THE EFFECT OF IMMOBILIZED MATERIALS ON SACCHAROMYCES CAREVISIAE

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A PROJECT SUBMITTED TO

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DEDICATION

With the personal conviction that the destiny of a child is in the hands of his parents, through the infinite mercies of the Almighty God. I humbly dedicate this project work to my Mother

MRS. ELIZABETH JOE OSUNWOKE

AND

THE ALMIGHTY GOD,

AMEN.

CERTIFICATION

I certify that this project was carried out by me OSUNWOKE MICHAEL JOE and was approved by the under signed for the Department of Chemical Engineering school of Engineering and Engineering Technology, Federal University of Technology, Minna, Niger State.

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COSMOS JOE, MISS. PEACE JOE and COMRADE JOSHUA JOE, they have been my greatest source of encouragement and joy.

Finally, my regards to all the members of Kings Club, F.U.T Minna, and my friends NDUBUISI, ***TOLU***, JONATHAN, ADEJO, ITUNU, KING, JACOB, IDRIS, JULIA, THOMAS, BIODUN, YINKA, MONSUN and to all my entire classmates and to all FCS members.

God in His infinite mercies will bless them all.

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CONCLUTION

1.1 AIMS AND OBJECTIVE

The objective of this work is to investigate the effect of immobilizing materials on saccharomyces cerevisiae. The project is therefore aimed at the following:

a.To find the efficiency of rice husk, palm wood and bamboo chips when used as immobilizers.

b.To determine the rate of production of ethanol when glucose is passed through the immobilizers.

1.2 SCOPE OF RESEARCH WORK

This project work focuses on the effects of immobilized materials on saccharomyces cerevisiae. The packing materials employed in this project research work are rice husk, palm wood and bamboo chips. Much emphasis is placed on analyzing the product from successive runs with respect to each parking material used and to acknowledge which of the parking materials gives better performance at work by determining the concentration and quality of ethanol yield.

The parking materials serve as supports. The substrate glucose composition is run through the parking materials to come in contact with the yeast cells. The extent to which the yeast cells are immobilized on each parking material

determines the rate of ethanol yield produced by running the glucose composition continuously through each of the parking materials in the column.

The yield of ethanol by passing the glucose through each of the immobilizers provides a basis for quantifying the success of performances at work.

At present, fermenting microorganisms are the dominant forms of industrial biological catalyst. But by comparison, immobilized enzymes or cells are preferable and more convenient to control and handle. As a country working to improve her economical status, it is of paramount importance for biochemical industries to adapt the use of immobilized enzymes or cells for the following reasons.

a. Improved quality control

b. Stability of enzyme in reactor

c. Re-use and containment of enzymes in a reactor.

d. Continuous operation and down stream process compatibility.

e. Reactor intensification (smaller reactors and lower residence time).

Much advancement is needed of the engineers of how to adapt the use of local materials in chemical processes to economically enhance production such as calcium alginate can be successfully placed with much more effective and economical materials such as palm wood, rice husk, bamboo wood etc. This saves the companies the stress of having to buy packing materials overseas, which is much more extensive.

2.1 SOURCE OF SACCHAROMYCES CEREVISAE

Saccharomyces cerevisiae was cultured using Baker's yeast. Yeast is simple called fungus, which is mainly saprophytic and usually grows on plant roots. Yeast cells may be spherical oval, or rod the shared under the microscope,

distinct nucleus visible. Most great reproduce as usually by simple process known as budding. In one part of the cell, the cytoplasm bulges out of the cell wall. The bulge bud grows in size and finally separates as new great cell (Campbell 1985).

The economic importance of great lies in its ability to great down carbohydrate food into alcohol and carbon dioxide. This process takes place under anaerobic condition. Yeast produces a number of by-products during alcohol fermentation. The most important of these are glycerol, higher aliphatic and aromatic alcohol, aldehydes, ketones, organic acids, and esters of alcohol organic acids (Campbell 1985). These by-products give alcoholic beverages pronounced flavor and nearby the entire flavor compounds, although in different concentrations.

Saccharomyces cerevisiae is the organism commonly referred to when the term yeast, if not further, specified despite that a large number of species belong to this group. It is also called Eugedura yeast, or Baker's yeast. This specie has been used for thousand of years by mankind in the preparation of wine, beer and bread and can thus be regarded as a generally regarded as regarded safe organism (GRAS) (Molly 1992) saccharomyces cerevisiae has long been used in the production of various products. It was also the first yeast to be used for the production of heterogeneous proteins such as human interferon. Furthermore, Harinsenula polymorph, pichia pastoris, yarrowia lytolytica and kuyveromyces lattes have been used successfully for

a. Ability to grow on a particular subscript

b. Ability to produce a satisfactory growth yield

c. Ability to produce some primary and secondary products reproductively.

d. Ability to carry out a specific chemical transformation.

e. Genetic stability.

There are instances when the stable mixture of organisms can provide effective process than by a single organism, as specialized methods are required for their production.

2.3 YEAST AND USES

The broad use of yeast was brought about by it's unique ability to ferment sugars, yielding ethanol and carbon dioxide (CO_2) as the end product for use in beverage and the preparation of bread. No yeast other than saccharomyces cerevisiae and only a few other microorganisms have a metabolism so specialized for alcoholic fermentation of mono and some disaccharide. It should be stressed again that saccharomyces cerevisiae is in extension among yeast in that it is extremely well adopted for fermentation. Thus even in the presence of oxygen, about 80% of glucose consumed is fermented and less than 5% contribute to respiration (Molly 1992). As it stands, 80% of the glucose consumed by saccharomyces cerevisiae are channeled through glycolysis and alcoholic fermentation. A side from the

The stability of the enzyme or cell is often increased by immobilization and the concentration of the enzyme is increased such that a much small reactor can be used to achieve results and in a much more continent fashion. Immobilization enables the enzyme or cell to use evenly distributed throughout the reactor so ensuring an even supply of substrate to each enzyme or cell.

Enzymes and cells are buffered by the support against dangers in pH, Temperature or ionic strength in the bulk solvent. Once the reaction has been completely followed by re-use immobilization also can be used as method of recovering an enzyme from solution.

Immobilized enzyme or cell processes are easier to handle, to automate and enable the advantages of various reactor configurations to be exploited. These include pH and temperature control, good gas transfer in stirred reactors and the minimization of product incubation in backed-bed reactors. As enzyme is used more frequently, fewer cells have to be grown and so less fermentation wastes have to be disposed off. When immobilized cells are used, growth rate of growing cells in a fermentation culture would be exceeded so that washout of the cells and undue increase in the viscosity of substrate can be avoided.

2.4.1 IMMOBILIZED BIOREACTOR

The remarkable catalytic properties of enzymes make them very attractive for use in processes where mild chemical conditions and high specificity are required. Cheese manufacture has traditionally used rennet, an enzyme preparation from calf stomach, as a specific protease that leads to the precipitation of protein from milk. Mashing in the matting of grain for brewing of beer makes use of B-amylase stage. In both of these examples the enzymes are not recovered from the reaction mixture and a fresh preparation is used for each batch. Similarly, in more modern enzyme reaction applications, such as in biological washing detergents, the enzyme is discarded after single use but these are, however, situations where it may be desirable to recover the enzyme. This may be because the product is required in a pure state or that the cost of the enzyme preparation is such that single use would be uneconomic. To this end, immobilized biocatalysts have been developed where the original soluble enzyme has been modified to produce an insoluble material that can be easily recovered from the reaction mixture (Coulson 1994).

Many industrial important microorganisms tend to agglomerate during their growth and form focus suspended in the culture medium or films adhere to the internal surface of the fermenter. This tendency may or may not be advantageous to the process and is dependent on a variety of parameters such as the pH and ionic strength of the medium and the shear rate

experienced in the growth vessel (Coulson 1994). In some cases, the formation of substantial flocks is essential to the proper operation of the process. In the case of the activated sludge wastewater treatment is relent on the formation of a film of organism on the surfaces is it's parking material. The operation is not that of a filter, in which material would be removed on the surfaces is it's parking material. The operation is not that of a filter, in which material would be removed on the basis of it's particle size, but that of biological reactor in which the waste material forms the substrate for the growth of the microbes in the zoolgleal film. The presence of the film provides a means of relating a higher microbial concentration in the reactor than would be retained in a comparable stirred-tank fermenter. The formation of flocks and films for the retention of high microbial densities or to facilitate separation of microbes from the growth medium may be desirable in other instances as well. However, in some cases the microbe used may neither be amenable to the natural formation of large flocks nor adhere as surface films, and recourse may be made to the artificial immobilization of microbes.

2.4.2 CHOICE OF IMMOBILIZATION METHOD

Immobilization is aimed at restricting completely the movement of enzymes, cells or organelles in space. So many factors are considered when deciding on the method of immobilization to be used. Immobilization methods and materials differ for different processes. The immobilization method

employed for a process may not be suitable for the second process. This is because the different properties of substrates and product and different use to which the product can be applied this is the reason why although many methods of immobilization techniques have been developed, non-can be regarded as universal. This is what led to the necessity to find produce, which is simple and inexpensive to perform, and which gives a product with good potent ion of activity and high operational stability is laid upon us.

Many factors must be considered when choosing an immobilization method. This includes the chemical nature and kinetic features of the reaction, the cost and the chemical and physical stability of the biocatalyst. The yield and purity of product required, bulk of surface density of carrier binding cites, binding sites accessibly for bio-catalyst, the hydrophilic and hydrophobic balances and the charge effects and distribution are specific effects and factors of concern in the choice of immobilization. Thus a compromise must be reached since the important variable namely act independently of each other and several minor often unrelated effects can combine to produce a very undesirable effect such as loss of stability or excessive formation of site product (Ichiro 1977).

Method employed in immobilization should be simple and reproductive. It has to be a method that is dependent on a variety of scientific, engineering and economic aspects of the process. It should be versatile, safe, mild, cheap and easy to use on a large scale. The actual method to be employed

should allow easy control of the amount of enzyme or cells immobilized and the cells or enzyme should not leak from the support during operation.

None of the methods of immobilization dace loped can be regarded as universal because of the variation in properties of substrates and product and the different use to which the products are applied. Therefore, it is necessary to find a procedure, which is simple and inexpensive to perform, which gives a product with good retention and activity and operational stability.

2.4.3 IMMOBILIZATION TECHNIQUES

There are various methods, which have been developed for enzyme and microorganism immobilization and some of these have found commercial application. The two largest scale industrial processes utilizing immobilized enzymes are the hydrolysis of benzene penicillin by penicillin amylase and the isomerisation of glucose to glucose – fructose mixture by immobilization The immobilization techniques used in general may be categorized as:

(a) Physical adsorption on to an inert carrier.

(b) Induction in the lattices of a polymer gel or in microcapsules

(c) Covalent bonding.

2.4.3.1 PHYSICAL ADSORPTION ON TO AN INERT CARRIER.

The first of these methods has the advantage of requiring only mild chemical conditions so that enzyme deactivation flocs and films may be used considered to be in the category, although the subsequent adhesion of the microbes to the surface may not be a simple phenomenon. Special materials may be used as supports that provide the microbes with environments, which are particularly amenable to their adhesive; such materials include foam plastics, which provide conditions of low shear in their pores. The process may also be relatively cheap but it does tend to have the draw back that adsorption of the enzyme may also occur readily or that of the grown medium. This process is dependent on the nature of the specific enzyme or microbe used and it's interaction with the carrier and, whilst it is common in the case of immobilized enzymes.

2.4.3.2 INDUCSION IN THE LATTICES OF A POLYMER GEL OR IN MICRO-CAPSULES

This method attempts to overcome the problem of leakage by endorsing the relatively large enzyme modules or microbes in a tangle of polymer gel, or to enclose them in a membrane, which is porous to the substrate. It is theoretically possible to immobilize any enzyme or microorganism using those methods but they too have their problems. Some leakage of the method species may still occur, although this tends to be minimal, particularly in the case of microencapsulated enzymes or respiring but non-

growing cells. The main problem is due to mass transfer imitations to the introduction of the necessarily small substrate molecules into the immobilized structure, and to the slow outward diffusion of the product of the reaction. If the substrate is it micro-molecules, such as a protein or a polysaccharide, then it will be effectively screened from the enzyme or microbes and little or no reaction will take place.

2.4.3.3 COVALENT BINDING

Biological catalysts may be made insoluble and hence immobilized by effectively increasing their size. This can be done either by chemically attaching them to otherwise inert carrier materials or by cross-linking the individuals to form large agglomerations of enzyme molecules or microorganisms. The chemical reagents used for the linking process are usually bifunctional, such as the carbon-di-imide, and many have been developed from those used in the chemical synthesis of peptides and proteins. The use of a carrier is the most economical in terms effect enzymes usage since the local enzyme activity in the cross linked enzyme will be less of a limitation than the rate of transfer of substrate to the active centers. This has the result that in many cases only about 10 percent of the original enzyme activity can be realized (Coulson 1994). The inert carriers used tend to be hydrophilic material, such as cellulose and it's derivatives, but in some cases the debris of the original cells has been used, the cells having been broken and then cross linked with the enzyme and each other to form

large particles. The latter technique has the advantage of missing out some purification steps (with their loss of total activity) that are normally associated with enzymes recovery and also avoids the need to satisfy the maintenance requirement of the living cells.

2.4.4 LOSS OF ACTIVITY

In general, when enzymes (or microbes) are immobilized for use in engineering systems a significant decrease in overall activity is disserved. The decrease may be ascribed to three factors

a. Loss due to de activation of the catalytic activity by the immobilizing procedure itself

   This includes destruction of the active sites of the enzyme by the reagents used and the obstruction of the active sites by the support material.

b. Loss of overall activity by diffusion limitation external to the immobilized system. This refers to the apparent loss in activity when the rate of reaction is controlled by transport of the substrate from the bulk of the solution to the surface of a carrier or the microbes from a very thin film, with negligible activity within the support.

c. Loss due to diffusion limitation within the immobilized catalyst matrix. This can clearly arise when gel entrapment is being considered but it can also occur when enzymes or microbes are covalently attached within pores in the inert carrier.

The consumption of biotransformation of substrate by immobilized microorganism's results in most cases in the growth of the microorganisms. The growth, which gives rise to a significant increase of thickness in an established bio-film, occurs at a rate that is essentially slow in comparison with rates of the diffusion processes. Simultaneously, the attrition of biofilms or flocks arising from the effects of fluid flow tends to maintain their thickness or size, and overall, the immobilized system can be considered to be in a quasi-steady state when short time intervals are involved. The mathematical similarity of enzyme and microbial kinetics then means that a common set of equations can be used to describe the behavior of both immobilized enzymes and microbial cells.

2.4.5 KINETICS OF IMMOBILIZED ENZYMES REACTIONS

Reactions involving the use of immobilized enzymes are basically affected by the new state of the enzymes. Hence, the reaction rates are not the same as for the free enzymes. As if is generally observed with chemical reaction in catalysis, the problem of mass transfer limitation sets in. The substrate would necessarily have to diffuse to the enzymes at its immobilized location before reactions can occur. Therefore, the observed transfer of substrate and products as well as the intrinsic of the reactions occurring at the enzyme site on the surface of the support matrix

2.4.6 EFFECT OF MASS TRANSFER ON KINETICS OF IMMOBILIZED ENZYME REACTIONS

The effect of the mass transfer processes is that the concentration at the enzyme location on the surface of the support would be much lower than the bulk concentration of the substrate, as corrosive and diffusive mass transfer process would necessarily move substrate from high bulk (Guenelle 1996). Concentrated to low concentration area at the surface.

The influence of mass transfer on the kinetics of the immobilized enzyme systems has been studied in analogy to the well-researched problems of mass transport in chemical catalysis. This means that once the substrate reaches the enzyme, the reaction is not affected by the enzyme immobilization. The effect of mass transfer, if significant can be examined through the use of a dimensionless parameter, the Dankohler number, Da defined as (Guneller 1996).

$$Da = \frac{V\max}{KsSo} = \frac{Maximum\,Reactionrate}{MaximumMassTransferrate}$$

Where,

Ks = mass transfer coefficient

So = bulk substrate concentration.

1.0 INTRODUCTION

The use of Immobilized cells enzymes and organelles in industries are increasingly common in recent times. Around the world, advanced effort has been made on the use of immobilized cells in industrial processes. In Companies like Khawa Hakko in Japan Columns of Saccharomyces cerevisiae entrapped in calcium alginate pellet are used (Campbell 1985).

To a lesser extent, soluble enzyme is of long standing interest, but there is a greater appreciation of the advantages of using immobilized enzymes and cells. In advanced industries, there have been records of Ethanol production from non-eternalized diluted cane molasses (Campbell 1985).

At present, fermenting microorganisms are catalysts. But by comparison, immobilized enzymes or cells as preferable and more convenient to control and handle, it permit easy separation of product from the catalyst and it is found to be less susceptible to microbial contamination. Industrialists have confirmed that the use of immobilized cells enables the condition for cell growth and a full optimization of feed for product formation.

Fermenting microorganisms have a great advantage of versatility as illustrated by the wide-range and sophistication of the processes in use

Hence, if Da <<1, the maximum mass transfer resistance and the effect of mass transfer is negligible on the reaction and the region where this happens is referred to as the reaction limited regime. On the other hand, if Da>>1, maximum mass transfer rate is for loss then the reaction rate that is, limiting the process. This is referred to as the diffusion limited

heterogeneous protein production. Yeast belongs to the class of fruit. They lack photosynthetic abilities and were first identified in sugar containing natural substrates such as barriers. The fact that different yeast species are found in different habitants can be used for their isolation. For these isolations, one can also take advantage of the fact that yeast can grow in a broad range of pH values preferable acidic media, (PH3.5 to 5.0). Thus yeast extract (0.3), malt extract (0.3%) peptone (0.5%) and glucose (1%) are used at pH 3.7 to 3.8 in the presence of antibiotics like chlorophenicol to avoid bacterial contamination (Molly 1992).

2.2 ISOLATION AND ESCREENING OF MICRO ORGANISIMS

The sources of microorganisms in large collections are held by national organizations. Also many industrial companies may take their own collections that are maintained through a continuous program of enrichment and isolation (Campbell 1985). Private companies usually secure some, which have been constructed genetically to carry out specific biosynthetic degradation, or patents may protect biocatalyst. Culture maintained for long period loose some of the characteristics, which distinguished them for isolation for this reason; it is often beneficial to return to natural habitants for fresh isolations when contemplating for a new process.

In most instances, culture of single organism (pure culture) is required and these are for various properties, started below (Campbell 1985)

$$\text{Glucose} + HSO_3^- \rightarrow \text{Glucose} + \text{Acetaldehyde} - HSO_3 + CO_2$$

Under alkaline condition still another type of fermentation patter is observed.

$$2\ \text{Glucose} \longrightarrow 2\ \text{glycerol} + \text{acetate} + \text{ethanol} + 2CO_2$$

2.6 ETHANOL

The action of immobilizers such as palm wood, bamboo chips and rice husk on saccharomyces Cerevisiae gives ethanol as a major product.

2.6.1 ECONOMIC IMPORTANCE OF ETHANOL

a. SOLVENT

Ethanol rank second only to water as an industrial solvent, solvent application include resins, pharmaceutical cosmetics, house hold cleaning products and industrial about 50 – 55% of this industrial (non-fuel) alcohol uses in for solvent application.

b. BEVERAGES

Ethanol is a constituent of alcohol beverages such as wine and beer which when taken in controlled quantity is claimed to give pleasure to the drinkers.

c.	Density	-	789 kg/m^3
d.	Boiling point	-	78.4^0c
e.	Melting point	-	112^0c
f.	Solubility in 100 part of water	-	Infinity

2.6.2.2 CHEMICAL PROPERTIES OF ETHANOL

a. Ethanol reacts with sodium to form salt (sodium alkoxide) and hydrogen.

$$2C_2H_5OH+2Na \longrightarrow 2C_2H_5ONa+H_2$$

b. Ethanol reacts with acid in excess and the mixtures is heated to about 140^0c,to give an ether.

$$C_2H_5OH + H_2SO_4 \longrightarrow C_2H_5\text{-}O\text{-}C_2H_5 + H_2O$$

This reaction occurs by displacement of the hydrogen sulphate group by the excess of the alcohol as in the SN_2 substitution of an alkyl halide.

c. Ethanol react with organic acid to form esters, the reaction is slow unless an acid catalyst is used e.g. H_2SO_4. H_2SO_4 is a suitable catalyst. The reaction is known as Etherification reaction.

$$CH_3\text{-}CH_2\text{-}OH+CH_3\text{-}C\text{-}OH \rightleftharpoons CH_3\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH + H_20$$

d. When Ethanol is headed with concentrated Tetraoxosulphate VI acid, it undergoes a dehydration reaction to produce Ethene.

$$CH_3\text{-}CH_2\text{-}OH + H_2SO_4 \longrightarrow CH_2 = CH_2 + H_2O$$

The reaction occurs via the alkyl hydrogen sulphate, for example.

$$C_2H_5\text{-}OH + H_2SO_4 \longrightarrow CH_3 - CH_2 - O - SO_2 - OH + H_2O$$

CHAPTER THREE

METHODOLOGY

3.0 MATERIALS

Palm wood blocks used as packing materials for this project work were obtained from Isu in Nwangele LGA of Imo State in the South Eastern part of Nigeria, from a species natively called Nkwu-Ojukwu. While the rice husk and bamboo chips were obtained from Bida LGA of Niger State in the Middle-Belt part of Nigeria.

3.1 PREPARATION OF CULTURE

1.3g of nutrient broth containing 0.2g yeast were added to 100ml of distilled water. The solution was autoclaved (sterilization) at 121^0c (1spsia) for 15 minutes and allowed to cool to temperature between 30^0c and 45^0c, 5g of saccharomyces cerevisiae was inoculated into the solution and the solution was stored in an incubator at 30^0c to enhance it's growth. Duration of storage in the incubator was 24hrs. The starter culture was then transferred to a refrigerator to inhibit its growth.

c. CHEMICAL INTEMDIATE

Ethanol is the starting material for many chemical products such as polystyrene for the manufacturing of plastics. Summarizes several of the important chemicals that can be derived from ethanol.

d. FUEL

Fuel used in internal combustion engines is the fastest growing application of fermentation ethanol. The original auto engine was developed during anhydrous ethanol fuel. 10% by volume ethanol added to gasoline result in a three point's increase in area octane rating. (Maidrolla 1981). Consequently ethanol can be used as fuels enhance, to increase the fuel performance.

2.6.2 PROPERTIES OF ETHANOL

Ethanol exhibits mainly to properties.

a. - Physical properties

b. - Chemical properties.

2.6.2.1 PHYSICAL PROPERTIES

a.	Molecular weight	-	46.07%
b.	Color	-	Colorless liquid

Composition of Nutrient Broth (Oxoid)

Lab-Len co	1.09/litre
Yeast Extract	2.09/Litre
Peptone	5.09/Litre
Sodium Chloride	5.09/Litre
pH	7 + 0.2

3.2 CALIBRATION OF SUBSTRATE GLUCOSCONCENTRATION

0.1g/Litre solution of glucose in distilled water was prepared by dissolving 0.1g of glucose in distilled water and made up to 100ml. The refractive index of the solution was measured with retract meter. This procedure was repeated for glucose concentration of 1.2, 4.0, 5.0, 7.0, 9.0, 11.0, 14.0, 17.0, 20.0, and 25.0g/Litre. The calibration curve for substrate concentration was then obtained by plotting refractive index against glucose concentration.

3.3 CALIBRATION OF YEAST CELL CONCENTRATION

10ml of the cultured yeast was diluted with 10ml of water. 10ml of the solution obtained was then mixed with another 10ml of water. This procedure was repeated until ten serially diluted samples were obtained. From each of the ten dilutions, 3ml were pipette into a corvette and their absorbent was measured at 540 um using a colorimeter.The calibration curve

of cell concentration was then obtained by plotting a graph of absorbance against cell concentration.

3.4 CALIBRATION OF ALCOHOL CONTENT

Mixing known weight of ethanol with glucose and making it up to 100g prepared standard solutions of ethanol in glucose solution. The specific gravity of these solutions was determined using specific gravity bottle. The calibration curve was obtained by plotting specific gravity against percentage ethanol in solution. Ethanol concentration was varied from 1.0 % to 10%.

3.5 FEED PREPARATION

The reactor feed is composed of the following per litter

a.	Glucose	-	100g
b.	Yeast Extract	-	3g
c.	Peptone	-	3.5g
d.	KH_2PO_4	-	2.0g
e.	$MgSO_4.7H_20$	-	1.0g
f.	$(NH_4)_2SO_4$	-	1.0g

3.6 IMMOBILIZED REACTOR SYSTEM

The reactor used for this research project is a cylindrical glass column of inner diameter 5.5cm. Wire gauze was used at the base of the cylindrical glass as a support for the parking materials. The palm wood blocks and the bamboo chips were cut into sizes (10cm x 10cm x 10cm) but the rice husk were left the way they are. The immobilizing materials were packed in the column at a height of 25cm. The column was maintained at room temperature of 36^0c. In the absence of pelstaltic pump, gravity method was used in introducing the cultured yeast into the column. The cultured yeast was introduced into the reactor system very slowly to enhance proper adsorption of yeast cells on packing materials. (Fig.1 is a schematic diagram of the immobilized reactor system used for this research project).

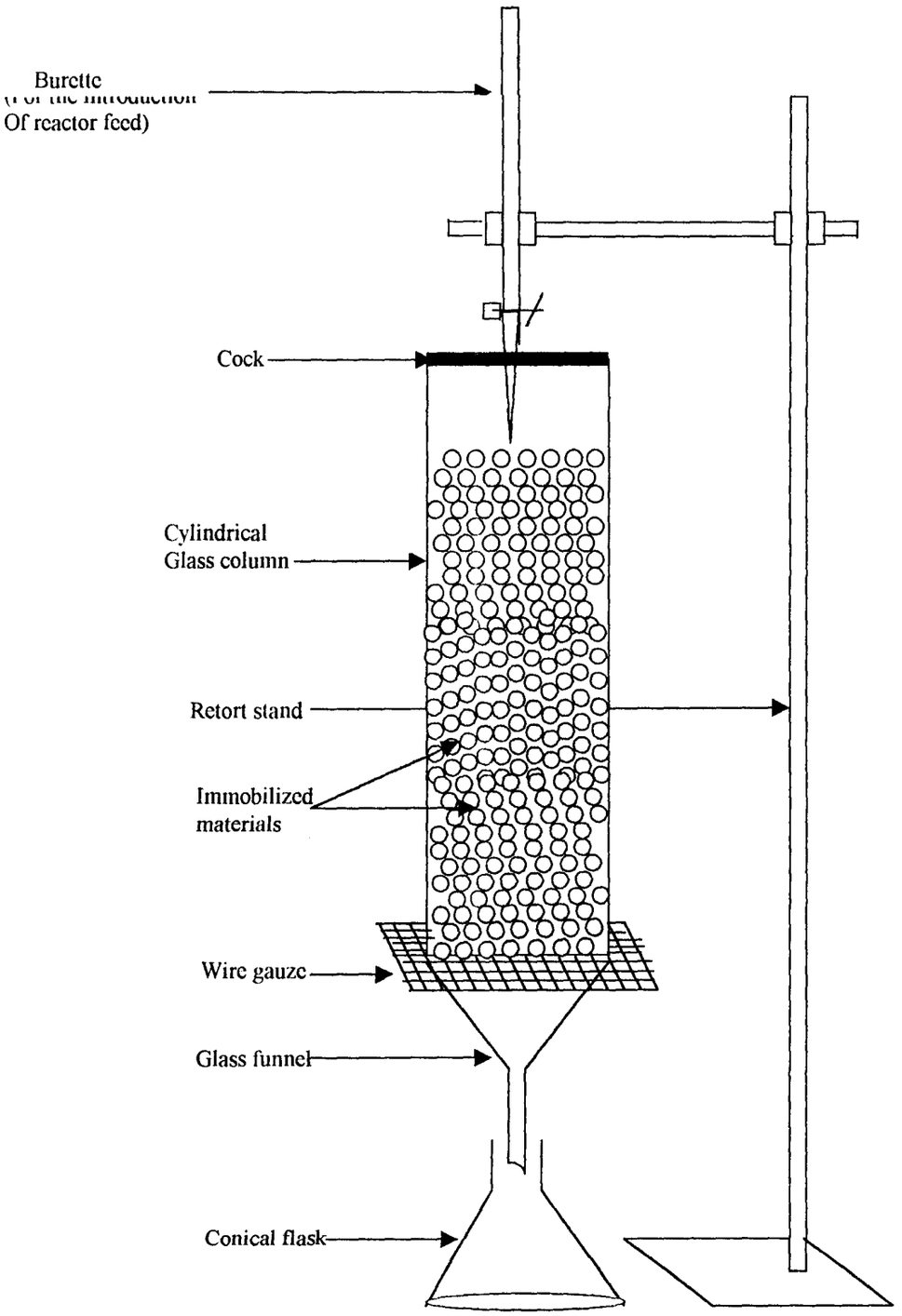

Fig 1. schematic diagram of the bioreactor system

3.7 EXPERIMENTAL PROCEDURE

The immobilization materials (packing materials) were first boiled to remove the lignin and fibbers present inside the material, which might react with yeast cells. They were then sterilized using an Autoclave maintained at 121^0c for 15 minutes.

The reactor column was packed with the packing materials (Palm wood) and sterilized using a solution of sodium metabisulphite. Sodium metabisulphite was also used in sterilizing the burette used for introducing this fed. In the absence of pelstaltic pump, gravity method was adopted in carrying out this experiment, 200ml of concentrated yeast suspension was re-circulated through the reactor at a flow rate of 0.12ml/sec.for 8 (eight) hrs in order to have the yeast cells absorbed into the palm wood blocks. Reactors feed containing glucose (100g), yeast extract (3.0g), peptone (3.5g), KH_2P0_4 (2.0g), $MgS0_4$. $7H_20$ (1.0g) and $(NH_4)_2S0_4$ (1.0g) was in to the reactor column from a burette in order to build up biomass as a result of the feed molecules moving into the pores of the palm wood blocks.

Samples of the exit stream (product) were taken at intervals of 30 minutes over a period of 4.5 hours. Specific gravity, refractive index and absorbance were measured using specific gravity bottle, refractometer and calorimeter in determining the ethanol concentration, glucose concentration and the yeast

cell concentration of the sample respectively. The same procedure was repeated using bamboo chips and rice husk in succession. Results are tabulated in chapter 4.

CHAPTER FOUR

RESULTS

4.0 RESULTS

TABLE 1.0 CALIBRATION OF GLUCOSE CONCENTRATION

CONCENTRATION OF GLUCOSE (g/l)	REFRACTIVE INDEX
0.2	1.332
1.2	1.333
4.0	1.337
5.0	1.338
7.0	1.341
9.0	1.344
11.0	1.346
14.0	1.350
17.0	1.353
20.0	1.357
22.0	1.359
25.0	1.362

Table 2.0 CALIBRATION OF CELL CONCENTRATION USING CALORIMETER

ABSORBANCE	CELL CONCENTRATION [PPM]
0.97	0.484
0.89	0.395
0.86	0.392
0.77	0.244
0.64	0.183
0.50	0.058
0.35	0.038
0.25	0.036
0.12	0.023
0.01	0.003

TABLE 3.0 CALIBRATION OF ETHANOLCONCENTRATION

% ETHANOL CONCENTRATION	SPECIFIC GRAVITY	WEIGHT OF ETHANOL (g)	WEIGHT OF GLUCOSE (g)	WEIGHT OF SOLUTION (g)
1.0	1.010	1.0	5.0	94.0
2.0	1.012	2.0	5.0	93.0
3.0	1.016	3.0	5.0	92.0
4.0	1.022	4.0	5.0	91.0
5.0	1.030	5.0	5.0	90.0
6.0	1.032	6.0	5.0	89.0
7.0	1.034	7.0	5.0	88.0
8.0	1.036	8.0	5.0	87.0
9.0	1.046	9.0	5.0	86.0
10.0	1.066	10.0	5.0	85.0

TABLE 4.0 TO TABLE 6.0 SHOWS THE STEADY STATE OF GLUCOSE CONCENTRATION.

Table 4.0

PALMWOOD

TIME(hr^{-1})	GLUCOSE CONCENTRATION (g/l)	REFRACTIVE INDEX
0.5	7.0	1.341
1.0	7.7	1.343
1.5	7.8	1.344
2.0	5.5	1.339
2.5	4.0	1.337
3.0	3.3	1.336
3.5	3.3	1.336
4.0	3.3	1.336
4.5	3.3	1.336

TABLE 5.0

BAMBOO CHIPS

TIME(hr^{-1})	GLUCOSE CONCENTRATION (g/l)	REFRACTIVE INDEX
0.5	5.6	1.339
1.0	7.5	1.342
1.5	7.0	1.341
2.0	5.0	1.338
2.5	4.5	1.337
3.0	3.3	1.336
3.5	2.8	1.335
4.0	2.8	1.335
4.5	2.8	1.335

Table 6.0

RICE HUSK

TIME(hr^{-1})	GLUCOSE CONCENTRATION (g/l)	REFRACTIVE INDEX
0.5	5.6	1.339
1.0	6.4	1.342
1.5	5.7	1.341
2.0	4.0	1.336
2.5	2.5	1.335
3.0	0.5	1.333
3.5	0.4	1.332
4.0	0.2	1.331
4.5	0.2	1.331

Table 7.0 to Table 9.0 shows steady state of Cell Concentration

Table 7.0

PALMWOOD BLOCKS

TIME(hr^{-1})	ABSORBANCE	CELL CONCENTRATION (PPM)
0.5	0.48	0.150
1.0	0.50	0.158
1.5	0.51	0.159
2.0	0.52	0.159
2.5	0.56	0.171
3.0	0.55	0.171
3.5	0.59	0.183
4.0	0.59	0.183
4.5	0.71	0.203

Table 8.0

BAMBOO CHIPS

TIME(hr^{-1})	ABSORBANCE	CELL CONCENTRATION (PPM)
0.5	0.43	0.110
1.0	0.47	0.142
1.5	0.55	0.171
2.0	0.53	0.164
2.5	0.49	0.153
3.0	0.57	0.170
3.5	0.66	0.175
4.0	0.65	0.189
4.5	0.65	0.185

TABLE 9.0

RICE HUSK

TIME(hr^{-1})	ABSORBANCE	CELL CONCENTRATION (PPM)
0.5	0.55	0.171
1.0	0.55	0.161
1.5	0.54	0.170
2.0	0.56	0.172
2.5	0.57	0.175
3.0	0.64	0.183
3.5	0.65	0.185
4.0	0.61	0.179
4.5	0.63	0.180

Tab e 10.0 to Table 12.0 shows steady state of Ethanol Con :entration

Tabl : 10.0

PALMWOOD BLOCKS

TIME(hr^{-1})	SPECIFIC GRAVITY	ETHANOL CONCENTRATION (g/l)
0.5	1.003	0.2
1.0	1.012	1.9
1.5	1.019	3.4
2.0	1.025	4.6
2.5	1.027	4.9
3.0	1.027	4.9
3.5	1.028	5.2
4.0	1.028	5.2
4.5	1.028	5.2

Table 11.0

BAMBOO CHIPS

TIME(hr^{-1})	SPECIFIC GRAVITY	ETHANOL CONCENTRATION (g/l)
0.5	1.004	0.3
1.0	1.009	0.3
1.5	1.018	3.2
2.0	1.027	5.0
2.5	1.030	5.7
3.0	1.032	6.1
3.5	1.032	6.1
4.0	1.032	6.1
4.5	1.032	6.1

Tab e 12.0

RICE HUSK

TIME(hr^{-1})	SPECIFIC GRAVITY	ETHANOL CONCENTRATION (g/l)
0.5	1.012	1.7
1.0	1.017	2.8
1.5	1.023	4.2
2.0	1.027	4.9
2.5	1.032	6.1
3.0	1.041	7.7
3.5	1.043	8.3
4.0	1.044	8.6
4.5	1.044	8.6

CHAPTER FIVE

CONCLUTION

5.0 DISCUSSION OF RESULTS

Immobilization is the process whereby the movement of enzymes, cells and organelles in space are restricted. Immobilization is profitably used in biochemical industries around the globe because of its ability to increase the stability of the cell, enable even cell distribution etc (CAMPBELL 1985). Immobilization techniques used in general can be categorized as; Physical adsorption to inert carrier, induction in the lattice of a polymer gel or microcapsules, covalent bonding (COULSON 1994).

When palm wood blocks were used as immobilized materials, the ethanol yield is 0.0583g/g and the glucose conversion is 0.967g/g. Using bamboo chips as immobilized materials under the same feed composition, ethanol yield and glucose conversion obtained was 0.0628g/g and 0.972g/g respectively. A higher ethanol yield and glucose conversion of 0.08617g/g and 0.998g/g was obtained respectively when rice husk is used as immobilization materials.

Tables 4.0,5.0 and 6.0 show that the glucose concentration of the medium decreases with time as the reaction progress. The graphs of glucose concentration versus time take a negative exponential curve pattern. The

yeast cells were observed to grow in an irregular pattern shown in tables 7.0, 8.0, 9.0. The graphs of cell concentration versus time are observed to take an irregular form because of the irregular and random pattern of cell growth in the medium. Ethanol concentration is shown in tables 10.0, 11.0, and 12.0 increase with time. The graphs of ethanol concentration versus time illustrate the exponential form of ethanol yield in the fermentation process.

5.1 CONCLUSION

Based on the results obtained this work, the following conclusions can be deduced.

a. Rice husk allowed more quantity of yeast cells to be absorbed into its pores compared to bamboo chips and palm wood blocks. This is because of its matrix porosity and pore size; hence higher concentration of ethanol was obtained when rice husk is used as immobilization materials.
b. As the cell grows, the consumption of glucose becomes faster and the rate of ethanol yield becomes more rapid as can be seen in tables 10.0, 11.0 and 12.0.
c. The substrate has to diffuse to the enzymes at their immobilized locations before reactions can occur. The overall rate of reaction is dependent on the rate of transfer of substrate and product as well as the kinetics of the reactions occurring at the enzyme site on the surface of the support matrix

5.2 RECOMMENDATION

a. To enhance good results and to improve the efficiency and accuracy of operation, the use of a pelstaltic pump should be employed.

b. Attempts should always be made to source locally cheap materials for use as immobilized materials. Industries in the country should take advantage of the immobilized enzyme process technology to improve the economy of their operations, thereby increasing productivity and overall material cost reduction.

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APPENDIX A

NOTATIONS

X_A	Glucose Conversion	g/g
G_O	Initial Glucose Concentration	g/l
G_F	Final Glucose Concentration	g/l
$Y_{E/G}$	Ethanol yield from Glucose	g/g
E_F	Final Ethanol Concentration	g/l
E_O	Initial Ethanol concentration	g/l
P	Ethanol Productivity	g/h/l
D	Dilution Rate	g-l
C	Specific Gravity	g/g
V	Reactor Volume	cm3
d	Diameter of Reactor	Cm
h	Height of Reactor	Cm
F	Flow rate	ml/hr
r	Radius of reactor	Cm
S	Built substrate concentration	g/l
K_S	Mass transfer coefficient	g-1
Vmax	Maximum reaction rate at support	g/g
I	Effectiveness factor	_
Da	Dankoller number	_

APPENDIX B

CALCULATIONS

$V = \pi r^2 h$, (where $r = d/2$)

$V = 3.142 \times (2.75)\ 2 \times 25$

$V = 594 cm^3$

$D = F/V$

$F = 0.14 ml/sec$

$$D = \frac{0.14 \times 3500}{594}$$

$D = 0.8485 g/hr$

For Palm wood blocks

$$X_A = \frac{Go - G_F}{G_O}$$

$$X_A = \frac{100 - 3.3}{100}$$

$X_A = 0.97g/g$

$P = D \times E_F$

$P = 0.8485 \times 5.2$

$P = 4.4122g/l/hr$

$$Y_{E/G} = \frac{E_F - E_O}{G_O - G_F}$$

$$Y_{E/G} = \frac{5.2 - 0}{100 - 3.3}$$

$Y_{E/G} = 0.0538g/g$

For Bamboo Chips

$$X_A = \frac{Go - G_F}{G_O}$$

$$X_A = \frac{100 - 2.8}{100}$$

$X_A = 0.972g/g$

$P = D \times E_F$

$P = 0.8485 \times 6.1$

$P = 5.1759g/l/hr$

$$Y_{E/G} = \frac{E_F - E_O}{G_O - G_F}$$

$$Y_{E/G} = \frac{6.1 - 0.3}{5.6 - 2.8}$$

$Y_{E/G} = 0.0628g/g$

For rice husk

$$X_A = \frac{G_O - G_F}{G_O}$$

$$X_A = \frac{100 - 0.2}{100}$$

$X_A = 0.998 g/g$

$P = D \times E_F$

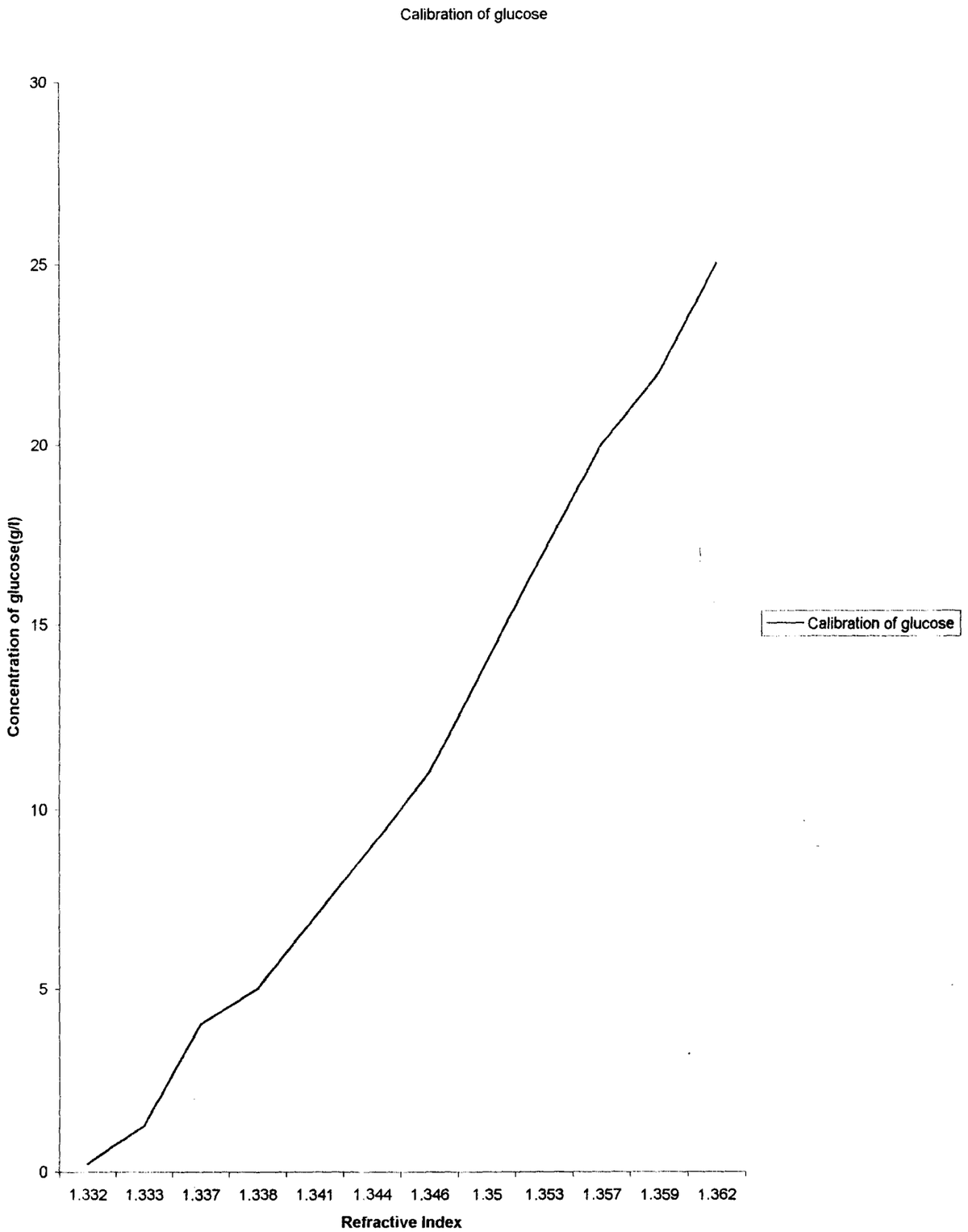
$P = 0.8485 \times 8.6$

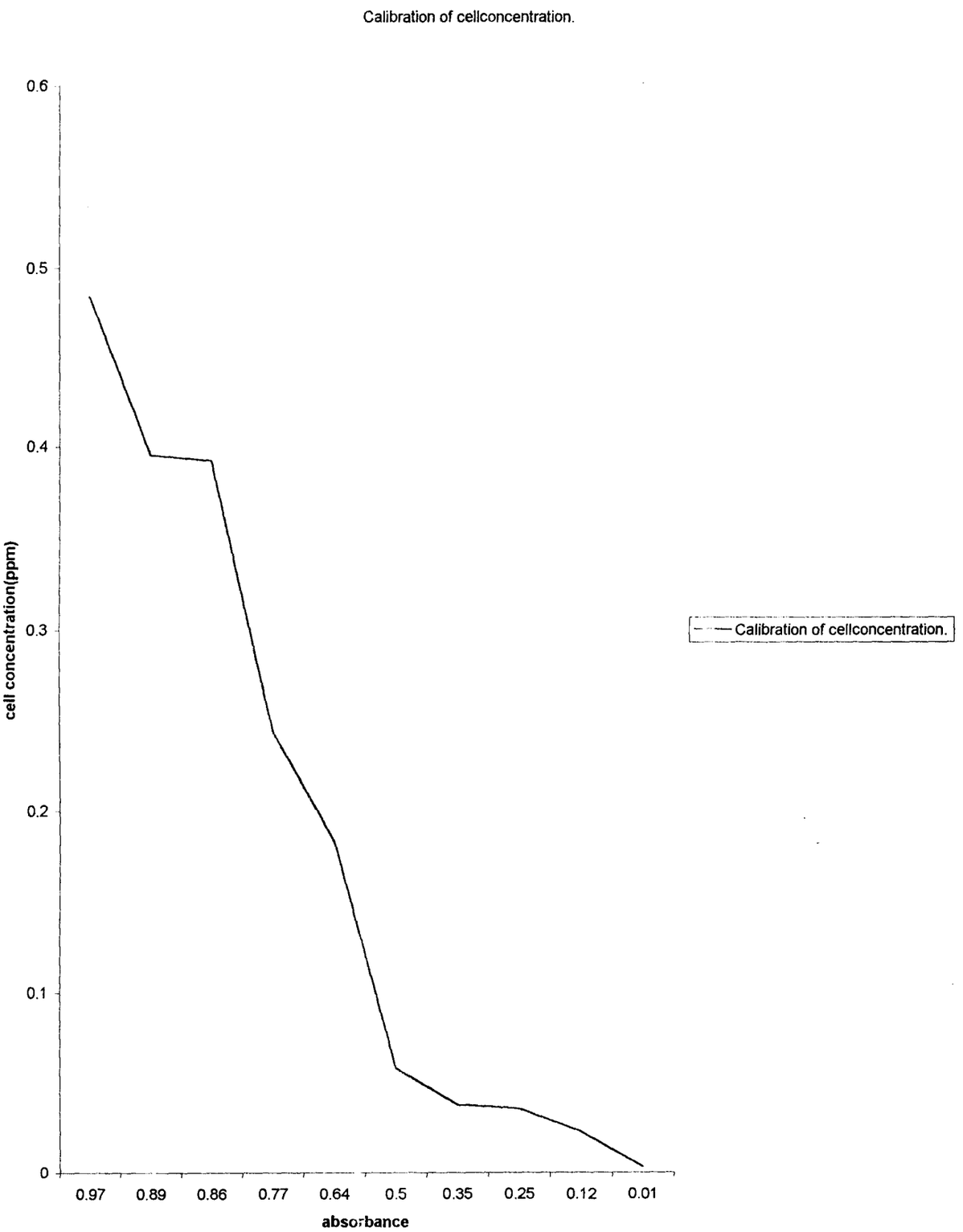
$P = 7.297 g/l/hr$

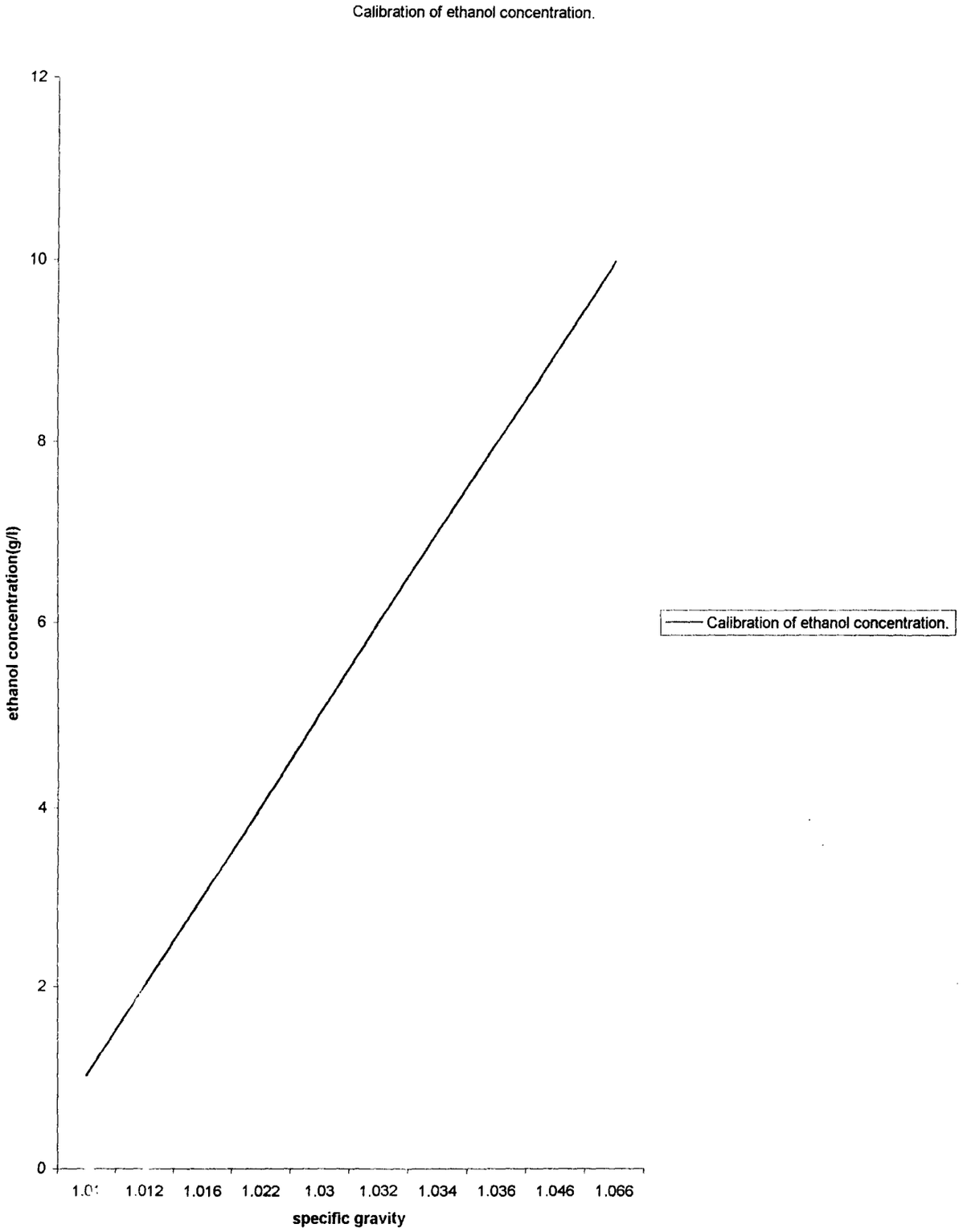
$$Y_{E/G} = \frac{E_F - Eo}{G_O - G_F}$$

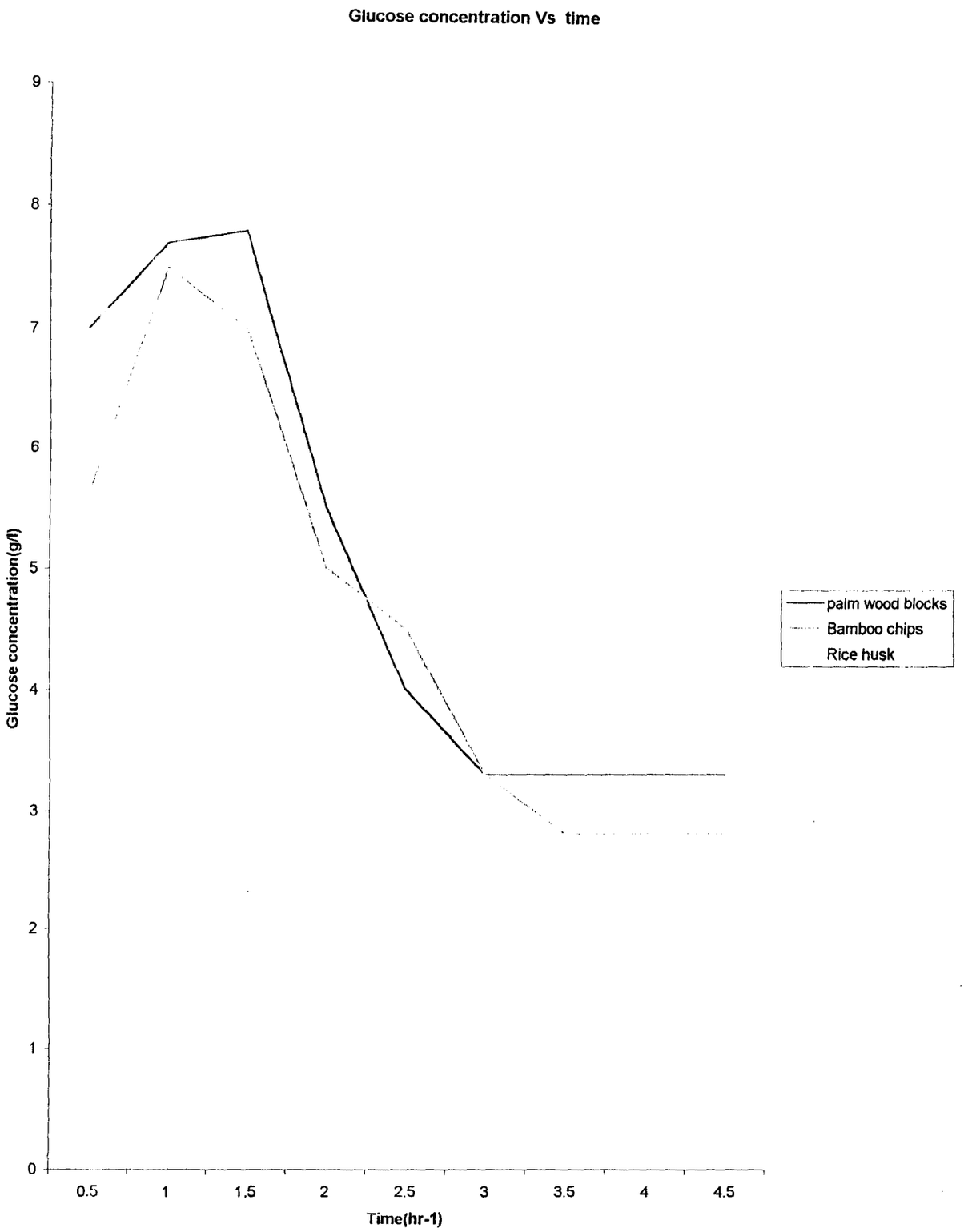
$$Y_{E/G} = \frac{8.6 - 0}{100 - 0.20}$$

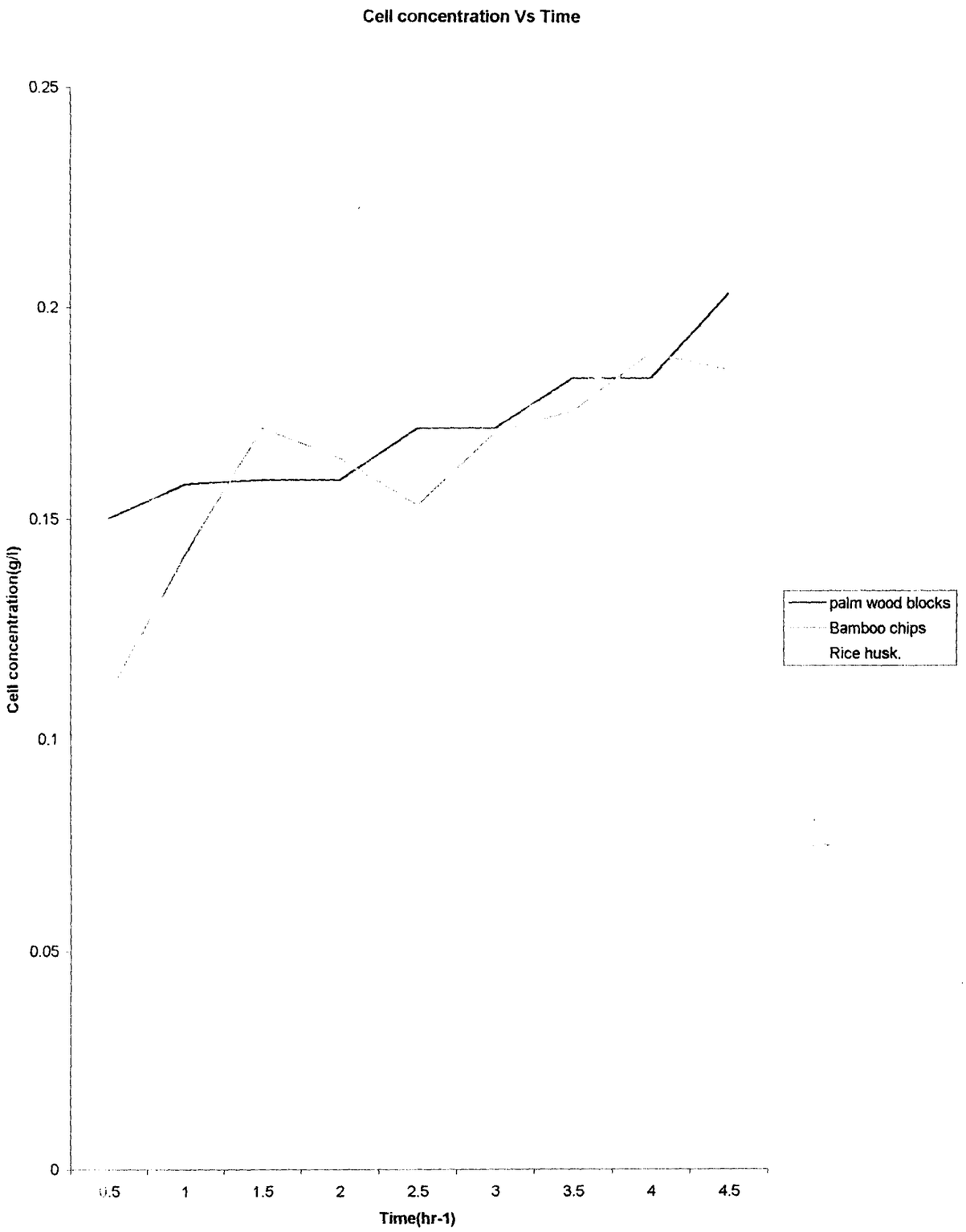
$Y_{E/G} = 0.8617 g/g$


Calibration of glucose
30
25
20
15
10
5
0
Concentration of glucose(g/l)
1.332
1.333
1.337
1.338
1.341
1.344
1.346
1.35
1.353
1.357
1.359
1.362
Refractive Index
Calibration of glucose


Calibration of cellconcentration.
0.6
0.5
0.4
0.3
0.2
0.1
0
cell concentration(ppm)
0.97
0.89
0.86
0.77
0.64
0.5
0.35
0.25
0.12
0.01
absorbance
Calibration of cellconcentration.


Calibration of ethanol concentration.
12
10
8
6
4
2
0
ethanol concentration(g/l)
1.012
1.016
1.022
1.03
1.032
1.034
1.036
1.046
1.066
specific gravity
Calibration of ethanol concentration.


Glucose concentration Vs time
9
8
7
6
5
4
3
2
1
0
Glucose concentration(g/l)
0.5
1
1.5
2
2.5
3
3.5
4
4.5
Time(hr-1)
palm wood blocks
Bamboo chips
Rice husk


Cell concentration Vs Time
0.25
0.2
0.15
0.1
0.05
0
Cell concentration(g/l)
0.5
1
1.5
2
2.5
3
3.5
4
4.5
Time(hr-1)
palm wood blocks
Bamboo chips
Rice husk.

Ethanol concentration Vs Time