

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.

BATCH CULTURE STUDIES OF *BIFIDOBACTERIUM BIFIDUM*

BY NOMEKO T. MLOBELI

**A thesis presented in partial fulfilment of the requirements for the degree of
Master of Technology in Biotechnology and Bioprocess Engineering**

MASSEY UNIVERSITY, PALMERSTON NORTH, NEW ZEALAND

ABSTRACT

The growth of *Bifidobacterium bifidus* on batch culture was investigated. Growth was studied with regard to growth rates, substrate utilisation rates, biomass yields and product formation rates. The organism was cultured on a variety of substrates and at different pH values. The objective was to determine whether different sugars or different medium pH values had any effect on specific growth rates, substrate utilisation rates, biomass yields and product formation rates.

B. bifidum (Hansen's strain) from a stock culture obtained from New Zealand Dairy Research Institute (DRI) was used for this study. The organism was grown on TPY medium supplemented with L-cysteine-HCl, sodium chloride, potassium chloride, magnesium chloride, potassium di-hydrogen phosphate, di-potassium phosphate and ammonium chloride. The experiments were carried out at uncontrolled and controlled pH.

At controlled pH, maximum growth of this organism was obtained on medium containing mixed sugars (glucose and lactose) at pH 5.5. On single carbohydrate sources, glucose, maltose and raffinose were readily utilised by this organism to give high rates of growth and formation of products. Very high concentrations of lactose were growth limiting. Lactulose and cellobiose were fermented but with low specific growth and product formation rates, while xylose was not fermented at all.

Growth of *B. bifidum* was pH-dependent. The pH below which this organism did not grow was $3.5 \leq \text{pH} < 4.1$. Optimum growth occurred at $4.9 \geq \text{pH} \geq 6.5$. It was demonstrated

that the A: L (acetic acid to lactic acid) ratio of products was different at different pH.

Accumulation of the fermentation products, in particular, acetic acid, caused limitations on growth. It was demonstrated that this inhibition was pH-dependent. Inhibition was higher at lower pH than at higher pH, and this was a result of the degree of dissociation of the acid at the different pH values.

ACKNOWLEDGEMENTS

I would like to sincerely thank my supervisor, Associate Professor Ian Maddox for giving the opportunity to work with him in this study. His constant guidance, supervision and encouragement was greatly appreciated.

I would also like to thank my co-supervisor, Dr. Noemi Gutierrez, for her guidance and supervision. I am deeply grateful to her assistance.

I would also like to thank Professor R. L. Earle (former Head of Department of Process and Environmental Technology) for allowing me the opportunity to do this work.

I am also grateful to the New Zealand Ministry of Foreign Affairs and Trade for giving me the scholarship during the period of this study.

I would also like to extend my appreciation and gratitude to the all the people who provided the technical and laboratory assistance, namely. Mrs. A-M Jackson, Mr. J Sykes, Mr. M Sahayam, Mr. M. Stevens, Mr. J. Alger, Mr. D. Maclean and Mr. B. Collins.

I would also like to thank my dear friends, Samuel Oppong for his friendship, encouragement and support, Kathy and Sam Hansen for their greatly appreciated friendship and support throughout my stay in New Zealand.

Last but not least, I would like to express my deepest love and gratitude to my parents, whose love, support and encouragement were felt from so many miles away.

TABLE OF CONTENTS	PAGE
Abstract	i
Acknowledgements	iii
Table of Contents	iv
List of Tables	ix
List of Figures	xi
CHAPTER 1 INTRODUCTION	1
1.2 OBJECTIVES OF THE STUDY	3
CHAPTER 2 LITERATURE REVIEW	4
2.1 DESCRIPTION	4
2.1.1 Historical background	4
2.1.2 Morphology	6
2.1.3 Identification	6
2.2 ECOLOGY	9
2.2.1 Bifidobacteria in humans	9
2.2.2 Colonisation in humans	12
2.3 PHYSIOLOGY	17
2.3.1 Respiration	17
2.3.2 Temperature	19
2.3.3 pH	19
2.3.4 Metabolism	19

2.3.5	Nutrient requirements	24
2.3.5.1	Nitrogenous matter	24
2.3.5.2	Trace elements	24
2.3.5.3	Growth factors	25
2.3.5.4	Lactoferrin	28
2.3.5.5	Lactulose and Lactitol	28
2.3.5.6	Oligoholositides and Oligosaccharides	29
2.3.5.7	Fructooligosaccharides (FOS)	29
2.3.6	Bile metabolism	30
2.3.7	Mucin metabolism	31
2.4	APPLICATIONS	31
2.4.1	Medical and health benefits	31
2.4.2	Bifidobacteria in food	35
2.4.2.1	Bifidobacteria in dairy products	39
2.4.2.2	Selection of Bifidobacteria for use as dietary adjuncts	40
2.5	CONCLUSION	42
CHAPTER 3	MATERIALS AND METHODS	43
3.1	MATERIALS	43
3.1.1	Organism	43
3.1.2	Bacteriological media	43
3.1.3	Chemical reagents	43
3.1.4	Growth substrates	44

3.1.5	Enzymes	44
3.1.6	Equipment	44
3.2	METHODS	45
3.2.1	Cultivation	45
3.2.2	100-ml scale fermentation	46
3.2.3	1.2-L scale fermentation	47
3.3	ANALYSES	49
3.3.1	pH	49
3.3.2	Biomass	49
3.3.2.1	Biomass calibration curve	49
3.3.2.2	Biomass concentration	50
3.3.3	Substrate concentration	50
3.3.4	Ethanol and Acetic acid	51
3.3.5	Lactic acid	51
3.3.5.1	Solution Preparation	52
3.3.5.2	Reaction mixture	53
3.3.5.3	Sample preparation	53
3.3.5.4	Standard curve	53
3.3.5.5	Assay	54
3.3.6	Formic acid	54
3.3.6.1	Reagents and Solutions	55
3.3.6.2	Procedure	56
3.3.6.3	Measurement	57

CHAPTER 4	RESULTS	58
4.1	MORPHOLOGY	58
4.2	GROWTH OF <i>Bifidobacterium bifidus</i>	58
4.3	INITIAL GROWTH AT DIFFERENT pH VALUES	59
4.4	GROWTH AT UNCONTROLLED pH	62
4.4.1	Specific rate of growth	63
4.4.2	Substrate utilisation	66
4.4.3	Product formation	70
4.4.4	Changes in pH during fermentation.	78
4.5	GROWTH AT CONTROLLED pH	80
4.5.1	Different substrates at pH 5.5	81
4.5.1	Rate of growth	81
4.5.2	Substrate utilisation	83
4.5.3	Product formation	87
4.6	GROWTH AT MIXED SUBSTRATES	93
4.6.1	Specific rate of growth	94
4.6.2	Substrate utilisation	94
4.6.3	Product formation	95
4.7	EFFECT OF pH VALUES ON GROWTH OF <i>Bifidobacterium Bifidum</i>	98
4.7.1	Specific rate of growth	98
4.7.2	Substrate utilisation	100
4.7.3	Product formation	103
4.8	PRODUCT INHIBITION	108

4.8.1	Product inhibition at pH 5.5	109
4.8.2	Product inhibition at pH 4.5	110
CHAPTER 5	GENERAL DISCUSSION AND CONCLUSIONS	112
REFERENCES.		116

List of Tables

Table 1: Distribution of <i>Bifidobacterium</i> species in the human colon	12
Table 2: Vitamin production by <i>Bifidobacterium</i>	23
Table 3: Characteristics of the main bifidogenic factors	27
Table 4: Fermented Milk Products containing bifidobacteria	40
Table 5: Rates of growth of <i>B. bifidum</i> in bottle fermentation	65
Table 6: Rate of substrate utilisation	69
Table 7: Rates of Product formation	75
Table 8: Specific rates of product formation	75
Table 9: Fermentation balance and carbon recovery for <i>B. bifidum</i>	76
Table 10: Rates of substrate utilisation and yield	82
Table 11: Rates of product formation	85
Table 12: Volumetric rates of product formation.	89
Table 13: Specific rates of product formation	89
Table 14: Fermentation balances in moles/mole of substrate and carbon recovery ...	91
Table 15: Growth parameters of <i>B. bifidum</i> growing in a mixture of glucose and lactose	96
Table 16: Specific rates of growth at different pH values	99
Table 17: Rate of glucose utilisation and yields at different pH values	101
Table 18: Rate of lactose utilisation and yields at different pH values	101
Table 19: Specific rates of production formation at different pH values	104
Table 20: Specific rates of production formation at different pH values	104
Table 21: Fermentation balance in moles/mole of substrate at different pH values.	105

Table 22: Carbon recovery at different pH values. 106

List of figures

Fig. 1: The metabolic pathway of *Bifidobacterium*. 21

Fig. 2: A picture of the fermentation apparatus used in the 1.2-L scale 48

Fig. 3: Plot of biomass produced versus time at different initial pH values 60

Fig. 4: Plot of products at t_{36} versus pH 60

Fig. 5: Log (x) versus time at 10 g/L glucose 63

Fig. 6: Log (x) versus time at 10 g/L glucose 63

Fig. 7: Log (x) versus time 50 g/L glucose 64

Fig. 8: Log (x) versus time 50 g/L glucose 64

Fig. 9: Log (x) versus time at 10 g/L lactose 64

Fig. 10: Log (x) versus time at 50 g/L lactose 64

Fig. 11: Substrate concentration versus time at 10 g/L glucose 66

Fig. 12: Substrate concentration versus time at 10 g/L glucose 66

Fig. 13: Substrate concentration versus time at 50 g/L glucose 66

Fig. 14: Substrate concentration versus time at 50 g/L glucose 66

Fig. 15: Substrate concentration versus time at 10 g/L lactose 67

Fig. 16: Substrate concentration versus time at 50 g/L lactose 67

Fig. 17: Substrate concentration versus biomass concentration at 10 g/L glucose . . . 68

Fig. 18: Substrate concentration versus biomass concentration at 10 g/L glucose . . . 68

Fig. 19: Substrate concentration versus biomass concentration at 50 g/L glucose . . . 68

Fig. 20: Substrate concentration versus biomass concentration at 50 g/L glucose . . . 68

Fig. 21: Substrate concentration versus biomass concentration at 10 g/L lactose 69

Fig. 22: Substrate concentration versus biomass concentration at 50 g/L lactose 69

Fig. 23: Acetic acid concentration versus time at 10 g/L glucose 71

Fig. 24: Acetic acid concentration versus time at 10 g/L glucose	71
Fig. 25: Acetic acid concentration versus time at 50 g/L glucose	71
Fig. 26: Acetic acid concentration versus time at 50 g/L glucose	71
Fig. 27: Acetic acid concentration versus time at 10 g/L lactose	72
Fig. 28: Acetic concentration versus time at 50 g/L lactose	72
Fig. 29: Lactic acid concentration versus time at 10 g/L glucose	72
Fig. 30: Lactic acid concentration versus time at 10 g/L glucose	72
Fig. 31: Lactic acid concentration versus time at 50 g/L glucose	72
Fig. 32: Lactic acid concentration versus time at 50 g/L glucose	72
Fig. 33: Lactic acid concentration versus time at 10 g/L lactose	73
Fig.34: Lactic acid concentration versus time at 50 g/L lactose	73
Fig. 35: Formic acid concentration versus time at 10 g/L glucose	73
Fig. 36: Formic acid concentration versus time at 10 g/L glucose	73
Fig. 37: Formic acid concentration versus time at 50 g/L glucose	74
Fig. 38: Formic acid concentration versus time at 50 g/L glucose	74
Fig. 39: Formic acid concentration versus time at 10 g/L lactose	74
Fig. 40: Formic acid concentration versus time at 50 g/L lactose	74
Fig. 41: Plot of pH versus time	78
Fig. 42: $\text{Log}_{10}(x)$ versus time at controlled pH	81
Fig. 43: Substrate concentration versus time at controlled pH	84
Fig. 44: Biomass produced versus substrate used	85
Fig. 45: Acetic acid concentration versus time	87
Fig. 46: Lactic acid concentration versus time	88
Fig. 47: Formic acid concentration versus time	88

Fig. 48: $\text{Log}_{10}(x)$ versus time	94
Fig. 49: Substrate concentration versus time	94
Fig. 50: Acetic acid concentration	95
Fig. 51: Lactic acid concentration	95
Fig. 52: Formic acid concentration versus time	95
Fig. 53: Plot of $\text{Log}_{10}(x)$ versus time at pH 4.9	98
Fig. 54: Plot of $\text{Log}_{10}(x)$ versus time at pH 6.5	98
Fig. 55: Plot of μ against pH	99
Fig. 56: Plot of substrate concentration versus time at pH 4.9	100
Fig. 57: Plot of substrate concentration versus time at pH 6.5	100
Fig. 58: Biomass concentration versus substrate used at pH 4.9	100
Fig. 59: Biomass concentration versus substrate used at pH 6.5	100
Fig. 60: Plot of Yield against pH	101
Fig. 61: Acetic acid concentration versus time at pH 4.9	103
Fig. 62: Acetic acid concentration versus time at pH 6.5	103
Fig. 63: Lactic acid concentration versus time at pH 4.9	103
Fig. 64: Lactic acid concentration versus time at pH 6.5	103
Fig. 65: Lactic acid concentration versus time at pH 4.9	104
Fig. 66: Lactic acid concentration versus time at pH 4.9	104
Fig. 67: Biomass versus time at different acetate concentrations	109
Fig. 68: Biomass versus time at different acetate concentrations	110