The Immobilization of

Kluyveromyces fragilis and *Saccharomyces cerevisiae*

in

Polyacrylamide Gel

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ABSTRACT

The search for new energy sources has indicated that biomass, in the form of green plant materials and biological wastes, can provide a perpetual energy source if converted to a useful form. This study investigated the production of ethanol by the fermentation of sugars using immobilized cells.

The experimental procedure involved the immobilization of two yeast species, Kluyveromyces fragilis NRRL Y 1109 and Saccharomyces cerevisiae NCYC 240, in polyacrylamide gel for the fermentation of lactose and glucose respectively. The gel methodology of two previous authors, Chibata et al. (1974) and Neuhoff (1973) was used. The former author's gel was used as a basis for batch experiments to determine the gel composition for maximum ethanol producing activity by both cell species as initial trials with this gel yielded encouraging results.

Variations in monomer, BIS and cell concentration revealed that a gel containing 15% ($\frac{w}{v}$) acrylamide, 1.5% ($\frac{w}{v}$) BIS and 25% ($\frac{w}{v}$) cells in addition to 0.6% ($\frac{w}{v}$) BDMAP and 0.25% ($\frac{w}{v}$) ammonium persulfate in tris-HCl buffer pH 7.1 polymerised at $0^{o}C$ produced the greatest activity in immobilized K. fragilis cells with an activity retention for immobilization of 80%. The gel composition for greatest activity in immobilized S. cerevisiae cells differed only slightly from that above containing 20% ($\frac{w}{v}$) acrylamide, 1.6% ($\frac{w}{v}$) BIS and 40% ($\frac{w}{v}$) cells and resulted in a 46% activity retention for immobilization. Further experiments at various substrate concentrations indicated that the gel imposed small or negligible limitations on the diffusion of substrate and product.

Experiments to increase the cell activity retention for the immobilization of S. cerevisiae using the Neuhoff (1973) gel were unsuccessful but produced some important results. It was found that exposure to gel components, especially to the acrylamide monomer, reduced the ethanol producing ability and the viability of the cells. The general protective agents Tween 80, glycerol, gelatin and dithiothreitol proved ineffective. To minimize this damage to the cells the gels were polymerised at $0^{o}C$ with rapid polymerisation being induced by high initiator and accelerant concentrations.

Repeated use of the immobilized cells indicated that the simple substrate medium, of the sugar in distilled water used previously, was not sufficient to maintain stable ethanol producing activity. Although trials involving supplementation with a salt solution were unsuccessful, the incorporation 0.5% ($\frac{w}{v}$) peptone in the medium and the use of protein-containing media, such as whey, was found to stabilize activity.

Experiments in continuous processing revealed that immobilized K. fragilis cells produced ethanol from deproteinised whey at an efficiency of 70 to 80% over extended periods with complete substrate utilization of full strength whey being achieved at flowrates of 0.15 SV. The half life of the activity of the immobilized cells was estimated to be at least 50 days.

The experimental results suggest that this approach to fermentation may be industrially acceptable for the production of ethanol. However, a costing exercise on the production of ethanol from whey indicates that unless the product is a highly priced commodity, such as a pharmaceutical, the process is unlikely to be economically feasible due to the high cost of the immobilization support monomer.

LIST OF ABBREVIATIONS

BDMAP	β-dimethylaminopropionitrile
BIS	N,N'-methylene-bisacrylamide
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
^{o}C	Temperature in degrees celcius
DO	Dissolved Oxygen
g	Gravitational force
HEMA	2-hydroxyethyl methacrylate
hr	hour
Km	Michealis Constant
M	Molar
mA	milliampere
MEA	Malt Extract Agar
ml	millilitre
mM	milliMolar
NAD^{+},NADH	β-nicotinamide adenine dinucleotide
PDA	Potato Dextrose Agar
%	percentage
rpm	Rotational speed, revolutions per minute
SV	Space Velocity. Working volumes per hour
TEMED	N,N,N',N'-tetramethyl-ethylenediamine
Vmax	Maximum velocity
% $(\frac{v}{v})$	component composition expressed as percentage volume per unit volume
% $(\frac{w}{v})$	component composition expressed as percentage weight per unit weight

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CHAPTER 1

INTRODUCTION

This study investigates an approach to industrial processing that may be applied to the energy production and waste disposal fermentations of the future, i.e. the use of immobilized cells.

The current energy crisis, which has been initiated by the diminishing supply of fossil fuels, has prompted a search for new energy sources. One plentiful and universal energy source is solar radiation. The collection of this radiation via plant photosynthesis provides a perpetual energy source in the form of plant biomass. Waste disposal can also be an energy producing process. The breakdown of biodegradable wastes, or biomass, can in some cases yield more energy than that required for the actual process.

A number of methods have been proposed for the conversion of the biomass to energy. Methane can be produced by pyrolysis, hydrogasification and anaerobic digestion. Some research has been directed towards the biological production of hydrogen and ethanol may be produced by fermentation of the sugars present.

The latter process is of interest in this study. With notable exceptions, such as the production of beer by continuous fermentation in New Zealand, fermentation traditionally has been a batch operation using intact cells or cell free extracts. In many cases the extent of processing has been limited by the high cost due to the catalyst being used only once and the labour intensive nature of the process. The significance of these factors may be reduced considerably by the use of continuous fermentation, a mode of operation made possible by the use of immobilized catalysts.

Recent developments in immobilization techniques have enabled the introduction of continuous processing in such areas as the production of pharmaceuticals and industrial chemicals, the treatment of specialized wastes and scientific analysis. A wide range of immobilization techniques have been used for both cells and enzymes. These techniques range from the mild adsorption, microencapsulation and entrapment procedures to more rigorous covalent and crosslinking procedures which may markedly change the properties of the cells and enzymes.

The most widely used method of whole cell immobilization, entrapment, was used in this study. This method of trapping cells in a polymer network is a stable form of immobilization which has a relatively mild effect on the cells. The polymer chosen for this study was polyacrylamide gel, a synthetic polymer which has been used for the successful immobilization of many enzymes, bacteria and fungi.

Energy production, in the form of alcohol fermentation from sugars, has been the topic of few investigations. In this study immobilized Kluyveromyces fragilis NRRL Y 1109 will be used for the production of ethanol from lactose and immobilized Saccharomyces cerevisiae NCYC 240 will be used to produce ethanol from glucose. For industrial processing it is envisaged that a supply of raw materials could be maintained using whey as a source of lactose and energy crops such as sugarbeet or wood as a source of carbohydrate.

The aim of this study was to assess the method of immobilization for each species and to determine its probable feasibility for industrial processing. Initially two similar polyacrylamide gel formulations were used for the immobilization of both cell species with the formulation producing the highest activity being used in further experiments to maximize this activity. Aspects of the enzymic reaction were also studied using immobilized cells in continuous reactors.