NHIBITION 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

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 intial 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 apreciated. 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:

LIST OF FIGURES

LIST OF TABLES