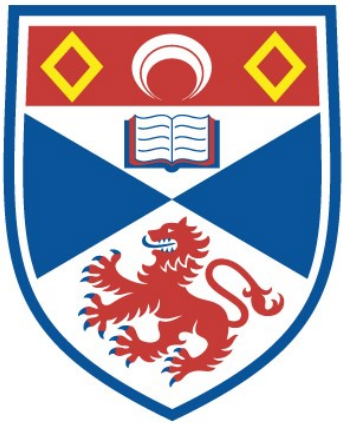
PRODUCTION OF ETHANOL FROM CASSAVA STARCH HYDROLYSATE BY IMMOBILISED CELLS OF 'ZYMOMONAS MOBILIS'

Robson Geraldo Costa

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1984

PRODUCTION OF ETHANOL FROM CASSAVA STARCH HYDROLYSATE BY IMMOBILISED CELLS OF ZYMOMONAS MOBILIS

A thesis presented by

ROBSON GERALDO COSTA

to the University of St. Andrews

in application for the degree of

Doctor of Philosophy

Biochemistry Department,

The University,

St. Andrews.

July, 1983.

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ABSTRACT

PRODUCTION OF ETHANOL FROM CASSAVA STARCH HYDROLYSATE BY IMMOBILISED CELLS OF ZYMOMONAS MOBILIS

Thesis presented by ROBSON GERALDO COSTA to the University of St. Andrews in application for the degree of Doctor of Philosophy.

Department of Biochemistry - July, 1983.

Cells of Zymomonas mobilis (ATCC 10988) were immobilised in calcium alginate beads and used to convert enzymatically hydrolysed cassava starch to ethanol. Optimum operating conditions were investigated in batch experiments. The optimum pH range and temperature were found to be 3.0 to 8.0 and 30°C, respectively. The maximum rates of glucose consumption and ethanol formation were obtained with an initial glucose concentration of 100 g/l. There was no fermentation inhibition below an initial ethanol concentration of 60 g/l. Ethanol productivity was the same using pure cassava hydrolysate or a medium composed of cassava hydrolysate, yeast extract and mineral nutrients.

The immobilised Zymomonas mobilis cells were studied in a packed-bed reactor system operating under optimised parameters from the batch experiments. Volumetric ethanol productivities of 8.91 g/l.h and 22.5 g/l.h were obtained at 100% and 75% of glucose utilization, respectively; these productivities correspond to 1.5 times that of a free cell reactor when glucose utilisation was 100% and 3 times that of a free cell reactor when glucose utilisation was 75%. The maximum specific ethanol formation rate and the maximum specific glucose uptake rate were found to be 1.4 g/g.h and 2.8 g/g.h, respectively. The immobilised-cell reactor was operated continuously at a constant dilution rate of 0.2 h^{-1} for 20 days resulting in only a 20% loss of the original fermentative activity corresponding to an estimated half- life of 63 days.

Based on experimental data, a mathematical analysis has been made and rate equations proposed.

CERTIFICATE

I hereby certify that Robson Geraldo Costa has spent nine terms engaged in research work under my direction and that he has fulfilled the conditions of Ordinance General No. 12 of the Resolution of the University Court 1967 , No. 1, and that he is qualified to submit the Accompanying thesis for the degree of Doctor of Philosophy.

DECLARATION

I hereby declare that this thesis is based on work carried out by me, that the thesis is of my own composition and that no part of it has been presented previously for a higher degree.

The research was conducted in the Department of Biochemistry, University of St. Andrews, under the direction of Dr W. M. Ledingham.

ACADEMIC RECORD

I graduated with the degree of Doctor of Medicine in 1973 from the Federal University of Alagoas, Maceio, Brazil, and with the degree of Master of Science (Biochemistry) in 1977 from the Federal University of Pernambuco, Recife, Brazil.

I matriculated as a research student in the Department of Biochemistry, University of St. Andrews, in October 1980.

ACKNOWLEDGEMENT

I should like to express my gratitude to Dr W. M. Ledingham for his advice during this work, and to my wife for her incentive.

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CONTENTS

1 INTRODUCTION

1.1 General introduction

1.1.1 Energy from biomass

The developing oil shortage, with the rapid rise in price, has speeded recognition of the need for solutions to fuel and raw material problems. A possible solution, at least for the near to medium term, is through use of the large amounts of biomass existing in the world as feedstock for conversion to fuels.

Biomass represents a mechanism of utilising solar energy. In a single day the sun sends more energy to Earth than the world consumes in an entire year, and although the vegetation captures only 1% or less of that energy, it stores 20 times as much energy in a year as the world uses (Peterson, 1978). The advantage of the biomass route of solar energy utilisation is that biomass energy is trapped in the form of chemical bonds in organic compounds and, therefore, it can be stored or transported as convenient.

The solar energy stored in plant material can be released by using different process, including:

(i) direct combustion to produce heat and electricity;

(ii) a number of thermal conversion processes, like pyrolysis, gasification and hydrogasification, to produce oils, methane, ethane, methanol and electricity;

(iii) biological conversion processes to produce oils, ethanol and methane.

Direct combustion and thermochemical conversion are processes that operate at high temperatures; in consequence, they waste many of the nutrients and minerals of biomass which could usefully be returned to the soil. Moreover, these processes require feedstock of relatively low water content, but biomass material has a high moisture content; therefore, in converting biomass to energy by these processes it is first necessary to remove the water. Advantages of biological conversion processes are that they operate at mild temperatures , they do not destroy the nutrients and minerals in biomass and they can handle feedstock of high water content.

1.1.2 Ethanol as a fuel substitute

The principal biological technologies, currently available for production of fuels derived from biomass, are the production of methane by anaerobic digestion and the fermentative production of ethanol.

Ethanol is produced by the action of microorganisms on specific sugars, generally hexoses, derived from starch, sucrose, inulin or cellulose. Any crop which yields these compounds could be, in principle, used as a feedstock for ethanol fermentation; for example: sugar cane, corn, sweet-sorghum, cassava, etc. Fermentative production of ethanol from cellulose materials is also technically feasible; however, at present many problems remain to be solved before this process becomes economically worthwhile..

The major potential use of ethanol is as a fuel in internal combustion engines, as a blend or in its own right. Use of ethanol for this purpose is technically feasible: although ethanol has a calorific content that is 39% lower than petrol, when it is burned in a properly designed engine it delivers 18% more power than petrol. Moreover, in an ethanol-burning motor the fuel is more completely combusted giving ethanol a slightly practical edge in the miles-per-litre figures and also lowering the amount of pollutants emitted (Stumpf, 1977).

1.1.3 The Brazilian Ethanol Program

The use of ethanol as a fuel substitute may allow oil - importing nations savings in foreign exchange.

In Brazil, a large scale government program was adopted in 1975 with the specific aim of replacing part of the imported oil with ethanol derived from the existing sugar cane acreage. The 1975 plan was for a fermentation production of $3 - 4 \times 10^6$ litres of ethanol by early 1980s, $7 - 8 \times 10^6$ litres by 1985, and 12×10^6 litres by 1990 (Lindeman and Rocchiccioli, 1979; Pimentel, 1980) The relative success achieved with the Brazilian Ethanol Program (PROALCOOL) has stimulated other countries to adopt similar measures: e.g., the United States National Program aimed at replacing petrol with gasohol (a 9:1 blend of petrol and ethanol) currently based on the fermentation of maize starch.

1.1.4 Ethanol fermentation from cassava and its potential

Brazil is currently the world leader in ethanol production. However, almost all the ethanol produced in Brazil today derives from sugar cane. Therefore, the need for an abundant and inexpensive alternative feedstock is somewhat urgent. Another substrate of great potential in Brazil today, in addition to sugar cane, is cassava.

Cassava or manioc (Manihot esculenta, Grantz) is a plant valuable for its large tuberous roots that are rich in starch. It is widespread throughout the world, mainly distributed among Africa, South America and South-East Asia. Brazil is presently the largest producer of cassava in the world; its production figure for 1978 was 25 thousand metric tons (FAO, 1979).

There are two varieties of cassava: bitter and sweet. The bitter variety is cultivated for industrial purposes because the roots have a higher starch content (20 - 30%); the remainder is moisture (60 - 75 %), protein (3%), cellulose (2%), other soluble and insoluble carbohydrates, fats and minerals. The bitter variety also contains a cyanogenic glycoside, linamarin, which is hydrolysed by the endogenous enzyme linamarase to hydrocyanic acid in damaged tissues after harvesting (Lages and Tannenbaum, 1978; Wood, 1956). When bitter cassava is used as a foodstuff, this toxic compound must first be removed by water leaching but it does not cause any trouble during fermentation processing because the cyanide is flashed off with the

steam during the mash cooking.

As previously stated, cassava is a very viable source of ethanol. Some of its advantages are listed (Araujo Filho, 1977; Kosaric et al., 1980):

(i) Ethanol yields from cassava are in the region of 165 - 180 litres/metric.ton, which, on a weight for weight basis, is a great improvement over sugar cane. Improvements in future agricultural technology will make available higher yields.

(ii) It has been found that in mash fermentation, no addition of acids or nutrients are necessary and a clean spirit, relatively free from congeneries, results. With a mash pH close to neutral, materials of construction for distillation equipment are not so prone to corrosive attack, allowing a subsequent saving in capital cost.

(iii) A much lower quality of soil, less fertiliser and rainfall are required than that for sugar cane, and therefore vast areas of little used land can be cultivated more profitably.

(iv) Cassava leaves and stems can produce a valuable animal foodstuff; the leaves have a crude protein content of 27% and can provide a protein supplement for animals and the fibrous residues can be used as forage for cattle.

(v) There are about 48 hours available before serious deterioration sets in between crop lifting and processing. This is up to five or six times greater than for sugar cane, where the sucrose content falls off considerably 8 hours after cutting. Therefore, transportation problems to the factory are eased and the radius of estate operation is increased.

(vi) Cassava chips can be dried to well below 20% moisture for stable storage. This will permit a distillery to operate during the non-cropping periods.

However, cassava starch can not be fermented directly into ethanol. As shown in Figure 1.1.4, the starch in cassava, as in any other source, needs to be hydrolysed to produce glucose, which is the fermentable substrate. This step is called saccharification and can be catalysed by acid (Samson, 1951) or by the combined action of the enzymes alpha-amylase and amyloglucosidase (Park and Papini, 1970). The conversion yield of the enzymatic method is known to be higher than the acid process (Araujo Filho, 1977). The amylose and amylopectin molecules, components of the starch, are initially broken down with the aid of alpha-amylase. This preliminary step, liquefaction,is required to decrease the viscosity of the starchy suspension and thus facilitate the cooking operation. Then, amyloglucosidase is used to catalyse the degradation of the resultant limit dextrins and oligosaccharides to achieve final conversion into glucose. After the saccharification step, the hydrolysed starchy

material is inoculated with the fermenting microorganism and the fermentation step takes place. Due to the slow rate of the saccharification step, a high energy input is necessary in the cassava processing prior to fermentation (for batch alcohol manufacture from cassava, the steam requirement is about 1.8 kg steam per kilogram of cassava). In addition to this, a number of other aspects of ethanol production from cassava starch still hinder the adoption of the process on an industrial scale:

(i) a high capital investment is required (perhaps as much as 40% over other independent sugar cane distilleries of the same size);

(ii) a high degree of material handling is involved, and consequently, higher operating costs.

Therefore, improvements in the process are needed in order to reduce the overall production cost and so make it an economically feasible one.

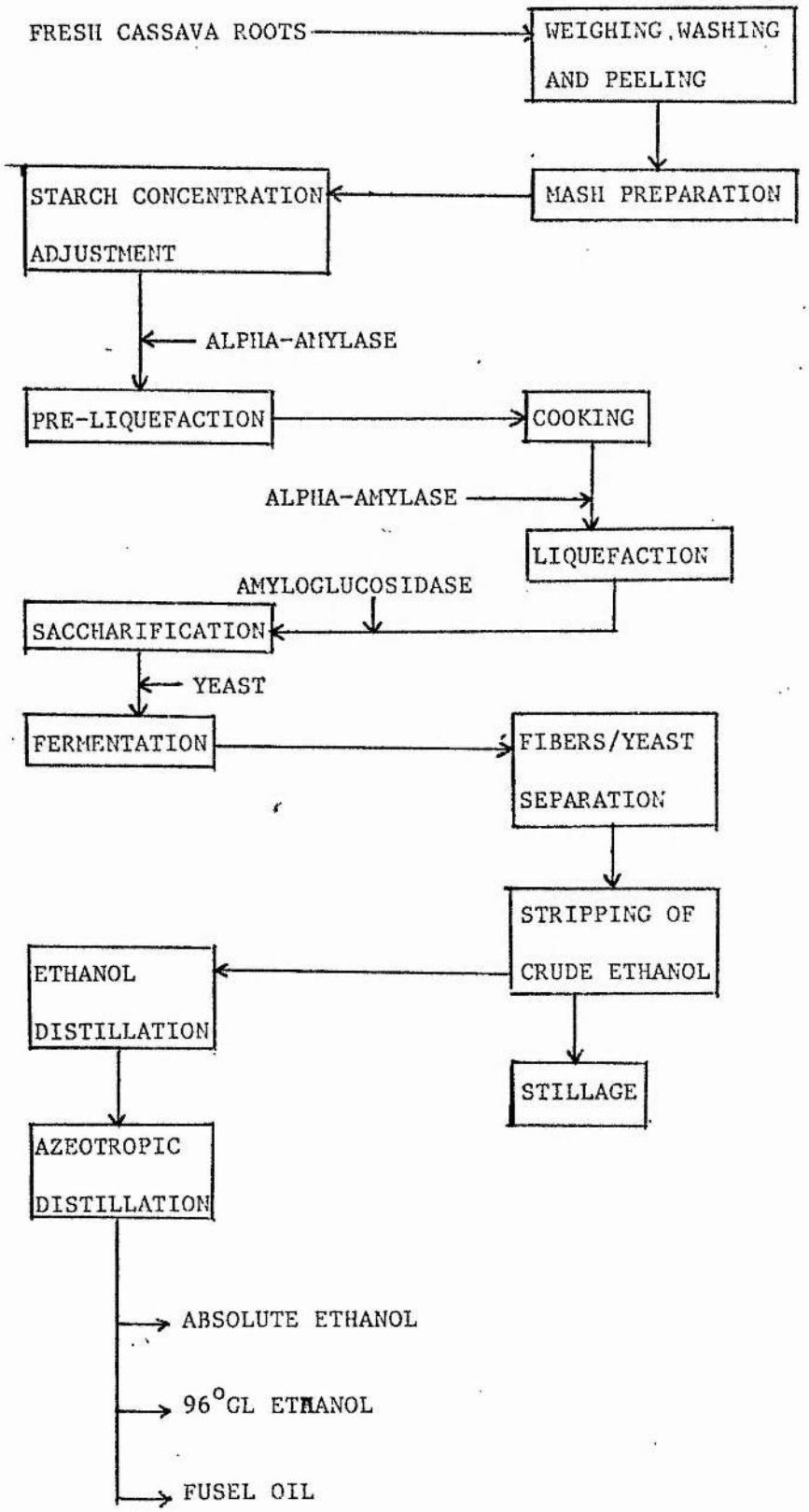

Fig. 1.1.4. Production of ethanol from cassava roots.

1.2 Fermentation procedures

1.2.1 Batch and continuous processes

Fermentation procedures for ethanol production can be divided into two broad categories:

(i) batch processes

(ii) continuous processes

In a batch process a vessel is filled with starting materials including the substrate (molasses, cane juice, cassava or grain hydrolysate), the microorganism and additional nutrients if required. Adjustment of the initial pH to values less than 5.0 is important to prevent contamination. The fermentation takes place over a period that can range from 36 to 72 hours. Fermentation times could be reduced by the use of large inocula. When fermentation is complete, ethanol makes up from 6 to 9% w/v of the spent medium, which also include by - products, unconsumed nutrients and cells (an increase in yeast population to about 1.5×10^8 cells per ml occurs). Ultimately, the fermenter is emptied, sterilised and a new batch is started (Jones

et al., 1981).

In a continuous process the nutrients are continuously added to the rermenter and the products of the reaction are continuously removed. The contents of the reactor are continuously stirred for ideal mixing, so the concentration of nutrients, oxygen and metabolic products, and ractors such as pH and temperature, are the same everywhere in the reactor. Therefore, in a continuous culture, microbial growth takes place under steady-state conditions; that is, growth occurs at a constant rate and in a constant environment (Herbert et al., 1956).

Continuous fermentation for industrial purposes has several advantages over batch processes namely, ease of control, greater uniformity, reduced reactor volumes and reduced down - time (harvesting, sterilising, recharging) all resulting in significantly higher productivities.

Because continuous reactors lose cells in the effluent liquid stream their productivity is limited by the growth characteristics of the organism and depends on the dilution rate employed. Steady-state cell-mass balance shows that the specific growth rate equals the dilution rate of system. High productivity requires high flow rates but under such conditions the dilution rate may exceed the maximum specific growth rate of the organism and then washing out of the culture from the reactor may occur.

One way to overcome the above limitation is recycling (feedback), i.e., taking cells from the effluent and returning them to the reactor. The maximum possible dilution rate in a recycled system is greater than in a conventional continuous system but on an industrial scale recycling is difficult to achieve: often damage to the cells is considerable and occasionally the reactor becomes contaminated with foreign organisms.

Another limiting factor in ethanol production is the inhibition effect of ethanol on cell growth and fermentation rate. Prolonged exposure of cells to high ethanol concentration results in a loss of viability (Nagodawithana and Steinkraus, 1976). So, recycled cells must be continually replenished or intermittently discarded completely. It was found that, in continuous culture with cell recycle, after three residences times, the rate of fermentation drops dramatically (Espinosa et al., 1978). Consequently, because conventional continuous fermentation depends critically upon cell growth, the susceptibility of the cells to ethanol inhibition constitutes a important limiting factor to productivity.

A number of different techniques have been investigated to overcome the above limitations. Cell recycle associated with vacuum fermentation was developed for continuous ethanol production by Cysewski and Wilke (1978) to achieve high cell densities and rapid ethanol fermentation rates. In this system, fermenter ethanol productivity increased as much as twelvefold over conventional

continuous fermentations. However, a high energy input is required to operate this system. It has been computed that the energy requirement in a vacuum process is nearly 29 times higher than normal fermentation (Ghose and Tyagi, 1979). Therefore, the vacuum process requires higher capital costs, more sophisticated control devices and higher operator skills. According to Ghose and Tyagi (1979), it seems unrealistic as a possible technology.

A much less costly and less complex way to reduce losses of cells from the reactor is available by cell immobilisation onto solid supports.

Processes based on immobilised cells have several advantages in industrial processing that can not be obtained with conventional continuous cultures (without feedback) (Abbot, 1977; Jack and Zajic, 1977; Durand and Navarro. 1978; Chibata, 1979, Venkatasubramanian and Vieth, 1979; Kolot, 1980). For example, dilution rates higher than the maximum specific growth rate of the organism can be achieved without cell-washout; and they allow a very high reactor cell loading capacity, and should thus also give small reactors a high fermentation capacity. Moreover, in immobilised cell reactors there is continuous removal of toxic metabolites while the microorganisms can be retained inside the reactor until a favourable environment is restored. Also, since the microorganisms are contained in a dense film attached to a solid support the viscosity of the liquid phase is reduced compared to a conventional continuous culture system thus providing better mass transfer and mixing properties in the reactor. Another mechanism by

which immobilisation may improve mass transfer is that microorganisms attached to solid supports are exposed to higher nutrients concentrations than exist in the bulk solution, therefore higher product formation rates are realised (Hattori, 1972; Martin et al., 1976).

1.2.2 Production of ethanol by immobilised cells

The application of immobilised-cell reactors to the production of ethanol is obviously a promising technology. Yeast and Zymomonas mobilis cells have been successfully immobilised in different supports. Some of the results reported in the literature will be presented here.

Ethanol production using Saccharomyces cerevisiae cells immobilised in k-carrageenan was studied by Wada et al. (1980). When the gel beads containing a small number of cells were incubated in a complete medium, the cells grew well in the gel and the number of living cells per ml increased to over ten times that of free cells per ml of culture medium. The living cells in the gel were maintained at the high level of 10^9 per ml of gel and continuous production of ethanol using a medium containing 100 g / l glucose was carried out with a retention time of 1 h. Ethanol production at the level of

50mg/ml was observed for more than three months and the conversion of glucose utilised to ethanol was 100% of theoretical yield.

Ghose and Bandyopadhyay (1980) carried out continuous ethanol fermentation in an immobilised yeast (Saccharomyces cerevisiae) cell packed reactor using molasses as substrate. The maximum productivity achieved was 24.9 g/l.h employing molasses containing 19.7% reducing sugars within a period of 2.9 h. The reactor performance was found to be steady for more than 75 days.

Kierstan and Bucke (1977) reported experiments with immobilised cells of Saccharomyces in calcium alginate fibers. The fibers were packed into a column. A solution of 10% (w/v) glucose was pumped upwards in the column with a residence time of 10 h. The system produced ethanol for 23 days with an efficiency of conversion of glucose to ethanol between 65-90% of the theoretical maximum.

Navarro (1978) studied ethanol production using Saccharomyces cerevisiae immobilised on brick or polyvinyl chloride chips. The maximum ethanol production was observed after 2 days of incubation and it was at the level of 120 g/l with 46% of conversion of glucose to ethanol being achieved. Navarro and Durand (1977) found a better yield for the conversion of glucose to ethanol with cells of Saccharomyces carlsbergensis adsorbed onto porous glass than with free cells.

Moo-Young et al. (1980) described ethanol productivities of 21.8 g/l.h obtained with wood-chip-adsorbed Saccharomyces cerevisiae cell cultures, which compares well with optimal values of 18 to 32 g/l.h obtained using free-suspension cultures in stirred-tank fermenters with cell recycle.

Larsson and Mosbach (1979) reported studies on immobilisation of Saccharomyces cerevisiae cells in calcium alginate gel together with iron oxide to produce magnetic immobilised yeast beads. Magnetisation had no adverse effect on yeast fermentation capacity, operational stability and ability to growth in presence of nutrients. Magnetic and non-magnetic immobilised yeast behaved almost identically, both having an ethanol production rate as well as a final ethanol yield approximately equal to that found for free yeast. Application of this technique could be advantageous where viscous or particle - containing media are used.

Linko et al. (1981) entrapped Kluveromyces fragilis yeast cells in calcium alginate gel beads at loadings of 4 to 16 g/g of sodium alginate. In a batch system, about 90% conversion in 48 h was obtained using demineralised whey of 5 to 10% (w/v) lactose content as substrate. In continuous packed-bed column operation , at 5% substrate lactose level, ethanol production remained nearly constant at about 2% (w/v) for at least one month, with 80 to 90% of lactose utilised.

Williams and Munnecke (1981) reported immobilisation of Saccharomyces cerevisiae in calcium alginate beads for use in continuous ethanol production. Maximum productivity was 53.8 g of ethanol/l.h. The activity of the immobilised yeast remained high during a monitoring period of 44 days.

Margaritis et al. (1981) used immobilised Zymomonas mobilis cells in the form of small beads (1 mm diameter). A maximum ethanol productivity of 102 g/l.h for an inlet substrate concentration of 100 g/l and 87% conversion was achieved.

Grote et al. (1980) carried out studies with a highly productive strain of Zymomonas mobilis in an immobilised cell reactor using both calcium alginate and k-carrageenan as support matrices. Productivities above 50 g/l.h have been found at ethanol concentrations in excess of 60 g/l using a synthetic fermentation media containing 150 g/l of glucose. There was a decline of approximately 30% in activity after 800 h of operation.

Arcuri et al. (1980) studied column reactors containing immobilised cells of Zymomonas mobilis for the continuous production of ethanol from glucose. Two different immobilisation strategies were investigated: in one case, cells were entrapped in borosilicate glass fiber pads, while in the other, cells were immobilised via flocculation. The reactors were operated in both the fixed-bed and expanded-bed manners. Ethanol productivities as high as 132 g/l.h

were achieved using a synthetic media containing 5% glucose as substrate.

Bland et al. (1982) described experiments with Zymomonas mobilis in an attached film expanded bed fermenter. Vermiculite was used as the material for attachment of the bacterial film. Maximum ethanol productivities were 105 g/l.h and 210 g/l.h based on total fermenter volume and bed volume, respectively.

By immobilising Saccharomyces cerevisiae to a fixed support (gelatin) using glutaraldehyde as a crosslinking reagent, Sitton (1979) found an ethanol productivity of 15.9 g/l.h which is 9 times the productivity measured in a conventional stirred-tank reactor. Hydrolysed corn residue containing 3% glucose was used as feedstock material.

An immobilised-cell reactor appears to be a good technique to improve the ethanol production from cassava starch hydrolysate. Higher productivities should be expected than those usually achieved in conventional reactors thus lowering the cost of production.

1.2.3 Methods for cells immobilisation

A large number of methods for microbial cell immobilisation have been devised. These methods can be classified into three basic groups (Jack and Zajic, 1977; Chibata, 1979; Kolot, 1980).

A first method is based on electrostatic interactions between charged cells and cationic or anionic materials. In this case the affinity of specific species for a given ion exchanger depends on the chemical nature of the cell wall constituents (providing the necessary sites for attachment to the charged support) and any others factors that can affect surface charges (such as the presence of ions). Cell immobilisation by this mechanism is a simple and mild technique and permits the retention of the original cell activity. However, disruption of cells can occurs with pH changes during metabolism or with other factors like cell multiplication or gas bubble production.

A second method for cell immobilisation is based on an attachment of cells to an activated support by covalent linkage. This method requires the use of a cross-linking agent like glutaraldehyde or isocyanate. The final immobilised cell preparation is usually very stable but cell fixation apparently interferes with its activity.

A third method of cell immobilisation is entrapment in a polymer matrix. The general procedure involves mixing the cells to be immobilised into a solution of appropriate monomers followed by polymerisation. The resultant complex is a gel with voids where the cells are trapped. Various biochemically inert hydrogels, such as collagen, gelatin, polyacrylamide, carrageenan and calcium alginate, have been used to entrap microbial cells. The calcium alginate immobilisation technique is straightforward and versatile allowing entrapment of many different types of cells and organelles; the resulting immobilised cell preparation is usually stable and with high activity. Moreover, calcium alginate provides little resistance to diffusion of neutral substrates up to a molecular weight of 5,000 and is extremely cheap. Difficulties arise in the system when phosphates are present as these tend to disrupt gel structure (Kierstan and Bucke, 1977; Cheetam et al., 1979). Calcium alginate will be used in the present study as the immobilisation matrix.

1.3 Description of microorganism

The microorganisms traditionally used for production of ethanol from sugars are yeasts of the genera Saccharomyces and Kluyveromyces. Several publications have recently appeared suggesting that the bacterium Zymomonas mobilis might be a useful organism in industrial alcohol production. (Lee et al., 1979, 1980, 1981, 1981a; Rogers et al., 1979, 1980, 1980a; Skotnicki et al., 1980; Lyness and Doelle, 1981).

Strains of Zymomonas mobilis are commonly used in tropical countries (Mexico, Brazil) for making various alcoholic drinks (Mexican pulpe made from agarve plant juice, "Caldo de cana picada", a fermented cane juice preparation from north-east Brazil and a variety of palm wines and native beers from African countries). In Europe, this bacterium is well-known as an occasional contaminant of beer, fermented apple juice (cider), and pear juice (perry) (Swings and De Ley, 1977).

Zymomonas mobilis became a very well studied microorganism by microbial biochemists since the discovery by Gibbs and Moss, in the early 1950s, that it uses the anaerobic Entner - Doudoroff pathway of degradation of glucose. Gibbs and De Moss (1951; 1954) and De Moss (1953) confirmed previous observations of Kluyver and Hoppenbrouwers (1931) that Zymomonas mobilis ferments one mole of glucose to approximately two moles of each of ethanol and carbon dioxide. Using

specifically labelled (^{14}C)glucose these authors showed that almost all the activity of (1-^{14}C)glucose accumulated in carbon dioxide; about half of the activity of (3,4-^{14}C)glucose was released in carbon dioxide, and (2-^{14}C)glucose yielded ethanol labelled in the carbinol carbon atom.

The presence of the Entner-Doudoroff pathway in Zymomonas mobilis was confirmed later by Stern et al. (1960) with a radio-respirometric method, and at the enzymic level by Dawes et al. (1966).

The basic mechanism of glucose breakdown and the destination of the individual carbon atoms in Zymomonas mobilis is represented in figure 1.3.a.

The key reactions of the Entner-Doudoroff pathway (see fig. 1.3.b.) are dehydration of 6-phosphogluconate, by the enzyme 6-phosphogluconate dehydratase, to 3-deoxy-2-oxo-6-phosphogluconate, which is split by a specific aldolase to yield one mole each of pyruvate and glyceraldehyde 3-phosphate per mol glucose; the triose phosphate is then metabolised to pyruvate by reactions common to Embden-Meyerhof pathway. Dawes et al. (1966) reported enzymic evidence supporting the operation of the Entner-Doudoroff pathway in anaerobic conversion of glucose into ethanol and carbon dioxide by Zymomonas mobilis. These authors observed that the enzymes necessary for the production, from 6-phosphogluconate, of 3-deoxy-2-oxo-6-phosphogluconate and its subsequent fission to pyruvate and glyceraldehyde 3-phosphate are present in extracts of the

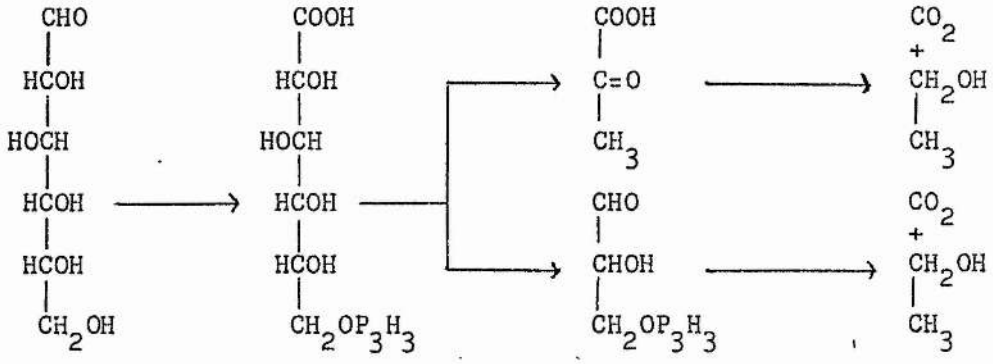

Fig.1.3.a. Mechanism of glucose breakdown in Zymomonas mobilis.

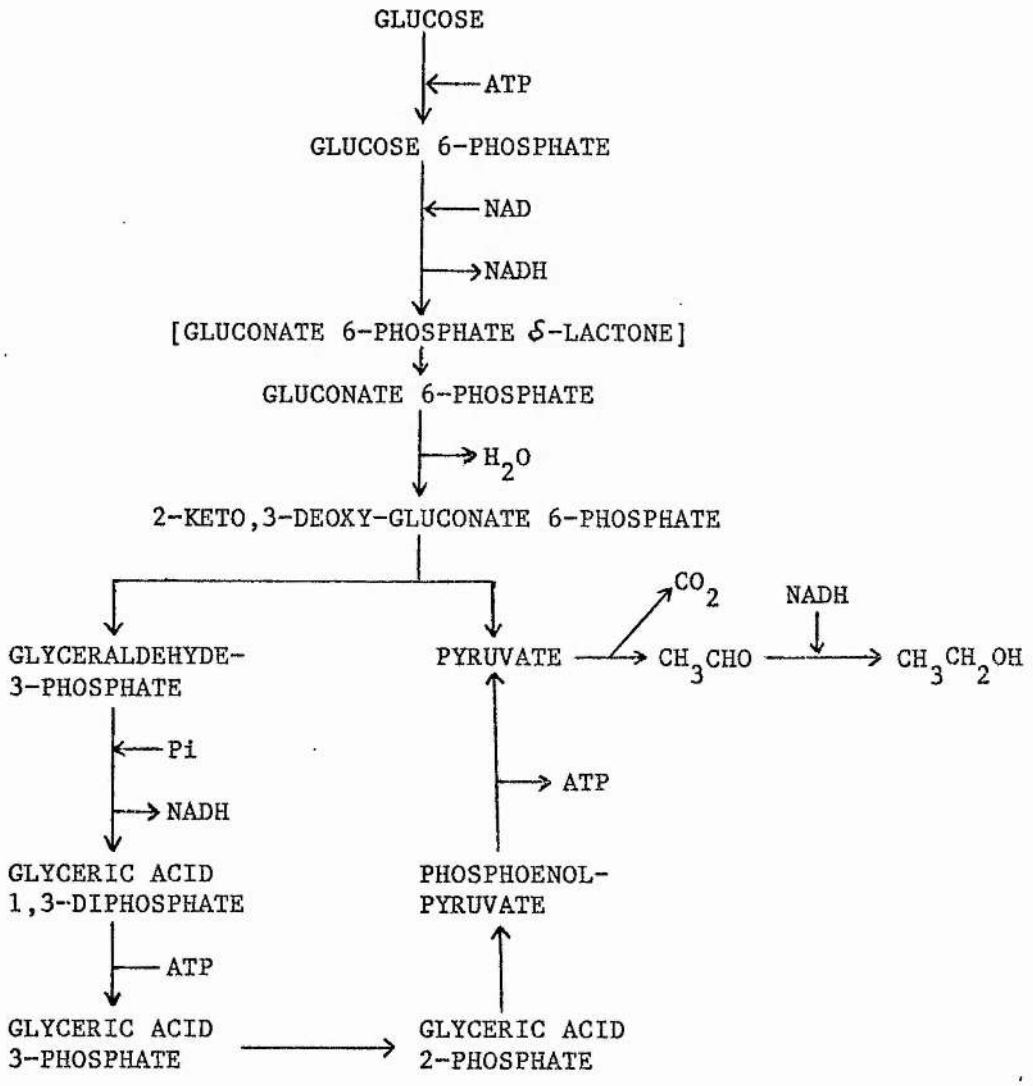

Fig. 1.3.b. Reactions of the Entner-Doudoroff pathway.

organism; further, the 3-deoxy-2-oxo-6-phosphogluconate finally present account for all of the 6-phosphogluconate metabolised. The other following enzymes were also detected: glucose-6-phosphate dehydrogenase (active with NAD and NADP, but only the former is the physiological electron carrier in the balanced oxidation-reduction involved in ethanol formation), ethanol dehydrogenase (active with NAD), glyceraldehyde 3-phosphate dehydrogenase (active with NAD), hexokinase, glucokinase, glucose dehydrogenase and pyruvate decarboxylase (carboxylase).

Bauchop and Elsden (1960) determined the relationship between growth and energy supply of some anaerobic bacteria. They demonstrated that the yield coefficient - defined as g.dry wt.organism/mole glucose - of Zymomonas mobilis was approximately one-half of the corresponding value obtained for Saccharomyces cerevisiae, which is accounted for by the provision of one mole of ATP per mole of glucose. These results are also consistent with the Entner-Doudoroff pathway of glucose degradation in Zymomonas mobilis.

Others important observations on the metabolism of Zymomonas mobilis are:

(i) It is able to grow on and ferment fructose and sucrose, as well as glucose, but on virtually no others sugars (Swings and De Ley, 1977). Aminoacids as sole carbon source are not fermented and they do not support growth when glucose is absent (Belaich, 1963).

(ii) The metabolism of sucrose in Zymomonas mobilis starts with the action of a specific enzyme (levansucrase, EC 2.4.1.10). Approximately 90% of sucrose in the medium is hydrolysed to glucose and rructose; the remaining sucrose is converted into a polyfructose (levan) and glucose (Dawes et al., 1966a). Subsequently, glucose and fructose are fermented to ethanol following the Entner-Doudoroff pathway (Swings and De Ley, 1977). Ethanol yields from sucrose are appreciably lower than those for equivalents concentrations of glucose plus fructose due to levan formation from sucrose (Dawes et al., 1966a). Recently, Lyness and Doelle (1983) studied some kinetic aspects of levansucrase purified from Zymomonas mobilis; they showed that the enzyme has a low substrate affinity (Km for sucrose of 8.0 x 10^{-3}M), an optimum pH for hydrolysing activity of 6.5 and that it is inhibited by glucose and ethanol at high concentrations. Since levansucrase plays such important role in the production of ethanol from sucrose, further work on this enzyme is warranted in order to fully understand the factors affecting this enzyme and the interplay between sucrose hydrolysis and ethanol production, if Zymomonas mobilis is considered for industrial production of ethanol from sugar cane.

(iii) 98% of the glucose consumed by Zymomonas mobilis is converted in ethanol, carbon dioxide, ATP (and heat), and only 2% it used for growth processes. Nevertheless, the 2% glucose incorporated accounts for about 50% of the cellular substance, the rest is derived from yeast extracts or peptone components. Pantothenate is an

essential factor for Zymomonas mobilis growth; with limiting pantothenate concentrations in defined media the molar growth yield falls considerably (Belaich et al., 1969,1972; Lazdunski and Belaich, 1972).

(iv) Zymomonas mobilis is not a strictly anaerobic organism. It can grow in the presence of oxygen. However, lower ethanol production rates and yields were observed when limited amounts of oxygen were supplied to the cultures (Swings and De Ley, 1977).

As was previously established, recent reports have showed that the bacterium Zymomonas mobilis has some potential advantages as an ethanol-producing microorganism for industrial fermentation (Lee et al., 1979, 1980, 1981, 1981a; Rogers et al., 1979, 1980, 1980a; Skotnicki et al., 1980; Lyness and Doelle, 1981). Some of these potential advantages would be :

(i) Significantly higher specific rates of glucose uptake and ethanol production are obtained than those reported for yeast.

(ii) Higher ethanol yields and lower biomass production than for yeast. When compared to yeast, the lower cell concentration would seem to be a consequence of the lower energy available for growth. Zymomonas cultures metabolise glucose via the Entner-Doudoroff pathway which yields one mole of ATP/mole glucose, while yeasts metabolise glucose anaerobically via the glycolytic pathway to give two moles ATP/mole glucose.

(iii) In contrast to yeast (Jones et al., 1981), Zymomonas mobilis can grow under strict anaerobic conditions. This would mean a significant saving in costs which would otherwise be required for providing a controlled oxygen environment within the fermenter.

(iv) Zymomonas mobilis tolerates high initial glucose concentrations (up to 25%).

(v) Ethanol tolerance of some strains of Zymomonas mobilis is comparable if not higher than for strains of S. cerevisiae. Ethanol concentrations of 70 - 80 g/l have been achieved in continuous culture and up to 130 g/l in batch culture using a strain of Zymomonas mobilis (Rogers et al., 1980).

1.4 The aims of this research

The work presented here was carried out with a view to studying the fermentation of cassava starch hydrolysate to ethanol by using cells of Zymomonas mobilis immobilised in calcium alginate beads.

In order to fulfil this objective, the following points were investigated:

i) physical properties of the alginate-cell gel (mechanical resistance, substrate mass transfer and cell leakage from the beads);

ii) batch fermentation of cassava hydrolysate using the immobilised-cell preparation for optimisation of the operating conditions (temperature, pH, initial glucose concentration and requirement for additional nutrients);

iii) performance of an immobilised-cell column reactor operated under the optimised parameters derived from batch experiments;

iv) comparison of the performance of the immobilised-cell reactor with the performance of a free-cell continuous stirred tank reactor operated under the same experimental conditions;and,

v)derivation of a mathematical model based on experimental data obtained in the immobilised-cell reactor experiments.

2 MATERIALS AND GENERAL METHODS

2.1 Chemicals

Sodium alginate type IV, glucose oxidase type I , amyloglucosidase grade IV and bacterial alpha-amylase type XI-A were obtained rrom Sigma London Chemical Co. Ltd., England. Other chemicals used were of analar grade.

2.2 Organism and media

Zymomonas mobilis, strain ATCC 10988, was obtained from the National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland, and used in this investigation.

The composition of the media used in this study were the following:

i)Maintenance media ,MM - agar, 20 g/l; yeast extract, 10 g/l; glucose, 20 g/l.

ii)Growth media,GM - glucose, 100 g/l; yeast extract, 10 g/l; KH_2PO_4, 2 g/l; $(NH_4)_2SO_4$, 2 g/l; $MgSO_4.7H_2O$, 2 g/l. The pH was adjusted to 5.0. Glucose solution was sterilised separately and added to the salt-yeast extract solution.

iii)Fermentation media - enzymatically hydrolysed cassava starch (unsupplemented) was used as the media for the fermentation experiments in this study. The hydrolysate was prepared as described in the section 2.4.

The media were sterilised by autoclaving at 120°C (15 p.s.i.) for 25 min.

2.3 Cultivation and immobilisation of cells

Zymomonas mobilis cells were maintained on agar slants in Universal bottles containing 15 ml of the medium MM. This culture was incubated at 30°C for 1 - 2 days and subsequently stored at 4°C. Stock cultures were subcultured every 2 or 3 weeks.

Inocula were prepared by transferring cells from the stock cultures to 50 ml of the media GM in 100 ml containers and allowed to develop at 30°C.

As soon as these cultures showed vigorous gassing, they were added to a 1-litre flask containing 500 ml of the media GM and the cells were cultured at 30°C in a thermostatted water bath with gentle magnetic stirring. These cultures were allowed to grow for 18-20 h, which corresponded roughly to the end of the exponential growth phase (Fig. 2.3). The cells were then harvested by centrifugation at 3,000 x g for 1.0 h ,washed in distilled water and immobilised (as described below).

The immobilisation procedure used in this study was a modification of that described by Kierstan and Bucke (1977). Sodium alginate solution (2%, w/v) was added to a washed cell suspension to give a final cell concentration of 20% (wet weight/v). The resulting alginate-cell mixture was pumped dropwise into a 0.1 M $CaCl_2$ solution with a Watson Marlow flow inducer . The slurry was extruded as

discrete drops to form pellets of ca. 2 mm radius. The pellets were stored in 0.1 M $CaCl_2$ solution for 2 h before used.

All steps of this procedure were carried out under aseptic conditions.

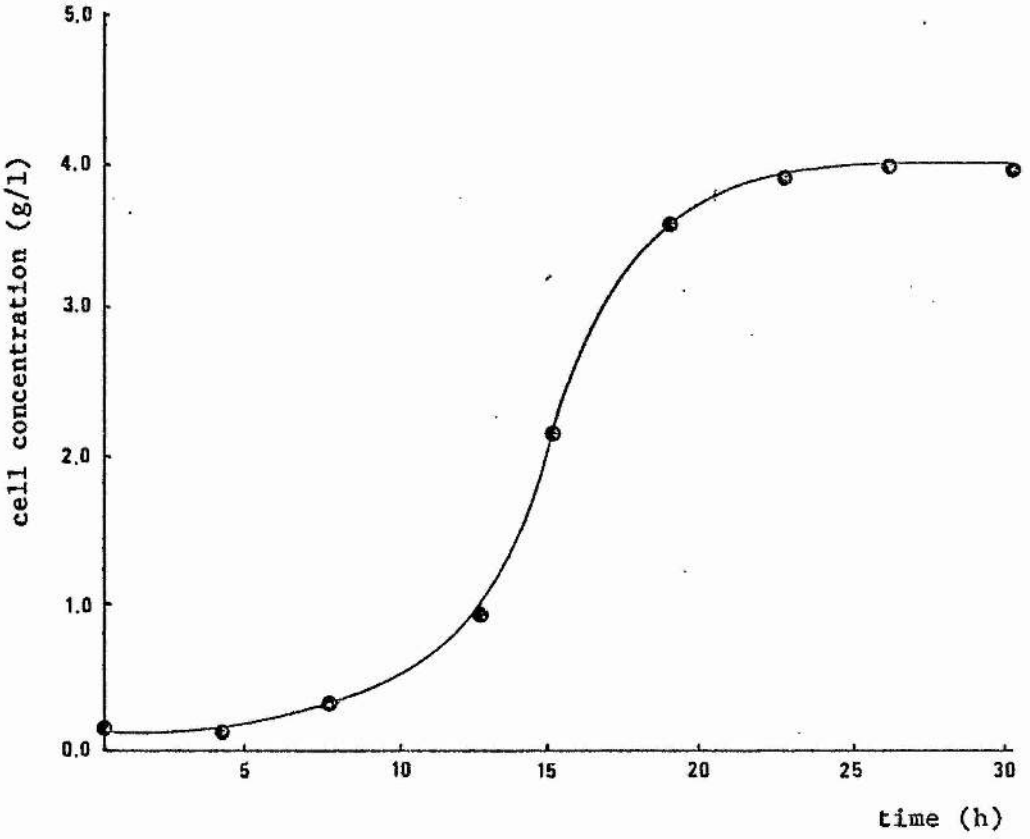

Fig. 2.3. Growth curve of Zymomonas mobilis on medium GM.

2.4 Cassava hydrolysis

Dried chopped cassava roots from Alagoas, Brazil, were pounded in a mortar vessel with a pestle. The ground cassava contained about 70% (w/w) starch and 10% (w/w) water; the remainder, unidentified, contained mainly ligneous material from the exterior parts of the root. A starch slurry containing 10% (w/v) starch was prepared by thorough homogenisation of ground cassava powder in a blender with distilled water.

Cassava starch hydrolysate was produced by liquefaction of the starch slurry using a modified two-step enzyme process followed by saccharification by amyloglucosidase described by Roushdi et al. (1979).

The slurry was poured into a 2-litre vessel. The content of the vessel was agitated by a magnetic bar. A constant temperature bath controlled the temperature of reaction mixtures at pre-set values. The cassava starch slurry was adjusted to pH 5.5. Bacterial alpha-amylase (50-100 units/mg solid, where one unit is defined as the amount of enzyme which produces 1.0 mg of maltose from starch in 3 min at pH 6.9 and 20°C). was then added to give a concentration of 0.2% (w/w of dissolved solids) and $CaCl_2$ to give a final concentration of 50 p.p.m. The temperature was maintained for 30 min at 50°C, then the mixture was heated and maintained at 120°C (15 p.s.i. steam) for 20 min. The temperature was then decreased to 50°C and alpha-amylase

added to give a concentration of 0.2% (w/w of dissolved solids). The reaction mixture was kept at 50°C for 30 min. The liquefied starch was adjusted to pH 4.5. Amyloglucosidase (670 AG units/ml; one AG unit is defined as the amount of enzyme that produces 1 mg of glucose from starch/3 min, at 55°C and pH 4.5) at a concentration of 0.3% (v/w of dissolved solids) was added and the temperature was maintained constant at 55°C with continuous stirring for 72 h. The glucose content of the reaction mixture was determined at the end of the saccharification step.

The liquefaction process with the addition of alpha-amylase took place in two steps giving products with relatively low viscosity. The final cassava starch hydrolysate contained more than 9% (w/v) glucose. It was then concentrated in a rotatory vacuum evaporator or diluted with distilled water to adjust the glucose concentration to the required levels used .

2.5 Fermentation procedures

2.5.1 Continuous stirred-tank fermenter (CSTF)

CSTF experiments were carried out in a 1 l fermenter with a 800 ml working volume (fig. 2.5.1). A peristaltic pump delivered a controlled volumetric flow of medium into the reactor. The temperature was controlled at 30°C via a contact thermometer/150 W heating lamp system. A magnetic stirrer was used for mixing the fermenter contents. Medium left the vessel by an overflow pipe inserted through a side arm.

The CSTF was sterilised by autoclaving the system at 120°C (15 p.s.i.) for 30 min. After cooling overnight, the system was filled with sterile cassava hydrolysate and allowed to stand overnight to check for sterility. 80 ml of a 18-20 h batch culture was added. The culture was then left in batch growth for 10 hours before the test flow rates started.

The CSTF operated at various flow rates from 8 to 160 ml/h. At each dilution rate the effluent was sampled at 12 h intervals and analysed for glucose, ethanol and cell concentration. Steady state was assumed when the cell and glucose concentrations were constant for three successive samples.

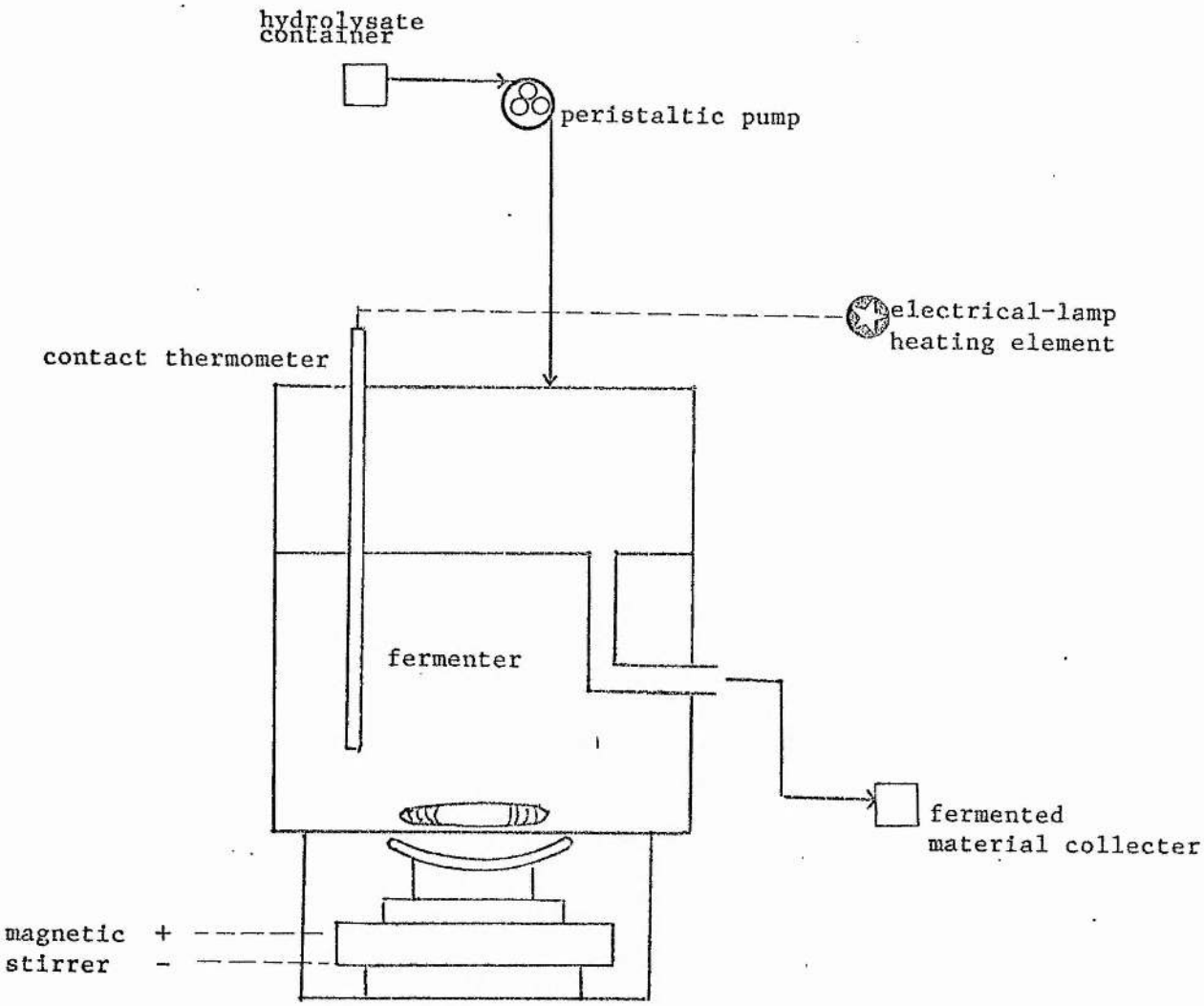

Fig. 2.5.1. Schematic diagram of CSTF.

2.5.2 Immobilised-cell system experiments

2.5.2.1 Batch experiments

Batch experiments were carried out in 250-ml flasks containing 5 g(dry weight)/l of immobilised Zymomonas mobilis cells and 100 ml of cassava hydrolysate. The flasks were incubated without agitation. Unless specified otherwise, the temperature was controlled at 30°C during fermentation. Samples of beads and liquid aliquots were periodically removed and biomass, glucose and ethanol contents were measured.

2.5.2.2 Continuous experiments

A column reactor was constructed to incorporate batch optimisation data for a continuous flow operation (Fig. 2.5.2.2.).

The column was constructed of a polyvinyl chloride tube of 2.3 cm diameter x 10 cm long. Five sample ports were installed at 2, 4, 6, 8 and 10 cm along the reactor length. A perforated plate was placed at the inlet of the column to distribute the feed solution radially. Sterilised cassava hydrolysate containing the equivalent of 100 g/l

glucose was pumped into the bottom of the column by peristaltic pump with a constant level maintained by an overflow device. Silicone rubber tubing was used to transport the feed solution from a 20 l container, reactor and tubing being sterilised by immersion in 70% (v/v) ethanol solution for 24 h. Incubation temperature was kept at 30°C.

Pellets containing immobilised Zymomonas mobilis cells were packed into a cylindrical plastic grid and placed into the column, this grid being used to facilitate the removal of carbon dioxide produced.

All operations were done under aseptic conditions.

The feed solution was pumped into the reactor and allowed to stand for 4 h before the test flow rates started.

The liquid volume phase was 36% of the total reactor volume for all experimental conditions; it was measured by draining the column.

The immobilised cell concentration inside the column was 19 g (dry weight)/l of liquid volume.

Glucose, ethanol and biomass concentrations were determined by analysing the effluent liquid taken at the top sample port of the column at each dilution rate at 12 h intervals. A steady state was assumed when the glucose and ethanol concentrations levelled off as

evidenced by assaying three successive samples.

During experiment investigating substrate consumption kinetics, glucose concentrations were determined by assaying bulk liquid samples taken from all the five sample ports along the reactor length at steady-state conditions.

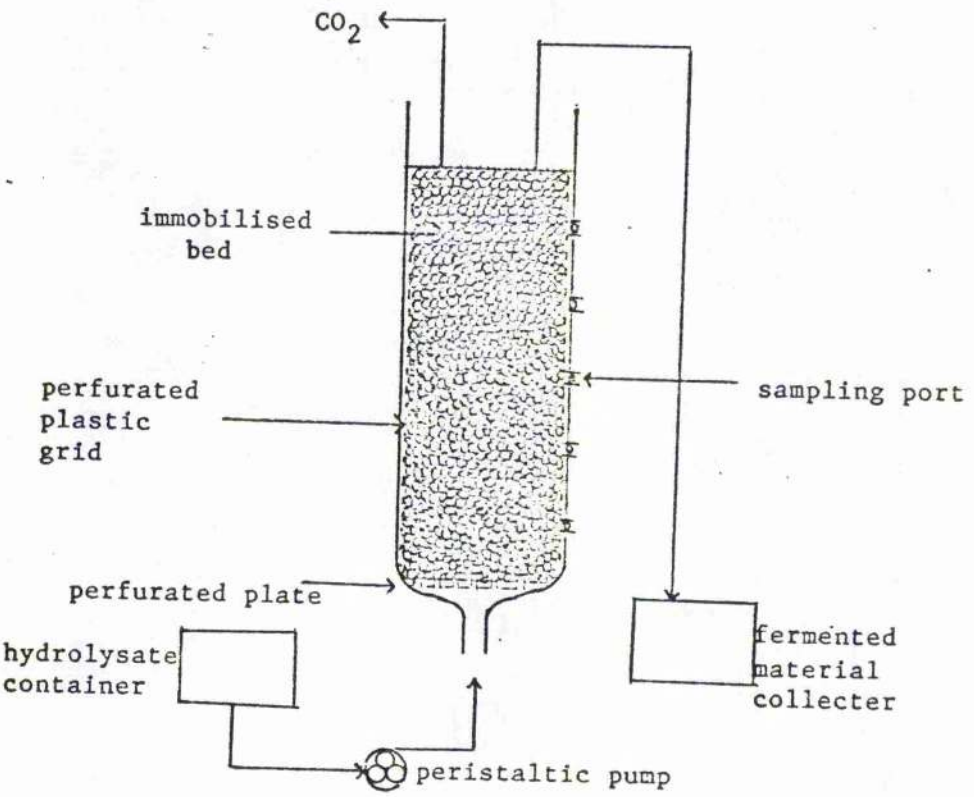

Fig. 2.5.2.2. Schematic diagram of the immobilised cell reactor system.

2.6 Analytical methods

2.6.1 Measurement of cell concentration

2.6.1.1 Absorbance-dry weight relationship (free cells)

Zymomonas mobilis organisms were harvested from 1 l culture by centrifugation, washed twice with distilled water, and resuspended to a completely homogenous suspension in distilled water. From this suspension, a series of suitable dilutions were made and their absorbances recorded at 610 nm with a Pye SP 600 Series 2 (Unicam Instruments, Cambridge, England) spectrophotometer. A sterile nutrient broth was used as the reference solution.

Three accurately measured 1 ml samples of the original suspension were reduced to dryness in weighting bottles of accurately known weight. The average weight of the cells in 1 ml samples were determined and a graph of absorbance against dry weight of cells per ml was constructed. Figure 2.6.1.1 presents these results.

2.6.1.2 Dry weight (Immobilised cells)

The cell concentration in immobilised cells was investigated by washing the beads containing cells with water and drying to constant weight at 105°C.

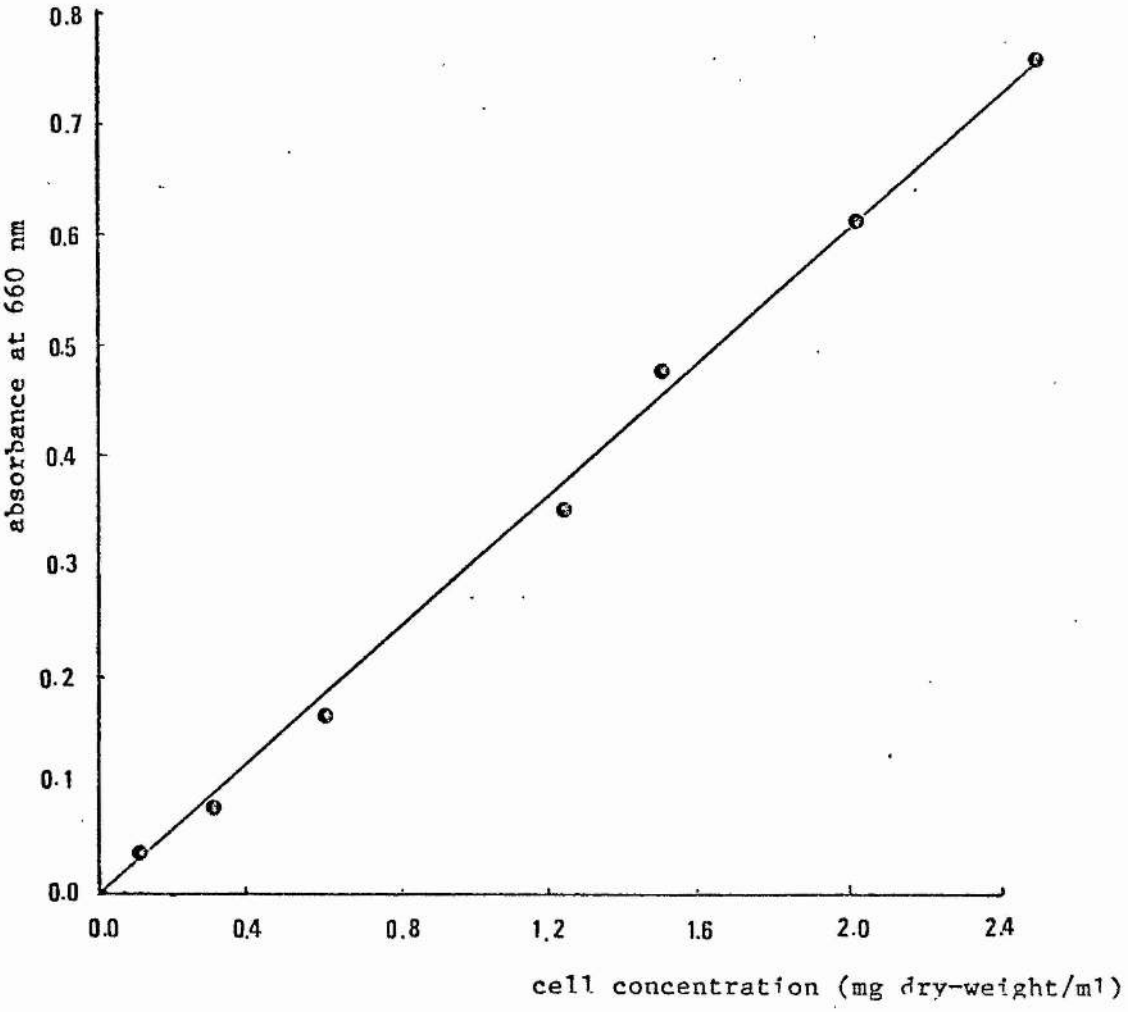

Fig. 2.6.1.1. Calibration curve of absorbance of culture against dry weight of cells/ml.

2.6.2 Measurement of ethanol concentrations

A gas-liquid chromatographic procedure was used to measure ethanol concentrations.

Measurements were made on a Pye Series 104 (Unicam Instruments, Cambridge, England) gas chromatograph. The column was packed with Porapak Q (Waters Associates, Inc., Milford, Mass., U.S.A.) 80-100 mesh and maintained at 200°C during operation. The carrier gas was nitrogen at a flow-rate of 37 ml/min. The flame-ionization detector was operated at 250°C.

Isopropanol was used as an internal standard. A series of aqueous standards were prepared containing ethanol in various concentrations from 1 to 9 mg/mg of isopropanol. For each standard, the ethanol/isopropanol peak-height ratio was calculated and plotted against the respective ethanol concentrations. Figure 2.6.2 presents the calibration curve obtained.

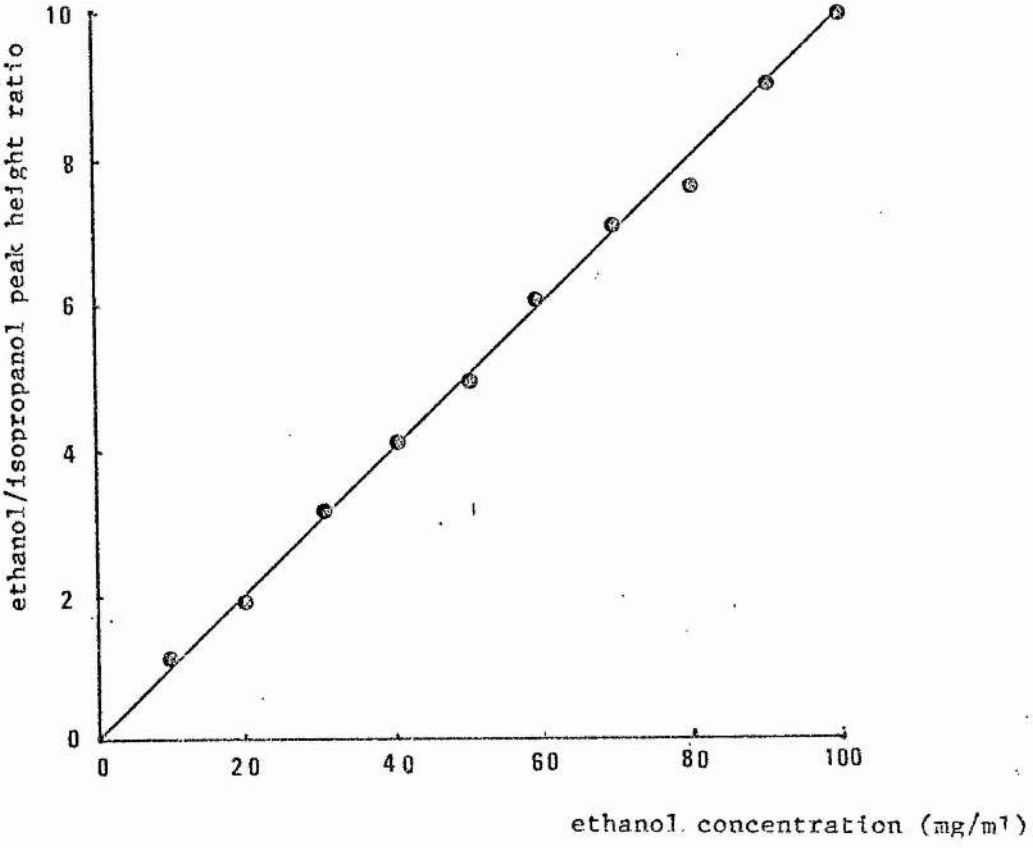

Fig. 2.6.2. Calibration curve for ethanol concentration measurements.

2.6.3 Measurement of glucose concentrations

A glucose oxidase/oxygen electrode method was used to measure glucose concentrations. The method is based on the oxidation of glucose catalysed by the action of glucose oxidase:

$$\text{glucose} + O_2 = \text{gluconic acid} + H_2O_2$$

The oxygen uptake can be followed using an oxygen electrode system. A Yellow Springs oxygen electrode system (Yellow Springs Instruments, Co., Ohio, U.S.A.) was used. The reaction vessel was surrounded by a thermostatted water jacket. The electrode output was used to drive an AR 45 chart recorder.

The following reagents were used:

(i) 25 mM phosphate/citrate buffer, pH 5.0 (PC buffer): 121.5 ml of 0.1 M citric acid and 128.5 ml of 0.2 M dibasic sodium phosphate were mixed, pH adjusted to 5.0 and the volume made up to 1 litre;

(ii) 10% (w/v) glucose made in PC buffer;

(iii) glucose oxidase reagent - 0.3 mg/ml glucose oxidase grade I made in PC buffer.

3 ml of PC buffer containing 0.1 to 1.0 mg of glucose were placed in the reaction vessel of the oxygen electrode at 40°C. 50 ul of glucose oxidase reagent were added through the hole in the plunger by using a microsyringe and the oxygen uptake recorded with a chart speed of 1 cm/min.

For oxygen solubility a value of 0.380 micromoles $O_2.ml^{-1}$ (air saturated buffer, 40°C) was used for calibration of reaction rates. Thus,

1 scale division of recorder =

$$= \frac{3 \text{ ml (assay volume)} \times 380 \text{ nmoles } O_2.ml^{-1}}{100 \text{ (scale divisions)}} =$$

= 11.4 nmoles of O_2

A calibration plot was prepared plotting rate of oxygen uptake against glucose concentrations (fig. 2.6.3).

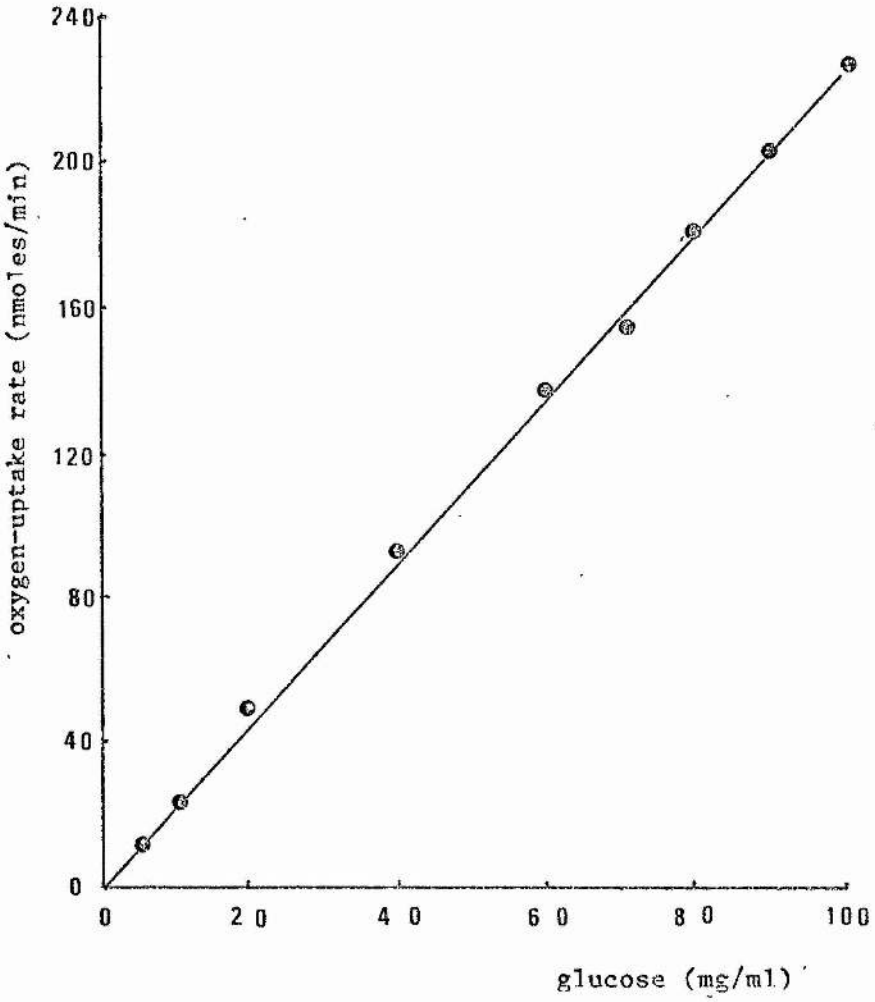

Fig. 2.6.3. Calibration curve for glucose concentration determination.

3 RESULTS AND DISCUSSION

3.1 Physical characterization of the immobilised-cell system

Desirable characteristics for immobilised cell systems for ethanol production should include the following:

(i) good mechanical resistance of the gel beads, otherwise the biocatalyst can be disrupted by excess gas production;

(ii) easy substrate penetration;

(iii) Gel pore size should be small enough so cells do not leak out of the gel lattice.

Therefore, an initial examination of the strength, porosity to glucose and capacity of retention of cells of the immobilised Zymomonas mobilis cell preparation to be used in the subsequent experiments was carried out.

The following sections describe and discuss these experiments and their results.

3.1.1 Mechanical resistance

The compression behaviour of a single biocatalyst particle has been the most common method for characterisation of the mechanical strength of immobilisation matrices (Washausen, 1979;Takata et al.,1978; Birbaum et al.,1981). Therefore, it was decided to follow this method for studying the mechanical resistance of the cell-gel beads to be used in subsequent experiments of this work.

For this purpose, the test unit illustrated in fig. 3.1.1 was made. One bead (4 mm diameter) was placed on the plate A and subjected to an axial load with measurements of the corresponding change in vertical diameter being made.

The system behaved as a plastic material, i.e., the bead did not regain its original dimension upon removal of the load.

The degree of irreversible compression (plastic deformation) increased as the load increased, but no fracture of the bead was observed within the measurement range examined.

The maximum load value, corresponding to the point where a completely irreversible compression was observed, was 7.8×10^4 N/m^2 (calculated from the applied force and the original surface perpendicular to the force); at this point the percentage of compression of the beads was 60%.

As can be seen in Table 3.1.1., the gel-strength of the Zymomonas mobilis cells/calcium alginate system is similar to that of k-carrageenan and polyacrylamide gel (Nishida et al., 1979), which already are immobilisation matrices in current industrial use, and fall in the range of maximum compression stress of a calcium alginate/yeast system using several combinations of alginate and cell concentrations (Washausen, 1979).

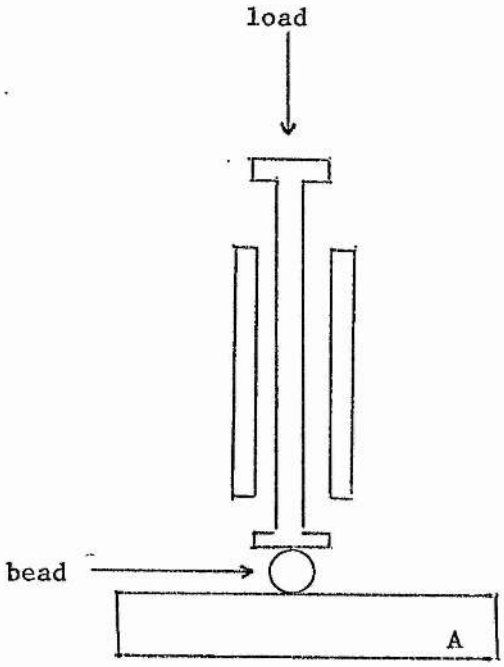

Fig. 3.1.1. Mechanical strength measuring unit.

Material	gel-strength (N/m^2)	reference
Immobilised preparation used in this work (2% Ca-alginate/200g/l *Zymomonas* cells.	7.8×10^4	present investigation
3.4% k-carrageenan	8.04×10^4*	Nishida *et al.*, 1979
Polyacrylamide gel	7.94×10^4*	Nishida *et al.*, 1979
Ca-alginate/5-25 g/ml cells	$4.2\text{-}14.6 \times 10^4$*	Washausen, 1979

Table 3.1.1. Comparison of the gel-strength of the immobilised preparation used in this work with some literature values for other systems.

* estimated load for gel crush.

3.1.2 Porosity

In order to evaluate the porosity of immobilised Zymomonas mobilis cell preparations the partition of glucose between the bulk solution and the beads (distribution coefficient) was measured by mass transfer experiments between a glucose solution and the beads.

2% (w/v) calcium alginate pellets containing 20% (wet weight) cells of Zymomonas mobilis were incubated in 200 mM $HgCl_2$ solution for 24 h and then placed in 500 ml of stirred (120 rpm/min) and unstirred 100 g/l glucose solution. At time intervals, beads were removed from the glucose solution, blotted dry and disrupted in 0.3 M K_2HPO_4. After suitable dilution they were assayed for glucose content. The results are presented in figure 3.1.2.

The time to reach equilibrium was the same (40 min) for both stirred and unstirred experiments indicating that there is no external limitation for glucose diffusion into the beads. When the equilibrium was reached the glucose distribution coefficient, C/C_o (where C is the glucose concentration inside the beads and C_o is the glucose concentration at the interface) was close to the theoretical maximum, indicating the presence of a highly porous structure in the immobilised-cell preparation.

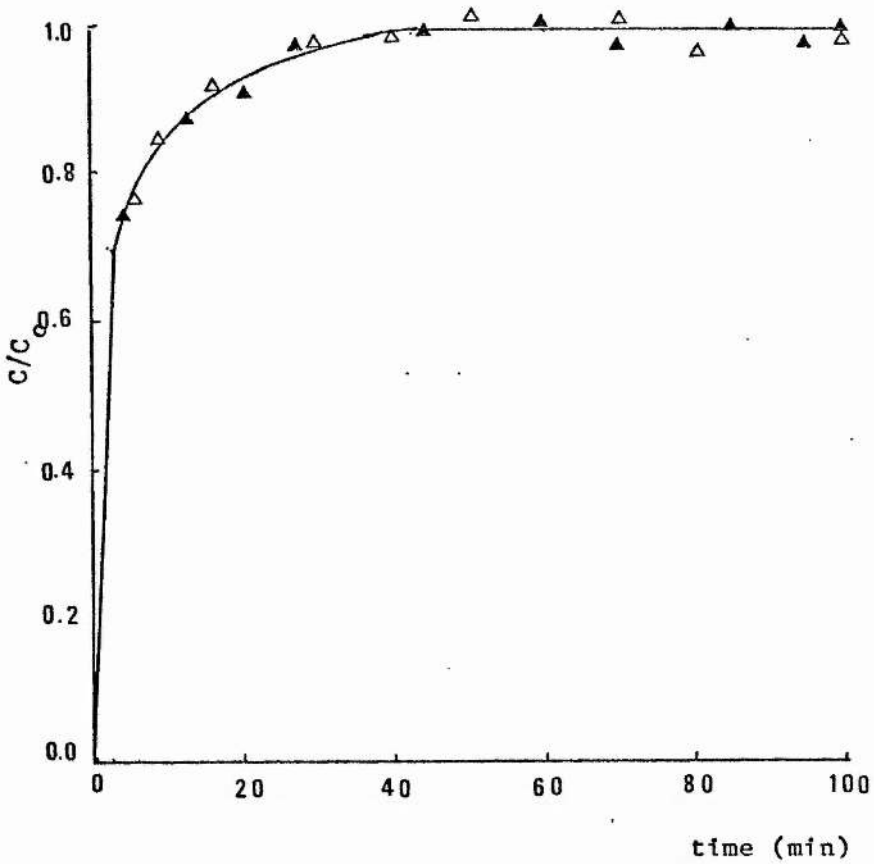

Fig. 3.1.2. Porosity of Ca-alginate/Zymomonas beads (2 mm radius) to glucose.

C=concentration of glucose inside the bead;

C_o=concentration of glucose at the liquid-solid interface.

(▲)stirred experiment; (△)non-stirred exp.

3.1.3 Effectiveness of the method of immobilisation

To test the effectiveness of the immobilisation procedure the extent of cell leakage from the beads under agitation for 48 h was investigated.

Calcium alginate beads containing entrapped Zymomonas mobilis cells were prepared as described (section 2.3), washed, and shaken in distilled water at 120 oscillations/min at 30°C on an orbital shaker for 48h and the cell concentration was measured in the supernatant at intervals.

No loss of cells occurred over the period of observation.

3.1.4 Conclusion

Based on the results outlined above it was concluded that the immobilised-cell preparation used in these studies (beads of 2% w/v of calcium alginate containing 20% (wet weight/v) of Zymomonas mobilis) is mechanically strong and allows easy access of substrate without cell leakage from the beads, and therefore constitutes a suitable

system for the purposes of the present investigation.

3.2 Batch experiments

This section of this work is an investigation of the operating parameters required for optimal production of ethanol by an immobilised Zymomonas mobilis cell system.

The experiments were carried out under batch conditions as described (section 2.5.2.1), and the system monitored for ethanol production rate at various initial glucose concentrations, temperatures, pH, medium composition and initial ethanol concentrations in the medium.

The following sections describe the results obtained from these experiments.

3.2.1 Effect of initial glucose concentration

Investigation of the glucose uptake rate and ethanol production rate for the immobilised Zymomonas mobilis cell system, using cassava hydrolysate containing 50, 100, 172 and 250 g/l glucose, was carried out. The results obtained from these studies are shown in Table 3.2.1.

The maximum glucose uptake and ethanol formation rates, (derived by measuring the maximum slope of glucose and ethanol concentrations plotted against time) were found to be highest with an initial glucose concentration between 50 to 100 g/l. Beyond this value, as the glucose concentration in the hydrolysate increased the value of the maximum substrate uptake rate and product formation rate decreased, with considerable elongation of the fermentation time. These results contrast with those reported by Rogers et al., (1979); these authors, working on fermentation of pure glucose to ethanol by free cells of Zymomonas mobilis, found that specific ethanol formation rate and specific glucose uptake rate were unnaffected by increasing initial glucose concentration in medium from 100 to 250 g/l. Therefore, it is apparent that the inhibitory effect of high concentrated cassava hydrolysates observed in the present study on the activity of the cells should be related to the presence of others compounds in hydrolysate. Another possible reason could be that the immobilisation procedure itself induced, in some way, structural changes of the cell wall leading to a higher susceptibility to osmotic effects produced by high glucose concentrations.

The overall efficiency of conversion of glucose to ethanol (defined as g of ethanol produced/g of glucose consumed) attained was about the same for all the initial glucose concentrations tested.

Parameter	glucose in hydrolysate (g/l)			
	50	100	172	250
max. glucose uptake rate (g/l.h)	15.4	16.0	11.2	8.5
max. ethanol formation rate (g/l.h)	7.5	7.6	5.8	4.0
total utilised glucose (g/l)	50	100	152	200
max. ethanol concentration (g/l)	25	49	74	97
ethanol yield (%)	97.8	95.9	95.3	94.8
fermentation time (h)	3-3.5	10-13	16-20	58-63

Table 3.2.1. Effect of initial glucose concentration in cassava hydrolysate on the production of ethanol by immobilised cells of Zymomonas mobilis.

From the analysis of the results presented above it was decided to use cassava hydrolysate containing 100 g/l of glucose as the standard medium in the subsequent experiments.

3.2.2 Batch fermentation pattern

Cell concentration, glucose concentration and ethanol concentration during the course of fermentation of cassava hydrolysate containing 100 g/l of glucose are plotted against time in fig. 3.2.2.a.

Ethanol concentration increased with the time reaching 49 g/l after 8 h of reaction. A linear relationship was found to exist between the rate of ethanol production and the rate of glucose utilisation (fig. 3.2.2.b.) which can be represented by the following equation:

$$dE/dt = 0.49\ (-dG/dt)$$

where E is the ethanol concentration in g/l, G is the glucose concentration in g/l and t is the time of fermentation.

The conversion of glucose to ethanol was 96% of the theoretical yield. The maximum theoretical yield was calculated by assuming complete conversion of glucose to ethanol via the Entner-Doudoroff pathway whereby 180 g of glucose (1 mol) gives 92 g of ethanol (2 moles). The ethanol produced was divided by the ethanol yield theoretically possible from the amount of glucose converted at the end of fermentation and expressed as a percentage yield.

Cell concentration remained constant over the fermentation time indicating that ethanol has been produced via the maintenance metabolism only. A possible explanation is that cell division was limitated by the space available inside the gel or, due to the high initial cell concentration used, by the availability of nutrients.

The beads remained intact for the duration of the experiment despite vigorous production of carbon dioxide .

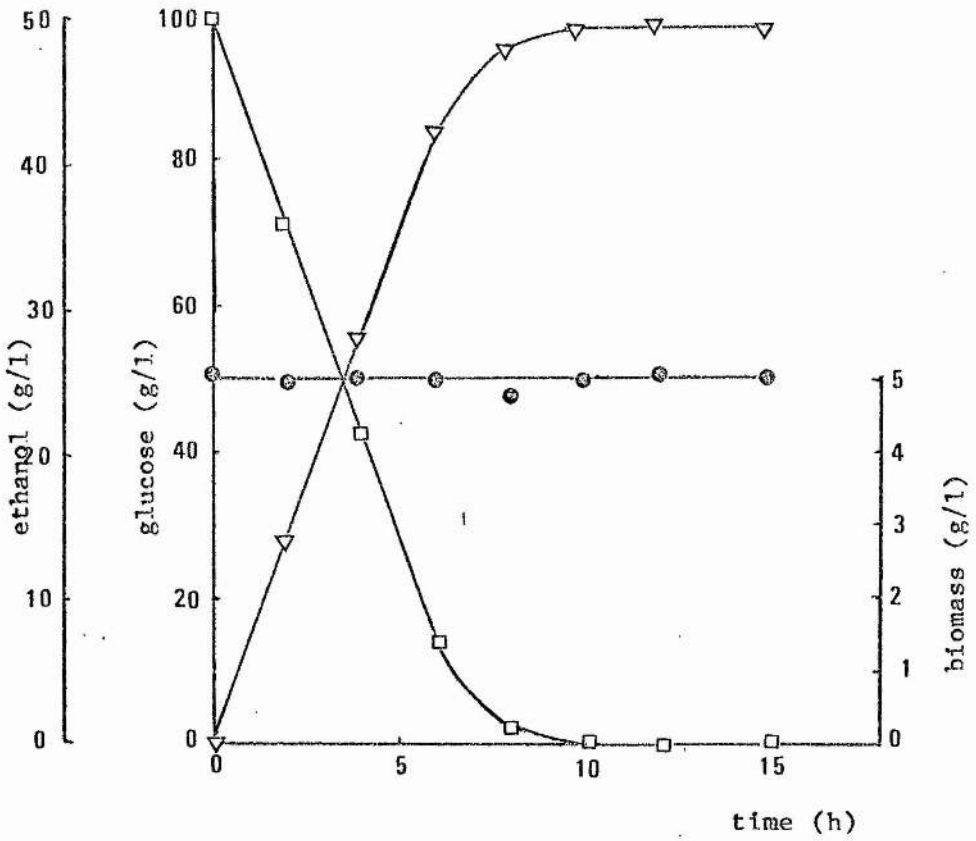

Fig. 3.2.2.a. Batch fermentation of cassava hydrolysate by immobilised cells of Zymomonas mobilis.

(□) glucose concentration

(▽) ethanol concentration

(●) biomass

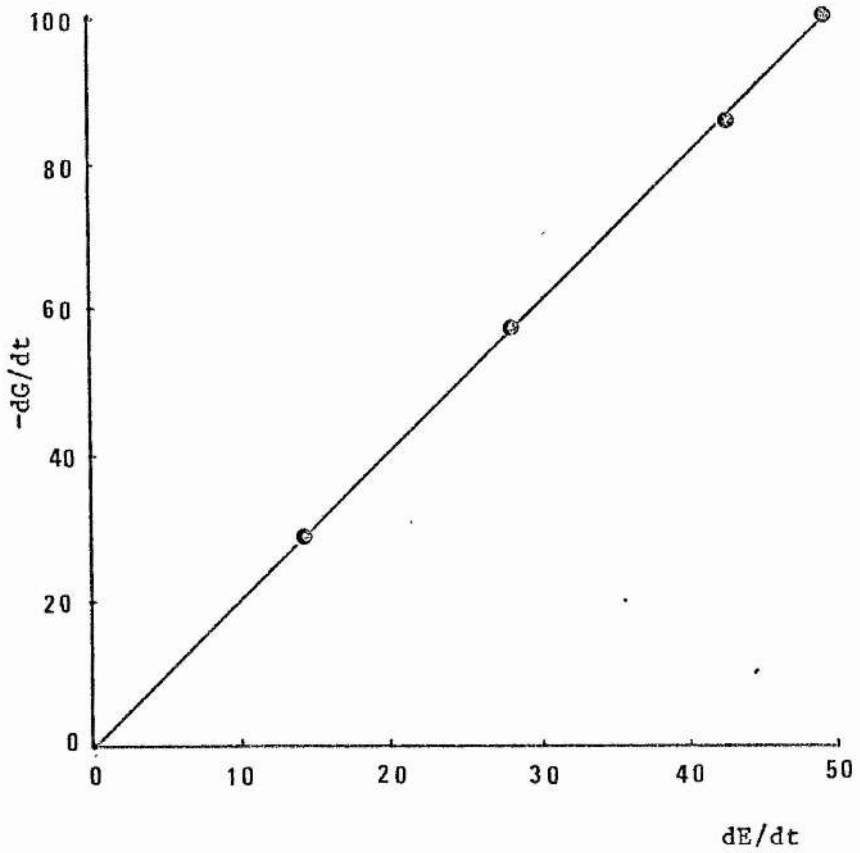

Fig. 3.2.2.b. Proportionality between the rate of glucose consumption (-dG/dt) and the rate of ethanol formation (dE/dt) during cassava hydrolysate fermentation in batch culture by immobilised cells of Zymomonas mobilis.

3.2.3 Temperature

The effect of temperature on the rate of ethanol production from cassava hydrolysate by immobilised Zymomonas mobilis cells was investigated under batch conditions described (section 2.3). The cells were incubated at different temperatures from 28 to 43°C.

The results are presented in figure 3.2.3. A decrease in ethanol production rate was observed as the temperature of fermentation increased from 30°C to 38°C; there was no fermentative activity at 43°C.

3.2.4 pH

The effect of pH on the rate of ethanol production was determined under the batch conditions described in section 2.3. The immobilised cells were incubated at 30°C with the media pH ranging from 3.0 to 8.0.

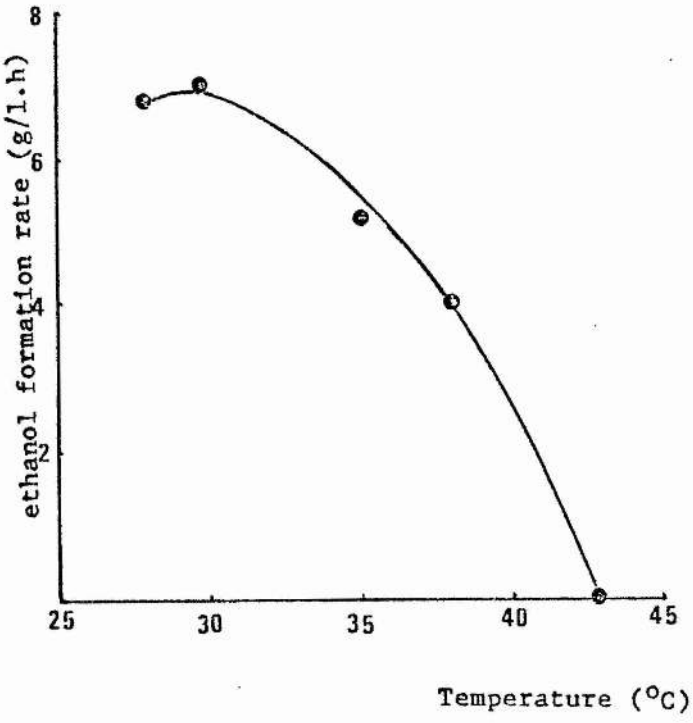

Fig. 3.2.3. Effect of temperature on the rate of ethanol formation by immobilised cells of Zymomonas mobilis. Initial pH of the medium=5.0

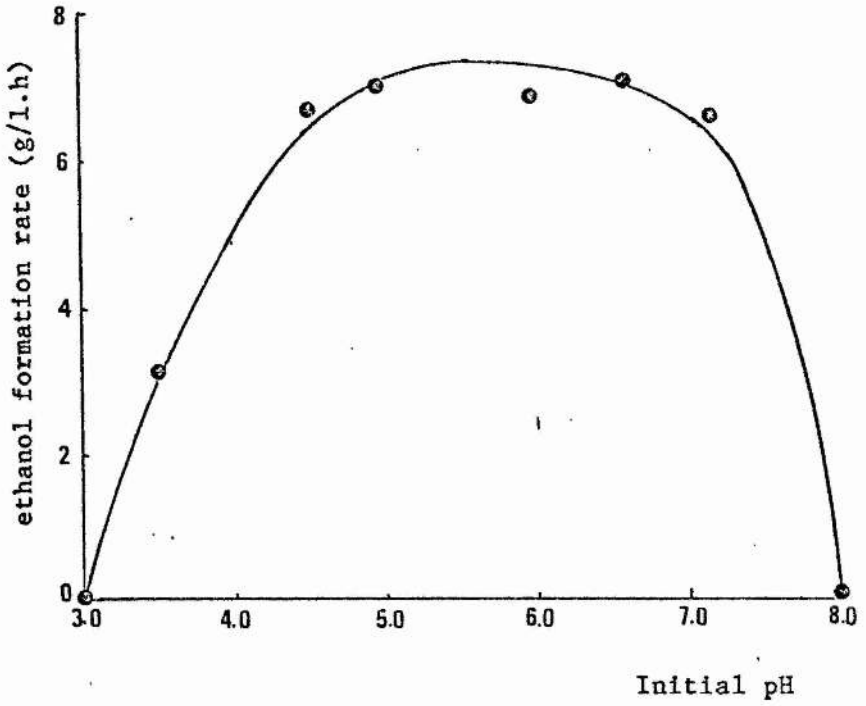

Fig. 3.2.4. Ethanol production by immobilised cells of Zymomonas mobilis from cassava hydrolysate (glucose 100 g/l) at several values of initial pH of medium. Incubation temperature=30°C.

The results showed a broad pH optimum with the best ethanol production rates between pH 4.5 and pH 7.2 (figure 3.2.4).

3.2.5 Effect of nutrients

The efficiency of ethanol production by growing cells of Zymomonas mobilis is dependent upon the presence of nitrogen, phosphorus, potassium and magnesium ions, as well as pantothenic acid, besides an carbon and energy source (Swings and De Ley, 1977; Cromie and Doelle, 1981; Kosaric et al., 1982). According to Kosaric et al. (1982), a fermentation medium for maximal ethanol production would contain: 15 g/l yeast extract, 1.5 g/l KH_2PO_4, 1 g/l $(NH_4)_2SO_4$ and 0.5 g/l $MgSO_4.7H_2O$ plus glucose.

However, it is to be expected that these nutrients, at least at the same levels, are unnecessary supplements to cassava hydrolysate for an efficient conversion of glucose to ethanol by the cells of Zymomonas mobilis used in this study, as no growth related activities are involved.

Fermentation medium	ethanol formation rate (g/l.h)
cassava hydrolysate*	7.4
cassava hydrolysate* plus 15 g/l yeast extract, 1.5 g/l KH_2PO_4, 1 g/l $(NH_4)_2SO_4$ and 0.5 g/l $MgSO_4.7H_2O$	7.6

* containing 100 g/l glucose.

Table 3.2.5. Effect of additional nutrients on the formation rate of ethanol from cassava hydrolysate by immobilised cells of Zymomonas mobilis.

In fact, an experiment was performed in order to evaluate the influence of adding these nutrients to cassava hydrolysate on the rate of ethanol production by the immobilised cell system. The experiment was carried out under the batch conditions described in section 2.5.2.1; the results are summarised in Table 3.2.5. These results indicate that cassava hydrolysate provides , for itself, the necessary nutrient requirements to allow efficient ethanol production by the immobilised (non-growing) cells of Zymomonas mobilis. These results are, from a industrial point of view, very promising if one consider the cost of adding nutrients on a large scale fermentation process. It was decided, therefore, to continue using pure cassava hydrolysate (100 g/l glucose) as the fermentation media in all subsequent experiments of this work.

3.2.6 Product inhibition

In order to investigate the extent to which ethanol concentration in the medium affects the fermentative activity of the immobilised-cell system, batch experiments were performed, as described previously, with different initial ethanol concentrations in basic medium.

The experimental values of v/V_{max} and respective initial ethanol concentration are showed in figure 3.2.6.

It can be seen that there was no inhibition of the fermentative activity below an initial ethanol concentration of about 60 g/l. Above this concentration the inhibition effect became stronger as the initial ethanol concentration in medium increased.

These results are not at all surprising because viable cells of Zymomonas can be isolated from alcoholic beverages containing up to 100 g/l ethanol and, therefore, should present a high ethanol tolerance (Swings and De Ley, 1977) .

From these results it was concluded that product inhibition effect on the fermentative activity of the immobilised-Zymomonas mobilis system at the substrate level used in this study is unimportant. In fact, with a initial glucose concentration of 100 g/l the theoretical maximum ethanol concentration which could be produced is 51 g/l which is lower than the threshold concentration at which the inhibition effect becomes apparent.

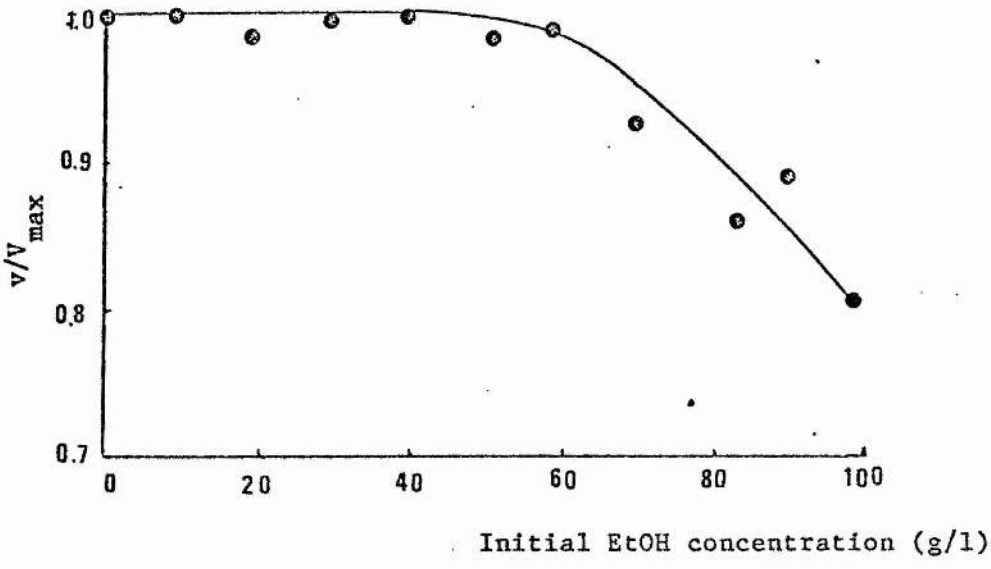

Fig. 3.2.6. Effect of initial ethanol concentration on the rate of ethanol formation from cassava hydrolysate by immobilised cells of Zymomonas mobilis.

v= observed rate of ethanol production

V_{max}= maximum rate of ethanol production

3.3 Continuous experiments

Continuous flow experiments were carried out to investigate the performance of a packed-bed reactor of immobilised Zymomonas mobilis cells. Experiments were conducted as described in section 2.5.2.2. The performance of the immobilised-cell reactor was compared to the performance of a conventional continuous stirred tank fermenter operated under identical conditions.

Dilution rates were calculated from the reciprocal of residence time based on liquid volume phase:

$$D = F/V$$

where D is the dilution rate (h^{-1}), F is the volume of inflowing substrate per time unit (ml/h) and V is the liquid volume phase (ml).

Volumetric ethanol productivity was defined on the basis of liquid phase residence time:

$$P = D \times E$$

where P is the volumetric ethanol productivity (g/l.h) and E is the ethanol concentration in the effluent stream (g/l).

The specific rates of glucose uptake (q_s) and ethanol production (q_p) of the immobilised-cell reactor were estimated based on the initial biomass loading.

The following sections describe the results obtained.

3.3.1 Immobilised-cell reactor performance

Figure 3.3.1.a. shows the volumetric ethanol productivity and percentage of utilised glucose as a function of the dilution rate.

Productivity ranged from a minimum of 8.9 g/l.h at a dilution rate of 0.18 h^{-1} to a maximum of 27.2 g/l.h at a dilution rate of 1.85 h^{-1}.

Almost 100% glucose conversion occurred at a dilution rate of 0.18 h^{-1}; beyond this value, glucose was not completely utilised.

An ethanol productivity of 22.5 g/l.h was obtained at a dilution rate of 0.6 h^{-1} with an efficiency of glucose utilisation of 73%. Higher productivities achieved by increasing the dilution rate resulted in greater losses of glucose in effluent liquid.

Figure 3.3.1.b shows the relationship of glucose and ethanol concentrations in the effluent liquid for different dilution rates.

Data show that as the dilution rate increased there was a decrease in ethanol concentration with a corresponding increase in glucose concentration in the effluent.

The efficiency of conversion of glucose to ethanol was close to 100 % of the theoretical maximum yield for all dilution rates tested.

Figure 3.3.1.c shows the specific ethanol formation rate (q_p) and the specific glucose uptake rate (q_s) of the immobilised cells as a function of the dilution rate.

A maximum q_s of 2.8 g/g.h and a maximum q_p of 1.4 g/g.h were found which agree very well with those reported by Margaritis et al. (1981) and Grote et al. (1980) for cells of Zymomonas mobilis immobilised in calcium alginate fermenting a 100 g/l glucose synthetic medium.

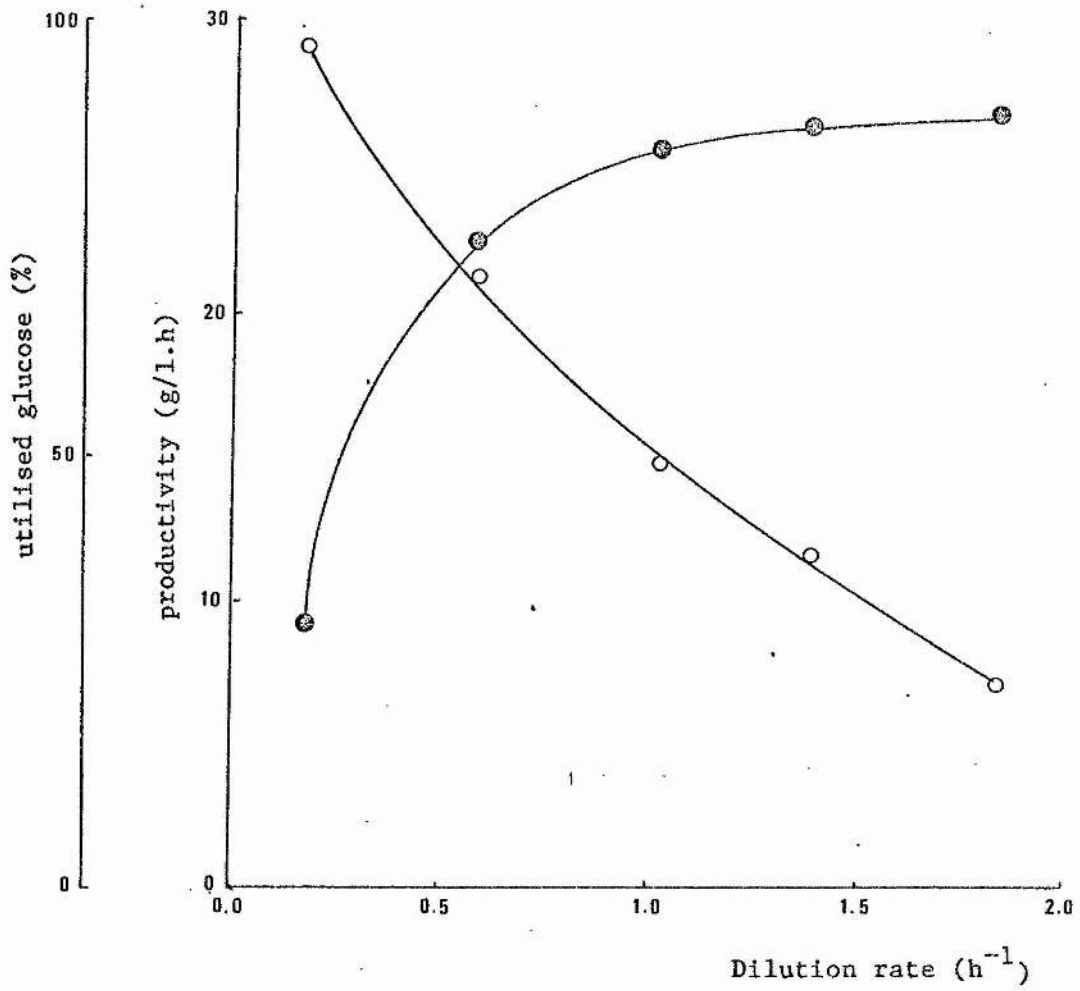

Fig. 3.3.1.a. Volumetric ethanol productivity and percent glucose utilisation as a function of dilution rate for the immobilised cell reactor.

(○) % glucose utilised

(●) ethanol productivity

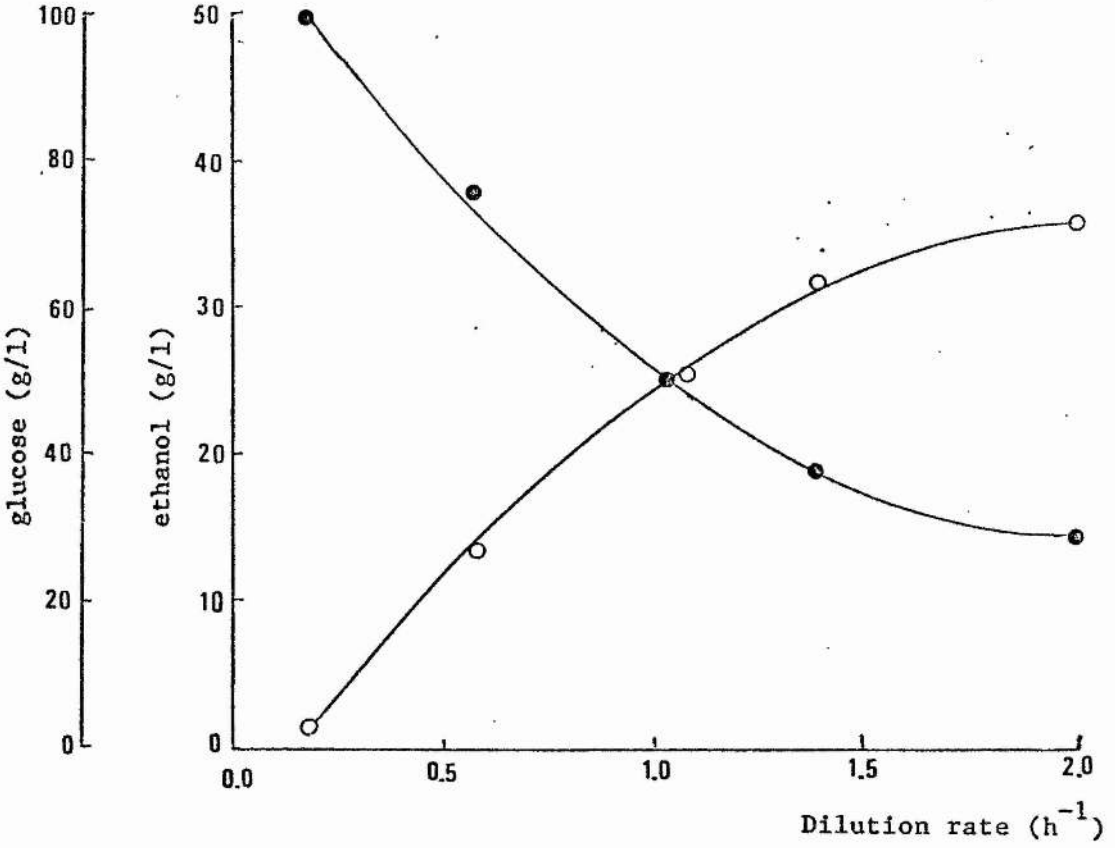

Fig. 3.3.1.b. Glucose and ethanol concentrations in the effluent liquid as a function of the dilution rate for the immobilised cell reactor.

(●) ethanol concentration
(○) glucose concentration

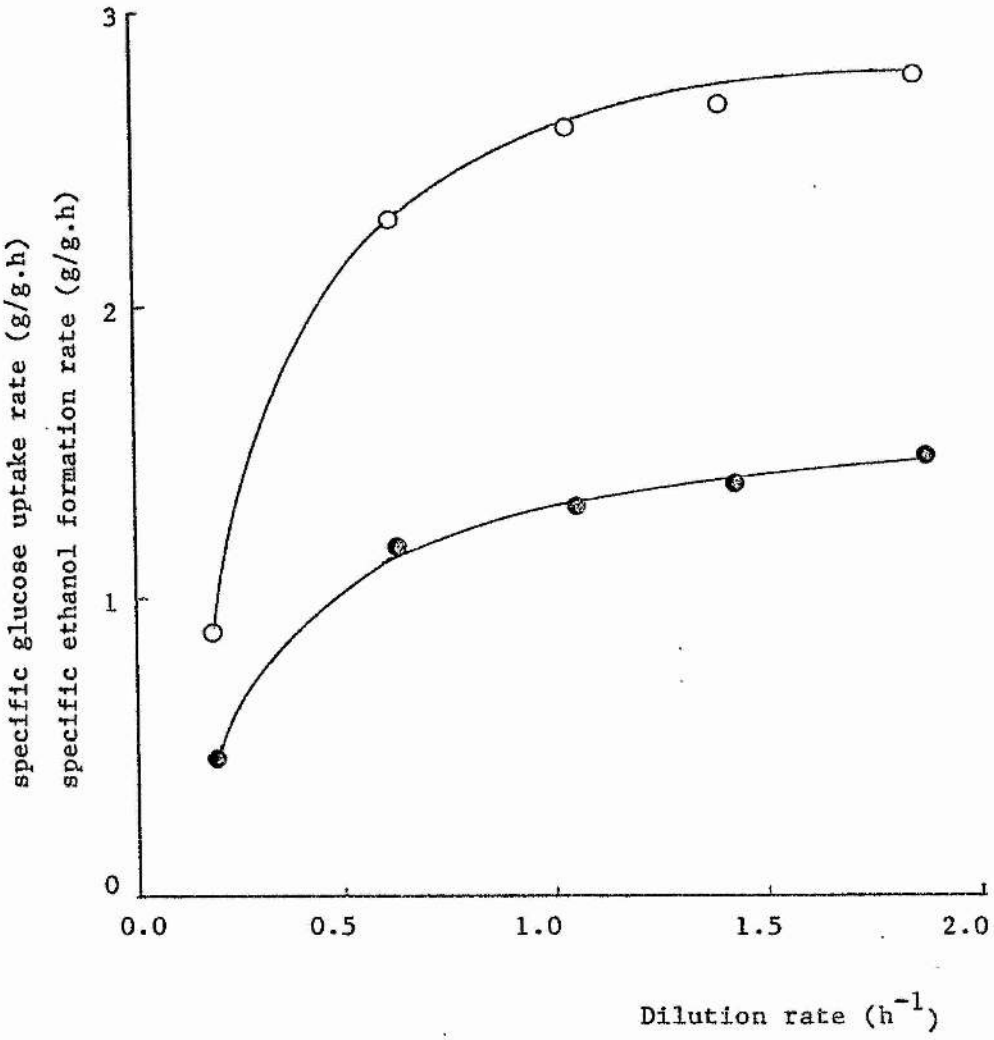

Fig. 3.3.1.c. Specific ethanol production rate (●) and specific glucose uptake rate (○) for immobilised cells of Zymomonas mobilis .

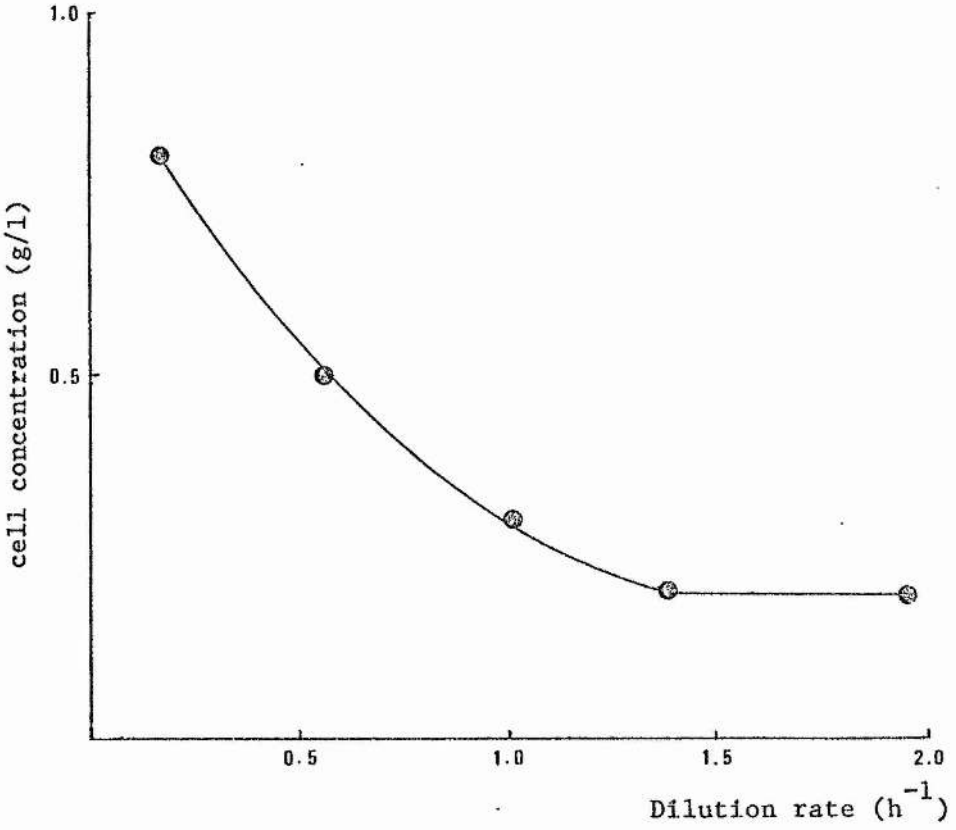

Fig. 3.3.1.d. Cell concentration in the effluent liquid as a function of dilution rate for the immobilised cell reactor.

It was also observed that the reactor loses cells with the effluent liquid. The free biomass in the effluent stream decreased with increasing dilution rates, varying from 0.8 g dry wt.cell/l at a dilution rate of 0.18 h^{-1} to 0.25 g dry wt.cell/l at a dilution rate of 1.85 h^{-1} (fig.3.3.1.d). Cells appearing in the effluent could be derived by leakage from entrapped cells - possibly enhanced in number by cell division after leakage. These present data are in agreement with published data on immobilised yeast cell reactors (Margaritis and Bajpai, 1982; Tyagi and Ghose, 1982).

3.3.2 Results of the continuous-stirred tank fermenter (CSTF)

Figures 3.3.2.a and 3.3.2.b show the steady-state data for ethanol production from cassava hydrolysate (100 g/l glucose) in a CSTF using free cells of Zymomonas mobilis.

In figure 3.3.2.a ethanol, glucose and cell concentrations are plotted against dilution rates.

The concentration of ethanol and amount of consumed glucose were maximum up to dilution rate of 0.12 h^{-1}; beyond this dilution rate ethanol concentration and the fraction of utilised glucose decrease with increasing dilution rate.

The cell concentration increased from 3.3 g/l at a dilution rate of 0.01h^{-1} to 4.3 g/l at a dilution rate of 0.085 h^{-1}, and then remained constant up the dilution rate of 0.2 h^{-1}.

Figure 3.3.2.b shows the values of the specific glucose uptake rate, specific ethanol formation rate and volumetric ethanol productivity for various dilution rates.

Maximum ethanol productivity was 7.3 g/l.h at a dilution rate of 0.2 h^{-1}.

Maximum specific glucose uptake rate and ethanol formation rate were 1.7 g/g.h and 3.5 g/g.h, respectively, at a dilution rate of 0.2 h^{-1}.

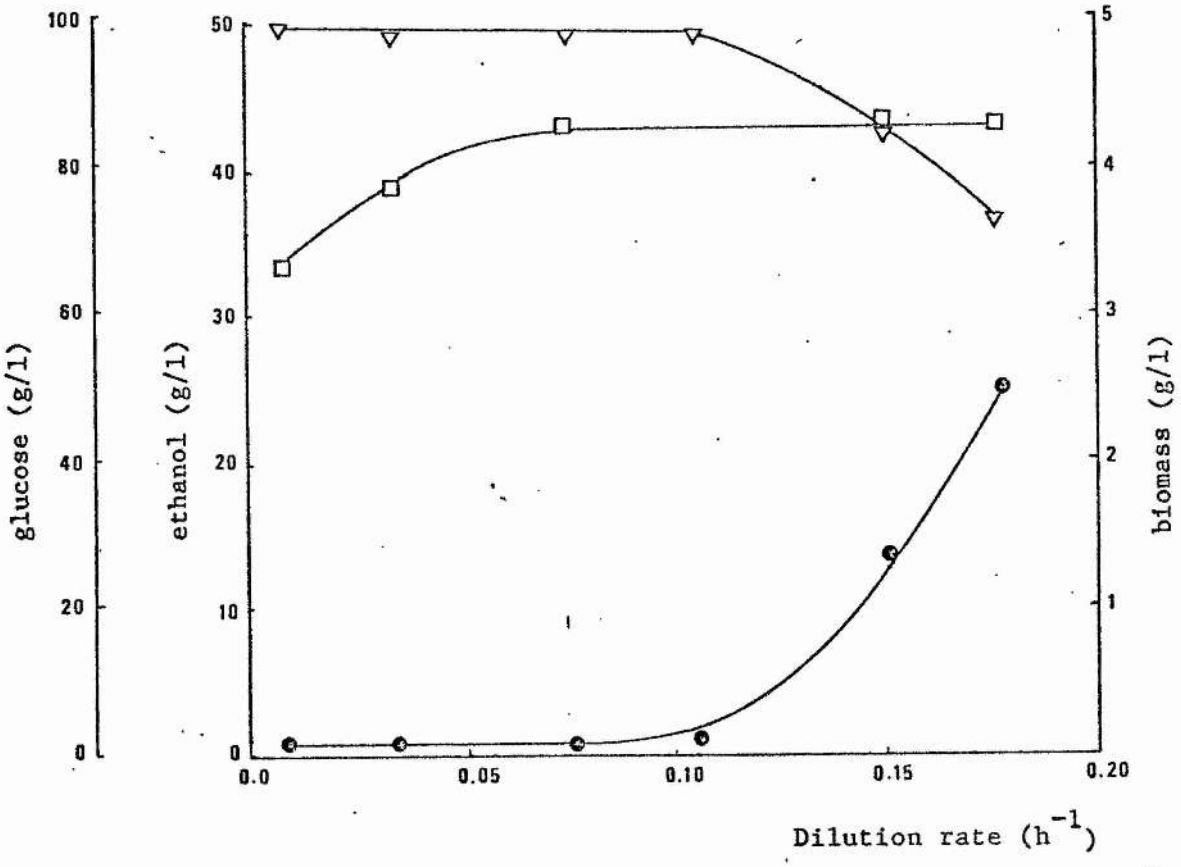

Fig. 3.3.2.a. Continous fermentation of cassava hydrolysate with feed glucose concentration of 100 g/l by free cells of Zymomonas mobilis.

(□) cell concentration

(▽) ethanol concentration

(●) glucose concentration

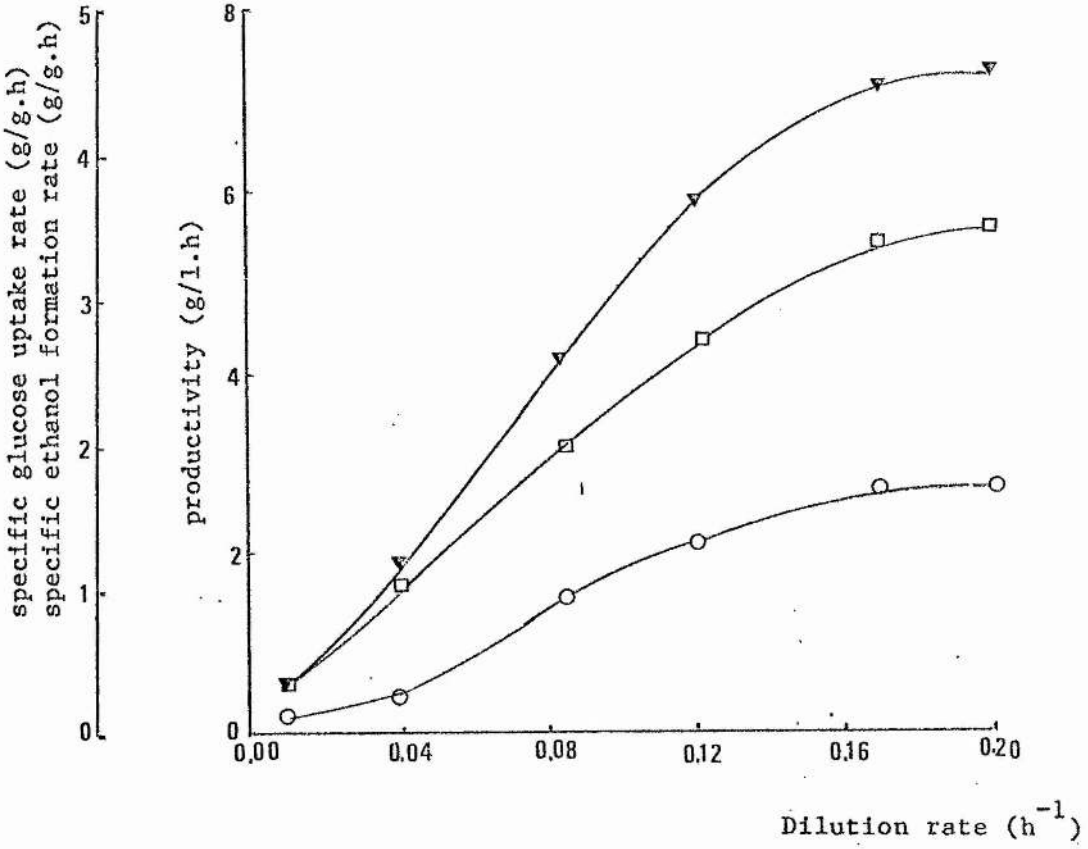

Fig. 3.3.2.b. Continuous fermentation of cassava hydrolysate with feed glucose concentration of 100 g/l by free cells of Zymomonas mobilis.

(▼) ethanol productivity
(□) specific glucose uptake rate
(○) specific ethanol formation rate

3.3.3 Comparison between the CSTF and immobilised-cell reactor

Table 3.3.3.a. shows a comparison between the data from the experiments with the CSTF and the data from the experiments with the immobilised-cell reactor. The data shown in table 3.3.3.a. correspond to dilution rates at which the percentage of glucose utilisation and effluent ethanol concentration are comparable.

The ethanol productivity of the immobilised-cell reactor was 1.5 times higher than the ethanol productivity of the free-cell CSTF at the respective dilution rates which gave maximum glucose utilisation, and the ethanol productivity of the immobilised-cell reactor was 3 times higher than that of the free-cell CSTF at the respective dilution rates which gave 75% of substrate utilisation.

The specific ethanol production rate and the specific glucose uptake rate of the free-cell system were found to be higher than those for the immobilised-cell system. Similar results were reported by Grote *et al.* (1980) and by Margaritis and Bajpai (1982) who worked with free- and immobilised-cells of *Zymomonas mobilis* and *Kluveromyces marxianus*, respectively; these authors suggested that the decrease of specific glucose consumption and ethanol formation rates for the immobilised-cell system could be related to mass transfer limitations (see Section 3.4.6).

The great disadvantage observed with the immobilised-cell reactor is the large gas hold-up which accounted for 16% of the total reactor volume for all the operating conditions tested (fig. 3.3.3.). It seems that this is a very common problem presently found in immobilised-cell reactors (Ghose and Bandyopadhyay, 1980; Tyagi and Ghose,1982). Nevertheless, as the tendency to scale-up immobilised-cell reactor continues, design progress will, hopefully, overcome this inconvenience and, therefore, improve the performance of these fermenters.

An immobilised-Zymomonas cell reactor also presents a viable alternative system to yeast cell reactors (with or without recycle) for the conversion of cassava hydrolysates to ethanol:

Table 3.3.3.b shows a comparison of ethanol productivity results from the present work with those reported by Bonomi et al. (1981).

It is evident that the immobilised cell reactor can match the productivities of the yeast feedback reactor which, because of the cell recycle system (for feedback), is a more complex and costly system.

Parameter	Free cells		Immobilised cells	
Dilution rate (h^{-1})	0.12	0.20	0.18	0.60
Feed glucose concentration(g/l)	100	100	100	100
Percent glucose utilised(%)	100	75	98	73
Effluent EtOH concentration(g/l)	49	36.4	49.5	37.5
Biomass (g/l)	4.3	4.3	19.6*	19.6*
EtOH yield (g/g.h)	0.50	0.48	0.51	0.51
Specific EtOH productivity(g/g.h)	0.96	1.7	0.46	1.17
Specific glucose uptake rate(g/g.h)	1.97	3.5	0.9	2.29

*Initial cell loading

Table 3.3.3.a. Continuous ethanol production from cassava hydrolysate using free and immobilised cells of Z. mobilis.

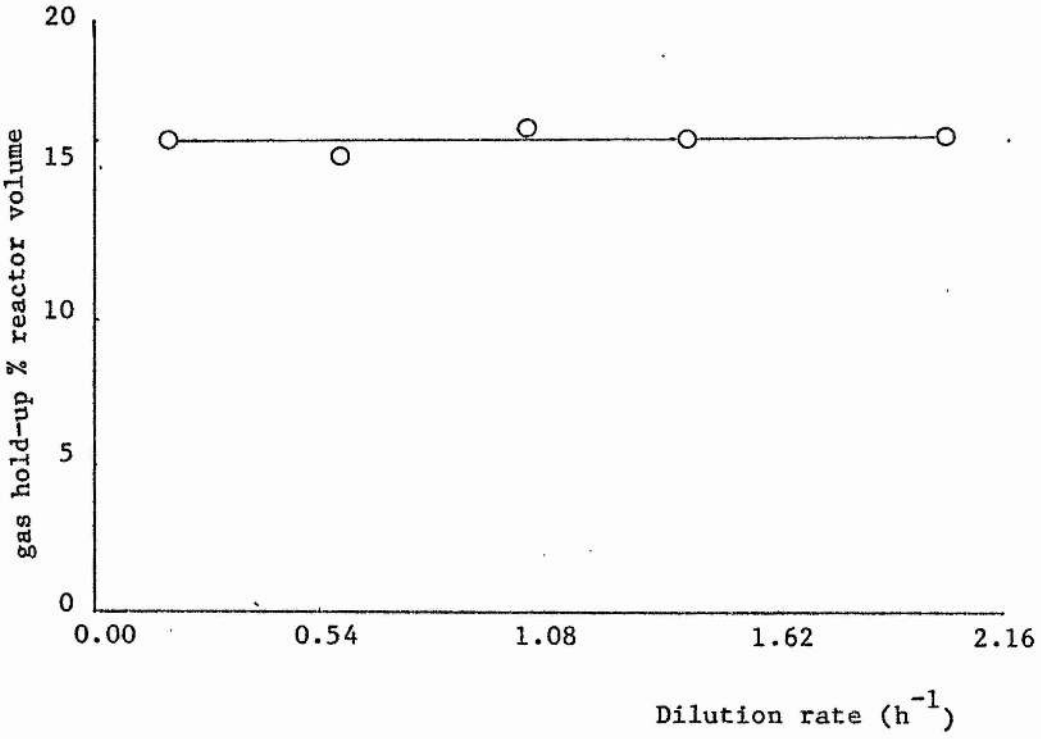

Fig. 3.3.3. Variation of the gas hold-up in the immobilised cell reactor at various dilution rates.

system	dilution rate (1/h)	feed glucose (g/l)	glucose conversion (%)	ethanol concent. (g/l)	ethanol productivity (g/l.h)
yeast* (without feedback)	0.10	150	57.5	34.9	3.49
	0.35	150	0.0	0.0	0.0(washout conditions)
yeast* (with feedback)	0.13	150	98.7	61.3	7.28
	0.44	150	94.1	56.7	22.3
Zymomonas** (immobilised)	0.18	100	98.0	49.5	8.91
	0.60	100	73.0	37.5	22.5

(Bonomi et al. * ; this investigation**)

Table 3.3.3.b. Comparison of ethanol productivity results between immobilised Zymomonas mobilis cells and free yeast cells using cassava hydrolysate as feedstock.

3.3.4 Operational stability of the immobilised-cell reactor

Operational stability is one of the most important parameters to evaluate in measuring the efficiency of a immobilised-cell system.

In order to evaluate the operational stability of the immobilised-*Zymomonas mobilis* cell reactor, the system was run at a fixed dilution rate ($D = 0.2h^{-1}$) for 20 days. The effluent was assayed for ethanol and glucose and efficiency of conversion was calculated.

The values obtained are presented in figure 3.3.4.

It was found that during the first nine days of operation, ethanol was produced from glucose at almost 100% of the theoretical yield and the concentration of glucose and ethanol remained almost constant. Beyond the ninth day, there was a slow decrease in fermentative capacity of the reactor with falling ethanol and rising glucose concentrations in the reactor output.

After 20 days, the capacity of the reactor had fallen by some 20%. On the assumption that capacity drop-off would be an exponential phenomenon, a reactor half-life of 63 days was estimated.

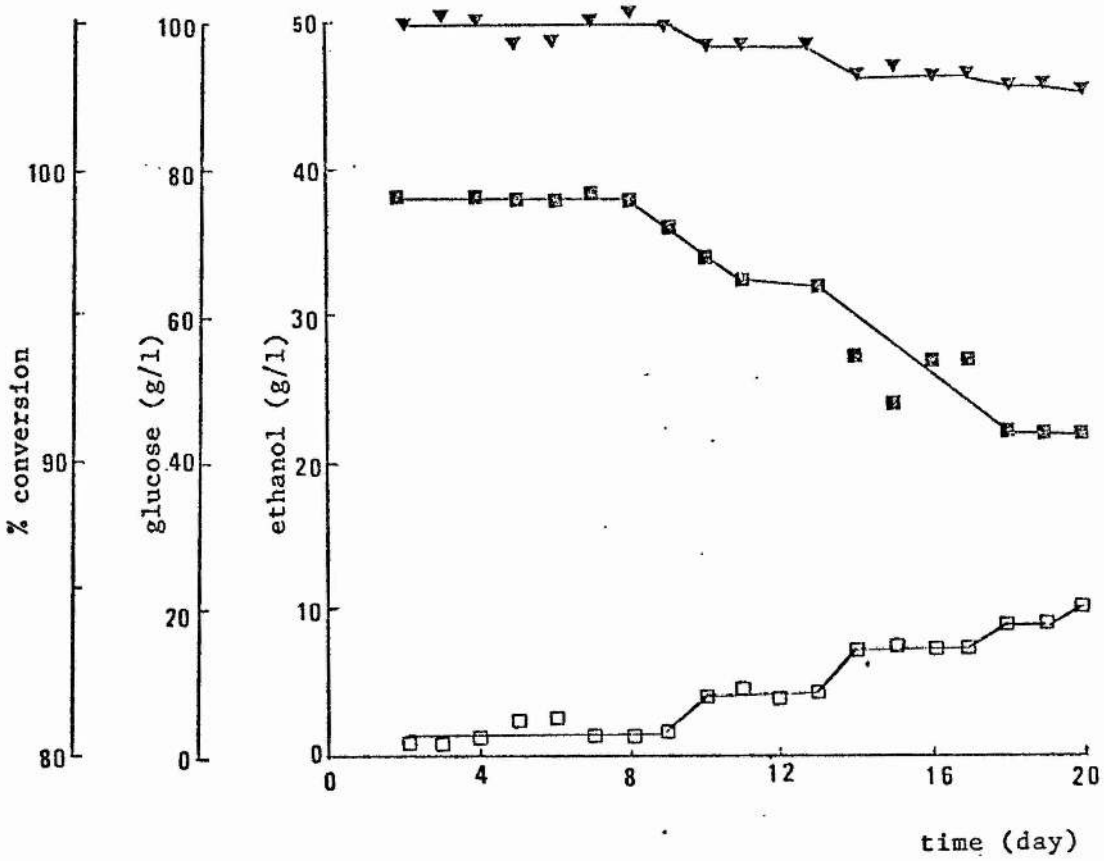

Fig. 3.3.4. Operational stability of the immobilised cell reactor.

(▼) ethanol concentration in effluent liquid.

(□) glucose concentration in effluent liquid.

(■) % percentage of conversion of glucose to ethanol.

3.4 Development of a mathematical model

To evaluate the potential of a fermentation process, such as ethanol production in an immobilised-cell reactor, it is desirable to have an adequate mathematical model to predict substrate consumption and productivities hence facilitating the projection from laboratory to industrial practice.

A mathematical analysis of the reaction kinetics and mass transfer aspects of the immobilised Zymomonas mobilis cells reactor converting cassava hydrolysate to ethanol is presented in this section.

3.4.1 Flow pattern characterization

In an ideal plug-flow tubular reactor all the fluid elements flow at constant velocity and without dispersion; however, in real plug-flow fermenters inevitably some degree of dispersion occurs.

If a small volume of a tracer is injected with the stream entering the plug-flow fermenter with a small extent of dispersion, a time record of the tracer leaving the fermenter will be a symmetrical curve, called a C curve, representing a normal distribution of residence times about the mean, t_r. According to Levenspiel (1972) the dimensionless number D/UL, where D is the axial diffusion coefficient (cm^2/h), U is the fluid velocity (cm/h) and L is the reactor length (cm), is one parameter of the C curve and can be evaluated by calculating its variance, σ^2:

$$2(D/UL) = \sigma^2/t_r^2$$

The term D/UL is called fermenter dispersion number and its value measures the extent of axial dispersion. As the fermenter approaches ideal plug-flow characteristics the dispersion number approaches zero (Levenspiel,1972).

The flow characteristics of the immobilised-cell reactor used in this study was evaluated by determining the value of fermenter dispersion number from the following tracer experiments.

1 ml of a highly concentrated solution of congo-red, a harmless dye to Zymomonas metabolic activity (Swings and De Ley,1977), was injected at the column inlet. The column effluent was sampled every 2 min and the absorbance measured at 380 nm. The test was repeated with

samples taken at half column height. The reactor was operated at a media flow rate of 27 ml/h and input glucose concentration of 100 g/l.

The resulting axial concentration profiles of the tracer are shown in figure 3.4.1.

The calculated fermenter dispersion number was:

$$D/UL = 0.019$$

The relation between the mean residence time in the total reactor length and the residence time in the lower half of the reactor (2.00) was not significantly different from the relation between the heights of the two sampling ports (1.96), indicating that the fluid velocity was constant through the reactor.

Therefore, the assumption of plug-flow characteristics for the immobilised-cell reactor used in this study is admissible and will be applied in the next section for the development of a mathematical model.

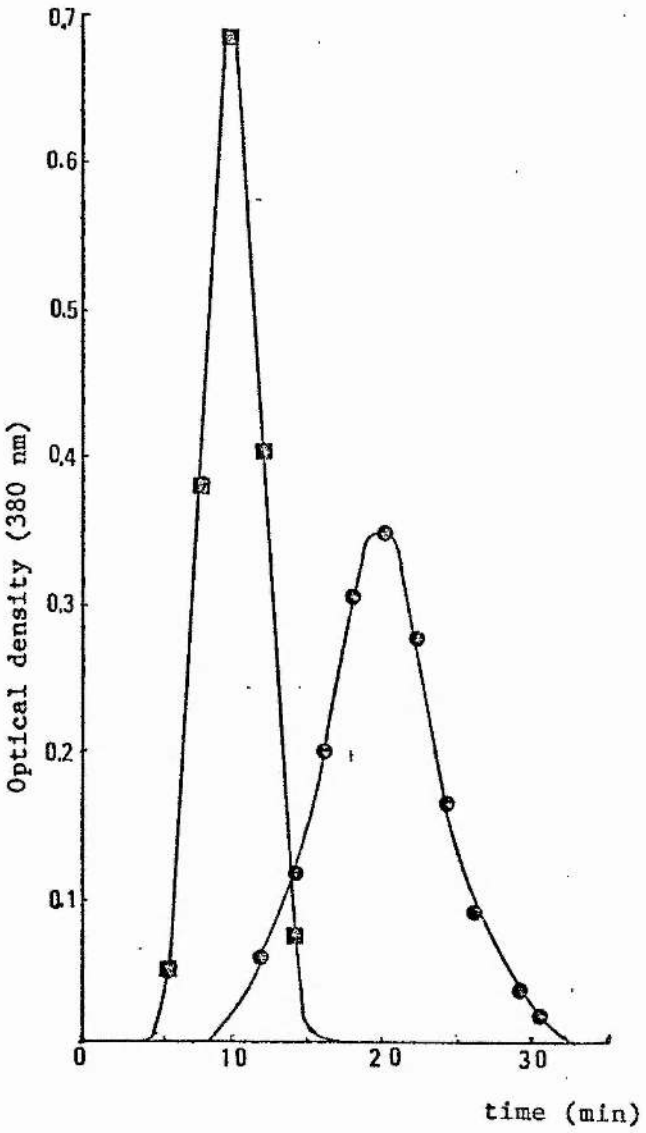

Fig. 3.4.1. C curve for the immobilised cell reactor.

3.4.2 Substrate mass balance

Consider a small element of the immobilised-cell reactor , dz, between the cross sections of z1 and z2 (figure 3.4.2) and take the material balance for the substrate:

INPUT = OUTPUT + ACCUMULATION + REMOVAL BY REACTION (1)

where:

INPUT = input bulk flow + input by axial dispersion

OUTPUT = output bulk flow + output by axial dispersion

input bulk flow = $G_{z1}.U.A$

output bulk flow = $G_{z2}.U.A$

input by axial dispersion = $D.A.(dG/dz)_{z1}$

output by axial dispersion = $D.A(dG/dz)_{z2}$

overall reaction rate = $-R.A.dz$

and, where G is the substrate concentration (g/l), U is the velocity of the liquid inside the column (cm/h), A is the cross section area (cm^2), D is the axial dispersion coefficient (cm^2/h) and R is the reaction rate (g/l.h).

Since ,

(i) at steady-state the accumulation term is zero;

(ii) for a continuous plug-flow reactor the dispersion term is absent;

the equation 1 reduces to:

$$G_{z1}.U.A = G_{z2}.U.A - R.A.dz \qquad (2)$$

Rearranging the equation 2 and taking the limit as dz -> 0:

$$U\ (dG/dz) = -R \qquad (3)$$

i.e., the bulk flow of substrate equates to the rate of consumption by the reaction.

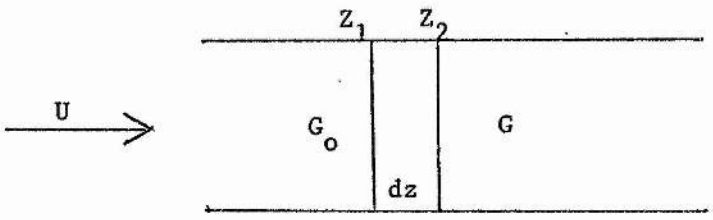

Fig. 3.4.2. An element, dz, of the immobilised cell column.

3.4.3 Reaction kinetics

Continuous alcoholic fermentation have been studied using starch-hydrolysate as feedstock material and first order kinetics was found to be adequate for description of sugar conversion rate (Yarovenko and Nakhmanovich, 1972). Also, Sitton (1979) has round that rirst-order reaction mechanism described the glucose to ethanol conversion in a immobilised-cell reactor using cellulosic hydrolysate as starting material.

Therefore, first-order reaction kinetics would be expected to occur in the present immobilised-cell system.

If this occurs, the rate of substrate conversion, R, in the reactor would be defined by:

$$R = k.G \qquad (4)$$

where k is a first-order rate coefficient with the dimension of $(time)^{-1}$.

Combination of equations 3 and 4, followed by integration leads to:

$$G/G_o = \exp(-k.z/U) \qquad (5)$$

It is obvious from equation 5 that for a given residence time in the reactor, the plot of log (G/G_o) vs. reactor length gives a straight line. If the height variable is scaled in relation to the total reactor length (z/Z), then the slope of the line is equal to kZ/2.303U, where Z is the total reactor length.

Figures 3.4.3.a to 3.4.3.c show the test of fitness of the proposed rate equation for three different experimental conditions (A, B and C) outlined in Table 3.4.3.a.

The results showed no significant difference between the slopes of the relation [log (G/G_o) vs (z/Z)] when the input substrate changed from 100 to 71 g/l; but when the media flow rate was increased the slope of the relation decreased which might be related to the change in the liquid velocity U.

The value of the first-order reaction rate can be calculated from the slope k.Z/2.303U. Table 3.4.3.b presents the reaction rate constants for the experimental conditions A, B and C described in Table 3.4.3.a.

3.4.4 Prediction of substrate consumption

The equation 5 provides an estimate of the performance of a fermenter of length Z with a liquid velocity U. If in equation 5 , G is interpreted as the concentration at any position z, the equation can be used to predict the variation of substrate concentration with position within the fermenter.

Data, summarised in table 3.4.3.a., also illustrate the extent of agreement between experimental and predicted substrate concentration at several positions in the immobilised-cell reactor used in this study.

3.4.5 Prediction of ethanol productivity

It has already been established that there is a linear relationship between the amounts of glucose fermented and ethanol produced by the immobilised cells of Zymomonas mobilis which follow the equation:

$$E = 0.49\ (G_o - G) \qquad (6)$$

where E is the ethanol concentration in g/l.

Combination of the equations 5 and 6 leads to the equation:

$$E = 0.49G_o[1-\exp(-kZ/U)] \qquad (7)$$

which expresses the relationship governing ethanol production in the immobilised-cell reactor on the basis of a first-order rate of substrate conversion with rate constant k. If information is available for k, equation 7 can be used to give the productivity for a given length and flow rate.

Table 3.4.5 shows good agreement between the model and the experimental results.

3.4.6 Rate-controlling mechanism

In an immobilised-cell reactor the substrate has to move from the liquid phase to within the beads where the reaction takes place and hence the rate of mass transfer could be an important factor affecting the overall rate of reaction.

One can determine whether film resistance to substrate mass transport is or is not affecting the rate of reaction by comparing the observed reaction rate with the mass transfer rate to within the particle. If the observed reaction rate is higher than the mass

transfer rate or if the two terms are of the same order of magnitude the film resistance affects the reaction rate. On the other hand, if the reaction rate is much smaller than the rate of movement of reactant into beads, we can ignore the resistance to mass transport (Levenspiel, 1972).

The diffusion coefficient of glucose in calcium alginate beads, D, was determined from the mass transfer experiments described in section 3.1.2 by using the following equation (Blakebrough, 1967):

$$C/C_o = 1 - (6/\pi^2)\exp(-\pi^2 tD/r^2)$$

where C/C_o is the partition coefficient of glucose at equilibrium, r is the sphere radius and t is the time to reach the equilibrium.

A value of $D = 7.0 \times 10^{-6}$ cm^2/s was found. Using this value for mass transfer coefficient of glucose in calcium alginate beads and the model constant for experiment A test conditions ($k = 0.67$ h^{-1}) it was found that the rate of reaction per bead volume is 1.8 times the rate of substrate transfer into the bead, therefore film resistance to substrate diffusion is influencing the rate of reaction.

The transport effect on the overall reaction kinetics offer an explanation to the observed lower specific substrate consumption and product formation rates in the immobilised- cell reactor when compared with those values for free cells continuous culture.

	EXPERIMENTS					
	A		B		C	
	initial gluc.100g/l flow rate 8.3 ml/h		initial gluc.71g/l flow rate 8.3 ml/h		initial gluc.100g/l flow rate 15.7 ml/h	
Z	G	G*	G	G*	G	G*
0	100.0	-	71.0	-	100.0	-
2	71.0	78.2	44.0	55.7	92.0	86.5
4	64.0	61.6	42.6	43.4	70.0	75.2
6	42.0	48.4	39.0	34.4	68.0	65.4
8	41.0	38.1	26.3	27.1	58.0	56.8
10	29.0	29.9	17.7	21.3	48.0	49.4

Table 3.4.3.a. Profile of substrate concentration in the immobilised cell column.

Z = height of the column in cm.

G = substrate concentration experimentally determined

G*= substrate concentration calculated

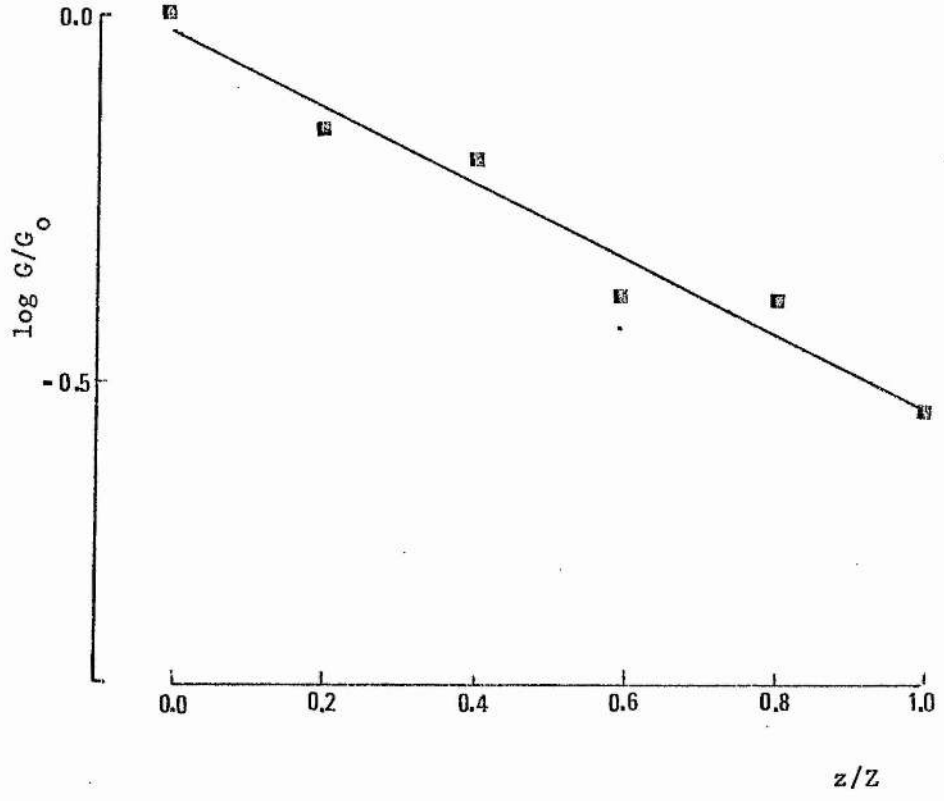

Fig. 3.4.3.a. Model fitting test for experiment A.

Coef. of correlation=0.9834

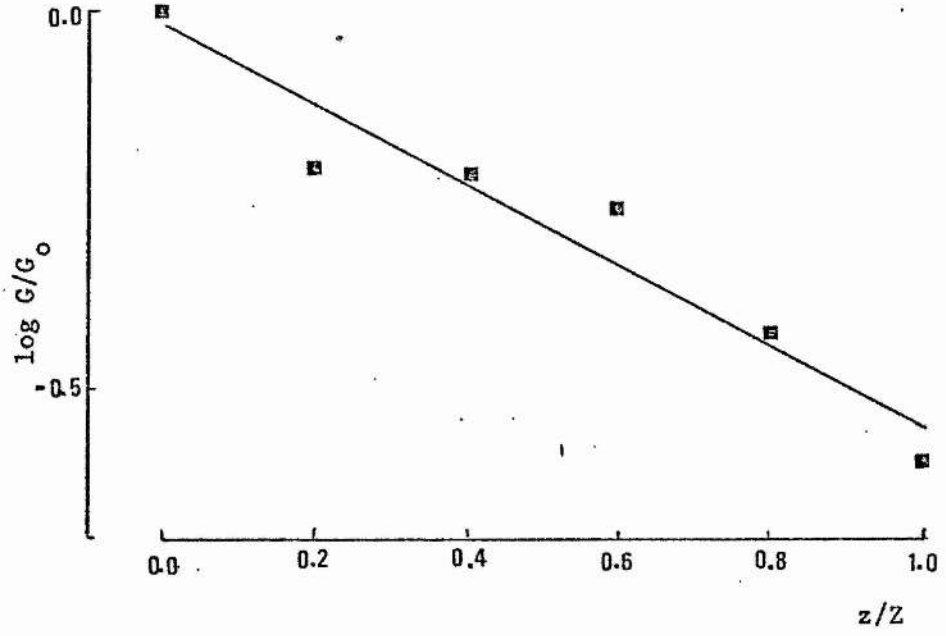

Fig. 3.4.3.b. Model fitting test for experiment B.

Coef. of correlation = 0.9606

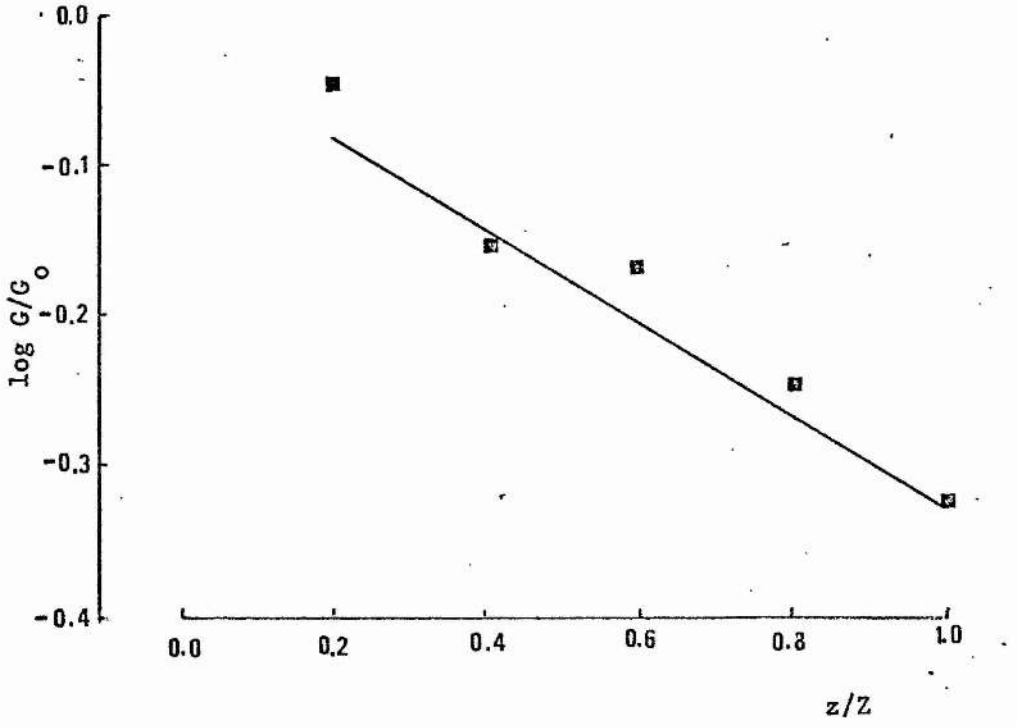

Fig. 3.4.3.c. Model fitting test for experiment C.

Coef. of correlation=0.9853

EXPERIMENT	k (h^{-1})
A	0.67
B	0.68
C	0.77

Table 3.4.3.b. Calculated values for kinetic rate coefficients for the experimental conditions A, B and C described in Table 3.4.3.a.

Liquid flow rate (ml/h)	Productivity (g EtOH/l.h)	
	Experimental	Calculated*
2.7	8.91	8.60
9.0	22.50	19.71
15.4	25.50	24.12
21.0	26.30	26.15
27.8	27.20	27.40

* Initial glucose concentration = 100 g/l.

Table 3.4.5. Test of fitness of the prediction model.

4 CONCLUSION

The results reported here show that an immobilised-cell reactor of Zymomonas mobilis is a good alternative process for the conversion of cassava hydrolysate to ethanol.

The immobilised-cell reactor presents productivities higher than those obtained with a conventional continuous fermentation system and with good operational stability.

The agreement between the experimental data and the mathematical model shows that the proposed equation looks satisfactory and encouraging further studies for determination of the characteristics of the immobilised-cell reactor (such as bead size, reactor height/diameter ratio, flow rate, etc.) so that its highest activity is revealed with minimal resistance to substrate diffusion.

Finally, an economic evaluation of the feasibility of the immobilised-Zymomonas mobilis cell reactor will also be necessary, as a complementary study, before the system become of practical importance.

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