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**AN IMMOBILIZED CELL BIOREACTOR
FOR THE MALOLACTIC FERMENTATION OF WINE**

A THESIS PRESENTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF TECHNOLOGY IN BIOTECHNOLOGY AT
MASSEY UNIVERSITY

DENISE E. JANSSEN

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ABSTRACT

Malolactic fermentation using immobilized cells of *Leuconostoc oenos* was investigated in order to improve this fermentation at an industrial-scale.

Three strains of bacteria were investigated in some detail, and one was chosen for further work. A satisfactory growth medium for the strain of bacteria used was found to be an apple juice broth. The effect, on both the growth and malic acid bioconversion for *Leuconostoc oenos* strain 1070, of having 6% v/v ethanol in the growth media was tested and found to cause a longer lag phase, and be slightly beneficial, respectively.

Oak chips were decided on as the immobilization media, in preference to bone char, and a synthetic, apple-juice based wine was used to determine operation parameters for a continuous culture bioreactor.

Temperature, pH, ethanol concentration, SO₂, malic acid concentrations, anaerobic conditions and dilution rate were investigated and it was shown that lower malic acid concentrations, and also an interaction between low pH, high temperature and high ethanol concentration affected the malic acid bioconversion adversely. Increasing the dilution rate above 0.35 h⁻¹ caused a 30% drop in the bioconversion rate. The pH level had no effect on bioconversion if the temperature was kept at 21°C or lower. Decreasing the temperature, increasing the ethanol concentration above 10% v/v and increasing SO₂ levels all caused a slight drop in bioconversion rates while strict anaerobic growth and bioconversion conditions caused an increase. The bioconversion rates ranged between 20 and 100 mg malic acid consumed/100ml oak chips/hour.

An industrial prototype bioreactor was built and used at Villa Maria Wineries, Auckland, during the 1991 vintage and successfully processed 200 litres of Chardonnay-style wine in 2 days. The bioconversion rate was between 25 and 30 mg malic acid consumed/100ml oak chips/hour. Informal taste tests showed satisfactory malolactic characteristics in the treated wine.

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1.0 INTRODUCTION

Malolactic fermentation (MLF) entails the conversion of L-malic acid to L-lactic acid via decarboxylation using a suitable microorganism. The primary uses of MLF are to lower the acidity in high-acid wine, to give wine enhanced sensory characteristics, especially diacetyl or "buttery" notes, and to improve microbiological stability (Eggenberger, 1988).

Currently, in cool climate wine areas, this fermentation is used for most red wines and some white wines, especially Chardonnays. Normal industrial practice is to use batch fermentation, either initiated naturally by bacteria occurring in the winery or following inoculation with commercially available preparations. Length of fermentation varies greatly with wine type and temperature, and may vary from one week for some red wines to up to one year for a Chardonnay-style wine. Delayed onset of fermentation is seen by winemakers as the primary problem with malolactic fermentation in New Zealand (Pilone, 1988).

Immobilized cells offer several advantages for MLF of wine:

- faster fermentation
- continuous operation
- improved temperature control
- greater tolerance to wines which have high alcohol and sulphur dioxide concentrations and low pH
- better control over the timing and extent of deacidification
- absence of flavour effects caused by bacterial growth in the wine.

Potential disadvantages of the technique include:

- the possibility of microbial contamination of the reactors
- transfer of taints from the reactor to the wine
- loss of activity on prolonged operation
- leakage of cells or immobilization substrate into the wine

(Davis *et al.*, 1985).

For this method to be attractive in commercial practice it must be:

- cheap
- simple
- easily performed in an industrial situation
- not liable to cause oxidation of the wine
- robust
- not susceptible to contamination
- able to impart correct flavour changes to the wine
- must use commercially acceptable supports and organisms

Investigation into any immobilization method must include the effects of major variations in substrate and environmental parameters so that the bioreactor throughput to give effective treatment can be calculated. These parameters include: SO₂, pH, ethanol concentration, temperature and flowrate.

This thesis deals with continuous malolactic fermentation of Chardonnay-style wines using immobilized cells, with the ultimate aim of developing and testing a prototype bioreactor in an industrial situation. Chardonnay-style wine was seen as providing the most difficult conditions for MLF and the style most in need of improved fermentation methodology.