

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.

INHIBITION OF THE LACTOSE TO ETHANOL FERMENTATION OF
KLUYVEROMYCES MARXIANUS Y113 AND ATTEMPTS AT ITS
ALLEVIATION
THROUGH MEDIA IMPROVEMENT.

BY

Christopher Francis Grubb

A thesis
presented to Massey University
in fulfillment of the
thesis requirement for the degree of
Master of Technology

Palmerston North, New Zealand 1991

FOR
Reference Only

NOT TO BE REMOVED FROM THE LIBRARY

1092042738



INHIBITION OF THE LACTOSE TO ETHANOL FERMENTATION OF
KLUYVEROMYCES MARXIANUS Y113 AND ATTEMPTS AT ITS
ALLEVIATION
THROUGH MEDIA IMPROVEMENT

ABSTRACT

Inhibition of the lactose to ethanol fermentation of *K.marxianus* Y113 was investigated. The use of initial lactose concentrations of 150 g/litre or greater resulted in less biomass accumulation, lower ethanol productivity and incomplete substrate utilisation.

Keeping the initial lactose concentration at 100 g/litre but increasing the medium osmolality by up to 5 times via the addition of non-utilised salt or maltose resulted in substantially reduced biomass accumulation and slightly lower ethanol productivity. This suggested that high medium osmolality inhibits the yeast in a non-specific way by increasing the energy required for cell maintenance at the expense of biomass production.

Keeping the initial lactose concentration at 100 g/litre but adding up to 5% (by weight) ethanol reduced the amount and rate of biomass accumulation and led to incomplete substrate utilisation, as well as dramatically lowering the amount of ethanol produced by the yeast itself. The detrimental effects of added ethanol became significant only when more than 2 to 3% (by weight) was added.

A maximum alcohol concentration of 4 to 5% (by weight) was observed in all cases, irrespective of the concentration of ethanol added initially. These results suggested that the ethanol inhibited the energy metabolism of the cell in some specific way and did not merely increase the requirement for cell maintenance energy.

In the concentrations tried supplementation of the medium with yeast extract, magnesium, calcium and chitin all failed to produce any change in the performance of the fermentation. Supplementation with still bottoms was found to be quite strongly inhibitory to the fermentation.

Demineralisation of the whey permeate medium reduced that the performance of the fermentation compared to that carried out on standard whey permeate medium. *K.marxianus* Y113 was able to ferment a medium of defined composition but the biomass growth, ethanol productivity and lactose utilisation were not as good as those achieved using complex media such as whey or lactose broth. increasing the concentration of nutrients in the defined medium was of small benefit but the performance was still well below that seen on complex media.

ACKNOWLEDGEMENTS

Sincere gratitude is expressed to Dr A J Mawson for his thoughtful and careful guidance and supervision during the course of these studies.

The help and encouragement of the staff and students of the Biotechnology Department, Massey University is gratefully acknowledged.

The support and encouragement of flatmates and friends of the author during the course of these studies is greatly appreciated. Without them the task would have been substantially more difficult.

Finally the author wishes to express sincere gratitude to his typist, Jenny Terry, whose extreme skill and speed saved him from several nervous breakdowns.

CONTENTS:

Abstract

Acknowledgements

Contents

List of Figures

List of Tables

1. Introduction
2. Ethanol Production from Whey - Literature Review
 - 2.1 General Aspects of Ethanol Production
 - 2.1.1 Introduction
 - 2.1.2 Biochemistry
 - 2.1.3 Microbiology and Substrates
 - 2.2 Whey as a Potential Substrate for Ethanol Production
 - 2.2.1 Introduction
 - 2.2.2 Types and Compositions of whey
 - 2.2.3 Whey and Whey Derivative Production
 - 2.2.4 Whey Utilisation
 - 2.2.4.1 Introduction
 - 2.2.4.2 Protein Recovery and Use
 - 2.2.4.3 Lactose Recovery and Use
 - 2.2.4.4 Fermentation Processes
 - 2.2.5 Ethanol Production From Whey
 - 2.2.5.1 Microbiology
 - 2.2.5.2 Biochemistry
 - 2.2.5.3 Effect of Temperature
 - 2.2.5.4 Effect of pH
 - 2.2.5.5 Effect of Ethanol Concentration
 - 2.2.5.6 Effect of Media Tonicity
 - 2.2.5.7 Effects of other Inhibitors and Promoters
 - 2.2.5.8 Commercial Aspects
 - 2.3 Summary
3. Materials and Methods
 - 3.1 Materials
 - 3.1.1. Organisms

- 3.1.2 Media
- 3.1.3 Chemicals
- 3.2 Methods
 - 3.2.1 Biological Methods
 - 3.2.1.1 Culture Maintenance
 - 3.2.1.2 Propagation and Inoculation
 - 3.2.1.3 Incubation and Sampling of Experiments
 - 3.2.1.4 Sterilisation of Media and Equipment
 - 3.2.2 Analytical Methods
 - 3.2.2.2 pH Measurement
 - 3.2.2.3 Biomass Measurement
 - 3.2.2.4 Lactose Measurement
 - 3.2.2.5 Ethanol Measurement
- 4. The Effects of Osmolality, Lactose, Salt and Ethanol Concentration on Ethanol Production from Lactose
 - 4.1 Introduction
 - 4.2 Lactose Concentration
 - 4.2.1 Introduction
 - 4.2.2 Results and Discussion
 - 4.3 Medium Tonicity
 - 4.3.1 Introduction
 - 4.3.2 Results and Discussion
 - 4.4 Ethanol Concentration
 - 4.4.1 Introduction
 - 4.4.2 Results and Discussion
 - 4.5 Summary
- 5. Effects of Medium Nutritional Status on Ethanol Production from Lactose by *K.marxianus* Y113.
 - 5.1 Introduction
 - 5.2 Effects of Yeast Extract Concentration
 - 5.2.1 Introduction
 - 5.2.2 Results and Discussion
 - 5.3 Comparison of Whey Permeate and Demineralised Whey Permeate for the Production of Ethanol.
 - 5.3.1 Introduction
 - 5.3.2 Results and Discussion

- 5.4 Effects of Supplementation with Still Bottoms
 - 5.4.1 Introduction
 - 5.4.2 Results and Discussion
 - 5.5 Effects of Supplementation with Chitin, Calcium or Magnesium
 - 5.5.1 Introduction
 - 5.5.2 Results and Discussion
 - 5.6 Attempted Development of a Defined Medium for use with *K. marxianus* Y113
 - 5.6.1 Introduction
 - 5.6.2 Results and Discussion
 - 5.6.2.1 Amino Acid Requirements
 - 5.6.2.2 Vitamin Requirements
 - 5.6.2.3 Trial of Defined Medium for Lactose Fermentation by *K. marxianus* Y113
 - 5.6.2.4 Attempted Improvement of the Defined Medium for Growth of *K. marxianus* Y113
 - 5.7 Summary of Nutritional Studies
 - 6. Conclusions
- References
- Appendix A. Calculation of Fermentation Parameters
 - Appendix B. Estimation of Concentration and Uncertainties
 - Appendix C. Relationship of Osmolality to Solute Concentration

LIST OF FIGURES

- 2.1 Chemical Derivatives from Ethanol
- 2.2 Embden-Meyerhof-Parnas Glycolytic Pathway
- 2.3 New Zealand Annual Whey Production; 1975 to 1989
- 2.4 Metabolism of Lactose Before Entry into the E.M.P. Glycolytic Pathway
- 2.5 Structure of Lipid Bilayer Membrane Concentration
- 2.6 Comparison of Distillation Costs and Ethanol Concentration.
- 4.1 Biomass Concentration Vs Time; Run 1
- 4.2 Ethanol Concentration Vs Time; Run 1
- 4.3 Lactose Concentration Vs Time; Run 1
- 4.4 Biomass Concentration Vs Time; Run 2
- 4.5 Ethanol Concentration Vs Time; Run 2
- 4.6 Lactose Concentration Vs Time; Run 2
- 4.7 Biomass Concentration Vs Time; Run 3
- 4.8 Ethanol Concentration Vs Time; Run 3
- 4.9 Lactose Concentration Vs Time; Run 3
- 4.10 Biomass Concentration Vs Time; Run 4
- 4.11 Ethanol Concentration Vs Time; Run 4
- 4.12 Biomass Concentration Vs Time; Run 4
- 4.13 Ethanol Concentration Vs Time; Run 4
- 4.14 Lactose Concentration Vs Time; Run 4
- 4.15 Biomass Concentration Vs Time; Run 5
- 4.16 Ethanol Concentration Vs Time; Run 5
- 4.17 Lactose Concentration Vs Time; Run 5
- 4.18 Comparison of Ethanol Production in 100 g/litre Lactose Broths
- 4.19 Comparison of Lactose Consumption in 100 g/litre Lactose Broths
- 4.20 Comparison of Ethanol Production in 150 g/litre Lactose Broths
- 4.21 Comparison of Lactose Consumption in 150 g/litre Lactose Broths

- 4.22 Plot of Lactose Concentration against Ethanol Concentration Showing Lines of Iso-Osmolality
- 5.1 Biomass Concentration Vs Time; Run 6
- 5.2 Ethanol Concentration Vs Time; Run 6
- 5.3 Lactose Concentration Vs Time; Run 6
- 5.4 Biomass Concentration Vs Time; Run 7
- 5.5 Ethanol Concentration Vs Time; Run 7
- 5.6 Lactose Concentration Vs Time; Run 7
- 5.7 Biomass Concentration Vs Time; Run 8
- 5.8 Ethanol Concentration Vs Time; Run 8
- 5.9 Lactose Concentration Vs Time; Run 8
- 5.10 Biomass Concentration Vs Time; Run 9
- 5.11 Ethanol Concentration Vs Time; Run 9
- 5.12 Lactose Concentration Vs Time; Run 9
- 5.13 Biomass Concentration Vs Time; Run 9
- 5.14 Ethanol Concentration Vs Time; Run 9
- 5.15 Lactose Concentration Vs Time; Run 9
- 5.16 Biomass Concentration Vs Time; Run 10
- 5.17 Ethanol Concentration Vs Time; Run 10
- 5.18 Lactose Concentration Vs Time; Run 10
- 5.19 Biomass Concentration Vs Time; Run 10
- 5.20 Ethanol Concentration Vs Time; Run 10
- 5.21 Lactose Concentration Vs Time; Run 10
- B1 Standard Curve for Biomass
- B2 Standard Curve for Lactose
- B3 Standard Curve for Ethanol
- B4 Effect of Ethanol Concentration on Confidence Interval
- C1 Osmolalities of Solutions of Salt, Maltose and Sucrose

LIST OF TABLES

- 2.1 Typical Chemical Analysis of Sulphuric Casein Whey and Cheese Whey of Commercial Origin
- 2.2 Analysis of Vitamin Content of Acid and Sweet Wheys
- 2.3 Production of Whey-Derived Products in New Zealand during the 1988/89 Season
- 2.4 Typical Dried Whey Permeate Compositions
- 2.5 Commercial Ethanol Production from Whey in New Zealand
- 4.1 Summary of Results; Run 1
- 4.2 Summary of Results; Run 2
- 4.3 Summary of Results; Run 3
- 4.4 Summary of Results; Run 4, Maltose Cultures
- 4.5 Summary of Results; Run 4, Salted Cultures
- 4.6 Summary of Results; Run 5
- 5.1 Summary of Results; Run 6
- 5.2 Summary of Results; Run 7
- 5.3 Summary of Results; Run 8
- 5.4 Summary of Results; Run 9
- 5.5 Constituents of Difco Yeast Base Media
- 5.6 Establishment of Essential Amino Acid Requirements of *K.marxianus* Y113
- 5.7 Establishment of Essential Vitamin Requirements of *K.marxianus* Y113
- 5.8 Summary of Results; Run 10
- 5.9 Biomass Yield Following Modification of the Defined Medium