

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**OPTIMISATION OF INDUSTRIAL WHEY ETHANOL
FERMENTATION PROCESS**

by

DIANESIUS DIMIN WONGSO

A thesis presented in partial
fulfilment of the requirements for the degree of
Doctor of Philosophy
in *Biotechnology* at Massey University,
Palmerston North, New Zealand

1993

ABSTRACT

Ethanol is produced from whey at four distilleries in New Zealand. The Anchor Ethanol Co. distillery at Tirau was established in the 1981 and employs a continuous fermentation process. The aim of this work was to characterise the production yeast employed at this plant and to evaluate possible methods by which this commercial fermentation could be optimised.

Fermentation and assimilation tests confirmed the production yeast (strain Fi) as a strain of *K. marxianus*. The kinetics of ethanol fermentation of lactic acid casein whey serum were examined in continuous culture. The data were best fitted with a Langmuir plot and gave μ_{\max} of 0.21 h^{-1} and K_s of 4.94 g/l . A maximum ethanol productivity of 1.27 g/l.h was achieved at a dilution rate of 0.10 h^{-1} . Experiments were conducted in shake flask cultures using semi-synthetic media and sulphuric acid casein whey permeate to investigate the effect of lactate on the fermentation performance of the yeast. Lactic acid is present naturally in lactic acid casein whey at a concentration of about 7 g/l and may be present at concentrations up to 30 g/l , if there is gross bacterial contamination or if the whey were concentrated. Lactate added up to 30 g/l in the presence of 50 or 100 g/l lactose had no effect on the ethanol production rate or yield, although the biomass yield was slightly reduced.

The yeast strain Fi was grown aerobically on the slops, the liquid remaining after ethanol distillation, which contains 7 g/l lactic acid as the major component. The biomass produced was used as an inoculum for the whey ethanolic fermentation and performed as well in this role as an inoculum pre-grown on lactic whey. Continuous culture of the yeast grown aerobically on slops was again best fitted with Langmuir plot to give μ_{\max} of 0.30 h^{-1} and K_s of 0.32 g/l .

The yeast by-product from whey ethanolic fermentation was autolysed in a batch or continuous systems. On the basis of α -aminonitrogen release, yeast grown aerobically autolysed more readily than yeast grown anaerobically. The optimum autolysis condition of 55°C and pH 5.5 for anaerobically grown yeast was established on the basis of the α -aminonitrogen released after 6 h and the stimulatory effect of the lysate on the ethanol fermentation. Continuous autolysis was conducted successfully and the autolysate produced at dilution rate of 0.10 h⁻¹ gave the highest stimulatory effect on the ethanol fermentation. Improvements in the ethanol productivity and production rate of 10-20 % were observed following the addition of autolysate. A direct relationship between the α -AN utilized and the ethanol volumetric productivity was established.

Overall this work has identified three potential areas for process intensification:

1. An increase in the operating dilution rate from the current value of 0.07 h⁻¹ to the optimum of 0.10 h⁻¹ will lead to an approximately 30% increase in throughput.
2. The use of distillation slops containing lactic acid as a growth medium for inoculum production. This will enable an extra of 62,000 kg of ethanol per year to be produced from the lactose currently used for growth of the inoculum.
3. The addition of a continuously produced autolysate to the ethanolic fermentation will improve the fermentation rate and allow the whey throughput to be increased further.

Each of these options could be implemented at the commercial plant with a payback period of one year or less.

ACKNOWLEDGEMENT

I sincerely thank my supervisor, Dr. John Mawson, for his remarkable guidance, and supervision. His encouragement, patience and enthusiasm for this project was deeply appreciated.

I also wish to thank my co-supervisors, Associate Professor Ian Maddox and Dr. Pak Lam Yu for their supervision.

I am thankful to Professor R.L.Earle, Head of the Department of Process and Environmental Technology, for his interest in this project.

I gratefully acknowledge the funding from TBG and Anchor Ethanol Co. and the following people who made the project possible : Mr Laurie Brockliss, General Manager of Anchor Ethanol Co., Mr Ron Hamilton and Colin Reid, Technical Manager and Assistant Technical Manager, Anchor Ethanol Co., Tirau and Mr Merv Joseph, Plant Manager of Anchor Products Ltd, Tirau.

I am also thankful to the New Zeland Ministry of Foreign Affairs for the award for fees scholarship.

Appreciation is also extended to the following people:

Mr M.Stevens, Mrs A.M.Jackson, Mrs J.Collins, Mr J.Sykes and Mr M.Sahayam for their excellent laboratory support.

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

Ms P.Ratumaitavuki, Mrs M.Oecmkee for their assistance in all the related

computer work and for the excellent office support.

Dr Noemi Gutierrez for being a big sister to me during my undergraduate and postgraduate studies at Massey. Her constant encouragement is extremely appreciated.

Dr Sunthorn Kanchanatawee for his friendship.

Ms Pinthita Mungkarndee, my officemate and also for being another big sister to me. Her cooking has kept my momentum going throughout these years.

Mr P.Susarla, Mr S.Susarla and his wife Gayatry for their friendship and those hot and spicy indian foods.

My postgraduates colleagues Ms C.Russel, Ms T.Ngapo, Mr R.Sharma, Mr S.Hing, Mr P.Chareonsudjai, Mr J.Tisnajaya, Mr S.Gelera, Mr S.Wu, Mr C.Ford, Mr J.Knitel for their friendship.

Dr D.Tambunan who introduced me into playing golf; Diko and Ling for their friendship and the competitive tennis games.

My kiwi family, Barry, Pam and Lance Paine who welcomed me into their life and provided a warm friendship during my brief stay at Tirau.

My uncle Yacob for his financial and moral support.

My mother, brother Beda and Jerome, my sister Lisa, Fransiska and her husband Halim for their constant support, love and encouragement.

Finally to Ratna Wijayanti for her understanding, patience, love and encouragement.

TABLE CONTENT

	Page
ABSTRACT	i
ACKNOWLEDGEMENT	iii
TABLE CONTENTS	v
LIST OF FIGURES	xiii
LIST OF TABLES	xx
ABBREVIATIONS	xxv
CHAPTER 1 INTRODUCTION	1
CHAPTER 2 LITERATURE REVIEW	5
Part I Ethanol production from whey	5
2.1.1 Introduction	5
2.1.2 Factors affecting the fermentation	9
1.2.1 temperature	11
1.2.2 pH	11
1.2.3 Supplementary nutrients	11
2.3.1 yeast extract	11
2.3.2 other nutrients	13
1.2.4 Oxygen and sterols	15
1.2.5 Inoculum size	17
1.2.6 Salt concentration	17
1.2.7 Osmotic pressure	18
1.2.8 Lactose and ethanol concentration	18
2.1.3 New technological approaches	21
1.3.1 Hydrolysed whey	21
1.3.2 Concentrated whey	22
1.3.3 Continuous culture	23
3.3.1 Continuous culture with free cells	25

	3.3.2 Continuous culture with cell recycle	26
	3.3.3 Continuous culture with immobilized cells	26
2.1.4	New biological approaches	27
1.4.1	Strain adaptation	27
1.4.2	Genetic manipulation	27
1.4.3	Mixed cultures	29
2.1.5	Feasibility assessment of ethanol production from whey	31
2.1.5	Industrial whey-based ethanol fermentation	32
2.1.6	Discussion	37
Part II	Utilization of lactic acid in whey	39
2.II.1	Introduction	39
2.II.2	Effect of lactic acid on the yeast alcoholic fermentation process	41
2.II.3	Use of lactic acid as a microbial carbon source	44
II.3.1	Lactate in whey as a substrate for biomass production	44
II.3.2	Lactate as a substrate for propionic acid production	47
2.II.4	Discussion	49
Part III	Yeast autolysis	50

2.III.1	Introduction	50
2.III.2	Factors affecting autolysis	51
III.2.1	Temperature	51
III.2.2	Growth conditions	54
III.2.3	pH	57
III.2.4	Effect of chemical agents	58
2.4.1	Salt and/or ethanol	58
2.4.2	Effect of solvents and other chemicals	60
2.III.3	Other lysis methods	62
2.III.4	The use of autolysate as a nutrient source in commercial fermentation process	63
2.III.5	Discussion	65
CHAPTER 3	MATERIALS AND METHODS	67
3.1	Materials	67
3.1.1	Microbiological media	67
3.1.1.1	Whey media	67
3.1.1.2	slops	69
3.1.1.3	Semi-synthetic media	69
3.1.1.4	Agar slopes and plates	69
3.1.2	Chemicals	69
3.1.3	Other materials	71
3.1.4	Organism	71
3.2	Cleaning and sterilization procedures	72
3.2.1	Cleaning of glassware	72

3.2.2	Media sterilization	72
3.2.3	Equipment sterilization	72
3.3	Analytical methods	72
3.3.1	pH measurement	72
3.3.2	Determination of biomass dry weight	73
3.3.2.1	Spectrophotometric method	73
3.3.2.2	Dry weight method	73
3.3.3	Determination of colony forming units	75
3.3.4	Ethanol analysis	75
3.3.5	Lactose analysis	76
3.3.5.1	Modified DNS method	76
3.3.5.2	Enzymatic method	76
3.3.6	Lactate analysis	77
3.3.6.1	L(+)-lactate	77
3.3.6.2	D(-)-lactate	78
3.3.7	Amino-nitrogen analysis	79
3.3.8	Protein analysis	79
3.3.9	Total nitrogen	80
3.3.10	Glucose analysis	80
3.4	Fermentation protocols	80
3.4.1	Shake flask cultures	80
3.4.2	Batch fermentation culture	81
3.4.2.1	2-litre fermentation apparatus	81
3.4.2.2	14-litre fermentation apparatus	81
3.4.2.3	Batch fermenter operation	82
3.4.2.4	Continuous fermenter operation	82
3.4.3	Pump calibration	85
3.5	Autolysis protocols	85
3.5.1	Yeast cream production	85

3.5.2	Batch autolysis	86
3.5.2.1	Flasks	86
3.5.2.2	2-litre vessel	86
3.5.3	Continuous autolysis	87
3.6	Data analysis	87
3.7	Discussion of method	89
CHAPTER 4	CHARACTERISATION OF ETHANOL PRODUCTION BY INDUSTRIAL KLUYVEROMYCES YEAST	91
4.1	Introduction	91
4.2	Materials and methods	92
4.2.1	Organisms	92
4.2.2	Yeast differentiation media	92
4.2.3	Fermentation media	92
4.2.4	Fermentation apparatus and operation	92
4.2.5	Chemical analysis	93
4.2.6	Data analysis	93
4.3	Results	94
4.3.1	Yeast identification and differentiation	94
4.3.2	Batch fermentation	94
4.3.2.1	Comparison of yeast performance	94
4.3.2.2	Mixed culture	97
4.3.2.3	Effect of lactic acid	99
4.3.3	Continuous fermentation	114
4.3.3.1	The effect of dilution rate on the fermentation of LACWP and LACWS	114
4.3.3.2	The effect of different types of	

	whey substrate	117
4.3.3.3	Comparison with other yeast strains	120
4.4	Discussion and Conclusion	122
CHAPTER 5	AEROBIC YEAST CULTIVATION ON STILLAGE	130
5.1	Introduction	130
5.2	Materials and Methods	131
5.2.1	Organism	131
5.2.2	Fermentation and growth media	131
5.2.3	Maintenance media	131
5.2.4	Chemical analysis	132
5.2.5	Fermentation operation	132
5.2.6	Data analysis	132
5.3	Results	133
5.3.1	Aerobic growth of Fi yeast on lactate in batch culture	133
5.3.1.1	Growth in semi-synthetic medium	133
5.3.1.2	Growth on slops supplemented with yeast extract	135
5.3.2	Aerobic growth of Fi yeast on slops in continuous culture	140
5.3.2.1	The effect of dilution rate and yeast extract	140
5.3.3	Growth of Fi yeast on slops as an inoculum for serum ethanolic fermentation	144
5.3.3.1	Slops-grown inoculum for batch fermentations	144
5.3.3.2	Slops-grown inoculum for continuous	

	whey fermentations	148
5.3.4	Continuous inoculum growth on slops coupled with continuous whey fermentation	148
5.4	Discussion and Conclusions	151
CHAPTER 6	YEAST AUTOLYSIS AND THE USE OF THE AUTOLYSATE AS A NUTRIENT SOURCE IN WHEY FERMENTATION	161
6.1	Introduction	161
6.2	Materials and Methods	162
6.2.1	Yeast cream	162
6.2.2	Autolysis condition	162
6.2.3	Fermentation media	163
6.2.4	Fermentation operation	163
6.2.5	Chemical analysis	164
6.2.6	Data analysis	164
6.3	Results	164
6.3.1	Effects of pH and temperature	164
6.3.1.1	Aerobically-grown yeast	164
6.3.1.2	Anaerobically-grown yeast	169
6.3.1.3	Effect of growth medium and of the autolysis solution	172
6.3.2	Effects of salt(NaCl) or ethanol	172
6.3.3	Comparison of all autolysis trials conducted at 55°C and pH 5.5	175
6.3.4	The use of yeast extract or yeast autolysate as a nutrient source in batch whey fermentation	179

6.3.4.1	Effect of yeast extract on LACWP fermentation	179
6.3.4.2	Effect of yeast extract on SACWP fermentation	179
6.3.4.3	Effect of various autolysis conditions on the quality of the autolysate	184
6.3.4.4	Effect of yeast autolysate on SACWP fermentation	188
6.3.4.5	Effect of lysed cell material in the autolysate on whey fermentation	193
6.3.5	Continuous autolysis	198
6.3.5.1	Effect of cool storage of yeast cream on autolysis	198
6.3.5.2	Effect of dilution rate	202
6.3.5.3	The use of continuously produced autolysate as a nutrient source in batch whey	202
6.4	Discussion and Conclusions	204
CHAPTER 7	FINAL CONCLUSION AND DISCUSSIONS	216
REFERENCES		220

LIST OF FIGURES

Figure	Page
2.1 Batch culture of <i>K. fragilis</i> NRRL 665 on whey permeate supplemented with 3.75 g/l yeast extract	10
2.2 Lineweaver-Burk plot of the continuous culture of <i>K. fragilis</i> NRRL 665 on permeate supplemented with 3.75 g/l yeast extract and with various amounts of ethanol added to the medium	20
2.3 Flow diagram of Carbery process	33
2.4 The different metabolic pathways of glucose in lactic acid bacteria	40
2.5 Change in protein and amino acids (a) and (b) carbohydrate levels in extract during autolysis	52
3.1 Coupling of aerobic yeast growth on slops and whey serum fermentation	84
3.2 Continuous autolysis process with the yeast storage tank shown on the left side of the photo and the autolysis vessel on the right	88
3.3 Four different plots used for the analysis of continuous culture data (a) Lineweaver-Burk, (b) Heijnen, (c) Eadie and Hofstee and (d) Langmuir	90
4.1 Comparison of the fermentation performance of five different yeast strains. (a) Ethanol production and (b) lactose utilization	96

Figure	Page
4.2 The fermentation performance of a pure culture and a mixed culture (with <i>C. tropicalis</i>) of the production yeast strain Fi. (a) Ethanol and lactose profiles and (b) lactic acid and pH profiles	98
4.3 The fermentation performance of a pure culture of strain Fi and a mixed culture of strain Fi and yeast 19	100
4.4 The effect of lactate on the fermentation performance of strain Fi. (a) Ethanol production and (b) lactose utilization	101
4.5 The effect of lactate on (a) ethanol and (b) biomass production by strain Fi on YEPLL medium	103
4.6 The effect of lactate on (a) lactose consumption and (b) culture pH	104
4.7 The effect of lactate on fermentation of YEPLL at a higher lactose concentration (100 g/l). (a) Ethanol production and (b) biomass concentration	106
4.8 The effect of lactate on (a) lactose utilization and (b) culture pH at a lactose concentration of 100 g/l	107
4.9 The effect of lactate in SACWP on (a) ethanol and (b) biomass production	110
4.10 The fermentation performance of concentrated SACWP supplemented with lactate. (a) Ethanol and lactose profiles. (b) Biomass and pH profiles	112

Figure	Page
4.11 Steady state parameters for continuous culture of <i>K. marxianus</i> Fi on LACWS	116
4.12 The plots of the continuous culture data on LACWS (a) Lineweaver-Burk and (b) Langmuir plots	118
5.1 Aerobic growth of strain Fi on YEP lactate with (a) lactose-pregrown yeast and (b) lactate-pregrown yeast	134
5.2 Aerobic growth of strain Fi on YEPLL lactate with (a) lactose-pregrown yeast and (b) lactate-pregrown yeast	136
5.3 Effect of addition of yeast extract on the growth of Fi yeast on slops (a) 1 g/l YE (b) 2 g/l YE	137
5.4 (a) The rate of L- and D-lactate utilization in the unsupplemented slops. (b) The effect of supplementation of yeast extract on the growth of yeast aerobically on slops	139
5.5 Steady state parameters of the continuous runs of aerobic yeast growth on unsupplemented slops	141
5.6 Lineweaver-Burk plot of continuous aerobic yeast growth on slops	143
5.7 Aerobic growth of strain Fi on slops and LACWS. (a) Lactate and lactose profiles. (b) Ethanol and biomass profiles	146
5.8 Fermentation of LACWS with yeast inoculum pregrown on slops and serum. (a) Ethanol and lactose profiles. (b) Biomass and	

Figure	Page
pH profiles	147
5.9 Continuous LACWS fermentation at $D = 0.10 \text{ h}^{-1}$ coupled to the aerobic yeast growth on slops	149
5.10 Fermentation process at Anchor Ethanol Co., Tirau	157
5.11 The proposed modification of the fermentation process	158
6.1 The effect of pH on the autolysis of yeast grown aerobically showing (a) α -AN release and (b) protein release. Yeast cream concentration was 70 g dry weight/l. Autolysis temperature was 50°C.	166
6.2 The effect of temperature on the autolysis of aerobically grown yeast at pH 5.5 (other conditions as per Figure 6.1). (a) α -AN release and (b) protein release	167
6.3 Three dimensional plot of the combined effects of pH and temperature on the autolysis of aerobically grown yeast (other conditions as per Figure 6.1). (a) Rate of α -AN release at 6 h and (b) 48 h	168
6.4 The effect of pH on the autolysis of yeast grown anaerobically in YEPL showing (a) α -AN release and (b) protein release. Yeast cream concentration was 70 g/l. Autolysis temperature was 55°C	170
6.5 The effect of temperature on the autolysis of anaerobically grown yeast at pH 5.5 (other conditions as per Figure 6.4). (a) α -AN release and (b) protein release	171

Figure	Page
6.6 Three dimensional plot of the combined effect of pH and temperature on the autolysis of anaerobically grown yeast (other conditions as per Figure 6.4) showing (a) rate of α -AN release at 6 h and (b) 48 h	173
6.7 The combined effect of pH and temperature on the autolysis of anaerobically grown yeast in LACWS on the rate of α -AN release at 6 h and (b) 48 h. The yeast concentration was 65 g/l and was autolysed was in fermented beer	174
6.8 The effect of addition of ethanol on (a) α -AN release and (b) protein release, during the autolysis of anaerobically grown yeast. Autolysis condition was 55°C and pH 5.5. Yeast cream concentration was 70 g/l	176
6.9 The effect of addition of NaCl for the autolysis of anaerobically grown yeast on the (a) α -AN release and (b) protein release. Other conditions as per Figure 6.8	177
6.10 Comparison of the α -AN profiles of five autolysis trials of yeast grown aerobically and anaerobically in YEPL and LACWS. The yeast cream concentration was 65-70 g/l, and autolysis was conducted at 55°C and pH 5.5	178
6.11 The effect of addition of commercial yeast extract (Difco Laboratories) on the fermentation performance of LACWS. (a) Ethanol profile and (b) lactose profile	180
6.12 The effect of supplementation of commercial yeast extract at various concentrations on the fermentation profiles of SACWP	

Figure	Page
(a) Ethanol production and (b) lactose utilization	182
6.13 The relationship between the α -AN utilized following supplementation with yeast extract and (a) volumetric ethanol productivity or (b) maximum ethanol production rate	185
6.14 The effect of various autolysis conditions on the quality of the 6 h autolysates when added to LACWS. (a) Ethanol production and (b) lactose utilization	186
6.15 The effect of various autolysis conditions on the quality of the 48 h autolysates when added to LACWS. (a) Ethanol production and (b) lactose utilization	189
6.16 The effect of 6 h lysate produced from the autolysis of anaerobically grown yeast at 55°C and pH 5.5 on the fermentation performance of SACWP. (a) Ethanol production and (b) lactose utilization	191
6.17 The effect of addition of 48 h lysate on the fermentation performance showing (a) ethanol production and (b) lactose utilization	194
6.18 The effect of addition of 6 h "double strength" lysate on the fermentation performance showing (a) ethanol production and (b) lactose utilization	196
6.19 The effect of cell debris in the autolysate on the fermentation performance of SACWP showing (a) ethanol production and (b) lactose utilization	199

Figure	Page
6.20 The effect of cool storage of yeast cream on the subsequent α -AN production. Autolysis conditions were 55°C and pH 5.5	201
6.21 The effect of autolysate continuously produced at $D = 0.33 \text{ h}^{-1}$ on the fermentation performance of SACWP showing (a) ethanol production and (b) lactose utilization	206
6.22 Relationship between the relative volumetric ethanol productivity and utilization of α -AN supplied by yeast extract (a) or (b) autolysates	214

LIST OF TABLES

Table	Page
2.1 Typical analyses of lactic acid casein, rennet casein and cheddar cheese whey	6
2.2 Whey and permeate production in New Zealand	7
2.3 Kinetic parameters for growth of <i>K. fragilis</i> NRRL 665 on whey permeate with different supplementation.	14
2.4 Kinetic parameters for growth of <i>C. pseudotropicalis</i> IP 513 on whey permeate with different supplementation	14
2.5 Summary of concentrated whey fermentation data	24
2.6 Summary of cell immobilization methods and fermenter performance for ethanol production from whey	28
2.7 Whey alcohol production in New Zealand	35
2.8 Two stages continuous culture of <i>L. bulgaricus</i> and <i>C. krusei</i> on lactic acid whey	45
2.9 Growth rate (μ , h^{-1}) of yeast species isolated from the Bel yeast SCP process	46
2.10 Characteristics of the four proteolytic enzymes released during yeast autolysis	55

Table	Page
2.11 Comparison of proteolytic enzymes from three different autolysing yeasts	55
2.12a Effect of growth condition of unaerated <i>S. carlsbergensis</i> on the subsequent autolysis	56
2.12b Effect of growth pH of aerated <i>S. carlsbergensis</i> on the subsequent autolysis process	56
3.1 Typical composition of lactic acid casein whey permeate and sulphuric acid casein whey permeate	68
3.2 Typical composition of Tirau slops for aerobic batch and continuous yeast cultivation	70
3.3 Yeast Extract Peptone Lactose (YEPL) used for anaerobic batch fermentation	70
3.4 Lactose recovery	77
4.1 Characterisation of industrial production yeast <i>K. marxianus</i> strain Fi	95
4.2 Effect of lactate on the ethanolic fermentation using YEPLL containing 50 g/l lactose	105
4.3 Effect of lactate on the ethanolic fermentation using YEPLL containing 100 g/l lactose	108
4.4 Effect of lactate on fermentation of SACWP at 50 g/l lactose	111

Table	Page
4.5 Effect of lactate on fermentation of SACWP at 100 g/l lactose concentration	113
4.6 Summarized steady state parameters from continuous culture of <i>K. marxianus</i> strain Fi in various whey media	115
4.7 Determination of μ_{\max} and K_s from continuous lactic whey serum fermentation with full data	119
4.8 Determination of μ_{\max} and K_s from continuous lactic whey serum fermentation without data from dilution rate of 0.05 h^{-1}	119
4.9 Summarized steady state parameters from continuous cultures	121
4.10 Summary of μ_{\max} and K_s values obtained from Lineweaver-Burk and Langmuir plots	128
5.1 Estimated μ_{\max} values for strain Fi grown on supplemented and unsupplemented slops	138
5.2 Summary of steady state parameters for continuous aerobic yeast growth of <i>K. marxianus</i> strain Fi on slops	142
5.3 Determination of μ_{\max} and K_s from continuous growth data on slops using four different approaches	144
5.4 Summarized steady state parameters from continuous lactic whey fermentation at $D = 0.10 \text{ h}^{-1}$	150

Table

Page

6.1	Total Kjeldahl Nitrogen during autolysis of yeast grown under different conditions	169
6.2	The effect of ethanol and NaCl on the α -aminonitrogen liberation (g/g yeast.h)	175
6.3	Effect of yeast extract on lactic acid casein whey permeate fermentation	181
6.4	Effect of different concentrations of yeast extract supplementation on SACWP fermentation	183
6.5	Effect of autolysis conditions, 6 h lysate on the LACWS fermentation	187
6.6	Effect of autolysis conditions, 48 h lysate on the LACWS fermentation	190
6.7	Effect of 6 h lysate on SACWP fermentation	192
6.8	Effect of 48 h lysate on SACWP fermentation	195
6.9	Effect of 6 h "double strength" lysate on the SACWP fermentation	197
6.10	Effect of cell debris on the batch whey fermentation performance	200
6.11	The effect of dilution rate on the production of α -aminonitrogen	202

Table

Page

6.12	Effect of continuously produced autolysate ($D = 0.111 \text{ h}^{-1}$) on whey fermentation performance	203
6.13	Effect of continuously produced autolysate ($D = 0.167 \text{ h}^{-1}$) on whey fermentation performance	205
6.14	Effect of continuously produced autolysate ($D = 0.33 \text{ h}^{-1}$) on the batch whey fermentation performance	207

ABBREVIATIONS

α -AN	α -aminonitrogen
$^{\circ}$ C	Degree Celcius
D	Dilution rate
EtOH	Ethanol
g	Gram(s)
h	Hour(s)
K_s	Saturation constant
l	Litre(s)
LACWP	Lactic acid casein whey permeate
LACWS	Lactic acid casein whey permeate
M	Molar
ml	Millitre(s)
nm	Nanometre
q_s	Specific substrate uptake rate
rpm	Revolutions per minute
SACWP	Sulphuric acid casein whey permeate
S	Residual substrate
μ l	Microlitre(s)
μ m	Micrometre(s)
μ_{max}	Maximum specific growth rate
% (w/v)	Percentage weight by volume
% (v/v)	Percentage volume by volume
% (w/w)	Percentage weight by weight
YEP	Yeast Extract Peptone
YEPL	Yeast Extract Peptone Lactose
YEPLL	Yeast Extract Peptone Lactose Lactate