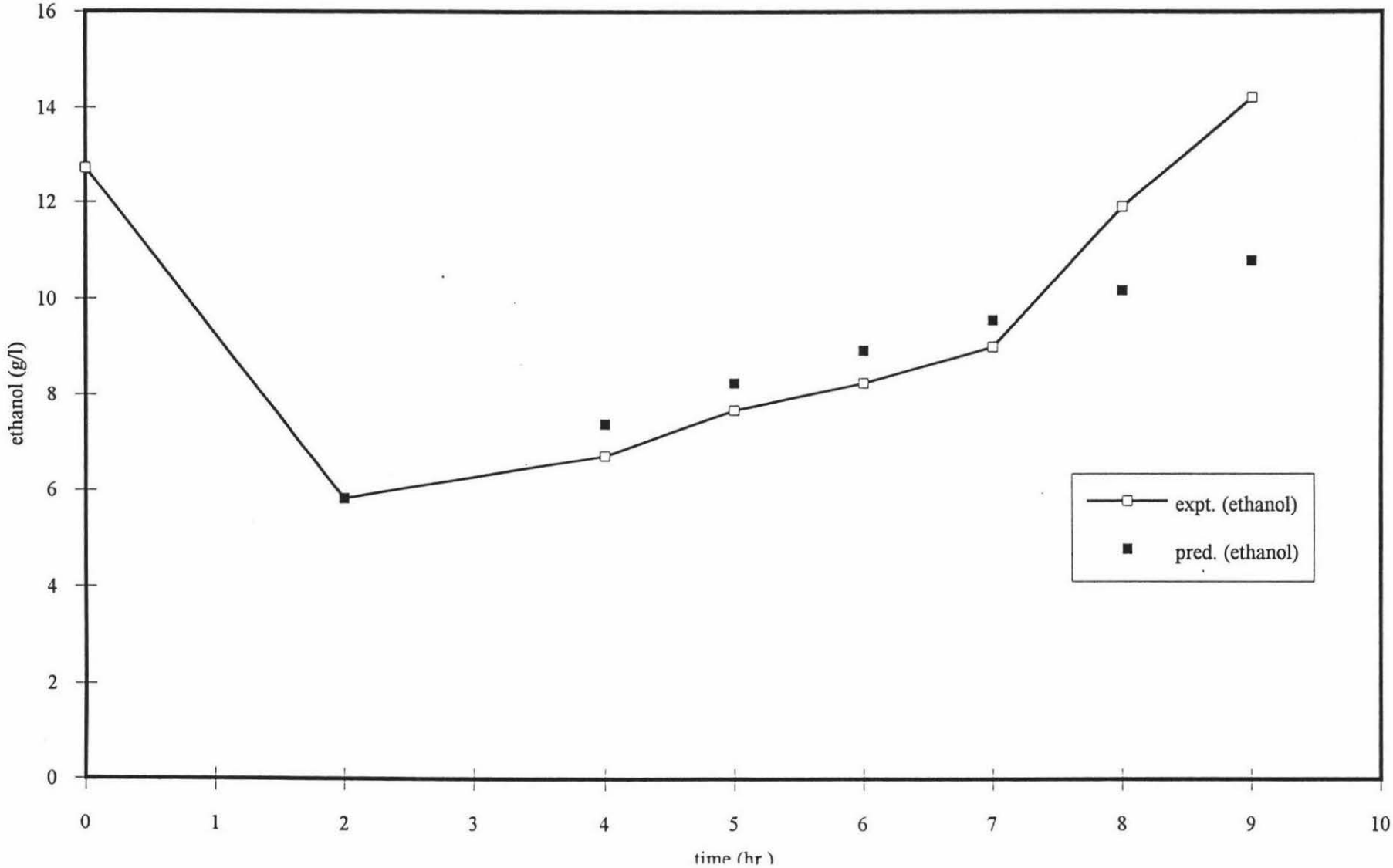


Figure 5.8 Plot of experimental and predicted ethanol conc. (g/l) vs time (hr.) (run 8)



5.4.2 Simulation series two

Figure 5.9, 5.10, 5.11 show the predicted concentration time profiles of run 5 using the analytical and predicted parameters. The experimental profiles are also shown in the figures. All the curves show similar trends. Table 5.6 summarizes the steady-state biomass, lactose and ethanol values. The steady-state experimental values are averages of data from t = 30.5 to 38.5 hour. The predicted and analytical solution values are the steady-state data from the simulations.

Table 5.6 Summary of the steady-state performance data of run 5

Data type	Biomass (g/l)	Lactose (g/l)	% error	Ethanol (g/l)	% error
experimental	9.7	12.4	NA	17.6	NA
predicted	10.0	9.9	20.2	20.7	17.6
analytical solution	10.0	11.3	8.9	17.9	1.7

Key :- NA - not applicable D - dilution rate (hr^{-1}) S_0 - feed lactose concentration (g/l)

Experimental condition :-

D = 0.335 S_0 = 64.26 g/l

Similar predicted and experimental lactose concentration curves were obtained for run 6 (Figure 5.12, 5.13, 5.14). The analytical solution curves also show similar profiles. Table 5.7 summarizes the steady-state performance data. The steady-state experimental values are averages of t = 11 to 15 hour.

Table 5.7 Summary of the steady-state performance data of run 6

Data type	Biomass (g/l)	Lactose (g/l)	% error	Ethanol (g/l)	% error
experimental	10.1	66.9	NA	16.3	NA
predicted	10	61.9	7.5	18.3	12.3
analytical solution	10	67.6	1.1	16.5	1.2

Key :- NA - not applicable D - dilution rate (hr^{-1}) S_0 - feed lactose concentration (g/l)

Experimental condition :-

D = 0.43 S_0 = 109.84

Figure 5.9 Plot of experimental and simulation biomass concentration-time profile (run 5)

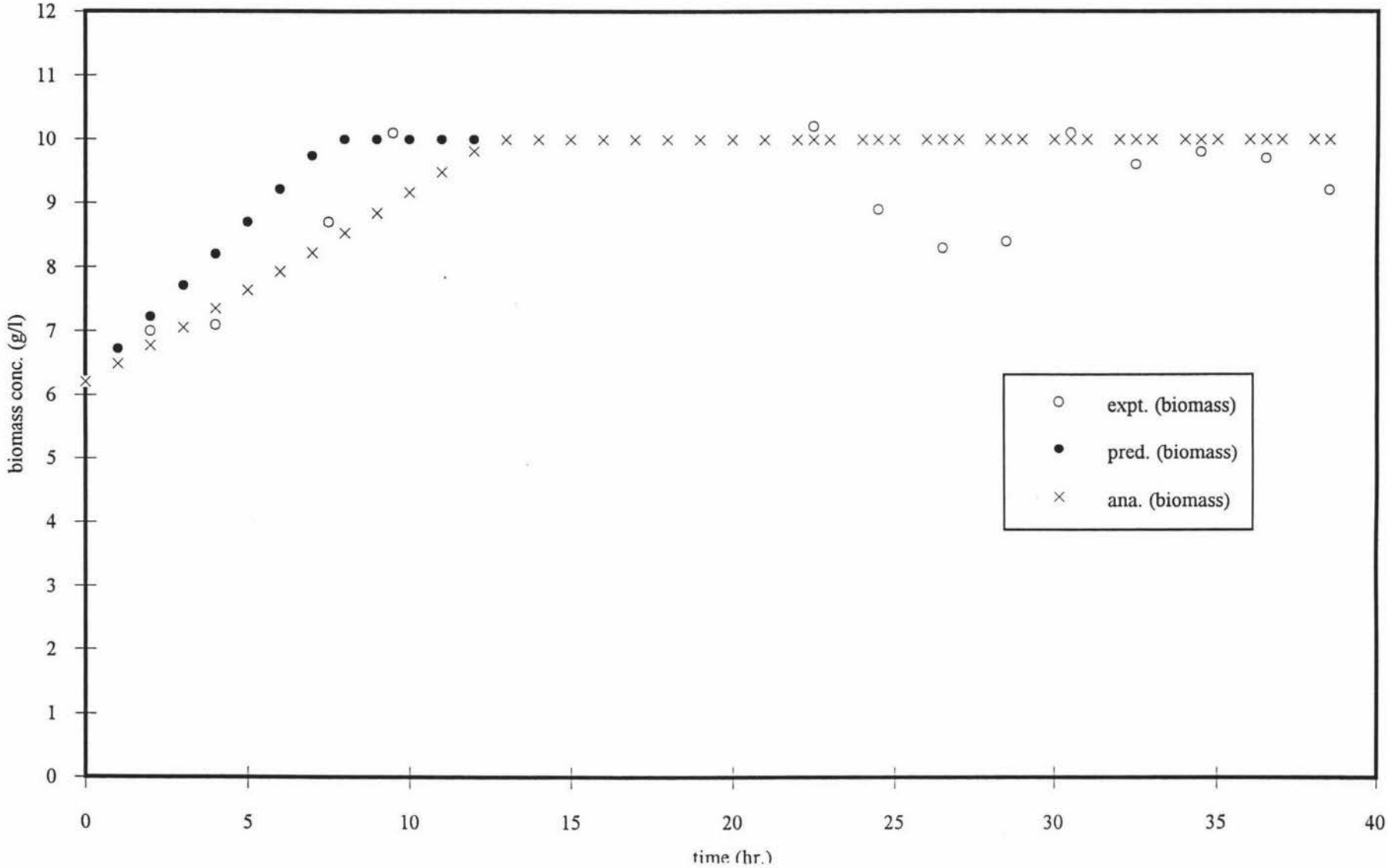


Figure 5.10 Plot of experimental and simulation lactose concentration-time profile (run 5)

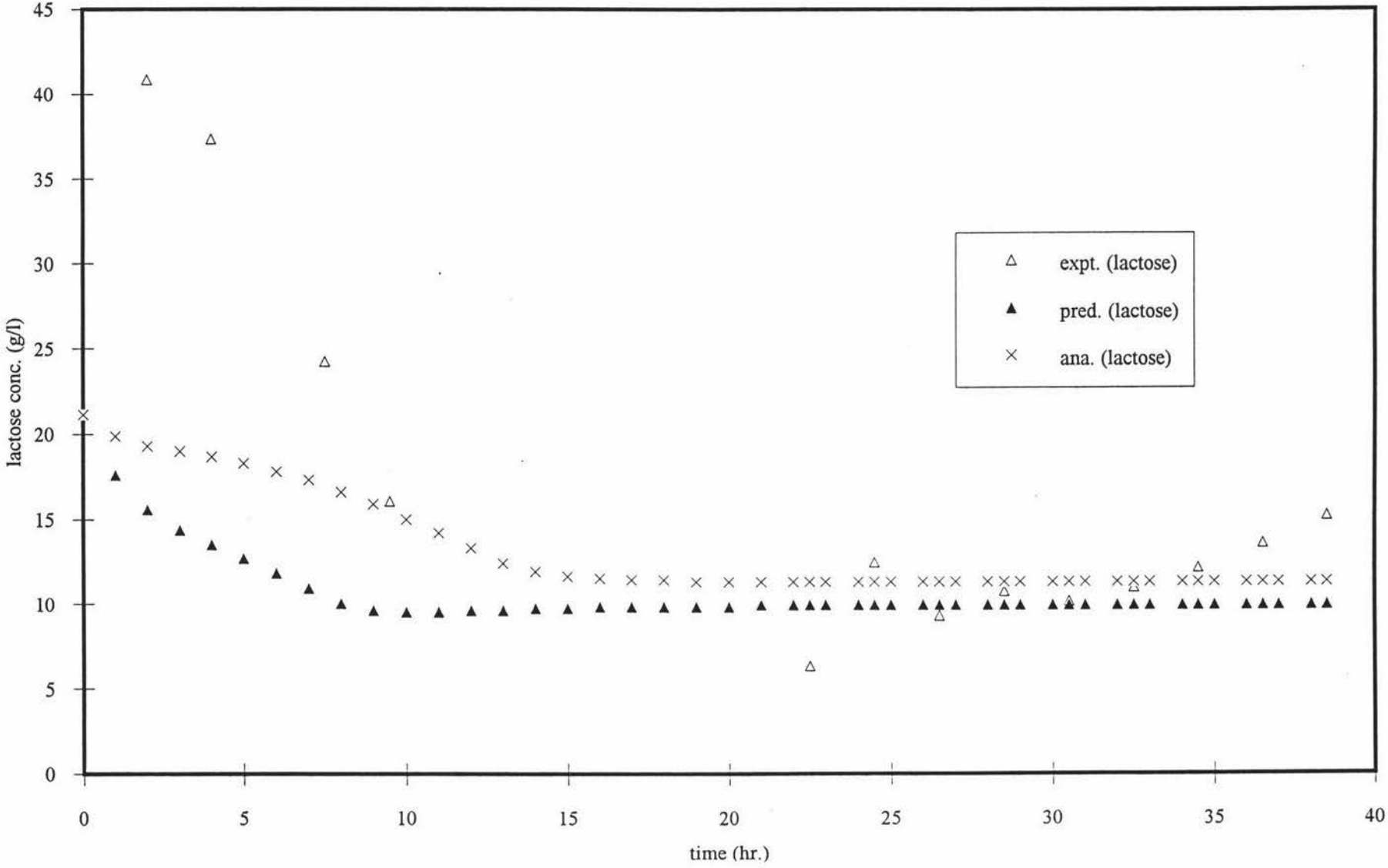


Figure 5.11 Plot of experimental and simulation ethanol concentration-time profile (run 5)

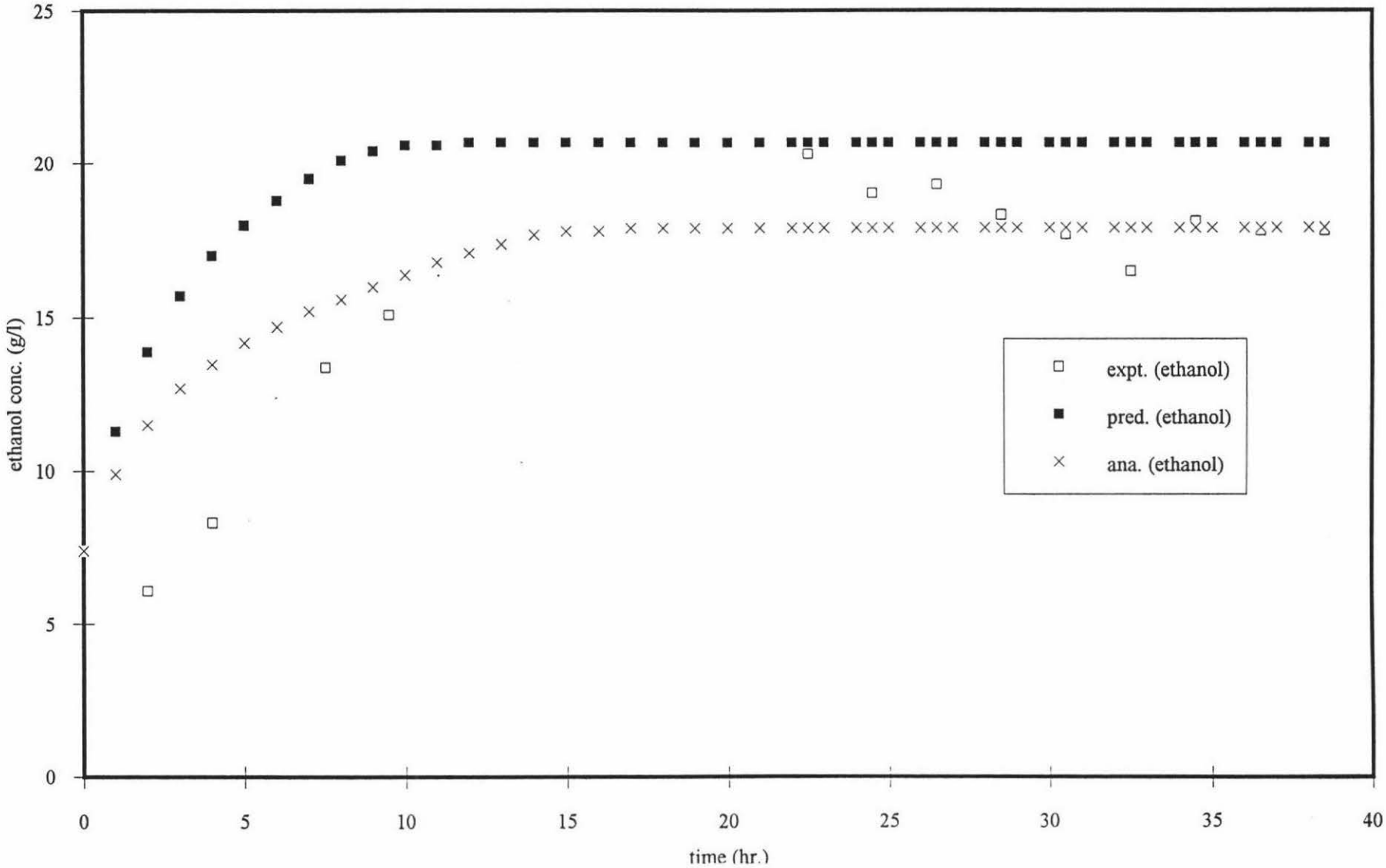


Figure 5.12 Plot of experimental and simulation biomass concentration-time profile (run 6)

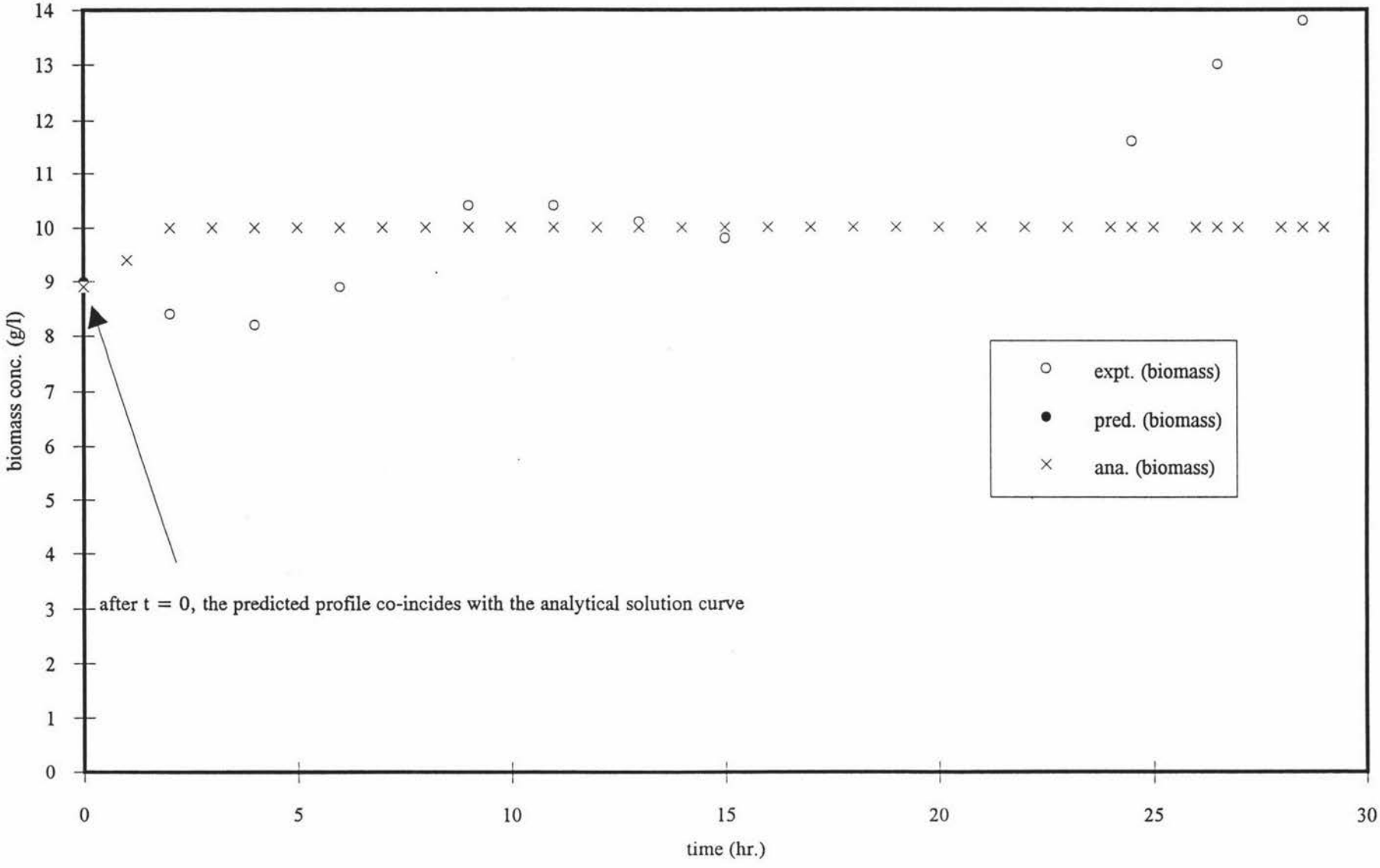


Figure 5.13 Plot of experimental and simulation lactose concentration-time profile (run 6)

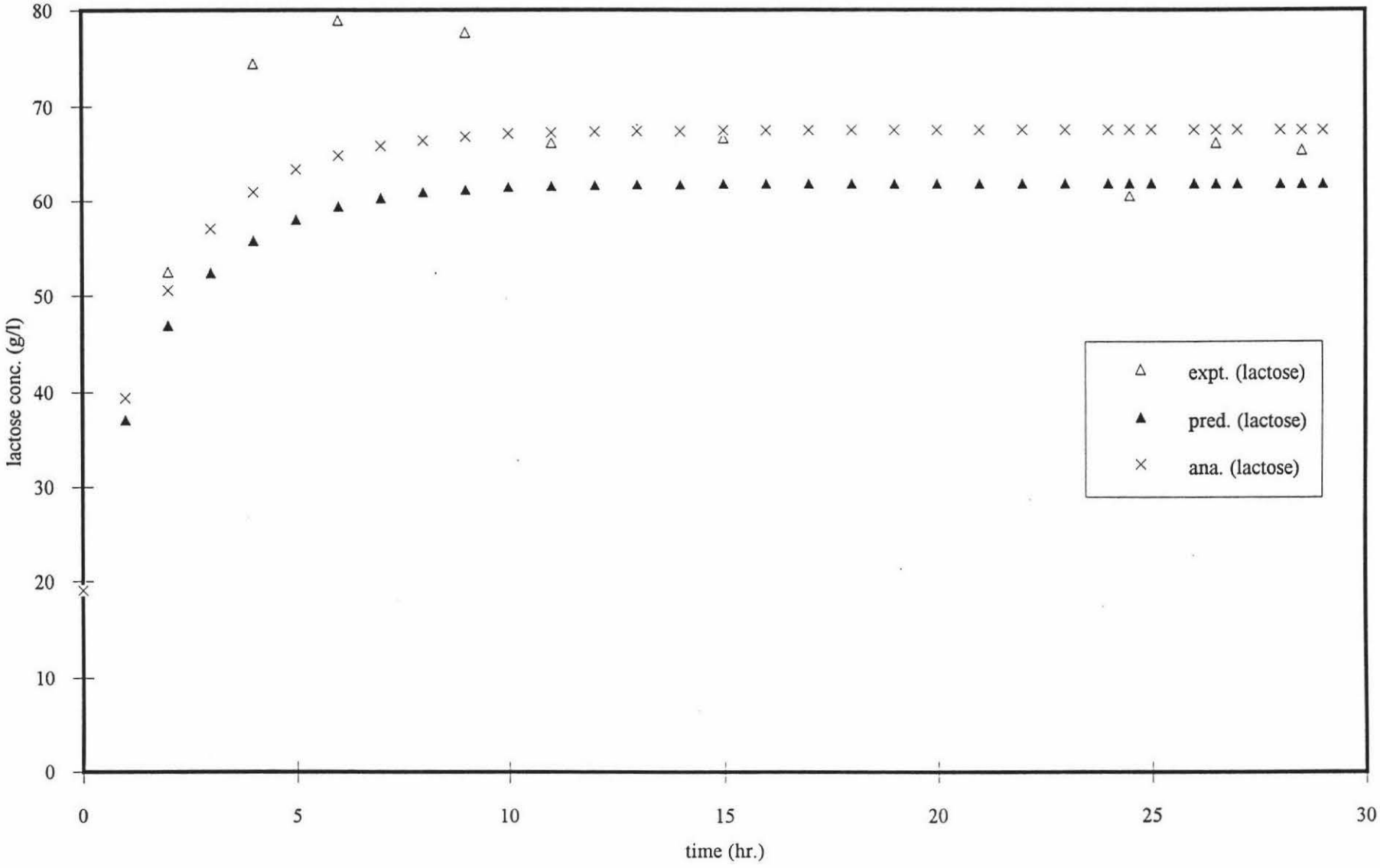
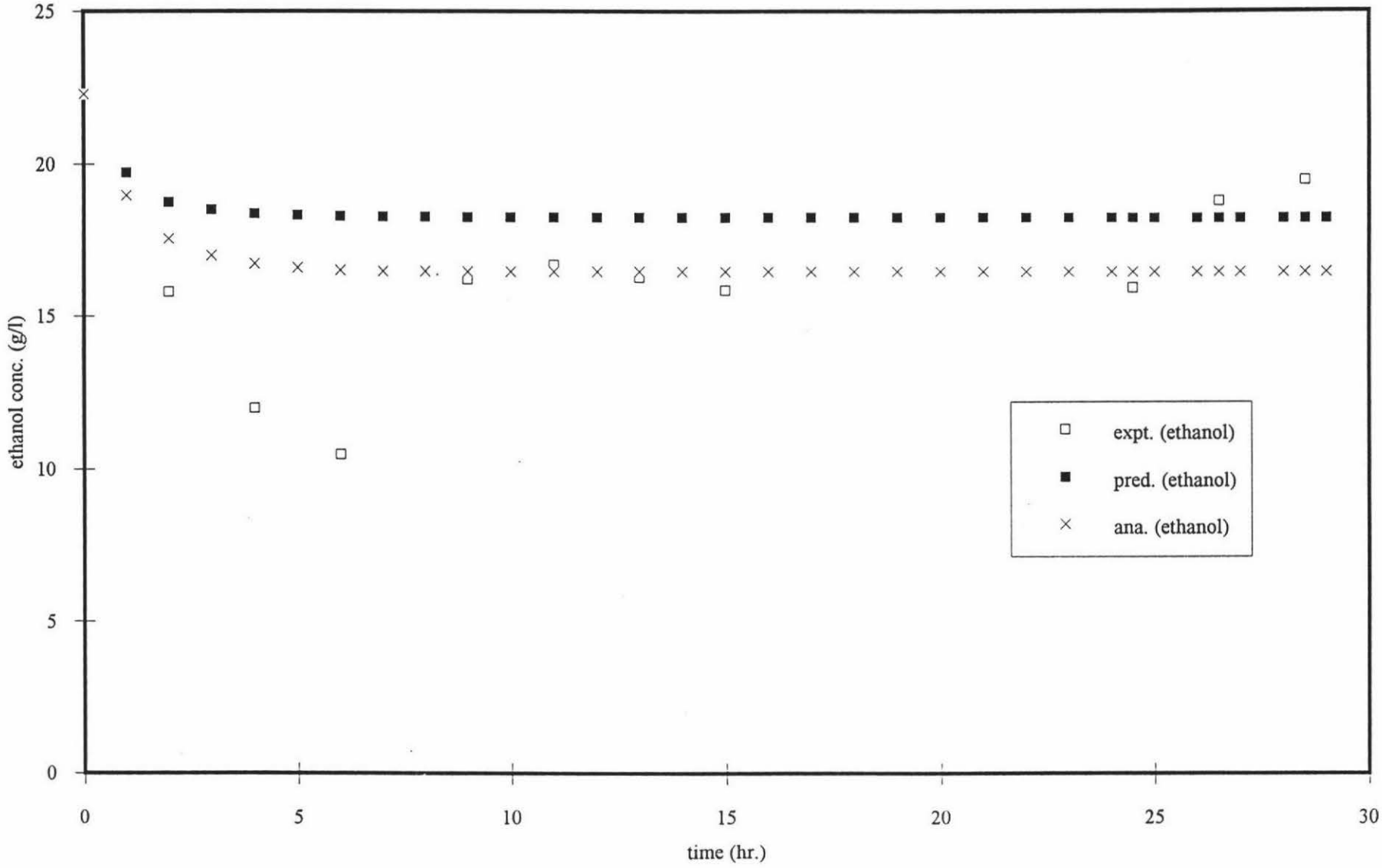


Figure 5.14 Plot of experimental and simulation ethanol concentration-time profile (run 6)



5.4.3 Sensitivity analysis simulations

Results for the predicted 12 hour biomass, lactose and ethanol concentration are shown in Table 5.3. The following equations were obtained by linear regression on the data. Insignificant predictors were screened by t-test and subsequently discarded from the analysis :-

(14) Biomass concentration = $58.37 + 1.68*\mu_m + 0.0766*Y_{xs} + 22.63*Y_{pm} + 0.767*Y_{px} + 0.00354*\mu_m*Y_{xs} + 1.125*\mu_m*Y_{pm} + 0.368*\mu_m*Y_{px} - 0.0174*Y_{xs}*Y_{px} + 0.0509*Y_{xs}*Y_{pm} + 0.00247*\mu_m*Y_{xs}*Y_{pm}$

(15) Lactose concentration = $-144.81 - 2.68*\mu_m - 0.43*Y_{xs} + 33*Y_{px} - 98.1*Y_{pm} - 0.0537*\mu_m*Y_{xs} + 0.55*\mu_m*Y_{px} - 1.601*\mu_m*Y_{pm} + 0.15*Y_{xs}*Y_{px} - 0.441*Y_{xs}*Y_{pm} + 0.0137*Y_{px}* \mu_m*Y_{xs} - 0.04*Y_{pm}*\mu_m*Y_{xs}$

(16) Ethanol concentration = $985.49 + 5.57*\mu_m + 0.00713*Y_{xs} + 416.84*Y_{px} + 460.4*Y_{pm} + 0.00032*\mu_m*Y_{xs} + 0.32*\mu_m*Y_{px} + 2.32*\mu_m*Y_{pm} + 196*Y_{px}*Y_{pm}$

Partial differentiations were performed on the equations (14), (15), (16). The sensitivity functions from the partial differentiations (D) were :-

(16) D(biomass concentration)/D(μ_m) = $1.68 + 0.00354*Y_{xs} + 1.125*Y_{pm} + 0.368*Y_{px} + 0.00247*Y_{xs}*Y_{pm}$

(17) D(biomass concentration)/D(Y_{xs}) = $0.0766 + 0.00354*\mu_m - 0.0174*Y_{px} + 0.0509*Y_{pm} + 0.00247*\mu_m*Y_{pm}$

(18) D(biomass concentration)/D(Y_{px}) = $0.767 + 0.368*\mu_m - 0.0174*Y_{xs}$

(19) D(biomass concentration)/D(Y_{pm}) = $22.63 + 1.125*\mu_m + 0.0509*Y_{xs} + 0.00247*\mu_m*Y_{xs}$

(20) D(lactose concentration)/D(μ_m) = $-2.68 - 0.0537*Y_{xs} + 0.55*Y_{px} - 1.601*Y_{pm} + 0.0137*Y_{px}*Y_{xs} - 0.04*Y_{pm}*Y_{xs}$

(21) D(lactose concentration)/D(Y_{xs}) = $-0.43 - 0.0537*\mu_m + 0.15*Y_{px} - 0.441*Y_{pm} + 0.0137*Y_{px}*\mu_m - 0.04*\mu_m*Y_{pm}$

(22) D(lactose concentration)/D(Y_{px}) = $33 + 0.55*\mu_m + 0.15*Y_{xs} + 0.0137*\mu_m*Y_{xs}$

(23) D(lactose concentration)/D(Y_{pm}) = $-98.1 - 1.601*\mu_m - 0.441*Y_{xs} - 0.04*\mu_m*Y_{xs}$

(24) D(ethanol concentration)/D(μ_m) = $5.57 + 0.00032*Y_{xs} + 0.32*Y_{px} + 2.32*Y_{pm}$

(25) D(ethanol concentration)/D(Y_{xs}) = $0.00713 + 0.00032*\mu_m$

(26) D(ethanol concentration)/D(Y_{px}) = $416.84 + 0.32*\mu_m + 196*Y_{pm}$

(27) D(ethanol concentration)/D(Y_{pm}) = $460.4 + 2.32*\mu_m + 196*Y_{px}$

The equations were simplified by substituting the predictors with the predicted values and nominalized by dividing with the corresponding concentrations and fermentation parameters values :-

(28) D(biomass concentration)/D(μ_m) = 7339.31

(29) D(biomass concentration)/D(Y_{xs}) = 1007.63

(30) D(biomass concentration)/D(Y_{px}) = 0.88

(31) D(biomass concentration)/D(Y_{pm}) = 8.47

(32) D(lactose concentration)/D(μ_m) = - 9711.46

(33) D(lactose concentration)/D(Y_{xs}) = - 10013.77

(34) D(lactose concentration)/D(Y_{px}) = 41.54

(35) D(lactose concentration)/D(Y_{pm}) = - 42.30

(36) D(ethanol concentration)/D(μ_m) = 14358.58

(37) D(ethanol concentration)/D(Y_{xs}) = 3.8

(38) D(ethanol concentration)/D(Y_{px}) = 8843.29

(39) D(ethanol concentration)/D(Y_{pm}) = 1153.97

5.5 Discussion

Large percentage errors were recorded on the unsteady-state run 7 lactose prediction (29.7%, Table 5.4). The model predicted the ethanol concentration with higher accuracy at a 2.6% error. The model predicted the run 8 lactose concentration with 11.7% error and the ethanol concentration at 19.7% error. When compared at 10 g/l biomass, no significant improvement on the prediction accuracy was observed (Table 5.9).

Table 5.8 Summary of percentage error of biomass, lactose, ethanol concentration prediction (run 7, 8 at t =9 hr. experimental)

Run	% error biomass conc.	% error lactose conc.	% error ethanol conc.
7	12.2	29.7	2.6
8	25	11.3	19.7

Table 5.9 Summary of percentage error of biomass lactose, ethanol concentration prediction (run 7, 8 at 10 g/l biomass)

Run	Data type	Biomass conc. (g/l)	Lactose conc. (g/l)	Ethanol conc. (g/l)
Run 7	predicted	9.7	27.5	10.8
	experimental	9.7	38.4	10.4
	% error	NA	28.5	3.8
Run 8	predicted	9.5	26.7	10.2
	experimental	9.2	35.9	11.9
	% error	NA	25.5	14.7

The large percentage error and consistent under or over-prediction of the product and substrate concentration suggested a deficiency in the model. A large percentage error was also obtained for the steady-state experiments, e.g. the largest percentage error was 20.2% for lactose concentration and 17.6% for ethanol concentration in run 5 (Table 5.10).

Table 5.10 Summary of the steady-state lactose and ethanol concentration prediction (run 5, 6)

Data type	% error lactose conc. Run 5	% error lactose conc. Run 6	% error ethanol conc. Run 5	% error ethanol conc. Run 6
predicted	20.2	7.5	17.6	12.3
analytical solution	8.9	1.1	1.7	1.2

A constant yield coefficient term was assumed for the model. However, the results of runs 1-4 showed a large variation of both product biomass yield coefficient (Y_{px}) and biomass substrate yield coefficient (Y_{xs}) as shown in Table 5.11..

Table 5.11 Summary of yield coefficients at various biomass (run 1-4)

Run	Biomass conc. (g/l)	Y_{xs}	Y_{px}
1	12.9	0.036	15.55
1	9.6	0.039	16.37
2	29.2	0.005	68.79
2	10.1	0.051	9.16
3	18.2	0.0024	15.92
3	9.3	0.045	9.56
4	18.8	0.016	11.45
4	10.3	0.013	9.91

Key : Y_{xs} - biomass substrate yield coefficient

Y_{px} - product biomass yield coefficient

Studies on the relationship of various operating condition and yield coefficients could thus help to improve the model accuracy. The need to obtain better yield coefficient estimate is supported by the analytical solution result. Ethanol was predicted within 2% error. The higher prediction accuracy could be attributed to the fact that the analytical solutions were calculated with the experimental data for run 5, 6 and therefore provided the best estimate on the yield coefficients.

Sensitivity analysis shows the maximum specific growth rate (μ_m) is one of the most important factors in the simulation as the biomass, substrate and ethanol level are all significantly affected (Table 5.12).

Table 5.12 Summary of sensitivity coefficients of various fermentation parameters on biomass, lactose and ethanol concentration (run 7)

Effect	Sensitivity Factor			
	μ_m	Y_{xs}	Y_{px}	Y_{ps}
Biomass conc.	7339.31	1007.63	NA	NA
Lactose conc.	-9711.46	-10013.77	NA	NA
Ethanol conc.	14358.58	NA	8843.29	1153.97

Additional experiments should therefore be carried out to determine the maximum specific growth rate (μ_m) value. The specific growth rate is not significantly affected by the substrate concentration and the dilution rate (section 4.2). However, it is affected by the biomass and ethanol concentration. A further possibility for improving the model accuracy is incorporating of a function accurately describting variation in the specific growth rate with biomass and ethanol. Another factor of significance to biomass and lactose prediction is biomass substrate yield coefficient (Y_{xs}). As discussed earlier (section 4.2), the biomass yield coefficient is affected by dilution rate and so further studies on how various conditions affect Y_{xs} are also necessary.

5.6 Conclusions and recommendations

It was concluded that the model provide s a prediction accuracy of up to 30% error on lactose concentration prediction and 20% on ethanol concentration prediction.

The accuracy is probably adequate in the context of providing a "preliminary analysis" of process economy and for process optimization. The model accuracy could be improved with more knowledge of how the yield coefficient and maximum specific growth rate (μ_m) vary with operating conditions.

CHAPTER 6

FINAL DISCUSSION

Approximately 2.5 million tonnes of whey is produced annually in New Zealand (New Zealand Dairy Board 1989). This results in large volumes of deproteinated whey, much of which is further processed into ethanol. Three of the four whey ethanol plants in New Zealand uses batch fermentation (section 2.4), which provides high lactose utilization and product stream ethanol concentration but only a low productivity (section 2.5.1.2.1). Further study of alternative fermentation systems with improved performance and potential for commercial application is therefore needed.

A continuous stirred tank-membrane fermenter was selected for further study as literature data showed improved productivity over 15 other fermenter configurations reviewed (section 2.6). Scaling-up of the fermenter is relatively easy as additional membrane area can be added by adding more modules. This design is also less mechanically complicated than other commercial cell recycle systems, such as those using continuous centrifuges.

Experiments on a laboratory-scale CMRF (crossflow-microfiltration recycle fermenter) recorded up to a 13 times increase in productivity over the current commercial batch fermentation process using the same organism. However, low substrate utilization and ethanol concentration were noted at high dilution rate. The productivity could be further increased by increasing the biomass concentration (Cheryan & Mehaia 1983). A value of 10 g/l biomass was selected for steady state runs and a maximum concentration of 29.2 g/l was obtained when biomass was allowed to accumulate constantly. However, much higher concentrations could conceivably be achieved and stably maintained. This is indicated by the literature, although long term operation is not proven and there are potential difficulties, as discussed below. With a high cell concentration, higher lactose utilisation could also be achieved with lower substrate concentration at higher dilution rates.

Optimization of the operating conditions is necessary to achieve higher values of productivity, lactose utilization and ethanol concentration. At lower inlet lactose concentrations, the lactose utilization increased but at the expense of both the productivity and ethanol concentration. Increasing the dilution rate

also increased the productivity but decreased the ethanol concentration. Thus, the work has indicated some of the important factors which should form part of an optimization program.

One approach would be to carry out simulation studies to provide a preliminary analysis of the effect of various operating conditions on system performance. Experiments on the predicted optimal conditions will then be necessary to confirm the fermenter performance. The model developed in this work (section 5.2) could be used as the basis for such a study. However, further refinement is needed to improve on the prediction accuracy and further experimental work is required to better understand the relationships between the various operating conditions and the metabolic parameters. The maximum specific growth rate has been identified as one of the most important factors in the simulation model. Future investigation should be directed to obtaining an accurate maximum specific growth rate (μ_m) value. Incorporation of a function relating specific growth rate to biomass and/or ethanol concentration could also improve the model accuracy. The model accuracy could also be improved with better estimation of the biomass yield coefficient (Y_{xs}). Sensitivity analysis demonstrated the significant effect of this parameter on biomass and substrate concentration prediction.

At higher biomass concentration the effect of cell volume fraction becomes significant. Yamane *et al* (1988) discussed the economic significance of increasing cell mass concentration for metabolite production. They showed the assumption that the volume occupied by cells has an insignificant effect on the overall substrate, product concentration was only justified with low cell mass concentrations (< 10 % v/v). But with a recycle fermenter configuration, high biomass concentrations are desirable, as noted above, and the effect of cell volume fraction must be considered.

The effect of an improved operating condition on fermenter design must also be considered. Higher biomass concentration may increase fouling of the membrane. In this case a mechanism to maintain the membrane flux is required. Backflushing or closing the permeate outlet valve could be used. In backflushing, permeate, water or air is passed back through the membrane in the reverse direction to permeate flow in order to dislodge adhering cells and other material. The effect of increased flux with backflushing was demonstrated for *Saccharomyces cerevisiae* separation (Matsumoto *et al* 1988). That a

similar effect to backflushing could also be achieved by closure of the permeate outlet valve was observed by Gabler (1985). However it should be noted that the above measures would require excess membrane capacity, i.e. some modules could be isolated for backflushing or cleaning while the remainder are in active service. This will increase the cost and both this, and the additional complexity with operation and control of these procedures, must be considered in an overall analysis.

At higher biomass concentrations and hence high productivities the microbial heat load will also become more significant. How to meet this increased cooling demand would then have to be considered in the fermenter design and economic analysis. The estimation of microbial heat load have been discussed by Bailey & Ollis (1986) and Luong & Volesky (1983). It can be showed that for the aerobic fermentation phase, the microbial heat load (kJ/m^3) is :-

amount of substrate utilized*16438.6 - amount of microbial cells produced*7480

For anaerobic fermentation, the microbial heat load is :-

amount of substrate utilized*16438.6 - amount of microbial cells produced*7480 - amount of the ethanol produced*29045.7

Therefore, at complete utilization of a 40 g/l lactose feed, the anaerobic heat load is approximately 158.5 MJ/m^3. This represents a potential increase of 7.7 °C of the fermenter temperature assuming that the specific heat of fermenter content is same as water. The problem is amplified with a recycle fermenter where the high productivity means a smaller fermenter volume, with a lower heat transfer area, is available. However, an external heat exchanger could be incorporated with the recycle loop. Foaming must also be considered with the much increased level of protein present.

The above mentioned points show the complexity of attempts to improve the productivity, lactose utilization or product concentration. To complicate the situation further, optimization of fermentation must be carried out in conjunction with the downstream product recovery system. Attention must be given to the need for high product concentration to reduce distillation costs.

Preconcentration of the feed is required for higher product concentration and this will affect the fermentation performance if only because of the inhibitory effects of the higher ethanol concentration. Overall, the key to further improvement in the fermenter performance lies in obtaining better understanding of the relationship between these various factors.

Over the course of the study, membrane fouling and foaming were the major operation difficulties. Other potential areas of concern include contamination, low packing density of tubular membrane, robustness of the membrane for industrial application, and cleaning. As mentioned earlier, the problem of membrane fouling could be improved by backflushing or permeate outlet closure. It is very important that in the situation of membrane fouling, the system is capable of automatically switching into the backflushing mode. Addition valves, by-pass piping, and controls are therefore necessary.

Foaming could be solved by installation of a foam breaker. The problem is worst during the aerobic phase of the fermentation although this period is relatively short. In an industrial scale fermenter, a foaming sensor would be incorporated and a mechanical defoamer used. Chemical antifoaming agent might also be required but consideration must then be given to its effect on the biomass activity and membrane fouling.

The risk of contamination could be reduced by pasteurization of the feed. This practise is followed by the New Zealand Distillery Co. at Edgecumbe. Alternatively, the pH of the fermentation can be lowered. The effect of pH on the specific yeast has to be determined. However, the risk of contamination will likely be lessened if operating with high yeast concentration.

The low packing density of tubular membrane results in large space requirement and hence additional costs. Other membrane designs such as the hollow fibre configuration has higher packing density but with a higher replacement cost, while spiral wound systems can be difficult to clean (Hanish 1986). Ceramic membranes have a longer life and greater chemical resistance, and these membranes are also steam sterilizable (Matsumoto *et al* 1988). The disadvantage is low transmembrane flux and high crossflow velocity required. Hence a larger filter area and high powered feed pump are required for industrial scale application (Kroner *et al* 1984). Further development in

membrane design and manufacturing technology is necessary for an improvement in this area, but this can be expected due to the increasing number of applications and size of the membrane market.

Membrane cleaning is another problem. Chemical or mechanical cleaning can be used. Chemical cleaning techniques are applicable to all membrane configurations but care must be taken to ensure compatibility with the membrane.

This study has successfully achieved high productivity with a CMRF (crossflow-microfiltration recycle fermenter) for ethanol production from acid casein whey using a commercial yeast strain. The relationship between various operating conditions on fermentation performance has also been examined and a mathematical model with sufficient accuracy for preliminary fermenter optimisation and feasibility study has been developed. Future investigation should concentrate on :-

1) study on the performance of the CMRF as compared to the hollow fibre and CSTR-hollow fibre configurations
2) determining the relation between various operating condition and the yield coefficients
3) more accurate prediction of the maximum specific growth rate (μ_m)
4) determine the relation between specific growth rate and the biomass and ethanol concentration
5) better estimation of biomass yield coefficient (Y_{xs})
6) methods for obtaining, and the effects of long term operation at, high biomass concentration

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APPENDIX 1

STATISTICAL ANALYSIS OF THE STANDARD CURVE DATA

A1.1 Statistical analysis on the dry weight-absorbance standard curve

A1.1.1 Minitab regression analysis printout

```
MTB >
MTB > regress 'drywt' on 1 predictor 'abs';
SUBC> residuals in 'res';
SUBC> predict of 'abs'.

The regression equation is
drywt = - 0.0298 + 0.586 abs

Predictor        Coef        Stdev     t-ratio        p
Constant    -0.029822     0.008475       -3.52    0.024
abs          0.58638      0.02269       25.85    0.000

s = 0.009641     R-sq = 99.4%     R-sq(adj) = 99.3%

Analysis of Variance

SOURCE        DF          SS          MS          F        p
Regression     1    0.062104    0.062104     668.09    0.000
Error          4    0.000372    0.000093
Total          5    0.062476

Obs.      abs      drywt         Fit  Stdev.Fit   Residual   St.Resid
  1     0.640    0.35600     0.34546    0.00804    0.01054       1.98
  2     0.330    0.15823     0.16368    0.00394   -0.00545      -0.62
  3     0.230    0.10304     0.10505    0.00455   -0.00201      -0.24
  4     0.135    0.05631     0.04934    0.00594    0.00697       0.92
  5     0.460    0.22689     0.23991    0.00491   -0.01302      -1.57
  6     0.190    0.08456     0.08159    0.00507    0.00297       0.36

     Fit  Stdev.Fit          95% C.I.             95% P.I.
 0.34546    0.00804  (0.32312,0.36780)  (0.31059,0.38033)
 0.16368    0.00394  (0.15275,0.17461)  (0.13476,0.19261)
 0.10505    0.00455  (0.09240,0.11769)  (0.07543,0.13466)
 0.04934    0.00594  (0.03285,0.06582)  (0.01790,0.08078)
 0.23991    0.00491  (0.22628,0.25354)  (0.20987,0.26996)
 0.08159    0.00507  (0.06751,0.09567)  (0.05134,0.11184)


MTB > plot 'res' 'abs'

          -
          -
   0.0080+
          -      *
res       -
          -            *
          -
   0.0000+
          -                  *
          -
          -                        *
          -
          -
  -0.0080+
          -
          -
          -                                 *
          -
          +---------+---------+---------+---------+---------+---
        0.10      0.20      0.30      0.40      0.50      0.60
```

Table A1.1 Raw data of the biomass dry weight-absorbance standard curve

Absorbance (620 nm)	Dry weight (g/l)
0.64	0.356
0.46	0.227
0.33	0.158
0.23	0.103
0.19	0.085
0.14	0.056

A1.1.2 Statistical analysis of the regression results

R^2 value :-

Very high R^2 value (99.4) was obtained with this regression equation.

Q-test on the regression :-

0.062104/0.000093 = 6677.849

This compared with F(0.05,1,4) = 7.71. Therefore, the model as a whole is significant.

t-test on the constant term :-

3.52 compared with t(0.025,4) = 2.776. Therefore, the term is significant.

t-test on the absorbance term :-

25.85 compared with t(0.025,4) = 2.776. Therefore, the term is significant.

A1.2 Statistical analysis on the ethanol concentration-nominal area standard curve

A1.2.1 Minitab regression analysis printout

```
MTB > noconst
MTB > regress 'gc' on 1 predictor '[etoh]';
SUBC> residuals in 'res';
SUBC> predict of '[etoh]'.

The regression equation is
gc =.0.0898 [etoh]

Predictor          Coef        Stdev    t-ratio         p
Noconstant
[etoh]       0.0897624    0.0008602     104.35     0.000

s = 0.02077
Analysis of Variance

SOURCE          DF          SS          MS           F         p
Regression       1      4.6974      4.6974    10889.07     0.000
Error           19      0.0082      0.0004
Total           20      4.7056

Obs.   [etoh]         gc         Fit  Stdev.Fit   Residual   St.Resid
   1      0.0    0.00000     0.00000    0.00000    0.00000       0.00
   2      1.0    0.07300     0.08976    0.00086   -0.01676      -0.81
   3      1.0    0.07600     0.08976    0.00086   -0.01376      -0.66
   4      1.0    0.07300     0.08976    0.00086   -0.01676      -0.81
   5      1.0    0.06600     0.08976    0.00086   -0.02376      -1.15
```

Table A1.2 Raw data of the nominal area-ethanol concentration standard curve

Nominal area	Ethanol conc. (g/l)
0	0
1	0.073
1	0.076
1	0.073
1	0.066
2	0.154
3	0.269
4	0.364
4	0.365
4	0.368
4	0.366
5	0.454
6	0.555
7	0.673
7	0.625
7	0.648
7	0.626
8	0.720
9	0.813
10	0.840

A1.2.2 Statistical analysis of the regression results

Q-test :-

4.6974/0.0004 = 11743.5 compared with F(0.05,1,19). Therefore, the model as a whole is significant.

t-test on the ethanol concentration coefficient :-

104.35 compared with t(0.025,19) = 2.093. Therefore, the term is significant.

Analysis of variance :-

F ratio (874.663) compared with F(0.05,9,9) = 3.18. Therefore, the means of the residuals of the replicates are the same.

A1.3 Statistical analysis on the lactose concentration-HPLC peak area standard curve

A1.3.1 Minitab regression analysis printout

```
MTB > name c1='area'
MTB > name c2='[lac]'
MTB > name c10='res'
MTB > read 'area' '[lac]'
     26 ROWS READ
MTB > end
MTB > regress 'area' on 1 predictor '[lac]';
SUBC> residuals in 'res';
SUBC> predict of '[lac]'.

The regression equation is
area = 4.63 + 0.971 [lac]

Predictor        Coef      Stdev    t-ratio      p
Constant      4.63258    0.02621     176.75  0.000
[lac]         0.97128    0.01836      52.89  0.000

s = 0.06345     R-sq = 99.1%     R-sq(adj) = 99.1%

Analysis of Variance

SOURCE          DF         SS         MS        F      p
Regression       1     11.262     11.262  2797.46  0.000
Error           24      0.097      0.004
Total           25     11.359

Obs.    [lac]      area       Fit  Stdev.Fit  Residual  St.Resid
   1     0.00    4.5330    4.6326     0.0262   -0.0996    -1.72
   2     0.00    4.5710    4.6326     0.0262   -0.0616    -1.07
   3     0.00    4.6510    4.6326     0.0262    0.0184     0.32
   4     0.48    5.2120    5.0959     0.0190    0.1161     1.92
   5     0.70    5.3240    5.3115     0.0161    0.0125     0.20
   6     0.70    5.3200    5.3115     0.0161    0.0085     0.14
   7     0.70    5.2830    5.3115     0.0161   -0.0285    -0.46
   8     0.85    5.4480    5.4533     0.0146   -0.0053    -0.09
   9     0.95    5.5600    5.5592     0.0136    0.0008     0.01
  10     1.00    5.5960    5.6039     0.0133   -0.0079    -0.13
  11     1.00    5.5980    5.6039     0.0133   -0.0059    -0.09
  12     1.00    5.6210    5.6039     0.0133    0.0171     0.28
  13     1.30    5.8930    5.8962     0.0125   -0.0032    -0.05
  14     1.30    5.8690    5.8962     0.0125   -0.0272    -0.44
  15     1.60    6.2170    6.1886     0.0140    0.0284     0.46
  16     1.70    6.3580    6.2828     0.0149    0.0752     1.22
  17     1.70    6.4220    6.2828     0.0149    0.1392     2.26R
  18     1.70    6.3930    6.2828     0.0149    0.1102     1.79
  19     1.78    6.3850    6.3595     0.0157    0.0255     0.41
  20     1.90    6.4700    6.4809     0.0172   -0.0109    -0.18
  21     2.00    6.5370    6.5751     0.0185   -0.0381    -0.63
  22     2.00    6.5670    6.5751     0.0185   -0.0081    -0.13
  23     2.00    6.5780    6.5751     0.0185    0.0029     0.05
  24     2.08    6.5370    6.6519     0.0196   -0.1149    -1.90
  25     2.08    6.6190    6.6519     0.0196   -0.0329    -0.54
  26     2.15    6.6060    6.7169     0.0205   -0.1109    -1.85
```

Table A1.3 Raw data of the peak area-lactose concentration standard curve

$\log_{10}$ (peak area)	$\log_{10}$ (lactose conc.) (g/l)
4.533	0
4.571	0
4.651	0
5.212	0.48
5.324	0.70
5.32	0.70
5.283	0.70
5.448	0.85
5.56	0.95
5.596	1.00
5.598	1.00
5.621	1.00
5.893	1.30
5.869	1.30
6.217	1.60
6.358	1.70
6.442	1.70
6.393	1.70
6.385	1.78
6.470	1.90
6.537	2.00
6.567	2.00
6.578	2.00
6.537	2.08
6.619	2.08
6.606	2.15

A1.3.2 Statistical analysis of the regression results

R^2 value :-

very high R^2 value was obtained with this regression equation

Q-test :-

11.262/0.004 = 2815.5

This compared to F(0.05,1,24) = 4.26. Therefore, the model is significant.

t-test on the constant term :-

176.75 compared with t(0.025,24) = 2.064. Therefore, the constant term is significant.

t-test on the lactose concentration term :-

52.89 compared with t(0.025,24) = 2.064. Therefore, the term is significant.

Analysis of variance :-

compare the F ratio = 5.327 with F(0.05,12,12) = 2.69. Therefore, the mean of the residuals of the replicates are the same.

APPENDIX 2

FERMENTATION AND SIMULATION DATA

Table A2.1 Raw data of fermentation trial 1

Time(hrs.)	Abs.(620 nm)	[Biomass] (dry wt.g/l)	[Biomass] 95% P.I.	hplc area	[Lactose] (g/l)	[Lactose] 95% P.I.	GC area ratio	[Ethanol] (g/l)	[Ethanol] 95% P.I.	Bleed vol. (l)	pH	Temp. (C)	air flowrate (l/min.)
0*	0.56(1:19)	6.0	1.0	749715	19.15	6.68	0.39	8.59	1.02	0	3.4	30.5	1.15
2*	0.56(1:19)	6.0	1.0	1527341	39.84	13.91	0.16	3.64	1.02	0	3.6	31.5	0.85
4*	0.6(1:19)	6.4	1.0	1533120	40.00	13.96	0.23	5.05	1.02	0	3.7	31.5	0.9
6	0.53(1:29)	8.4	1.5	1284244	33.33	11.63	0.29	6.46	1.02	0	3.8	32.5	0
8	0.595(1:29)	9.6	1.5	1148744	29.71	10.37	0.37	8.18	1.02	0	3.9	31.5	0
10	0.65(1:29)	10.5	1.5	1193279	30.90	10.78	0.43	9.56	1.02	0	4.1	31.5	0
12	0.6(1:39)	12.9	2.0	967216	24.89	8.69	0.47	10.51	1.02	0	4.3	31.5	0
0(feed)				1694042	44.33	15.47							
8(feed)				1714445	44.88	15.66							
12(feed)				1858993	48.78	17.02							
			Mean [Lactose] =		46.00	16.05							

Comment : unless stated, anaerobic fermentation condition with recycle
working volume 11 feed pump flowrate = 1.2257 l/hr.
* - aerobic recycle fermentation
0(feed) - feed at time t = 0 hr.
0.6(1:39) - after 1:39 dilution, the sample absorbance(620 nm) = 0.6

Table A2.2 Raw data of fermentation trial 2

Time(hrs.)	Abs.(620 nm)	[Biomass] (dry wt.g/l)	[Biomass] 95% P.I.	hplc area (1:9 diln)	[Lactose] (g/l)	[Lactose] 95% P.I.	GC area ratio	[Ethanol] (g/l)	[Ethanol] 95% P.I.	Bleed vol. (l)	pH	Temp. (C)	air flowrate (l/min.)
0*	0.355(1:19)	3.6	1.0	416699	104.56	36.49	0.24	5.33	1.02	0	3.75	32.5	0.85
2*	0.415(1:19)	4.3	1.0	426252	107.03	37.35	0.30	6.69	1.02	0	3.75	32	0.65
4*	0.5(1:19)	5.3	1.0	380692	95.27	33.25	0.41	9.21	1.02	0	3.6	32	0.8
6	0.42(1:29)	6.5	1.5	389989	97.67	34.09	0.53	11.77	1.02	0	3.65	32	0
8	0.49(1:29)	7.7	1.5	387370	96.99	33.85	0.58	12.82	1.02	0	3.9	30	0
10	0.54(1:29)	8.6	1.5	454112	114.25	39.87	0.64	14.30	1.02	0	4	32	0
12	0.48(1:39)	10.1	2.0	378030	94.59	33.01	0.70	15.62	1.02	0	4.15	31	0
20.5	0.585(1:49)	15.6	2.5	319692	79.59	27.78	0.92	20.53	1.02	0	4.5	32.5	0
22.5	0.67(1:59)	21.8	3.0	313148	77.91	27.19	0.89	19.73	1.02	0	4.5	33.8	0
24.5	0.55(1:69)	20.5	3.5	289590	71.88	25.09	0.93	20.75	1.02	0	4.3	32	0
44	0.55(1:99)	29.2	5.0	305052	75.84	26.47	0.97	21.58	1.02	0	4	32	0
0(feed)				500931	126.39	44.11							
2(feed)				514616	129.95	45.35							
4(feed)				499341	125.98	43.97							
6(feed)				547864	138.61	48.37							
12(feed)				512829	129.49	45.19							
20.5(feed)				539061	136.31	47.57							
44(feed)				541700	137.00	47.81							
			Mean [Lactose] =		131.96	46.05							

Comment : unless stated, anaerobic fermentation condition with recycle
working volume = 0.86 l, pump flowrate = 0.4 l/hr.
* - aerobic recycle fermentation
0(feed) - feed at time t = 0 hr.
0.42(1:29) - after 1:29 dilution, the sample absorbance(620 nm) = 0.42

Table A2.3 Raw data of fermentation trial 3

Time(hrs.)	Abs.(620 nm)	[Biomass] (dry wt.g/l)	[Biomass] 95% P.I.	hplc area	[Lactose] (g/l)	[Lactose] 95% P.I.	GC area ratio	[Ethanol] (g/l)	[Ethanol] 95% P.I.	Bleed vol. (l)	pH	Temp. (C)	air flowrate (l/min.)
0*	0.545(1:19)	2.8	1.0	627634	15.94	5.56	0.46	10.21	1.02	0	/	30	1
2.5	0.56(1:19)	6.0	1.0	1199757	31.07	10.84	0.35	7.67	1.02	0	/	30	N2
4	0.385(1:29)	5.9	1.5	1491655	38.88	13.57	0.40	8.97	1.02	0	4.13	29	N2
6	0.435(1:29)	6.7	1.5	998468	25.72	8.98	0.44	9.69	1.02	0	4.2	29	N2
8	0.39(1:39)	7.9	2.0	786956	20.13	7.02	0.52	11.53	1.02	0	4.24	28	N2
10	0.45(1:39)	9.3	2.0	480034	12.10	4.22	0.62	13.76	1.02	0	4.15	30	N2
12	0.52(1:39)	11.0	2.0	331116	8.25	2.88	0.68	15.11	1.02	0	3.98	30	N2
23	0.57(1:59)	18.2	3.0	182313	4.46	1.56	0.88	19.48	1.02	0	4.33	32	N2
0(feed)				1929064	50.67	17.69							
6(feed)				2080630	54.78	19.12							
23(feed)				1987350	52.25	18.24							
			Mean [Lactose] =		52.57	18.35							

Comment : unless stated, anaerobic fermentation condition with recycle
working volume = 0.93 l, pump flowrate = 0.405 l/hr.
* - aerobic recycle fermentation
0(feed) - feed at time t = 0 hr.
0.52(1:39) - after 1:39 dilution, the sample absorbance(620 nm) = 0.52

Table A2.4 Raw data of fermentation trial 4

Time(hrs.)	Abs.(620 nm)	[Biomass] (dry wt.g/l)	[Biomass] 95% P.I.	hplc area (1:9 diln)	[Lactose] (g/l)	[Lactose] 95% P.I.	GC area ratio	[Ethanol] (g/l)	[Ethanol] 95% P.I.	Bleed vol. (l)	pH	Temp. (C)	air flowrate (l/min.)
0	0.54(1:29)	8.6	1.5	474010	119.40	41.67	0.25	5.62	1.02	0	4.18	32	N2
1	0.45(1:39)	9.3	2.0	380443	95.21	33.23	0.20	4.44	1.02	0	4.23	31	N2
2	0.46(1:39)	9.6	2.0	308798	76.80	26.80	0.25	5.46	1.02	0	4.21	30	N2
3	0.49(1:39)	10.3	2.0	387422	97.01	33.86	0.31	6.86	1.02	0	4.23	30	N2
4	0.5(1:39)	10.5	2.0	279126	69.21	24.15	0.29	6.51	1.02	0	4.23	31	N2
5	0.45(1:49)	11.7	2.5	300463	74.66	26.06	0.33	7.36	1.02	0	4.26	30	N2
7	0.55(1:49)	14.6	2.5	331113	82.52	28.80	0.44	9.87	1.02	0	4.35	30	N2
8	0.47(1:59)	14.7	3.0	335043	83.53	29.15	0.48	10.62	1.02	0	4.41	30	N2
9	0.53(1:59)	16.8	3.0	301725	74.99	26.17	0.49	10.87	1.02	0	4.46	30	N2
10	0.53(1:59)	16.8	3.0	265488	65.73	22.94	0.48	10.66	1.02	0	4.55	29	N2
11	0.51(1:69)	18.8	3.5	271636	67.30	23.49	0.52	11.54	1.02	0	4.5	29	N2
1(feed)				529983	133.95	46.75							
8(feed)				556406	140.83	49.15							
11(feed)				543413	137.45	47.97							
			Mean [Lactose] =		137.41	47.96							

Comment : anaerobic condition, with nitrogen sparging
working volume = 0.91 l, feed pump flowrate = 1.045 l/hr.
0.51(1:69) - after 1:69 dilution, the sample absorbance(620 nm) = 0.51
N2 - no air flow, fermenter purged with nitrogen

Table A2.5 Raw data of fermentation trial 5

Time(hrs.)	Abs.(620 nm)	[Biomass] (dry wt.g/l)	[Biomass] 95% P.I.	hplc area	[Lactose] (g/l)	[Lactose] 95% P.I.	GC area ratio	[Ethanol] (g/l)	[Ethanol] 95% P.I.	Bleed vol. (l)	pH	Temp. (C)	air flowrate (l/min.)
0	0.58(1:19)	6.2	1.0	825611	21.14	7.38	0.33	7.3879948	1.02	0		29	N2
2	0.45(1:29)	7.0	1.5	1565777	40.88	14.27	0.28	6.1233131	1.02	0	4.44	31	N2
4	0.455(1:29)	7.1	1.5	1436082	37.39	13.05	0.38	8.3350989	1.02	0	4.47	32	N2
7.5	0.545(1:29)	8.7	1.5	942743	24.24	8.46	0.60	13.358469	1.02	0	4.71	31	N2
9.5	0.48(1:39)	10.1	2.0	633054	16.09	5.61	0.68	15.070557	1.02	0	4.66	30	N2
22.5	0.485(1:39)	10.2	2.0	255770	6.33	2.21	0.91	20.327216	1.02	0.78	4.16	30	N2
24.5	0.43(1:39)	8.9	2.0	494071	12.46	4.35	0.86	19.018696	1.02	0.12	4.15	31.5	N2
26.5	0.405(1:39)	8.3	2.0	372406	9.31	3.25	0.87	19.261632	1.02	0.12	4.18	32	N2
28.5	0.41(1:39)	8.4	2.0	427402	10.73	3.75	0.82	18.313817	1.02	0.012	4.19	32	N2
30.5	0.48(1:39)	10.1	2.0	405353	10.16	3.55	0.80	17.71396	1.02	0	4.13	32	N2
32.5	0.46(1:39)	9.6	2.0	437015	10.98	3.83	0.74	16.453361	1.02	0.0955	4.12	31	N2
34.5	0.47(1:39)	9.8	2.0	481475	12.13	4.23	0.81	18.069949	1.02	0.088	4.17	30	N2
36.5	0.465(1:39)	9.7	2.0	538188	13.61	4.75	0.80	17.827182	1.02	0.085	4.15	30	N2
38.5	0.445(1:39)	9.2	2.0	600159	15.23	5.31	0.80	17.799156	1.02	0.082	4.22	30	N2

0(feed)	2377876	62.86	21.94
4(feed)	2506568	66.36	23.16
7.5(feed)	2444872	64.68	22.57
22.5(feed)	2325734	61.44	21.44
36.5(feed)	2405515	63.61	22.20
38.5(feed)	2516840	66.64	23.26
Mean [Lactose] =		64.26	22.43

Comment : anaerobic condition, with nitrogen sparging
working volume = 1.06 l, feed pump flowrate = 0.355 l/hr.
0.445(1:39) - after 1:39 dilution, the sample absorbance (620 nm) = 0.445
0(feed) - feed at time = 0 hr.

Table A2.6 Raw data of fermentation trial 6

Time(hrs.)	Abs.(620 nm)	[Biomass] (dry wt.g/l)	[Biomass] 95% P.I.	hplc area (1:9 diln)	[Lactose] (g/l)	[Lactose] 95% P.I.	GC area ratio	[Ethanol] (g/l)	[Ethanol] 95% P.I.	Bleed vol. (l)	pH	Temp. (C)	air flowrate (l/min.)
0	0.555(1:29)	8.9	1.5	79830	19.07	6.65	1.00	22.31	1.02	0	4.06	30	N2
2	0.41(1:39)	8.4	2.0	213477	52.51	18.33	0.71	15.79	1.02	0	4.31	31.5	N2
4	0.4(1:39)	8.2	2.0	299699	74.47	25.99	0.54	12.00	1.02	0	4.39	31	N2
6	0.43(1:39)	8.9	2.0	317277	78.97	27.56	0.47	10.50	1.02	0	4.43	30	N2
9	0.495(1:39)	10.4	2.0	312304	77.70	27.12	0.73	16.21	1.02	0	4.7	32	N2
11	0.495(1:39)	10.4	2.0	267667	66.29	23.13	0.75	16.67	1.02	0.0975	4.87	30	N2
13	0.48(1:39)	10.1	2.0	273125	67.68	23.62	0.73	16.28	1.02	0.111	5	30	N2
15	0.47(1:39)	9.8	2.0	269760	66.82	23.32	0.71	15.86	1.02	0.108	4.88	30	N2
24.5	0.38(1:59)	11.6	3.0	245197	60.56	21.14	0.72	15.92	1.02	0.071	4.73	30	N2
26.5	0.42(1:59)	13.0	3.0	267370	66.21	23.11	0.85	18.82	1.02	0	4.64	31.5	N2
28.5	0.445(1:59)	13.8	3.0	264549	65.49	22.86	0.88	19.55	1.02	0	4.57	30	N2
0(feed)				441135	110.89	38.70							
4(feed)				421109	105.70	36.89							
9(feed)				409392	102.68	35.83							
13(feed)				438786	110.28	38.49							
24.5(feed)				462978	116.54	40.67							
28.5(feed)				449060	112.94	39.42							
			Mean [Lactose] =		109.84	38.33							

Comment : anaerobic condition, with nitrogen sparging
working volume = 0.84 l, feed pump flowrate = 0.36 l/hr.
0.43(1:39) - after 1:39 dilution, the sample absorbance (620 nm) = 0.43
0(feed) - feed at time = 0 hr.
N2 - no air flow, fermenter purged with nitrogen

Table A2.7 Raw data of fermentation trial 7

Time(hrs.)	Abs.(620 nm)	[Biomass] (dry wt.g/l)	[Biomass] 95% P.I	hplc area (1:9 diln)	[Lactose] (g/l)	[Lactose] 95% P.I	GC area ratio	[Ethanol] (g/l)	[Ethanol] 95% P.I	Bleed vol. (l)	pH	Temp. (C)	air flowrate (l/min.)
0	0.435(1:29)	6.7	1.5	159837	38.98	13.60	0.30	6.73	1.02	0	4.39	28	N2
1	0.46(1:29)	7.2	1.5	159036	38.78	13.53	0.31	6.80	1.02	0	4.38	29.5	N2
2	0.45(1:29)	7.0	1.5	180814	44.26	15.45	0.27	5.96	1.02	0	4.37	29	N2
3	0.5(1:29)	7.9	1.5	174135	42.57	14.86	0.34	7.54	1.02	0	4.39	29	N2
4	0.5(1:29)	7.9	1.5	162878	39.74	13.87	0.37	8.15	1.02	0	4.42	30	N2
5	0.445(1:39)	9.2	2.0	151938	36.99	12.91	0.41	9.19	1.02	0	4.53	30	N2
6	0.465(1:39)	9.7	2.0	157584	38.41	13.41	0.47	10.41	1.02	0	4.55	33	N2
7	0.46(1:39)	9.6	2.0	140077	34.02	11.87	0.48	10.73	1.02	0	4.65	30	N2
8	0.495(1:39)	10.4	2.0	140243	34.07	11.89	0.51	11.36	1.02	0	4.72	31.5	N2
9	0.51(1:39)	10.8	2.0	130812	31.71	11.07	0.56	12.42	1.02	0	4.85	31	N2
0(feed)				223593	55.07	19.22							
4(feed)				222177	54.71	19.10							
8(feed)				232887	57.43	20.04							
			Mean [Lactose] =		55.74	19.45							

Comment : anaerobic condition, with nitrogen sparging
working volume = 0.91 l, feed pump flowrate = 0.8022 l/hr.
0.43(1:39) - after 1:39 dilution, the sample absorbance (620 nm) = 0.43
0(feed) - feed at time = 0 hr.
N2 - no air flow, fermenter purged with nitrogen

Table A2.8 Raw data of fermentation trial 8

Time(hrs.)	Abs.(620 nm)	[Biomass] (dry wt.g/l)	[Biomass] 95% P.I.	hplc area (1:9 diln)	[Lactose] (g/l)	[Lactose] 95% P.I.	GC area ratio	[Ethanol] (g/l)	[Ethanol] 95% P.I.	Bleed vol. (l)	pH	Temp. (C)	air flowrate (l/min.)
0	0.4(1:29)	6.1	1.5	90253	21.64	7.55	0.57	12.72	1.02	0	4.27	28	N2
2	0.41(1:29)	6.3	1.5	179325	43.88	15.31	0.26	5.83	1.02	0	4.44	30	N2
4	0.475(1:29)	7.5	1.5	178748	43.73	15.26	0.30	6.74	1.02	0	4.39	30	N2
5	0.37(1:39)	7.5	2.0	166601	40.68	14.20	0.35	7.70	1.02	0	4.4	30	N2
6	0.385(1:39)	7.8	2.0	166777	40.72	14.21	0.37	8.27	1.02	0	4.45	31	N2
7	0.41(1:39)	8.4	2.0	159884	38.99	13.61	0.41	9.01	1.02	0	4.52	31	N2
8	0.445(1:39)	9.2	2.0	147450	35.87	12.52	0.54	11.93	1.02	0	4.69	31.5	N2
9	0.43(1:39)	8.9	2.0	109423	26.38	9.21	0.64	14.23	1.02	0	4.91	34	N2
0(feed)				219938	54.15	18.90							
4(feed)				207888	51.09	17.83							
8(feed)				223165	54.97	19.18							
			Mean [Lactose] =		53.40	18.64							

Comment : anaerobic condition, with nitrogen sparging
working volume = 0.91 l, feed pump flowrate = 0.86 l/hr.
0.43(1:39) - after 1:39 dilution, the sample absorbance (620 nm) = 0.43
0(feed) - feed at time = 0 hr.
N2 - no air flow, fermenter purged with nitrogen

Table A2.9 Raw data of simulation trial run 5 (analytical solution as simulation parameters)

initial fermenter biomass conc. g/l =6.200
initial feed stream abiotic-phase substrate conc. g/l =64.265
initial fermenter abiotic-phase substrate conc. g/l =21.140
initial feed stream abiotic-phase product conc. g/l =0.000
initial fermenter abiotic-phase product conc. g/l =7.388

feed flow rate l/hr. = 0.355
fermenter working vol. l = 1.060
maximum biomass conc. g/l = 10.000
anaerobic state biomass yield coeff. =0.0184000000
aerobic state biomass yield coeff. =0.0184000000
aerobic state product biomass yield coeff. =18.3800000000
anaerobic state biomass yield coeff. =18.3800000000
maximum abiotic phase product conc. g/l =40.0000000000
maximum specific growth rate hr-1 =0.0590
Monod half saturation constant l/g = 0.0000
Time step s =3.000E+01
Total number of steps = 4800
Number of steps between printout = 120

Time(hr.)	Biomass(g/l)	Substrate(g/l)	Product(g/l)	Bleed(l/hr.)
0.00	6.200	21.140	7.388	0.00000
1.00	6.493	19.898	9.856	0.00000
2.00	6.779	19.305	11.522	0.00000
3.00	7.064	18.965	12.685	0.00000
4.00	7.349	18.666	13.536	0.00000
5.00	7.638	18.305	14.195	0.00000
6.00	7.931	17.844	14.735	0.00000
7.00	8.229	17.274	15.202	0.00000
8.00	8.533	16.606	15.624	0.00000
9.00	8.843	15.854	16.019	0.00000
10.00	9.159	15.038	16.396	0.00000
11.00	9.481	14.172	16.760	0.00000
12.00	9.809	13.271	17.117	0.00000
13.00	10.003	12.395	17.449	0.01462
14.00	10.003	11.873	17.652	0.03479
15.00	10.003	11.602	17.763	0.03455
16.00	10.003	11.463	17.823	0.03442
17.00	10.003	11.394	17.856	0.03435
18.00	10.003	11.362	17.874	0.03432
19.00	10.003	11.347	17.884	0.03429
20.00	10.003	11.342	17.889	0.03428
21.00	10.003	11.341	17.892	0.03428
22.00	10.003	11.341	17.894	0.03427
23.00	10.003	11.343	17.895	0.03427
24.00	10.003	11.344	17.895	0.03427
25.00	10.003	11.345	17.895	0.03427
26.00	10.003	11.346	17.896	0.03427
27.00	10.003	11.347	17.896	0.03427
28.00	10.003	11.347	17.896	0.03427
29.00	10.003	11.348	17.896	0.03427
30.00	10.003	11.348	17.896	0.03427
31.00	10.003	11.348	17.896	0.03427
32.00	10.003	11.349	17.896	0.03427
33.00	10.003	11.349	17.896	0.03427
34.00	10.003	11.349	17.896	0.03427

Table A2.10 Raw data of simulation trial run 5 (regression solution as simulation parameters)

initial feed stream abiotic-phase substrate conc. g/l =64.265
initial fermenter abiotic-phase substrate conc. g/l =21.140
initial feed stream abiotic-phase product conc. g/l =0.000
initial fermenter abiotic-phase product conc. g/l =7.388

feed flow rate l/hr. = 0.355
fermenter working vol. l = 1.060
maximum biomass conc. g/l = 10.000
anaerobic state biomass yield coeff. =0.027800000
aerobic state biomass yield coeff. =0.027900000
aerobic state product biomass yield coeff. =13.700000000
anaerobic state biomass yield coeff. =13.700000000
maximum abiotic phase product conc. g/l =40.000000000
maximum specific growth rate hr-1 =0.1050
Monod half saturation constant l/g = 0.0001
time step s =3.000E+01
Total number of steps = 4800
Number of steps between printout = 120

Time(hr.)	Biomass(g/l)	Substrate(g/l)	Product(g/l)	Bleed(l/hr.)
0.00	6.200	21.140	7.388	0.00000
1.00	6.717	17.626	11.298	0.00000
2.00	7.217	13.644	15.895	0.00000
3.00	7.709	14.420	17.675	0.00000
4.00	8.203	13.510	18.764	0.00000
5.00	8.703	12.677	17.934	0.00000
6.00	9.211	11.810	18.766	0.00000
7.00	9.730	10.870	19.469	0.00000
8.00	10.004	9.958	20.063	0.02651
9.00	10.004	9.566	20.389	0.03451
10.00	10.004	9.471	20.562	0.05386
11.00	10.004	9.494	20.633	0.05356
12.00	10.004	9.558	20.675	0.05337
13.00	10.004	9.626	20.694	0.05329
14.00	10.004	9.686	20.703	0.05325
15.00	10.004	9.735	20.709	0.05323
16.00	10.004	9.773	20.711	0.05322
17.00	10.004	9.801	20.713	0.05321
18.00	10.004	9.823	20.713	0.05321
19.00	10.004	9.838	20.714	0.05321
20.00	10.004	9.849	20.714	0.05321
21.00	10.004	9.857	20.714	0.05321
22.00	10.004	9.863	20.714	0.05321
23.00	10.004	9.867	20.714	0.05321
24.00	10.004	9.870	20.714	0.05321
25.00	10.004	9.873	20.714	0.05321
26.00	10.004	9.874	20.714	0.05321
27.00	10.004	9.875	20.714	0.05321
28.00	10.004	9.876	20.714	0.05321
29.00	10.004	9.877	20.714	0.05321

Table A2.11 Raw data of simulation trial run 6 (analytical solution as simulation parameters)

initial feed stream biomass conc. g/l =0.000
initial fermenter biomass conc. g/l =8.940
initial feed stream abiotic-phase substrate conc. g/l =109.840
initial fermenter abiotic-phase substrate conc. g/l =19.070
initial feed stream abiotic-phase product conc. g/l =0.000
initial fermenter abiotic-phase product conc. g/l =22.308

feed flow rate l/hr. = 0.360
fermenter working vol. l = 0.840
maximum biomass conc. g/l = 10.000
anaerobic state biomass yield coeff. =0.0345000000
aerobic state biomass yield coeff. =0.0345000000
aerobic state product biomass yield coeff. =11.3000000000
anaerobic state biomass yield coeff. =11.3000000000
maximum abiotic phase product conc. g/l =40.0000000000
maximum specific growth rate hr-1 =0.1060
Monod half saturation constant l/g = 0.0001
Time step s =2.000E+01
Total number of steps = 5400
Number of steps between printout = 180

Time(hr.)	Biomass(g/l)	Substrate(g/l)	Product(g/l)	Bleed(l/hr.)
0.00	8.940	19.070	22.308	0.00000
1.00	9.416	39.389	18.945	0.00000
2.00	9.978	50.638	17.529	0.00000
3.00	10.003	57.027	16.973	0.04834
4.00	10.003	60.948	16.709	0.05129
5.00	10.003	63.387	16.575	0.05172
6.00	10.003	64.919	16.513	0.05192
7.00	10.003	65.890	16.483	0.05202
8.00	10.003	66.510	16.469	0.05207
9.00	10.003	66.907	16.461	0.05209
10.00	10.003	67.163	16.458	0.05210
11.00	10.003	67.328	16.456	0.05211
12.00	10.003	67.435	16.455	0.05211
13.00	10.003	67.504	16.455	0.05211
14.00	10.003	67.549	16.455	0.05211
15.00	10.003	67.578	16.455	0.05211
16.00	10.003	67.597	16.455	0.05211
17.00	10.003	67.610	16.454	0.05211
18.00	10.003	67.618	16.454	0.05211
19.00	10.003	67.623	16.454	0.05211
20.00	10.003	67.626	16.454	0.05211
21.00	10.003	67.629	16.454	0.05211
22.00	10.003	67.630	16.454	0.05211
23.00	10.003	67.631	16.454	0.05211
24.00	10.003	67.632	16.454	0.05211

Table A2.12 Raw data of simulation trial run 6 (regression solution as simulation parameters)

initial feed stream biomass conc. g/l =0.000
initial fermenter biomass conc. g/l =8.970
initial feed stream abiotic-phase substrate conc. g/l =109.840
initial fermenter abiotic-phase substrate conc. g/l =19.070
initial feed stream abiotic-phase product conc. g/l =0.000
initial fermenter abiotic-phase product conc. g/l =22.308

feed flow rate l/hr. = 0.360
fermenter working vol. l = 0.840
maximum biomass conc. g/l = 10.000
anaerobic state biomass yield coeff. =0.0278000000
aerobic state biomass yield coeff. =0.0278000000
aerobic state product biomass yield coeff. =13.7000000000
anaerobic state biomass yield coeff. =13.7000000000
maximum abiotic phase product conc. g/l =40.0000000000
maximum specific growth rate hr-1 =0.1050
monod half saturation constant l/g = 0.0001
Time step s =2.000E+01
Total number of steps = 5400
Number of steps between printout = 180

Time(hr.)	Biomass(g/l)	Substrate(g/l)	Product(g/l)	Bleed(l/hr.)
0.00	8.970	19.070	22.308	0.00000
1.00	9.433	37.076	19.725	0.00000
2.00	7.963	46.666	18.709	0.00000
3.00	10.003	52.386	18.472	0.04356
4.00	10.003	55.842	18.361	0.04732
5.00	10.003	58.009	18.302	0.04752
6.00	10.003	59.397	18.273	0.04761
7.00	10.003	60.287	18.262	0.04765
8.00	10.003	60.860	18.257	0.04767
9.00	10.003	61.231	18.254	0.04767
10.00	10.003	61.471	18.253	0.04768
11.00	10.003	61.627	18.253	0.04768
12.00	10.003	61.728	18.252	0.04768
13.00	10.003	61.794	18.252	0.04768
14.00	10.003	61.837	18.252	0.04768
15.00	10.003	61.865	18.252	0.04768
16.00	10.003	61.883	18.252	0.04768
17.00	10.003	61.895	18.252	0.04768
18.00	10.003	61.902	18.252	0.04768
19.00	10.003	61.907	18.252	0.04768
20.00	10.003	61.911	18.252	0.04768
21.00	10.003	61.913	18.252	0.04768
22.00	10.003	61.914	18.252	0.04768
23.00	10.003	61.915	18.252	0.04768
24.00	10.003	61.916	18.252	0.04768

Table A2.13 Raw data of simulation trial run 7 (regression solution as simulation parameters)

nitial feed stream biomass conc. g/l =0.000
nitial fermenter biomass conc. g/l =7.010
nitial feed stream abiotic-phase substrate conc. g/l =55.740
nitial fermenter abiotic-phase substrate conc. g/l =44.260
nitial feed stream abiotic-phase product conc. g/l =0.000
nitial fermenter abiotic-phase product conc. g/l =5.960

eed flow rate l/hr. = 0.802
ermenter working vol. l = 0.910
aximum biomass conc. g/l = 50.000
naerobic state biomass yield coeff. =0.0278000000
erobic state biomass yield coeff. =0.0278000000
erobic state product biomass yield coeff. =13.7000000000
naerobic state biomass yield coeff. =13.7000000000
aximum abiotic phase product conc. g/l =40.0000000000
aximum specific growth rate hr-1 =0.1050
onod half saturation constant 1/g = 0.0001
ime step s =1.000E+01
otal number of steps = 3600
umber of steps between printout = 360

ime(hr.)	Biomass(g/l)	Substrate(g/l)	Product(g/l)	Bleed(l/hr.)
0.00	7.010	44.260	5.960	0.00000
1.00	7.640	35.911	8.210	0.00000
2.00	8.291	31.901	9.352	0.00000
3.00	8.976	29.425	10.135	0.00000
4.00	9.699	27.479	10.810	0.00000
5.00	10.463	25.709	11.457	0.00000
6.00	11.267	23.993	12.099	0.00000
7.00	12.113	22.292	12.742	0.00000
8.00	13.001	20.595	13.387	0.00000
9.00	13.930	18.901	14.031	0.00000
0.00	14.900	17.216	14.673	0.00000

Table A2.14 Raw data of simulation trial run 8 (regression solution as simulation parameters)

initial feed stream biomass conc. g/l =0.000
initial fermenter biomass conc. g/l =6.310
initial feed stream abiotic-phase substrate conc. g/l =53.400
initial fermenter abiotic-phase substrate conc. g/l =43.880
initial feed stream abiotic-phase product conc. g/l =0.000
initial fermenter abiotic-phase product conc. g/l =5.830

feed flow rate 1/hr. = 0.860
fermenter working vol. 1 = 0.910
maximum biomass conc. g/l = 50.000
anaerobic state biomass yield coeff. =0.0278000000
aerobic state biomass yield coeff. =0.0278000000
aerobic state product biomass yield coeff. =13.7000000000
anaerobic state biomass yield coeff. =13.7000000000
maximum abiotic phase product conc. g/l =40.0000000000
maximum specific growth rate hr-1 =0.1050
Monod half saturation constant 1/g = 0.0001
Time step s =1.000E+01
Total number of steps = 3600
Number of steps between printout = 360

Time(hr.)	Biomass(g/l)	Substrate(g/l)	Product(g/l)	Bleed(l/hr.
0.00	6.310	43.880	5.830	0.00000
1.00	6.886	36.246	7.390	0.00000
2.00	7.493	32.564	8.269	0.00000
3.00	8.136	30.259	8.943	0.00000
4.00	8.820	28.419	9.565	0.00000
5.00	9.546	26.724	10.179	0.00000
6.00	10.315	25.063	10.800	0.00000
7.00	11.127	23.400	11.429	0.00000
8.00	11.984	21.727	12.064	0.00000
9.00	12.885	20.045	12.704	0.00000
10.00	13.830	18.357	13.347	0.00000

APPENDIX 3

SIMULATION PARAMETERS ESTIMATION

A3.1 Sample printout of the non-linear regression analysis on the unsteady-state trial

```
BMDPAR--DERIVATIVE-FREE NONLINEAR REGRESSION

Copyright (C) Regents of University of California.

  BMDP Statistical Software, Inc.
  1964 Westwood Blvd.  Suite 202          Phone (213) 475-5700
  Los Angeles, California  90025            Telex  4972203

Program Version:  April 1985

This version of BMDP has been converted for use on PRIME computers by
  Systems Applications Inc.          Phone: 415/472-4011
  101 Lucas Valley Road              Telex: 469287
  San Rafael, California 94903       Cable: SYSTEMS SAN RAFAEL CA


Manual  Edition:  1983, 1985 reprint.  State NEWS in the PRINT
                  paragraph for a summary of new features.

WED, NOV 08 1989     AT 14:32:36

PROGRAM CONTROL INFORMATION

/PROBLEM      TITLE IS 'BATCHFERM'.
/INPUT        VARIABLES ARE 2.
              UNIT IS 11.
              FORMAT IS FREE.
/VARIABLE     NAMES ARE TIME,BIOMASS.
/FUN          F=P1/(1+EXP(P2+P3*TIME)).
/REGRESS      DEPENDENT IS BIOMASS.
              INDEPENDENT IS TIME.
              PARAM=3.
/PARAM        INITIAL ARE 10,1,-0.1.
/PLOT         RESID.
              VAR=TIME.
              NORM.
              DNORM.
/END

PROBLEM TITLE IS
BATCHFERM

NUMBER OF VARIABLES TO READ IN. . . . . .          2
NUMBER OF VARIABLES ADDED BY TRANSFORMATIONS.      0
TOTAL NUMBER OF VARIABLES . . . . . .              2
NUMBER OF CASES TO READ IN. . . . . .  TO END
CASE LABELING VARIABLES . . . . . .
MISSING VALUES CHECKED BEFORE OR AFTER TRANS.  NEITHER
BLANKS ARE. . . . . .  MISSING
INPUT UNIT NUMBER . . . . . .             11
REWIND INPUT UNIT PRIOR TO READING. DATA. . .     YES
NUMBER OF WORDS OF DYNAMIC STORAGE. . . . . .   65530

VARIABLES TO BE USED
     1 TIME          2 BIOMASS

INPUT FORMAT IS
FREE

MAXIMUM LENGTH DATA RECORD IS    80 CHARACTERS.


  VARIABLES TO BE PLOTTED
            1  TIME

  PLOT OF PREDICTED VALUES VERSUS RESIDUALS . . .      YES
  NORMAL PROBABILITY PLOT OF RESIDUALS. . . . . .      YES
  DETRENDED NORMAL PROBABILITY PLOT OF RESIDUALS.      YES
1PAGE   2  BMDPAR BATCHFERM


  REGRESSION TITLE
BATCHFERM

  REGRESSION NUMBER . . . . . .          0
  DEPENDENT VARIABLE. . . . . .    BIOMASS
  WEIGHTING VARIABLE. . . . . .
  NUMBER OF PARAMETERS. . . . . .        3
  NUMBER OF CONSTRAINTS . . . . . .      0
  TOLERANCE FOR PIVOTING. . . . . .  1.0E-08
  TOLERANCE FOR CONVERGENCE . . . . . .  1.0E-05
  MAXIMUM NUMBER OF ITERATIONS. . . . . .       50
  MAXIMUM NUMBER OF INCREMENT HALVINGS. . . . .  5

***** FUN       PARAGRAPH IS USED *****

PARAMETERS TO BE ESTIMATED

                  P1                P2                P3
MINIMUM   -0.212676E 38     -0.212676E 38     -0.212676E 38
MAXIMUM    0.212676E 38      0.212676E 38      0.212676E 38
INITIAL       10.000000          1.000000         -0.100000


  USING THE ABOVE SPECIFICATIONS THIS PROGRAM COULD USE UP TO 4319 CASES.

NUMBER OF CASES READ. . . . . .          8

  VARIABLE                          STANDARD
  NO.  NAME          MEAN           DEVIATION        MINIMUM          MAXIMUM

   1   TIME       8.187498          7.161093        0.000000        23.000000
   2   BIOMASS    8.485722          4.647292        2.787400        18.241199

ITER.  INCR.   RESIDUAL SUM      PARAMETERS
 NO.   HALV.    OF SQUARES        P1              P2              P3

   0     0     203.324604      10.000000       1.100000       -0.100000
   0     0     189.167225      10.000000       1.000000       -0.100000
   0     0     173.708452      10.000000       1.000000       -0.110000
   0     0     155.537875      11.000000       1.000000       -0.100000
   1     0      45.962785      24.688761       2.532184       -0.140358
   2     0      16.544581      22.487297       1.748559       -0.174138
   3     0      10.084487      23.901966       1.766843       -0.113615
   4     0       2.360153      24.809432       1.710435       -0.121325
   5     0       2.296673      24.188233       1.663365       -0.123101
   6     0       2.236083      23.681700       1.656641       -0.125293
   7     0       2.228813      23.288268       1.642349       -0.126838
   8     0       2.228251      23.338809       1.643055       -0.126605
   9     5       7.690744      20.787731       1.552667       -0.124142
  10     0       2.228148      23.322944       1.643221       -0.126800
```

A3.2 Fermentation parameters estimation

A3.2.1 Unsteady-state parameters calculation (based on the fitted logistic equations)

A3.2.1.1 Maximum specific growth rate estimation

The maximum specific growth rate equals to the fitted P1 value of the logistic equation.

A3.2.1.2 Biomass yield coefficient estimation

$Y_{xs} = (dX'/dt)/(ds'/dt)$ (1)

where dX'/dt = rate of change of biomass produced (g/hr)

dS'/dt = rate of change of substrate utilized (g/hr)

$dX'/dt = V*dX/dt$ (2)

where dX/dt = rate of change of biomass concentration (g/l.hr)

$dX'/dt = V*d(P_1/(1+e^{P_2+P_3*t}))/dt$ (3)

$= -V*P_1*(1+e^{P_2+P_3*t})^{-2}*P_3*e^{P_2+P_3*t}$ (4)

$dS'/dt = F*S_o - F*S(t) + (-V*dS(t)/dt)$ (5)

where S_o = feed substrate concentration (g/l)

S(t) = substrate concentration at time = t hr

$dS'/dt = F*S_o - F*P_1/(1+e^{P_2+P_3*t}) + -V*P_1*(1+e^{P_2+P_3*t})^{-2}*P_3*e^{P_2+P_3*t}$ (6)

Therefore, Y_{xs} = (4)/(6)

A3.2.1.3 Product yield coefficient estimation

$Y_{px} = (dP'/dt)/(dX'/dt)$ (7)

where dP'/dt = rate of change of product produced (g/hr)

dX'/dt = rate of change of biomass produced (g/hr)

$dP'/dt = F*C_p(t) + V*dC_p(t)/dt - F*C_{po}$ (8)

where F = feed stream flowrate

V = fermenter working volume

$C_p(t)$ = product concentration at time t

C_{po} = product concentration at time

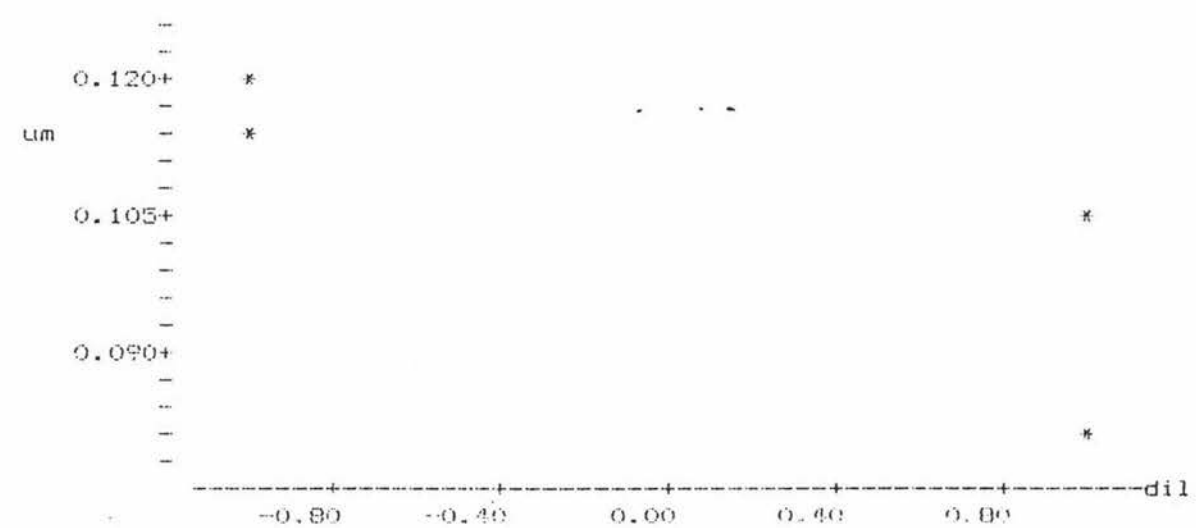
$dP'/dt = F*P_1/(1+e^{P_2+P_3*t}) + -V*P_1*(1+e^{P_2+P_3*t})^{-2}*P_3*e^{P_2+P_3*t} - 0$ (9)

Therefore, the product yield coefficient = (9)/(4)

A3.3 Sample linear regression analysis on the estimated unsteady-state fermentation parameters

A3.3.1 Minitab printout of the maximum specific growth rate regression analysis

```
MTB > regress 'um' on 2 predictors c2 c3;
SUBC> residuals in 'res'.

The regression equation is
um = 0.105 - 0.0125 dil + 0.00700 l*D

Predictor       Coef        Stdev     t-ratio        p
Constant    0.105500    0.004000       26.37    0.024
dil        -0.012500    0.004000       -3.12    0.197
l*D         0.007000    0.004000        1.75    0.330

s = 0.008000     R-sq = 92.8%     R-sq(adj) = 78.3%

Analysis of Variance

SOURCE        DF          SS          MS          F        p
Regression     2  0.00082100  0.00041050       6.41    0.269
Error          1  0.00006400  0.00006400
Total          3  0.00088500

SOURCE        DF      SEQ SS
dil            1  0.00062500
l*D            1  0.00019600

MTB > plot 'um' c2
```



A3.3.2 Statistical analysis of the regression results

R^2 value :-

high R^2 value (92.8%)

Q-test :-

0.0004105/0.000064 = 6.414 compared with F(0.05,2,1) = 199.5. Therefore, the model as a whole is not significant.

t-test on the constant term :-

26.37 compared with t(0.025,1) = 12.706. Therefore, the constant term is significant

t-test on the dilution rate term :-

3.12 compared with 12.706. Therefore, the term is not significant.

t-test on the lactose concentration-dilution rate interaction term :-

1.75 compared with 12.706. Therefore, the term is not significant.

A3.4 Sample linear regression analysis on the sensitivity test results of the biomass level on fermentation parameters

A3.4.1 Minitab regression analysis printout

```
MTB > name c20='UYYY'

MTB > multi c1 by c2 in c40
MTB > multi c40 by c3 in c41
MTB > multi c41 by c5 in c20
MTB > regress '[bio]' on 16 predictors in c1 c2 c3 c4 c5 c10 c11 c12 c13 c14 c15
    >  c16 c17 c18 c19 c20;
SUBC> residual in 'res'.
```

The regression equation is

[bio] = 14.3 + 3.40 Um + 0.325 Yxs - 1.39 Ypx + 0.0006 Ks + 1.49 Ypm
+ 0.326 UmYxs - 1.03 UmYpx + 1.07 UmYpm - 0.160 YxsYpx
+ 0.169 YxsYpm + 0.0009 YpxYpm - 0.160 UmYxsYpx + 0.169 UmYxsYpm
- 0.107 UmYpxYpm - 0.0034 YYY - 0.0034 UYYY

Predictor	Coef	Stdev	t-ratio	p
Constant	14.3335	0.0537	266.96	0.000
Um	3.40429	0.05967	57.05	0.000
Yxs	0.32547	0.05967	5.45	0.000
Ypx	-1.39103	0.05967	-23.31	0.000
Ks	0.00065	0.05967	0.01	0.991
Ypm	1.49138	0.05967	24.99	0.000
UmYxs	0.32562	0.06151	5.29	0.000
UmYpx	-1.03169	0.06151	-16.77	0.000
UmYpm	1.07469	0.06151	17.47	0.000
YxsYpx	-0.16025	0.06151	-2.61	0.015
YxsYpm	0.16875	0.06151	2.74	0.011
YpxYpm	0.00094	0.06151	0.02	0.988
UmYxsYpx	-0.16031	0.06151	-2.61	0.015
UmYxsYpm	0.16881	0.06151	2.74	0.011
UmYpxYpm	-0.10687	0.06151	-1.74	0.095
YYY	-0.00344	0.06151	-0.06	0.956
UYYY	-0.00338	0.06151	-0.05	0.957

s = 0.3480 R-sq = 99.5% R-sq(adj) = 99.2%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	16	617.293	38.581	318.65	0.000
Error	25	3.027	0.121		
Total	41	620.320			

A3.4.2 Statistical analysis of the regression results

R^2 value :-

high R^2 value (99.5%)

Q-test :-

38.581/0.121 = 318.85 compared with F(0.05,16,25) = 2.09. Therefore, the model is significant.

t-test (compared against t(0.025,25) = 2.06) :-

Constant term : 266.96, significant

μ_m	: 57.05, significant
Y_{xs}	: 5.45, significant
Y_{px}	: 23.31, significant
K_s	: 0.01, not significant
C_{pm}	: 24.99, significant
$\mu_m * Y_{xs}$	: 5.29, significant
$\mu_m * Y_{px}$	: 16.77, significant
$\mu_m * C_{pm}$	: 17.47, significant
$Y_{xs} * Y_{px}$	: 2.61, significant
$Y_{xs} * C_{pm}$	: 2.74, significant
$Y_{px} * C_{pm}$	: 0.02, not significant
$\mu_m * Y_{xs} * Y_{px}$	: 2.61, significant
$\mu_m * Y_{xs} * C_{pm}$	: 2.74, significant
$\mu_m * Y_{px} * C_{pm}$	: 1.74, not significant
$Y_{xs} * Y_{px} * C_{pm}$	: 0.06, not significant
$\mu_m * Y_{xs} * Y_{px} * C_{pm}$	: 0.05, not significant

APPENDIX 4

SIMULATION PROGRAM

A4.1 Printout of the simulation program

```
      begin
      B:=Vr*(Cx+Ks-Cx)/Cx;
      Cx:=(Vr*Cx-B*Cx)/Vr;
      end
      else
      begin
      B:=0;
      Cx:=Cx;
      end;

      Bnew:=Bnew+B;

      biomass(df,F,Cx0,B,Cxw,Cx,Yp,Ypm,mum,Ys,Vr,Ks,a1);
      sub(df,F,Ys0,B,Ys,Yp,Ypm,mum,Cx,Vr,Ks,Yxs,b1);
      product(df,F,Yp0,B,Yp,Ypm,mum,Ys,Cx,Vr,Ks,Ypx,c1);

      biomass(df,F,Cx0,B,Cxw,Cx+a1/2,Yp+c1/2,Ypm,mum,Ys+b1/2,Vr,Ks,a2);
      sub(df,F,Ys0,B,Ys+b1/2,Yp+c1/2,Ypm,mum,Cx+a1/2,Vr,Ks,Yxs,b2);
      product(df,F,Yp0,B,Yp+c1/2,Ypm,mum,Ys+b1/2,Cx+a1/2,Vr,Ks,Ypx,c2);

      biomass(df,F,Cx0,B,Cxw,Cx+a2/2,Yp+c2/2,Ypm,mum,Ys+b2/2,Vr,Ks,a3);
      sub(df,F,Ys0,B,Ys+b2/2,Yp+c2/2,Ypm,mum,Cx+a2/2,Vr,Ks,Yxs,b3);
      product(df,F,Yp0,B,Yp+c2/2,Ypm,mum,Ys+b2/2,Cx+a2/2,Vr,Ks,Ypx,c3);

      biomass(df,F,Cx0,B,Cxw,Cx+a3,Yp+c3,Ypm,mum,Ys+b3,Vr,Ks,a4);
      sub(df,F,Ys0,B,Ys+b3,Yp+c3,Ypm,mum,Cx+a3,Vr,Ks,Yxs,b4);
      product(df,F,Yp0,B,Yp+c4,Ypm,mum,Ys+b4,Cx+a4,Vr,Ks,Ypx,c4);
Cx:=Cx+(a1+2*a2+2*a3+a4)/6;
Ys:=Ys+(b1+2*b2+2*b3+b4)/6;
Yp:=Yp+(c1+2*c2+2*c3+c4)/6;

      if Cx<=0 then Cx:=0;
      if Ys<=0 then Ys:=0;
      if Yp<=0 then Yp:=0;

if nn>=nprint then
begin
Bprint:=Bnew*(nprint*ts/3600);
writeln(time:5:2,'  ',Cx:14:3,'   ',Ys:14:3,'   ',Yp:14:3,'    ',Bprint:12:5);
writeln(results,time:5:2,'  ',Cx:14:3,'   ',Ys:14:3,'   ',Yp:14:3,'      ',Bprint:12
,5);
writeln(results);
nn:=1;
Bnew:=0;
end
else
begin
nn:=nn+1;
Bnew:=Bnew;
end;
end;
flush(results);
close(results);
end.
```

```
      write('number of steps between printout ?');
      readln(nprint);
      end;

begin
    assign(results,'results.res');
    rewrite(results);
startup(Cx0i,Cxi,Ys0i,Ysi,Yp0i,Ypi);
    writeln(results);writeln(results);
    writeln(results,'initial feed stream biomass conc. g/l =',Cx0i:5:3);
    writeln(results,'initial fermenter biomass conc. g/l =',Cxi:5:3);
    writeln(results,'initial feed stream abiotic-phase substrate conc. g/l =',Ys
0i:5:3);
    writeln(results,'initial fermenter abiotic-phase substrate conc. g/l =',Ysi:
5:3);
    writeln(results,'initial feed stream abiotic-phase product conc. g/l =',Yp0i
:5:3);
    writeln(results,'initial fermenter abiotic-phase product conc. g/l =',Ypi:5:
3);
    writeln(results);writeln(results);
    writeln;writeln;
sys(F,Vrset,Cxset,Yxsan,Yxsa,Ypxa,Ypxan,Ypm,mum,Ks,ts,num,nprint);
    writeln(results);writeln(results);
    writeln(results,'feed flow rate l/hr. =',F:10:3);
    writeln(results,'fermenter working vol. l =',Vrset:10:3);
    writeln(results,'maximum biomass conc. g/l =',Cxset:10:3);
    writeln(results,'anaerobic state biomass yield coeff. =',Yxsan:10:10);
    writeln(results,'aerobic state biomass yield coeff. =',Yxsa:10:10);
    writeln(results,'aerobic state product biomass yield coeff. =',Ypxa:10:10);
    writeln(results,'anaerobic state biomass yield coeff. =',Ypxan:10:10);
    writeln(results,'maximum abiotic phase product conc. g/l =',Ypm:10:10);
    writeln(results,'maximum specific growth rate hr-1 =',mum:5:4);
    writeln(results,'Monod half saturation constant l/g =',Ks:10:4);
    writeln(results,'Time step s =',ts:9);
    writeln(results,'Total number of steps =',num:10);
    writeln(results,'Number of steps between printout =',nprint:10);
    writeln(results);writeln(results);
    writeln;writeln;

    nn:=1;
    dl:=ts/3600;
    Cx0:=Cx0i;
    Cx:=Cxi;
    Cxw:=0;
    Ys0:=Ys0i;
    Ys:=Ysi;
    Yp0:=Yp0i;
    Yp:=Ypi;
    Vr:=Vrset;
    Yxs:=Yxsan;
    Ypx:=Ypxan;


    time:=0;
    b:=0;
    Bnew:=0;
    writeln('Time(hr.)   Biomass(g/l)   Substrate(g/l)   Product(g/l)   Bleed(l/
hr.)');
    writeln(time:5:2,'  ',Cx:14:3,'  ',Ys:14:3,'  ',Yp:14:3,'  ',Bnew:12:5);
    writeln(results);
    writeln(results,'Time(hr.)   Biomass(g/l)   Substrate(g/l)   Product(g/l)
Bleed(l/hr.)');
    writeln(results,time:5:2,'  ',Cx:14:3,'  ',Ys:14:3,'  ',Yp:14:3,'  ',Bnew:
12:5);
    writeln(results);
    for i:=1 to num do
    begin
    time:=ts*i/3600;
    if (x>=b)and then
```

```
program simterm (input,output,results);
var Cx,CxO,Cxw,rs,YsO,Ysw,Yp,YpO,Ypw,F,Vr,B,Ypx,Ypxa,Ypxan,Ypm,mum,Yxs,Yxsan,Yxs
a,Ks,
    dT,dCx,dYs,dYp,a1,a2,a3,a4,b1,b2,b3,b4,c1,c2,c3,c4,Cxset,Vrset,CxOi,
    Cxi,YsOi,rsi,YpOi,Ypi,time,ts,bgate,Bi,Bnew,Bprint:real;
    i,num,nn,nprint:integer;
    results:text;

procedure biomass(dT,F,CxO,B,Cxw,Cx,Yp,Ypm,mum,rs,Vr,Ks:real;var dCx:real);
    begin
    dCx:=dT*(F*CxO-(F-B)*Cxw-B*Cx+(1-Yp/Ypm)*mum*Ys*Cx*Vr/(Ks+Ys))/Vr;
    end;

procedure sub(dT,F,YsO,B,Ys,Yp,Ypm,mum,Cx,Vr,Ks,rxs:real;var dYs:real);
    begin
    dYs:=dT*(F*YsO-(F-B)*Ys-B*Ys-((1-Yp/Ypm)*mum*Ys*Cx*Vr/(Ks+Ys))/Yxs)/Vr;
    end;

procedure product(dT,F,YpO,B,Yp,Ypm,mum,Ys,Cx,Vr,Ks,Ypx:real;var dYp:real);
    begin
    dYp:=dT*(F*YpO-(F-B)*Yp-B*Yp+((1-Yp/Ypm)*mum*Ys*Cx*Vr/(Ks+Ys))*Ypx)/Vr;
    end;

procedure startup(var CxOi,Cxi,YsOi,Ysi,YpOi,Ypi:real);
    begin
    write('initial feed stream biomass concentration g/l ?');
    readln(CxOi);
    write('initial fermenter biomass concentration g/l ?');
    readln(Cxi);
    write('initial feed stream abiotic-phase substrate concentration g/l ?');
    readln(YsOi);
    write('initial fermenter abiotic-phase substrate concentration g/l ?');
    readln(rsi);
    write('initial feed stream abiotic-phase product concentration g/l ?');
    readln(YpOi);
    write('initial fermenter abiotic-phase product concentration g/l ?');
    readln(Ypi);
    end;

procedure sys(var F,Vrset,Cxset,Yxsan,Yxsa,Ypxa,Ypxan,Ypm,mum,Ks,ts:real;
    var num,nprint:integer);
    begin
    write('feed stream flowrate l/hr ?');
    readln(F);
    write('fermenter working volume l ?');
    readln(Vrset);
    write('maximum fermenter biomass conc. g/l ?');
    readln(Cxset);
    write('anaerobic state biomass yield coeff. g biomass/g substrate ?');
    readln(Yxsan);
    write('aerobic state biomass yield coeff. ?');
    readln(Yxsa);
    write('aerobic state product biomass yield coeff. g product/g biomass ?');
    readln(Ypxa);
    write('anaerobic state biomass yield coeff. ?');
    readln(Ypxan);
    write('maximum product conc. g/l ?');
    readln(Ypm);
    write('maximum specific growth rate hr-1 ?');
    readln(mum);
    write('Monod half saturation constant l/g ?');
    readln(Ks);
    write('time step s ?');
    readln(ts);
    write('number of steps ?');
    readln(num);
```