Performance of a Stirred Catalytic Basket Reactor for the production of Bio-ethanol

By

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Amir Hussain

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ABBREVATION AND SYMBOLS

ATP	Adenosine Tri Phosphate
C	concentration (mol/m^3)
C_0	Initial concentration
C_{AB}	Substrate concentration in bulk
C_{Ai}	Substrate concentration in surface
cAMP	Cyclisches Adenosinmonophosphat
D	mass diffusivity (m^2/s)
D_{Ae}	effective diffusivity
DEAE	Diethylaminoethylcellulose
DHAP	dihydroxy-acetone-phosphate
DMAA	dimethl acrylamide
DNS	Dinitro salicylic acid
E	Energy dissipation
EM	Eddy momentum
ENO	Enolase
f	Friction factor
FADH2	Flavin-Adenin-Dinukleotid
FBPA	Fructose bisphosphate aldolase
F_d	Drag force
FID	Flame ionization detector
FID	flame ionization detector
G-3-P	Glyceraldehydes-3-phosphate
G3-P	glyceraldehydes-3-phosphate
GAPDH	Glyceraldehydes-3-phosphate dehydrogenase
GC	gas chromatography
GMA	Glycidyl methacrylate
HCl	Hydro chloric acid
HK	Hexokinase
J	mass flux (mol/m^2 .s)

JD	Chilton Colburn mass transfer factor
Kl	Mass transfer coefficient.
$Kr_{(obs)}$	Observed rate of reaction
Lc	Characteristics length
M	Momentum
MPEP	Macroporous polyethylene polymer
N	Impeller revolution per minute
NADH	Nicotinamidadenindinukleotid
Dp	Particle diameter
Ni	impeller rotation speed
N_p	power number
P	Product concentration
PBR	Packed Bed Reactor
PDC	Pyruvate decarboxylase
PEC	polyelectrolyte complex
PFK	Phosphofructokinase,
PGI	Phosphoglucoisomerase
PGK	Phosphoglycerate kinase
PGM	Phosphogleceromutase
Pl	Product concentration at specific time
εJD	Mass transfer factor with respect to porosity
Po	Product concentration at time zero
Ppi	pores per inch
PUF	Polyurethane foams
PVA	Polyvinyl alcohol
PYK	Pyruvate kinase,
r	radius of sphere or pore
Re	Reynolds Number
Re_p	Particle Reynolds Number
Reε	Reynolds number with respect to energy dissipation
Res	Reynolds number with respect to specific surface area.

RIGP	Radical initiated polymerization
rpm	Revolution per minute
Sc	Schmidt Number
SCBR	Stirred Catalytic Basket Reactor
Sh	Sherwood Number
Sl	Substrate concentration at a specific time
So	Initial substrate concentration (g/l)
So	Substrate concentration at time zero (g/l)
STR	Stirred Tank Reactor
TPI	Triose phosphate isomerase
U	velocity (m/s)
V	velocity of fluid.
v	kinematic viscosity (m^2/s)
Ve	Effective Velocity
V_l	Volume of liquid in reactor
W	Angular velocity
Y(p/s)	Ethanol yield

Greek Words

δ	Thickness of hydrodynamic boundary layer
δc“	Thickness of concentration boundary layer
ε	Porosity
τ	Tortuosity

THESIS SUMMARY

SUMMARY

Current environmental and health problems are sourced from the usage of fossil fuel. This issue has encouraged the scientific community to find environmentally friendly alternatives, such as biofuel. Ethanol, also called ethyl alcohol (CH_3CH_2OH) used as a beverage, a solvent, a germicide, and as a fuel (U.S. Department of Energy, 2005). The most preferred method for bioethanol production is the use of yeast *Saccharomyces cerevisiae* fermentation process in bioreactors. For ethanol production, the design and scale-up of a bioreactor are important factors. The Stirrer Catalytic Basket Reactor (SCBR) presents some of the advantages of a packed bed reactor (PBR) and seems to be specifically well suited to produce high value fine biochemical and ethanol as a fuel. The study of fluid-side mass transfer influence on productivity as well as the performance of bioreactors is still needed. The yeast physiology and mass transfer behaviors in PBR were monitored according to parameters such as glucose concentration, medium flow rate, and different support materials like alginate beads with and without chitosan coating. The maximum time of lag phase was found to be 290 min at a lower flow rate of 1 ml/min and 190 min at a higher flow rate of 90 ml/min when using 40 g/l of glucose. By decreasing the glucose concentration from 40 to 10 g/l, the lag phase decreased too. After the lag phase, no significant change was observed in both types of beads on glucose consumption with the same flow rate. It can be concluded that the lag phase is not due to the physiology of yeast, but it may be due to the resistance in the internal diffusion of glucose. By varying the flow rate from 1 to 90 ml/min, the time for glucose consumption decreases. The time for glucose consumption by chitosan coated beads at 30 and 90 ml/min is rather equal when using a higher glucose concentration i.e. 40 and 20 g/l as compared to lower glucose concentrations 10, 4, and 2 g/l. It was also observed that there was no significant difference in ethanol productivity for both *S. cerevisiae* strains at lower flow rates i.e. 4 ml/min, while higher productivity was obtained at higher flow rates (90 ml/min).

Further, in the PBR study, the purpose was also to understand the mechanism of internal mass transfer (Particle-side mass transfer) effects and the performance of PBR. Four operational parameters, glucose concentration, flow rate, alginate beads (chitosan-coated and without chitosan beads), and size of beads have significant effects on the internal mass transfer. The duration of the lag phase decreases by increasing the flow rate and decreasing the size of the beads. The chitosan also affects the intra-particle transfer of the substrate that can be reduced by reducing the size of beads and increasing the flow medium around the beads which enhances the molecular diffusion in pores. The time for glucose consumption is reduced by reducing the size of beads and this might be due to the reduction of the concentration gradient inside the beads. An insignificant difference of the glucose consumption time was noticed between

chitosan-coated and non-coated alginate beads at higher flow rates (30 and 90 ml/min), especially for a smaller bead size (1mm). This might be due to the reduction of solute diffusional distance from the surface to the site of reaction. The higher ethanol productivity was achieved by increasing the flow rate, glucose concentration, and varying the size of beads to improve mass transfer properties or reduced internal substrate diffusional resistance. After the study of external and internal mass transfer in PBR as a prototype reactor, the performance of SCBR with immobilized cells in alginate beads and DEAE sponges were evaluated and compared with Stirrer Tank Reactor (STR) with free cells. In SCBR, no lag phase was observed even at higher substrate concentrations because of the well-mixed environment of this bioreactor. The difference of time in glucose consumption between free and immobilized cells (alginate and sponges) tended to increase by increasing the glucose concentration (100 and 200 g/l) in the medium. The magnitude of mass transfer resistance has an inverse relation with the stirring speed. The reason behind it is that the stirring speed controls the internal diffusion of the substrate. The sponges showed lower internal diffusion resistance as compared to alginate beads due to fluid flow through macro-pores. The diffusivity of glucose through the boundary layer surrounding the biocatalyst particle has a major role to achieve a maximum ethanol yield that is directly controlled by the structure of the immobilizing matrix, stirring speed, and design of a bioreactor (Najafpour 2015).

Flow behavior between immobilized particles has a tremendous effect on mass transfer. As the flow regime is laminar at lower Rep, momentum transfer is by viscosity and velocity fluctuation while the mass transfer is through molecular diffusion and concentration fluctuation. If fluid cross over a porous particle with higher velocity, vortices or eddies are produced behind and carry solute particles to diffuse into the surface of the porous particle. In a packed bed by using smaller particles, interstitial velocity increases due to the reduction of channel diameter consequently mass transfer is increased. In a turbulent flow with higher Rep a point comes where the mass transfer coefficient becomes independent on the Schmidt number or molecular diffusion in the boundary layer. Our results for Sherwood & Schmidt numbers as a function of Rep are presented and seems experimental data are in good agreement with the model for sponges. Further by using DEAE sponges as catalysts support at higher Rep, Sherwood number becomes independent of substrate concentration. Pressure has a great role in fluid motion or mass transport in porous materials. Our results indicate the correlation of pressure drop with mass and momentum transfer. Further, it is depicted from SCBR results, liquid-solid mass transfer is a function of energy input per unit mass which is the application of Kolmogoroff theory of isotropic turbulence.

From all the results, our two hypotheses were evaluated: 1). DEAE sponges are better than Alginate beads. 2). SCBR performs better than PBR. In the case of the first hypothesis, from results, it has been observed that even at 200 rpm with a 100 g/l glucose concentration, the sponges consume glucose in 9 h, while alginate beads and free cells consume it in 19 h and 20 h, respectively. Alginate beads take a longer time to consume than polymers. This might be due to the increase of concentration gradient because of the thick layer around beads. But this is not the case of DEAE polymer because polymers have a larger pore size that can enhance the flow inside the particle. At a higher glucose concentration of 200 g/l, the volumetric productivity of polymer was higher as compare to alginate beads. These polymers are presumed as best immobilizing materials than alginate beads with higher ethanol productivity and lower glucose consumption time. This might be due to a lower difference in concentration between bulk and inside polymer and lower film diffusion barrier in sponges.

Hydrodynamic factors have a significant influence on convective mass transfer. The mass transfer coefficient factor (εJD) value may not undergo larges changes by increasing Rep and falls consistently even appear nearly flat. In a turbulent regime, the mechanism of mass transfer will be by convection or motion of eddy from fluid turbulent regime to film boundary. The increasing trend in Sh against Rep is due to a particular relationship of turbulent motion and its diffusing power. Due to turbulence alginate beads can have a colloidal motion that can enhance the micro-convection by eddy motion with a consequent increase in mass transfer (Sedahmed, Farag, et al. 1998). Macro-porous foams have excellent properties of higher porosity and a three-dimensional pore network for higher radial mixing, lower pressure drop that enhance heat and mass transfer as compared to alginate beads (Inayat 2013). Macro-porous foams have excellent properties of higher porosity and a three-dimensional pore network for higher radial mixing, lower pressure drop that enhance heat and mass transfer as compared to alginate beads (Inayat 2013). Sherwood number increases with an increase in Rep due to higher interstitial velocity in pores. Open pores of macro-porous sponges optimize the contact area and minimize the internal diffusion limitations. This novel DEAE sponge prevents plugging and allows higher flow inside the pores due to the action of pressure drop created at higher stirring effect or turbulence. Further by using DEAE sponges as catalysts support at higher $Re\epsilon$, Sh becomes independent of substrate concentration due to no concentration gradient. It is concluded that convection of reactants through pores of the sponge is directly proportional to Rep and pressure drop as it was observed by De Lathouder in 2007.

The second hypothesis is also proved correct as in SCBR complete conversion of 200g/l glucose, dissolved in medium, has been observed in a short time (5h) as compare to Packed Bed. In SCBR, no lag phase was observed even at higher substrate concentrations because of the well-mixed

environment of this bioreactor. At higher stirrer speed 500rpm, the time for glucose consumption (5h) was approximately the same for 50 g/l, 100 g/l, or 200 g/l glucose. It is depicted that sponges have no external or internal mass transfer limitations or due to macro-pores fluid flow is improved therefore there is no difference in consumption time at a higher speed. SCBR provides an efficient radial & axial mixing system. The SCBR with cell immobilized in sponges is more attractive and economical, especially on an industrial scale.

CHAPTER 1

Introduction:

(CELL IMMOBILIZATION IN BIOTECHNOLOGY)

SUMMARY

Fossil fuel is the major cause of environmental and health problems because of huge usage in transportation and industries. Bio-ethanol is an alternative environmentally friendly fuel introduced in the market, produced by microorganism (yeast) fermentation process. Understanding yeast cells' physiology, metabolism, and cell growth phase (lag, exponential, stationary, and death phase) are the most important factors. Cellular metabolism (anabolic & catabolic) is the sum of all energy conversion processes inside the cell. Yeast utilizes ATP (energy source), produced by the glycolysis pathway, to complete the fermentation reaction and yeasts convert pyruvate into ethanol. Free cells (Homogeneous reaction) are more sensitive to nutrient depletion, pH variation, an inhibitory compound (reactants or products) while by immobilized cells (Heterogeneous reaction) these problems can be overcome. Several advantages have been observed in immobilized cells in terms of higher productivity and high inhibition tolerance.

The immobilized cell's survival and its efficiency depend on mass transfer properties. Mass transfer is an important phenomenon because the consumption of substrate by cells encapsulated (in alginate beads or polymers) depends on three steps: the diffusion of the substrate from the bulk medium to the capsule and the diffusion within the capsule that follows the third step of the biochemical reaction (Lestari 2010). The bioreactor is the equipment in which the substrate is converted to the desired product(s). For the immobilization technology in ethanol production, the design and scale-up of a bioreactor are important factors. Four kinds of mode of operations in bioreactors are used for ethanol production: batch, fed-batch, continuous, and semi-continuous. A well-mixed operation is a very important factor to ensure an efficient bioprocess. The mixing possibilities in the stirred tank reactor are more or less the same as the (SCBR). The SCBR presents some of the advantages of a fixed bed reactor and seems to be specifically well suited for the production of high value fine biochemical and ethanol as a fuel. The SCBR can run as a batch as well as a continuous process with very high mass transfer rates and yields even at a small scale. Many configurations are possible in this type of bioreactor having a basket attached to the agitator shaft. The basket has the advantage of permitting greater contact between reactants and biocatalysts, which in turn increases the reaction rate and efficiency of the bio-catalytic reaction.

1. INTRODUCTION

1.1. FERMENTATION OF YEAST CELLS TO BIOFUELS AND BIOCHEMICALS

Biotechnology is a technique that uses living organisms to produce valuable commercial products. With the advances made in biochemistry, microbiology, cell physiology, immunology, and reaction engineering. As a result, many improvements have been seen in various fields, such as the pesticide industry, processing of fruit juices (i.e. clarification), antibiotics and human medicine formulations, culturing of human organs, etc.

In the last century, Louis Pasteur (1822-1895) had identified the role of microorganisms in fermentation processes. The fermentation (Latin verb '*fervere'*) illustrates the use of yeast to degrade sugar or other organic nutrients i.e. a biochemical transformation of organic compounds using different enzymes to produce different products under aerobic or anaerobic conditions. Microorganisms have the capability to utilize inexpensive nutrients, such as wastes of sugar industries, corn crops, municipal wastes to produce valuable products like antibiotics and fine chemicals for industries (Najafpour 2015). Fermentation is used nowadays in food processing (production of cheese, milk, yogurt, bread, etc.), alcoholic beverages production (wine, beer, etc.), antibiotics, vaccines, organic acids, and fine chemical industries (alcohol and acetone production) are an important application of this biotechnology (Najafpour 2015) (Doran 2012).

1.1.1. Bio-Fuels

Current environmental and health problems are sourced from the usage of fossil fuel. Nowadays, most countries are oil-dependent, especially in the transportation and domestic sectors. This issue has encouraged the scientific community to find environmentally friendly alternatives, such as bio-fuel. Bio-fuel is a generic term, which means any liquid fuel that is not derived from mineral reserves and is commonly named as bio-ethanol and bio-diesel (EECA, 2005).

Several products (bio-ethanol and organic solvents) are produced via bioprocesses by using cheaper raw materials compared to traditional processes. Ethanol, also called ethyl alcohol (CH_3CH_2OH), is a volatile, flammable, colorless liquid, which is strongly dependent on the hydroxyl group and used as a beverage, a solvent, a germicide, and as a fuel (Bickel and Friedrich 2005). It is the most important substitute for conventional fuel (petroleum) and has several advantages, such as enhancing vehicle performance and reducing the environmental pollution. Ethanol, when mixed with gasoline, is more environmentally friendly as this reduces the toxicity by producing less air born pollutants and greenhouse gases. Greenhouse gases emissions and carbon monoxide in the air are caused largely by the transportation sector. Bio-ethanol is a bio-degradable and even a less toxic fuel (Um 2002), which highlights again the importance of these bioprocesses that are an essential part of the fine chemical and pharmaceutical industries.

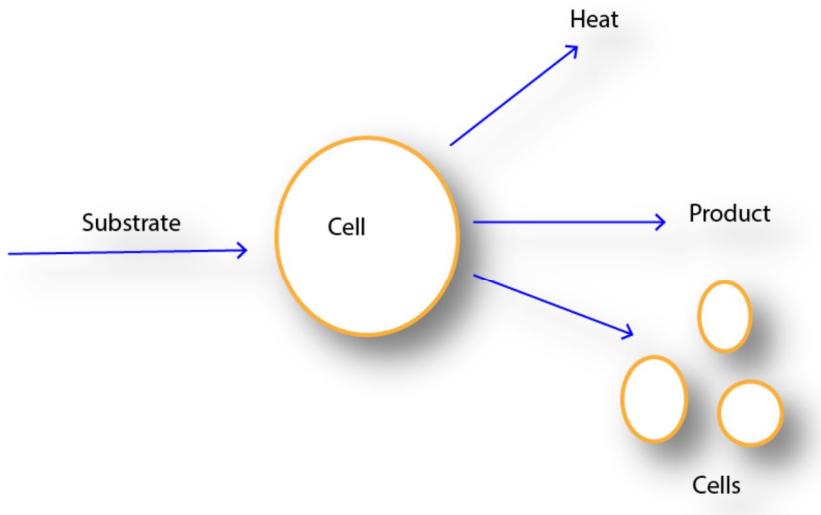
They rely on producing new products and destroying of harmful wastes by using microbial, animal, and plant cells or its components like enzymes.

1.1.2. Fine chemicals

The literature provides extensive information about the bio-fine-chemicals production via bioprocesses/fermentation processes (Carberry 1964); These authors covered the adequate work based on the fermentation process, but little attempts were made on improving the biochemical process. Enormous expensive commercial products like antibodies, proteins, and vaccines have been developed by this biochemical process (Galaction, Rotaru et al. 2011); (Doran 2012).

The most preferred method for bio-ethanol and bio-fine-chemicals production is the use of yeast *Saccharomyces cerevisiae* fermentation process in bioreactors (Pscheidt and Glieder 2008); (de Jong, Siewers et al. 2012); (Cha, Yang et al. 2014); (Djordjević, Gibson et al. 2015). For many centuries, yeast whole cells have profoundly been used as a workhorse in the production of bio-ethanol. Yeast is currently the most used microorganism due to its extensively high rate of fermenting sugars and its high tolerance to by-products produced during fermentation (Matsushika, Inoue et al. 2009); (Hasunuma and Kondo 2012); (De Bari, De Canio et al. 2013); (Borovikova, Scherbaka et al. 2014). The glucose is one of the best convertible sugar in the form of carbohydrates and cellulose, therefore most of the world is producing ethanol from corn the best source of carbohydrates (Wyman 1996).

Figure 1.1. View of a cell as an open system (Wyman 1996)



1.1.3. Cell Growth

Understanding the concept and characteristics of cell growth, especially cell physiology and metabolism, is important to interpret the fermentation process. Many factors affect cell growth and the concentration of cells is usually represented with respect to time by a growth curve (Figure 1.2). (Parolini and Carcano 2010). This curve has four distinct phases: lag, exponential, stationary, and death phase.

- **Lag Phase**:this is the first phase of cell growth. There are some factors that affect the lag phase and the major factors that affect the lag phase duration are related to the initial cell concentration, substrate concentration, growth phase of inoculums, and change of environment (nutritional or physical change) or transfer of nutrients to the site of action. This is the phase where cells need to adjust to a new environment. This adjustment ranges from a few minutes to several hours, thus affecting the cost of the process running.
- **Exponential/Growth Phase**: this phase starts when cells start to rapidly multiply after adjusting to the new environment and end due to different factors: exhaustion of the essential nutrients, accumulation of toxic by-products, or saturation points reached by cells. The growth rate here depends on the cell concentration at different times.
- **Stationary Phase**: in this phase cells are still growing and dividing, but at the same time, an equal number is dying. This happens when cells have no more carbon i.e. an energy source left. Accumulation of toxic metabolites could be one reason for the stationary phase.
- **Death Phase**: it is the loss of viable cells due to the lack of nutrients or to the inhibition of toxic metabolites.

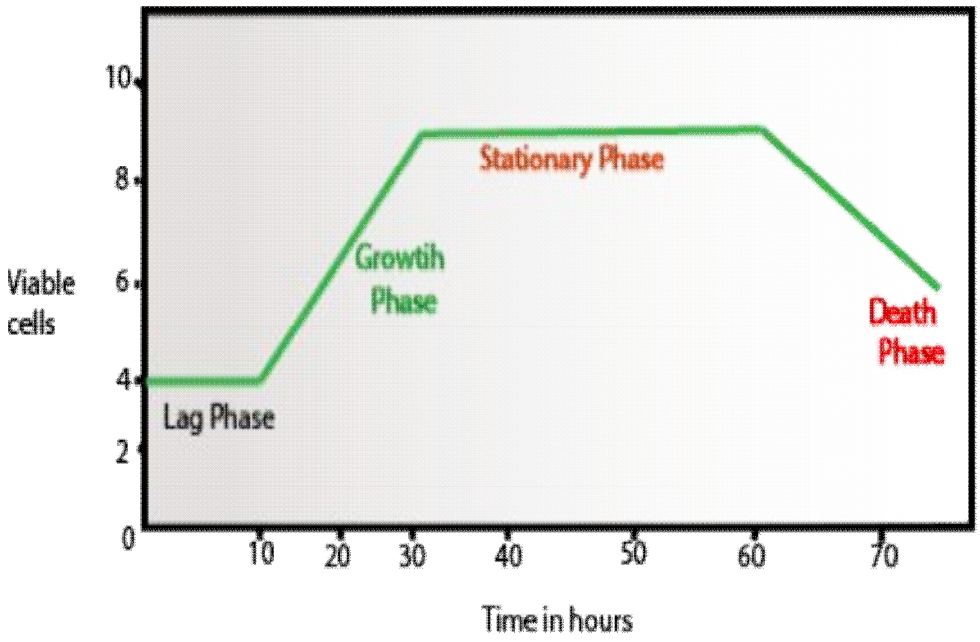
Normally, the fermentation process is interrupted when the stationary phase is attained i.e. a maximum cell concentration is reached (Parolini and Carcano 2010).

1.1.4. Cellular Metabolism

Cellular metabolism is the sum of all energy conversion processes taking place in a cell. A metabolic pathway is the sequence of a controlled chemical reaction. Two major metabolic pathways are considered anabolic and catabolic. The anabolic pathway requires energy to synthesize biomolecules like protein, nucleic acid, and membranes while the catabolic pathways release energy by breaking down bigger molecules (glucose) into smaller molecules (ethanol). Carbohydrates are the major energy sources for all living cells and they are also used for biochemical reactions. Yeast utilizes ATP (energy source) to complete the fermentation reaction, produced by a breakdown of organic compounds. Glucose is the primary source of energy for the yeast (*Saccharomyces cerevisiae*) and a lot of energy (ATP) is produced by the glycolysis pathway,

which is the first step of cellular respiration. Glycolysis is an anaerobic process, where glucose is converted into pyruvate (Parolini and Carcano 2010); (McKee and McKee 2012). Two pathways are adopted by pyruvate, such as respiration and fermentation. In the respiration pathway, it is converted due to the presence of oxygen into acetyl CoA that further releases CO_2, NADH, and FADH2. While in the anaerobic fermentation process, CO_2 and ethanol are produced from pyruvate when yeast is grown (Um 2002).

Figure 1.2. Cell growth phases in batch process (viable cells x10 [10])



The glycolysis is also referred to as the Embden-Meyerhof-Parnas pathway. The glycolytic pathway has 10 reactions as follows:

1. Glucose-6-phosphate Synthesis. A phosphorylation process occurs when glucose enters the cell. Hexokinase enzymes catalyze this irreversible process and as a result, glucose-6-phosphate is synthesized.

2. Glucose-6-phosphate to fructose-6-phosphate Conversion. By the reaction of phosphoglucose isomerase (PGI), glucose-6-phosphate converts to fructose-6-phosphate. This reaction is reversible.

3. Fructose-6-phosphate phosphorylation. Fructose-6-phosphate is phosphorylated by phosphofructokinase-1 and fructose-1 to synthesize 6-bisphosphate. During this process of phosphorylation, ATP is extensively used resulting in a decrease in free energy.

4. Cleavage of fructose-1,6-bisphosphate. By the reaction of the aldolase enzyme, fructose-1,6-bisphosphate is cleavaged into two molecules like glyceraldehydes-3-phosphate (G-3-P) and dihydroxy-acetone-phosphate (DHAP).

5. Glyceraldehydes-3-phosphate and dihydroxy-acetone-phosphate Interconversion.

G-3-P can be used as a substrate and triose phosphate isomerase enzyme acts as a catalyst to convert DHAP to G-3-P. One molecule of glucose is converted to two G-3-P molecules.

6. Glyceraldehydes-3-phosphate Oxidation. During this step, glyceraldehydes-3-phosphate undergoes an oxidation process by a catalytic complex reaction of glyceraldehydes-3-phosphate dehydrogenase, which forms glycerate 1,3-bisphosphate.

7. Transfer of Phosphoryl Group. This step is called substrate-level phosphorylation. ATP is synthesized by the transfer of the phsophoryl group from the substrate. Two molecules of glycerate-1,3-bisphosphate are generated by one glucose molecule.

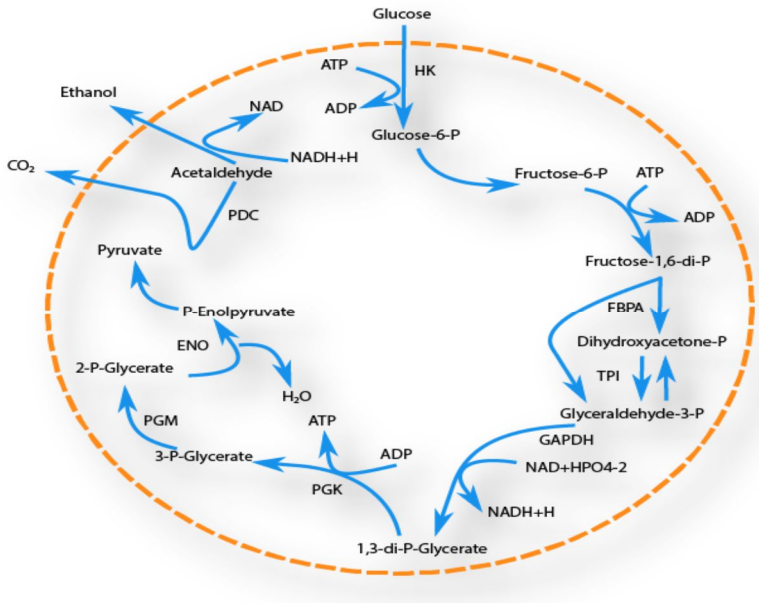
8. Conversion of 3-phosphoglycerate to 2-phosphoglycerate. The cells convert the C-3 phosphorylated compound to C-2 phosphorylated.

9. Dehydration Process. 2-phosphoglycerate is dehydrated to form phosphoenolpyruvate, which has a high phosphoryl-transfer potential. This transfer is valuable for the production of pyruvate.

10. Pyruvate Synthesis. In this step, a phosphoryl group is transferred from PEP to ADP by catalyzing the pyruvate kinase. Two molecules of ATP are produced from one glucose molecule.

In glycolysis, pyruvate is an energy-rich compound and in anaerobic conditions, yeasts convert pyruvate into ethanol. In all this process, the main purpose is the regeneration of NAD+ .from NADH, which is referred to as fermentation.

Figure 1.3. Metabolic Pathway of ethanol fermentation by *S. cerevisiae* (Bai, Anderson et al. 2008)



1.2. CELL IMMOBILIZATION

Cell immobilization technology is the localization of cells, enzymes, or proteins to a defined region of space by solid support or matrix, while the catalytic activity of the biochemical process is improved or preserved. Yu, Zhang, (2007); Willaert (2009); Duarte, Rodrigues. (2013);Krasňan, Stloukal. (2016). The impact that immobilized cell technology could have on the fuel ethanol industry is stimulating. Balance and Herryman et al. have compared the economics of the production of ethanol to conclude that cell immobilization could be up to ten times more productive and cost-effective than conventional free cell systems (Blanco and Herryman (1987). Several advantages have been observed in immobilized cells in terms of higher productivity, a higher ethanol yield, lower production of byproducts, a short lag phase, and a high inhibition tolerance. The immobilization, especially the encapsulation of cells, is called as an immunoprotective device in which biologically active cells are enclosed in semipermeable polymers and therefore protected from mechanical and physical damage. By immobilization, especially in the encapsulation of cells in alginate beads, higher cell concentration in cultivation media could be possible that can decrease the fermentation time and increase the inhibitor's tolerance (Lestari 2010).

In modern biotechnology, biocatalysts like living cells as well as enzymes have been utilized to produce a variety of fine chemicals and ethanol. The successful use of enzymes and microbial whole cells as low-cost biocatalysts to produce fine chemicals and ethanol has proven to be the most efficient and applicable approach as it indicated to be both ecological and economical as compared to traditional manufacturing. The use of cell immobilization can improve the process economy by increasing reusability and/or feasibility.

1.2.1. Advantages of Immobilization: Immobilization has numerous advantages over free cells, including:(Ennis 1987); (Buchholz, Kasche, et al. 2005); (Krasňan, Stloukal et al. 2016)

- Productivity by increasing the cell density per unit reactor volume.
- Easiness of product separation.
- Reutilization of entrapped cells especially in repeated or continuous processes.
- Maintaining of specific growth.
- Insensitivity to contaminants and the ability to be used in a non-sterile condition in some cases.
- Modified intrinsic behavior can be used for increasing productivity.
- Protection of attached cells from the environmental effect.
- Lower susceptibility to substrate inhibition effects and pH variations.
- Improved the yields by the increase of substrate uptake, reduction of product inhibition, and better process stability.

- Ability to be Regenerated and reused without removing from bioreactor for an extended period.
- Maturation time can sometimes be reduced.
- Longer activity time due to the regeneration of enzymes and co-factors.
- Protection from the effect of toxic substances like metal ions and aromatics.
- Ability to undergo a complex reaction sequence because cells remain viable and protected from the shear effect of impellers when immobilized.
- Ability to run a continuous fermentation with short residence time and high volumetric productivity.

1.2.2. Disadvantages of Immobilization

- Few disadvantages exist for cell immobilization, the notable negatives are listed below:
- Loss of biocatalyst (cells or enzyme) stability and its metabolic activity.
- Mostly diffusion resistance problems may occur that can slow down the process, with results substrate/product inhibition, an increase of lag phase or deactivation of immobilized catalyst can occur.
- Diffusion problems for a substrate/product with large size or molecular weight.
- Mass transfer limitations for cells grown in an immobilizing matrix.
- Mass transfer gradients develop for immobilized cell substrates or products.
- Cells may not maintain a physiological state, resulting in a decrease in productivity.
- A higher investment cost is required.

1.2.3. Free vs immobilized cells

In the traditional ethanol fermentation technology, *Saccharomyces cerevisiae* has been exploited to convert glucose into ethanol and freely suspended yeast cells were used in batch fermentation technology. The use of *Saccharomyces cerevisiae* can give higher ethanol yields. The growth of the microorganism can be observed by the activities of living cells like cell dry weight, cell density, turbidity, different growth phases adopted by cells, metabolic rates, substrate consumption, and product formation (Najafpour 2015), (Lestari 2010). However, several drawbacks exist in this technology, such as more fermentation time needed coupled with low volumetric productivity. Free cells are more sensitive to nutrient depletion, pH variations, and certain inhibitory compounds. This inhibition might be due to glucose, product (ethanol), or yeast itself and could be due to toxification by physical, chemical, or biological reasons. Different microorganisms have different abilities to tolerate such inhibitors. It has been observed that free cells are not able to ferment hydrolyzates even at 24 hours (with a longer lag phase), while

immobilized cells can ferment in less than 10 hours with a very short lag phase time (Lestari 2010). (Hilge-Rotmann and Rehm 1990); (Angelova, Pashova 2000).

Free cell reactions are called homogeneous catalytic reactions, where the reactant (glucose) and the catalyst are in one phase (mass transfer effect is negligible). When cells are immobilized in different matrices, such as cross-linked alginate beads or chemically modified sponges, the reaction is called heterogeneous, where reactants and catalysts are in different phases. The catalyst normally is in the solid phase and reactants are in the liquid phase (mass transfer effect exists) (Klaewkla, Arend 2011).

Yeast cell growth, physiology, and metabolism are severely affected by mass transfer properties. The glucose and other nutrients in the fermentation medium must diffuse in the yeast cells for the product or other metabolites to come out into the medium, otherwise, the latter could be toxic or show inhibition to cells, which decreases productivity (Pilkington, Margaritis 1998).

1.2.4. Cell Immobilization Techniques

Cell immobilization techniques hold many advantages for the industry but are not yet fully capitalized on. The ethanol industry, for example, could benefit from developing methods involving cell immobilization. Several microorganisms have an innate ability to stick on natural surfaces, but immobilization techniques presented by the biotechnologist have additional advantages (Kourkoutas, Bekatorou, et al. 2004). An Immobilizing matrix can be made either into natural or synthetic materials. Porous structures, such as wooden scobs, charcoal, cellulose, and wood, are examples of natural immobilizing matrices used in ethanol and vinegar production. Natural polymers like agarose, alginate, carrageenan, and pectate are used for their properties of diffusion, as well as, other biological and chemical characteristics. (Krasňan, Stloukal, et al. 2016).

Based on physical attachment, four types of immobilization techniques exist:(Pilkington, Margaritis et al. 1998).

- Adsorption
- Self-aggregation
- Membrane confinement
- Entrapment

1.2.4.1 Adsorption

Cells can be immobilized on solid surface by adsorption. Two adsorption methods are proposed: electrostatic binding and covalent binding to the solid surface. Several types of solid materials can be used, including wood, sawdust, and DEAE-cellulose. In this method, there is a possibility of cell detachment which is related to the strength of the adsorption and the size of the cell film.

The cells can be treated using polycation, chitosan, and other chemicals to enhance their adsorption to the solid surface (Kourkoutas, Bekatorou et al. 2004); (Norton and D'Amore 1994).

1.2.4.2. Self-aggregation

Self-aggregation is a natural and simple method of immobilization.is The cells aggregate to form a larger unit like flocs or biofilm, in which a mixed population of organisms serves as the matrix. There are some artificial aggregating agents or crosslinkers that can boost up the cell's aggregation. For stable aggregation multivalent ions (Ca-2) and polyelectrolytes (Chitosan) is used as an aggregating agent. Different parameters or cultural conditions have a big role in stabilizing the aggregation of microorganisms (Buchholz, Kasche, et al. 2005).

Biological wastewater treatment is possible with this method by immobilizing the bacteria. This method is attractive as it has a low cost, high efficiency, and operational stability. Moreover, this kind of aggregation is an important phenomenon in mold, fungi as well as in Nitrosomonas and Nitrobacter sp. especially for the oxidation of ammonia to nitrate in water (Buchholz, Kasche et al. 2005). Fungi is a mycelial organism and can aggregate. It has two steps for aggregation or pellets formation: first at the time of inoculation and second during germination or hyphal growth. However, the yeast Saccharomyces cerevisiae, which is used in the production of alcoholic beverages production, does not have the natural ability to self-aggregate. As a result, the artificial aggregation has to be induced using a flocculating agent or cross-linkers. (Kourkoutas, Bekatorou, et al. 2004), Jin and Speers, 1998). Inducing aggregation improves the productivity of ethanol production.

1.2.4.3. Membrane Confinement

Membrane confinement can be accomplished using microporous membrane filters, cell entrapment in the microcapsule, or by immobilizing cells on interaction surfaces of two immiscible liquids. Mass transfer limitations are a major disadvantage of this technique(Kourkoutas, Bekatorou, et al. 2004).

1.2.4.4. Cells Entrapment

Cell entrapment uses a porous starting material to trap cells within a matrix. The substrate, product, and other metabolites can diffuse into the matrix. Polysaccharide gels, like alginate, k-carrageenan, agar, chitosan, gelatin, collagen, and polyvinyl alcohol, are the best starting material for this method. Complete retention of cells is also dependent on the solvent used. The solvent should be nontoxic for the cell's catalytic activity. Several gels have been studied and applied. (Buchholz, Kasche, et al. 2005).

1.2.4.5. Ionotropic gel: This type of gel is prepared with polyelectrolytes. The gel is prepared with low cost and natural polysaccharides and used under mild conditions.

1.2.4.6. Gelation: In this method, cells are suspended in polymer solutions with a specific temperature. After cooling the solution, networks of cross-linked polymeric gels with cation (Ca^{+2} and K^{+}) and anions (polyphosphates) is formed. Suitable examples are alginate, pectin, carrageenan, chitosan, and many more polysaccharides. Alginic acid has been used as a cell immobilizing agent in the research. Alginic acid is a polychronic acid and composed of D-mannuronic (M) and guluronic acid (G). The acids are arranged in block patterns of GG blocks, MM blocks, and MG blocks. The strength of the gel depends on the G blocks. The cation (e.g. Ca^{+2}) also prefers to bind to GG blocks resulting in a gel, which is mechanically stable and inert or non-toxic especially for foods or biotechnological research.

The porosity and cell biomass inside the matrix can influence the growth of the cells by creating diffusion limitations. Therefore, cells near the surface of support behave differently than the cells inside and the cells could potentially diffuse into the medium. This is the major problem in this type of immobilization technique and could be avoided by developing a double layer of different polysaccharides (Kourkoutas, Bekatorou, et al. 2004).

1.2.5. Types of support materials

For the application of cell immobilization in industry, the support material should be safe to use, cost-effective, easy, and abundantly available and should be approved by consumers. Through recent decades, natural support materials like clay, bricks, corn cobs, and organic and inorganic polymers have been used in industries. Supports used for the fermentation process are classified as organic (polysaccharides, cellulose, alginates, chitosan, carrageenan, agar, and pectic acid), inorganic (porous alumina and mineral kissiris), membrane systems and natural supports (wood or fruits (Svec and Gemeiner 1996).

1.2.5.1. Beads

Currently, natural and synthetic polymers are used for cell immobilization. Calcium alginate beads are widely used in the immobilization of bacteria, yeast, fungi, and algae for different bioprocesses (Gòdia, Casas et al. 1987); (Kourkoutas, Bekatorou, et al. 2004); (Pacheco); (Galaction, Kloetzer et al. 2012); (Duarte, Rodrigues et al. 2013). These polymers have possible applications in bioethanol production, vinegar production, and wastewater treatment due to its simplicity, low cost, non-toxicity to cells, and good mechanical properties. Alginate beads, as supporting materials, were tested on a large industrial scale and proven to have operational stability, less maturation time, and required less investment. Although they also have low

mechanical stability, high mass transfer limitations and the cells are poorly regenerated. On the other hand, DEAE-cellulose has higher mechanical stability and less mass transfer limitations (Kourkoutas, Bekatorou, et al. 2004). Ciani and Ferraro performed some experiments by immobilizing cells on Ca-alginate for the production of glycerol and found in the fermentation rate doubled and the product yield increased, in comparison to free cells (Ciani and Ferraro 1996). Similar observations were reported by a different scientist for the production of different products (Hilge-Rotmann and Rehm 1990), (Angelova, Pashova et al. 2000);(Jamai, Sendide et al. 2001); (Kourkoutas, Koutinas et al. 2002) and (Iconomopoulou, Kanellaki et al. 2003); (Kourkoutas, Bekatorou et al. 2004), (Hussain, Kangwa et al. 2015).

The disadvantages of the alginate beads can be eliminated with a combination of chitosan, a polycationic polymer, and alginate, a polyanionic polymer. The polymer is diffused into the alginate beads to provide a strong ionic interaction between the chitosan amino groups and the carboxyl groups of alginate which forms a polyelectrolyte complex (PEC), giving more mechanical support to cells (Yu, Zhang et al. 2007); (Galaction, Kloetzer et al. 2012); (Duarte, Rodrigues et al. 2013). DEAE-chitosan was proposed as the most suitable hydrogel support for alcohol making (Kosseva, Beschkov, et al. 1998); (Spagna, Barbagallo, et al. 2001)and (Maicas, Pardo, et al. 2001).

1.2.5.2. Synthetic Polymers

Synthetic polymers have been prepared which overcome the drawbacks of natural polymers. Some notable examples are polyurethane, polyacrylamide, and polyvinyl alcohol. These kinds of polymers are very common for cells and enzyme immobilization. By chemical reaction of polycondensation and polyaddition, a stable network for cells, have been developed with characteristics of chemical and mechanical stability e.g. Polyurethanes are developed for conversion of hydrophilic and hydrophobic substances but not yet used on industrial scale.

1.2.5.3. Membranes

Membranes are mostly used in the downstream process and membrane bioreactors. These allow certain kinds of substances to pass between two phases. The best examples are polyethylene, polypropylene, polysulfone, polyamides. It is common for these materials to be prepared as an inert material. However, activation of the surface is required for covalent immobilization of biocatalyst (Svec and Gemeiner 1996).

1.2.5.4. Cellulose

Cellulose is known as polysaccharide and has crosslinking structure. This structure was formed by a polymerization reaction of monomers like divinylbenzene, methylene bis acrylamide, ethylene dimethacrylate, and trimethylolopropane trimethacrylate. Cellulose cannot be easily soluble

even at high temperature because it has hydrogen bonds in its structure (Svec and Gemeiner 1996).

1.2.5.5. Fibers

For the immobilization of cells and enzymes, many types of fibers like polyvinyl alcohol and polyethylene co acrylic acids and many more are being used. Such fibers have no diffusional restriction like other membranes (Svec and Gemeiner 1996).

1.3. HOMOGENEOUS BIO-CATALYSIS

1.3.1. Engineering Aspects of Biotechnology

The bioreactor is assimilated with the heart of biotechnological processes, being the equipment in which the substrate is converted to the desired product(s) as described above. The range of a laboratory reactor is 2-100 L and for commercial large-scale reactors, the volume ranges up to 100,000 L. Bioreactors are an important part of the upstream process that consists of cell storage, media preparation, sterilization, inoculation, and cells growth (Baltaru, Galaction, et al. 2009); (Najafpour 2015). On the other hand, much less interest has been given to the design of new types of bioreactors with increased volumetric productivities.

Moreover, the concrete applications of these developments to the biochemical processes industries are scare. The design of the bioreactor is important especially to satisfy fluid dynamic conditions and efficient transfer of substrate in and out of the biocatalyst surface. Impellers could create shear effects on the cells as well as on the immobilizing matrix. In the case of higher shear effects, the cells could leak the immobilized matrix.

1.3.2. Mode of Operations of Bioreactors (Fermentation Systems)

There are four types of industrial operations used for ethanol production: batch, fed-batch, continuous, and semi continuous (Jain and Chaurasia 2014).

1.3.2.1. Batch Fermentation

In this operational configuration, the substrate, microorganism, and nutrients (growing media) are placed all together into the closed system of a bioreactor. In this system, the microorganism gradually grows by introducing inoculums into the medium. The growth of the cells is determined as they propagate and consume the nutrients to form the product. Some advantages and disadvantages are highlighted in the literature (Najafpour 2015), (Jain and Chaurasia 2014), (Kadic and Heindel 2014). The advantageous aspect of a batch fermentation process lies in its simplicity and well-controlled system. As the growth of the microorganism proceeds, the concentration of nutrients, cells, and products can be easily determined with time. Physical and

chemical parameters influence substrate consumption and product formation can be determined in a short time of a growth cycle. This batch system is flexible for various specific products and the ease of its sterilization.

The disadvantages, however, are the fact that this is a closed system i.e. no effluent comes out from the reactor, which could become toxic for cells. As a result, the growth of cells does not follow all the phases in the growth curve if there is (i) a toxic environment produced due to by-products, (ii) accumulation of products (iii) depletion of nutrients. While the cells are growing, the medium composition changes and thus shortens the exponential growth phase. Within the working volume of this medium, there is a major problem of nutrients and substrate limitation/depletion. As a result, the death phase of cell growth may be reached quickly. Due to the above-mentioned drawbacks, the batch process seems to be highly laborious and to required more down-time.

1.3.2.2. Fed-Batch Fermentation

In fed-batch fermentation, the substrate, microorganisms, and the growing medium are fed at constant intervals. This system improves the process of productivity by using a low substrate concentration. This system can overcome substrate limitations and substrate inhibition could be prevented by feeding at constant intervals. Substrate limitations can occur in such a system that can be the reason for the repression of the growth rate. The reaction can be limited by heat if it is not controlled well.

1.3.2.3. Continuous Fermentation

In the continuous system, the media is continuously flowing to the bioreactor, while the converted solution is coming out with a product (s) as well as with microorganisms simultaneously. The objective of improving fermentation productivity by reducing the cost of the process makes this operation mode attractive. Sequential continuous cultures are also designed to enhance productivity and utilize the remaining unused substrate if left in the medium.

Continuous fermentation offers many advantages over batch fermentation because it offers flexible operational control that makes the product more profitable. This system can be automated (i.e. reduces labor costs) and there is less need for a sterilization process. In this operation mode, optimization is possible by varying the inputs and growth conditions, where a maximum growth rate can be obtained by adjusting essential nutrients in the medium. Higher productivity can be achieved here per unit volume and does not require more downtime. At the maximum flow rate, the substrate, product(s) concentration, and cell density remain constant.

The optimization of these above-mentioned variables can maximize the production time leading to an economical and consistent steady-state operation production. By using immobilized cells in

this system, free excess cells are removed. Besides, old cultures are replaced by fresh cultures that can enhance the continuous production of cells. Some drawbacks of the continuous process are related to contamination problems and by-product(s) formation that can reduce the quality of the desired product. A longer process like this one can reduce the steady growth of cells and cause cell aggregation on the bioreactor walls. High viscosity can also develop creating difficulties in the steady growth of filamentous microorganisms (Najafpour 2015), (Jain and Chaurasia 2014), (Kadic and Heindel 2014).

1.4. HETEROGENEOUS BIO-CATALYSIS

The homogenous reaction takes place when the reactants, products, and catalysts are present in the same phase. Biological reactions are supposed to be homogeneous in a single-phase system. Mostly these reactions take place in the liquid phase, but also in the gaseous phase. Some examples are the acid-base catalysis, esterification of alcohols, hydrolysis of esters, and inversion of sugars. (Klaewkla, Arend, et al. 2011); Falconer J.L. 1998).

The heterogeneous reaction takes place when catalysts are in a different phase as compared to reactants and products (Klaewkla, Arend, et al. 2011). Some examples are the production of ammonia, ethanol, methanol, organic polymers, and many more organic and inorganic chemicals (Falconer J.L. 1998). In heterogeneous reactions, catalysts are normally in the solid phase while reactants and products might be either in the liquid or the gaseous phases. Therefore, due to the presence of different phases, the reaction inside the catalyst depends on the mass transfer process. On the other hand, in multi-phase systems, the substrate and product concentration difference exists. The biological reaction takes place in the presence of a large concentration gradient. Heterogeneous reactions are common in bioprocesses and have more importance using solid biocatalysts, that can be produced by some bacteria and fungi like cell flocs, clumps, biofilms, pellets, and immobilized cells.

1.5. BIOREACTOR DESIGN

Traditional setups like membrane, airlift, and stirrer tank bioreactors have been used in bio-ethanol production. These have some drawbacks of low product yield due to a low mass and heat transfer, inefficient conversion of the substrate, uneven mixing and distribution of flow, and shear stress on biocatalysts. Therefore, there is a need to utilize a reactor that can sustain an excellent hydrodynamic regime coupled to reduce the overall mass transfer limitations (Saini and Vieth 1975); (Pilkington, Margaritis et al. 1998); (Karagöz and Özkan 2014).

1.5.1. Packed Bed Bioreactor

For the immobilization technology in ethanol production, the design and scale-up of a bioreactor are important factors. For lab-scale bioreactors, immobilized cells with batch or continuous processes are proposed, while most efforts on the industrial scale are the development of a bioreactor and its application (Kourkoutas, Bekatorou, et al. 2004). The industrial production of ethanol or fine chemicals requires the development of new bio-catalytic reactors and immobilizing materials for cells to achieve economically viable processes by eliminating heat and mass transfer effects. Several traditional setups are available for the heterogeneous catalytic reaction. These include membrane bioreactor, airlift bioreactor, tubular flow or plug flow reactor, and packed bed bioreactors. These have some drawbacks of low product yield due to a low mass and heat transfer, inefficient conversion of the substrate, uneven mixing and distribution of flow, and shear stress on biocatalysts (Hussain, Kangwa, et al. 2015).

Packed bed bioreactor can be used as a reference reactor for heterogeneous catalytic reactions and the diffusional or mass transfer effects can be predicted accurately. Different flow characteristics have been observed in many bioreactors that influence mass transfer between different phases. The mass transfer between phases is a critical problem that can influence the catalyst activity. In this reaction, reactants are in the liquid phase, which diffuses through the boundary layer called external diffusion or external mass transfer and then diffuse through the pores into the internal catalytic surface called internal diffusion of internal mass transfer. When products are formed, they should desorb or diffuse to the bulk liquid (Klaewkla, Arend, et al. 2011). To overcome the problems in mass transfer and improve the efficiency of a bioreactor, the most important factor needed is the design of a bioreactor, which can be a difficult task in ethanol-producing industries through fermentation using immobilized cells in different matrices (Klaewkla, Arend, et al. 2011).

Packed bed bioreactor (PBR) was most popular, especially for higher substrate conversion and productivity. Mass transfer is an important parameter for improving substrate consumption and productivity in PBR. To consider an external mass transfer, the choice of bioreactor and hydrodynamic conditions used are very important. On the industrial level, many problems regarding external mass transfer have been observed using PBR, especially channeling due to the use of different types of immobilizing materials, pressure drop due to different size of matrix or beads, and reactor plugging due to a difficulty in evacuating the CO_2 produced during the fermentation process (Norton and D'Amore 1994).

A packed bed bioreactor (PBR) with one bed containing immobilized beads was used as a prototype to understand mass transfer or flow through alginate beads. It contains a vessel for

culture medium, in which the culture medium is circulated from the vessel through the fixed bed and back (Figure 1.4a & b). A medium enriched with glucose re-enters the packed bed where it can be re-utilized to convert glucose into ethanol. Toxic metabolites and other by-products are diluted; oxygen and pH can be adjusted to optimal levels. It has several advantages over other bioreactors like low manufacturing and operating costs, automation process, and the ability to operate at low temperatures.

This work focuses on the operational performance of the immobilized packed-bed bioreactor during physiological and biochemical studies on the substrate uptake of immobilized yeast cells. The reactor was operated in a batch mode fermentation operation. The yeast physiology and mass transfer behaviors in PBR were monitored according to parameters such as glucose concentration, medium flow rate, and different support materials like alginate beads with and without chitosan coating.

Figure 1.4a. Schematic illustration of a packed bed reactor (PBR) of 100 ml media volume and a 20 ml capacity bead column.

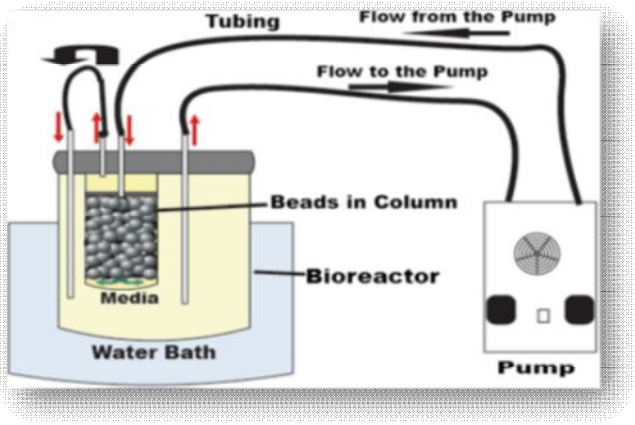


Figure 1.4b. Packed Bed Bioreactor from Medorex.e.K

It has also been shown that the immobilization of yeast cells in alginate beads is one of the best strategies for improved industrial ethanol production and an easy scale-up of the bioreactor (Chen, Wang, et al. 2012); (Chien and Sofer 1985). The main problem in a bioreactor system is the inadequate transfer of the substrate, product, and other metabolites towards cells and out of the immobilizing matrix (Núñez and Lema 1987). Recently, scientists have shown great interest in the application of PBR for bio-ethanol production due to its low manufacturing and operating costs (Galaction, Kloetzer, et al. 2012); (Rivaldi, Sarrouh et al. 2008).

A comprehensive understanding of mass transfer in an immobilized system using PBR as a prototype is required to achieve maximum productivity and bioreactor performance (Warnock, Bratch, et al. 2005); (de Jong, Siewers et al. 2012). There are two major steps involved in the substrate transfer to immobilized alginate beads: (1) Transfer of substrate from a homogeneous bulk liquid to the external surface of beads passing through a hypothetically stagnant liquid film around the beads (external mass transfer). Transfer of substrate to microorganisms through pores (internal mass transfer).

The study on PBR with immobilized *S. cerevisiae* cells in alginate beads shows that it is possible to have efficient external mass transfer without any loss of cell growth and physiology by selecting an optimum flow rate. It is possible to continue investigating the operational performance of the immobilized packed-bed bioreactor during physiological and biochemical studies on the substrate uptake of immobilized yeast cells (Hussain, Kangwa, et al. 2015).

The reactor was operated in a batch mode fermentation operation. The yeast physiology and mass transfer behaviors in PBR were monitored according to parameters such as glucose concentration, medium flow rate, and the different sizes of support materials. The use of PBR in the research is as a lab-scale reactor to understand how the system works in case of mass transfer

1.5.2. Stirrer Tank Reactor (STR)

For many reasons from a technological and cost-effective nature, the production of biochemicals needs a reactor that can meet several different running conditions, such as varying viscosity, aeration rates, varying agitation intensity, and changing broth volume. All of these can be achieved in a stirred tank-type reactor, as in the SCBR reactor, but not so easily in other types of reactors. Liquid-Solid reactions to produce ethanol or fine chemicals are common in the biochemical industry that is heterogeneously catalyzed.

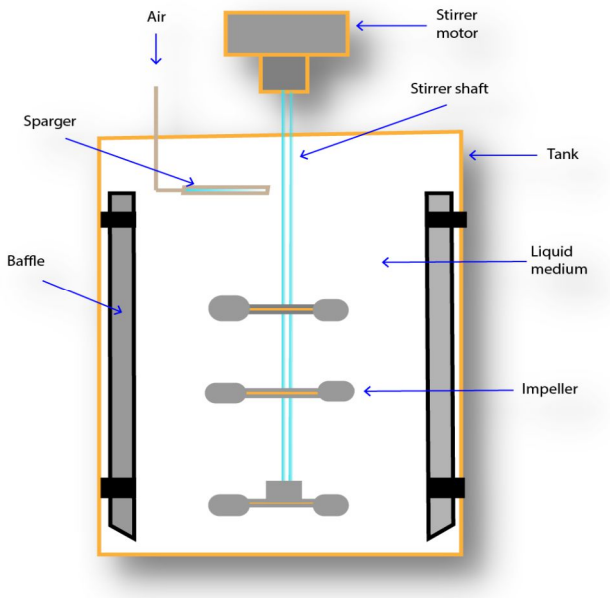
A well-mixed operation is a very important factor to ensure an efficient bioprocess. Overall, the distribution of substrate and product(s) can be determined by the flow and mixing conditions of a bioreactor that ultimately affects the reaction kinetics (Nedovic and Willaert 2013). Mixing

helps to homogenize the temperature of the culture medium, break up bubbles, eliminate dispersion and concentration gradient, and eliminate mass transfer resistance. Mixing creates the optimal environment for cells to access the substrate to make the desired product.

Different bioreactors have different methods of mixing operation. Here the mixing possibilities in the stirred tank reactor will be discussed because these properties are the same as the stirred catalytic basket bioreactor (SCBR). Mixing could occur using baffles that are strips of metal against the wall of the tank. They help to create turbulence in the liquid and stop liquid swirling and vortexing. A sparger mixing system could also be used and it improves gas mixing: when a gas bubble is dispersed from a sparger to the impeller, bigger bubbles will breakdown into smaller bubbles through the shear effect of the impeller making it easy for microorganisms to use gas (especially oxygen) to form product(s).

The usage of a stirrer shaft is also one option that helps to support and rotating the impeller in a specific torque. A stirrer shaft should reach to the base of the vessel to avoid mechanical stress. This is usually used for aerobic fermentation. Impellers are also important in mixing operations and can be attached with a stirrer shaft that is driven by an electric motor. Impeller helps in creating regular flows of liquid by pumping liquid from the bottom to the top and vice versa.

Figure 1.5. Configuration of stirred tank reactor (Rushton J.H., 1950)



1.5.2.1. Flow Patterns in Bioreactors

There are three types of flow patterns: rotational flow (movement of liquid around the stirrer shaft), radial flow (liquid moves from the central point of the reactor to the sidewalls, and then

back to center), and axial flow (up and down movements). Impellers are classified according to the flow regime of liquid.

1.5.2.1.1. Rotational Flow

All types of impellers generate a rotational flow inside the reactor, but it should be avoided by using different shapes of impellers. In this kind of liquid flow, upper and lower mixing is not possible and can generate vortexing and turbulence due to mechanical stress.

Figure 1.6 (a). Circular flow in an unbaffled stirred tank (Rushton J.H., 1950).

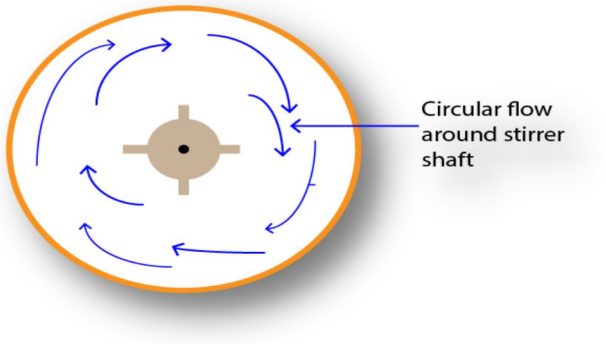
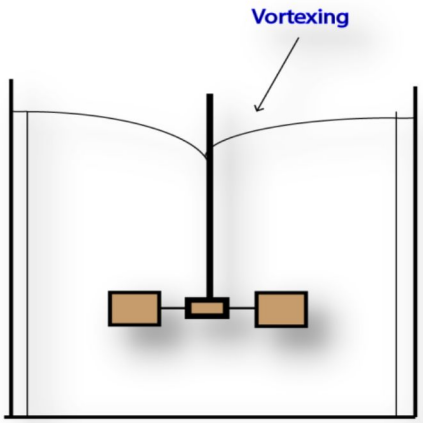


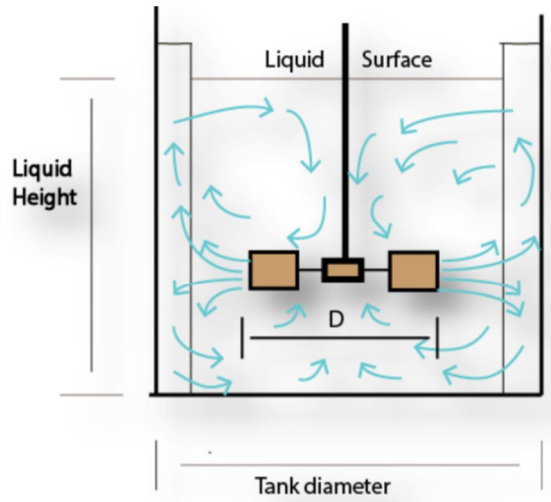
Figure 1.6 (b). Vortex formation during circular flow (Rushton J.H., 1950).



1.5.2.1.2. Radial Flow

Radial flow can be generated if impellers have blades parallel to the motor shaft. Such kind of flow is shown in Figure 1.7, where liquid moves from the impellers to the direction of vessel walls, and after striking on the walls, some of the liquid moves upwards, and some move downwards to eventually reach the central axis of the impeller. This kind of impellers also generates rotational flows, but such a flow can be reduced using baffles (Rushton J.H., 1950).

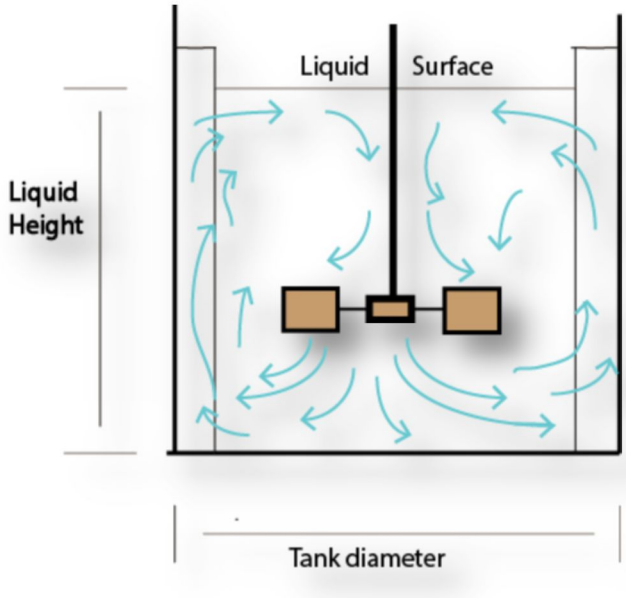
Figure 1.7. Flow pattern produced by a radial flow impeller in a baffled tank .



1.5.2.1.3. Axial Flow

For the mixing of up and down regions from the central axis of the impeller, axial flow is necessary that can be achieved by using impellers having inclined blades. Such kind of liquid flow is necessary to enhance the mass transfer properties of a reactor. As shown in figure 1.8, fluid moves from the downside to the bottom of the vessel and after deflecting, it moves up to the walls of the vessel and deflects to the direction of the impeller. With such impellers, rotational flows can be generated that is avoided using baffles (Rushton J.H., 1950).

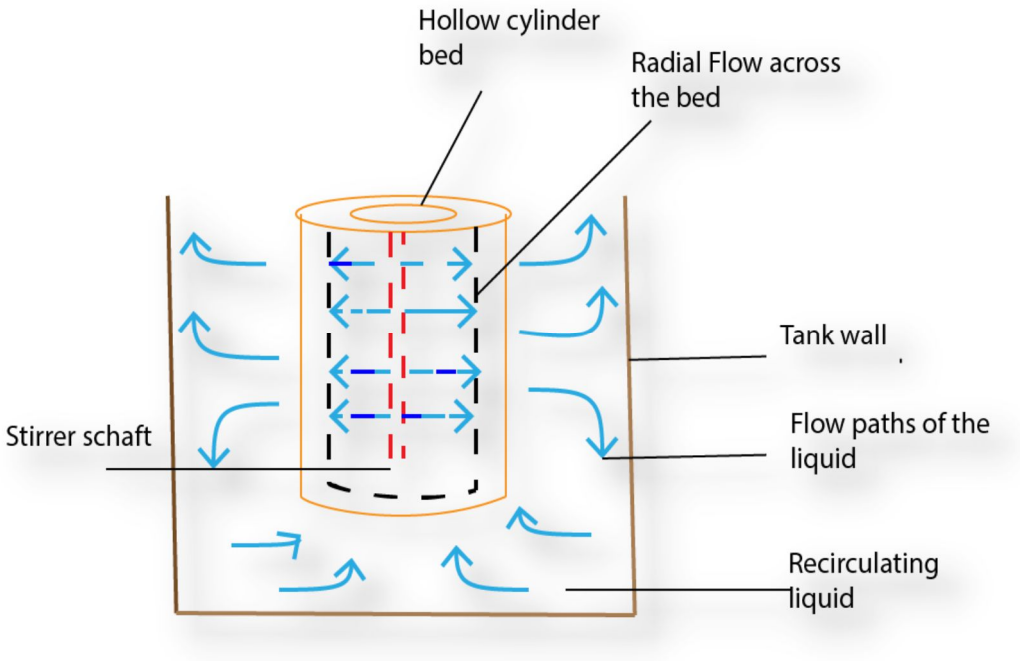
Figure 1.8. Flow pattern produced by an axial flow impeller in a baffled tank .



1.5.3. Stirred Catalytic Basket Reactor (SCBR)

In reality, SCBR behaves as a plug flow reactor in terms of the internal recycle of the broth/media created by the stirring. In effect, the blades of the agitator operate like a centrifugal pump that imposes a radially directed flow of liquid through the hollow bed as shown in Figure 1.9. This stream, after getting in contact with the wall of the fermenter, returns to the center of the hollow cylinder bed (basket) and, in this way, the broth recycles internally many times ensuring an easy and very accurate control of the pH and temperature. Consequently, the hydrodynamics of the broth in and around the basket shows an important effect on the transfer processes involved in substrate conversion. The SCBR is a simple and modified form of a stirred tank reactor that presents some of the advantages of a fixed bed reactor. This reactor does not depend on the superficial liquid velocity, but rather on the impeller rotation speed. It can be operated continuously at a low feed rate and high residence time. Due to the high residence time and high mass transfer coefficient caused by the impeller rotation speed, the substrate conversion increases (Mowena, Zaatout, et al. 2013).

Figure 1.9. Flow pattern produced by a radial and axial flow impeller in SCBR.



1.5.3.1. Use of Stirred Catalytic Basket Reactor

The Stirred Catalytic Basket Reactor (SCBR) was first designed by (Carberry 1964) and has been employed in a variety of applications in conventional chemical catalysis. It is also known as a "gradient less" reactor and has mainly been used for kinetic studies in the field of gas-solid heterogeneous catalytic reaction engineering. The SCBR seems to be specifically well suited to produce high value fine biochemical and ethanol as a fuel. These fine biochemicals are expensive products with low volumes and it is difficult to continuously produce them in high throughput with fluidized or fixed beds. The SCBR can run as a batch as well as a continuous process with very high mass transfer rates and yields even at a small scale. Many configurations are possible in this type of bioreactor having a basket (filled with immobilized catalysts) attached to the agitator shaft. The basket has the advantage of permitting greater contact between reactants and biocatalysts, which in turn increases the reaction rate and efficiency of the bio-catalytic reaction. The biocatalyst is separated from the reaction mixture simply by draining the circulating liquid (Baltaru, Galaction, et al. 2009)

The **advantages of the SCBR** can be summarized as follows:

1. It is versatile and can be operated batch-wise, continuously, or in a repeated batch mode.
2. It has easy and very accurate control of the pH and temperature due to a rapid circulation and recycles of the broth through the bed. In tubular fixed beds, longitudinal gradients of pH and temperature along the reactor are developed in such a way that much of it works in sub-optimal conditions. The control of this problem requires a complicated pumping system that adds to the costs and complicates the entire process. In the SCBR, pH is controlled by a simple titration with acid and base.
3. The SCBR is inherently more stable than tubular fixed-bed such as bed compaction, bed plugging, fluid by-passing, and short-circuiting and can reduce conversions, and complicate the handling of the entire plant. Plugging problems are especially noticeable in the case of immobilized cell systems. Fluidized beds may avoid some of the limitations of tubular fixed beds. However, since fluidized beds require a high fluid flow rate, they impose to have a very low residence time. In immobilized cell systems or enzyme technologies, very soft and breakable materials are used that are unable to support the violent liquid/solid movements and the attrition that characterizes the fluidized state. A continuous replacement of the catalyst, as in the petrochemical industry, is hardly possible. Furthermore, in the case of ethanol manufacture, back mixing is kinetically undesirable.

4. The SCBR seems to be especially well-suited to produce high value-added fine biochemicals, such as hormones, proteins, pharmaceuticals, secondary metabolites of plant cells, etc. In effect, the production of fine biochemical must comply with very rigorous quality control requirements and those can only be fulfilled in batch processing. Since fine biochemicals are low in volume and they are expensive products, their continuous production in high throughput fluidized or fixed beds is hard to justify. Instead, the batch processing capability of the SCBR and the very high mass transfer rates it can achieve combined with high productivity at a small scale seems to be an attractive option,.

1.5.4. Role of Fluid Dynamics on Mass Transfer (Dimensionless Correlation)

All fermentation broths have different rheological properties, such as high viscosity or flow behavior that can influence the turbulence of the fluid, dispersion of nutrients, as well as the broth homogeneous composition. Rheological properties can also reduce the turbulence of liquid that is most important for effective mixing. Some other rheological properties like fluid velocity, turbulence kinetic energy, eddy diffusion, shear effect of impellers, and the design of bioreactors can affect the efficiency of the process especially in case of nutrients consumption by microorganisms and product formation (Doran, 2012).

1.5.4.1. Efficient Mixing Mechanism

For efficient mixing of the fluid in a bioreactor, the optimal velocity of the fluid should provide the fluid ability to circulate and carry out materials from the impeller to all regions of the vessel. Poor mixing can affect the mass transfer properties of a bioreactor as well as on the physiology of cells (free or immobilized). Three physical processes affect the mixing efficiency of bioreactor: (i) distribution can be called as macromixing (ii) diffusion is a kind of micro-mixing, and (iii) dispersion, which depends on the movement of liquid broth, which can be micro as well as the macro-level of mixing. Distribution is an important but slow process. If the speed of the impeller is high, it affects the distribution process that creates turbulence. Turbulence is also necessary, but it allows fluid movements on a smaller scale (Doran, 2012).

1.5.4.2. Impellers Shear Effect in Bioreactors

The fluid mixing and turbulence of fluids depend on the system geometry and the design of impellers. Multiple impellers especially a combination of Rushton turbine and a downward pumping of pitched blade turbines are most important to reduce the shear effect of impellers especially on microorganism metabolism. The effect of impeller shear on cells is still not

understood, particularly its effect on nutrients consumption, cell behavior, product yield, and mass transfer properties of a bioreactor. All these parameters are discussed in our studies (Doran, 2012).

1.5.4.3. Flow in porous matrices

Mixing or fluid flow behavior is an important operation that creates an optimal environment for the consumption of nutrients by cells and reduces the non-uniformity of temperature, concentration, velocity, and pressure gradients (Mowena, Zaatout et al. 2013). Laminar flow through pores of porous materials is characterized in the form of streamlines where viscous forces are greatly involved as compared to inertial forces. Fluid elements follow the same path from one point to another point throughout its course.

As fluid flows through pores it adheres to the surface with the result fluid velocity become zero or 'No-slip' velocity occurs. Due to this and the viscosity of the fluid, drag forces are exerted on solid. In laminar steady flow lateral forces that are responsible for microscopic variation in velocity expected to zero as compared to macroscopic variation. It can be expected that inertial forces cannot be zero only negligible at laminar flow.

Laminar flow changes into turbulent flow due to the higher flow rate in pore channels. As fluid flows through pores tortuous paths, velocity, and forces on fluid elements change from one point to another point. Further due to the structure of pores, higher flow rate variation occurs, and inertial forces become dominant over viscous forces. In turbulent flow, forces exerted over fluid (mechanical or pressure difference) can change the direction as well as the magnitude of velocity. Such random variation will be uniform in the whole flow path.

The convective mass transfer has importance in the case of flow inside a stirred catalytic basket bioreactor which involves the transfer of mass between boundary surface and circulating fluid (Welty, Wilson, et al. 2008). Kolmogorov has proposed such a theory of isotropic turbulence that gives information about turbulence intensity around the particle. Further shows that mass transfer rate depends on the particle diameter and physical properties (Lal, Kumar, et al. 1988).

1.6. PROBLEMS STATEMENTS AND RESEARCH OBJECTIVES

Some solutions are mentioned here to solve the mass transfer problems statements:

I. Stirrer Catalytic Basket Reactor is the best reactor to minimize mass transfer limitations in immobilized system.

II. DEAE, newly developed polymer is best to use for enhancing productivity.

The immobilization technique offers numerous advantages over free cells in the ethanol industry. Further, Packed bed bioreactors have been used for a long time for ethanol production due to higher productivities achieved using a continuous fermentation process. On the other hand,

many disadvantages like the low operational stability and the non-viability of yeast cells during the process have been observed. Even on the industrial level, several engineering problems (mass transfer) have been seen, such as channeling in between carriers, drop in pressure, plugging of the bioreactor by a widespread growth, and higher production of product content or Co_2 **Therefore, Packed bed bioreactor as prototype reactor with different size of alginate beads will be tested to understand the external & internal mass transfer limitations.** Furthermore, research objectives are further briefly discussed here:

1. Understanding of Fluid-side and Particle-side Mass Transfer in PBR

I. Monitoring the influence of operating parameters on fluid-side mass transfer, ethanol yield, productivity, and performance of packed bed bioreactor (PBR).

II. Monitoring the influence of operating parameters on particle-side mass transfer behavior, yeast physiology, ethanol yield, productivity and performance of packed bed reactor.

2. Mass Transfer Evaluation of SCBR and Newly developed Polymer (Dimensional Correlation)

Testing the performance of Stirred Catalytic Basket Reactor (SCBR) with immobilized cells in different matrixes (alginate beads and DEAE polymers) and comparing it to free cells in a STR-stirred tank reactor in terms of the effect of mass transfer on cells physiology, process completion time and volumetric productivity, ethanol yield.

3. Role of fluid dynamics in Reactor Performance (Dimensionless Correlation)

I. Role of fluid dynamics in mass transfer using alginate beads in Packed Bed

II. Role of fluid dynamics in mass transfer using alginate beads in SCBR Basket

III. Role of fluid dynamics in mass transfer using Macro-porous polymer in SCBR Basket

1.7. RESEARCH METHODOLOGY

1.7.1. Part I (Chapter 3)

The 1st part of this study focuses on the operational performance of the immobilized packed-bed bioreactor as a prototype in the course of physiological and biochemical studies on the substrate uptake of immobilized yeast cells. The traditional support material for cell immobilization i.e. alginate beads were selected where a simple entrapment method of immobilized cells is used. The influence of operating parameters (fluid flow rate, glucose concentration, size of beads, and immobilizing matrix) on fluid-side and particle-side mass transfer (transfer of reactants to and products from fluid to surface of the particle and from the surface to inside the particle), yeast physiology, the appearance of lag phase, glucose consumption time, ethanol yield, productivity, and performance of bioreactor. The packed bed reactor was operated in a batch mode fermentation operation because batch culture provides the best benchmark for comparison of parameters. Batch culture can be the best development strategy in terms of both time and money and even most conventional ethanol production plants work in this mode.

Further large-scale productions of these carriers require complex and sophisticated equipment making this immobilization method expensive. After understanding the external and internal mass transfer using Packed Bed Bioreactor as a prototype, a newly produced macro-porous polymer was tested in scaled-up reactor.

1.7.2. Part II (Chapter 4)

The 2nd part of this work was to observe the performance of stirred catalytic basket reactor (SCBR) with immobilized cells in different matrixes (alginate beads and grafted polymers) to compare them to free cells in an STR-stirred tank reactor. The performance of the Stirred Catalytic Basket reactor (SCBR) was evaluated by monitoring the influence of operating parameters (impeller stirrer speed, glucose concentration, and immobilizing matrix) on mass transfer, yeast physiology, the appearance of lag phase, glucose consumption time, ethanol yield, productivity.

The variation of ethanol production and diffusion of the substrate in the fermentation process are co-related with the stirrer speed and initial glucose concentration. Also, the diffusion of nutrients to the cells and product into the media is dictated by the immobilization method of the cells (i.e. using alginate beads or chemically grafted inert sponges). Furthermore, the effect of mass transfer on cell physiology and ethanol productivity was studied. For evaluating its performance, the effect of glucose concentration and agitation speed on the characteristics of glucose consumption was measured using the ‘‘DNS’’ method and ethanol productivity was

determined using gas chromatography. In SCBR and STR reactors, a complete mixing with the help of agitation controls the pH and temperature.

1.7.3. Part III (Chapter 5)

For selecting, designing, and developing bioreactors, it is necessary to have basic information about the effect of hydrodynamic behavior on mass transfer in a two-phase system (liquid-solid). Further alginate beads and sponges results were evaluated by correlating mass transfer with dimensionless numbers. From results it has been observed that newly developed sponges enhance the mass transfer due to its properties of short contact time with high reaction rate and low-pressure drop, high surface area, and radial mixing in tortuous pathways, Collins 1976).

The 3rd part of this work was to understand the role of fluid dynamics in mass transfer using an immobilized matrix (Alginate beads & DEAE polymer) in a packed bed reactor and Stirrer Catalytic Basket Reactor. It was evaluated by calculating dimensionless number (Re_p, εJD, Sh, Sc, ϵ) and their correlation with fluid flow, immobilizing matrix that ultimately effects on external and internal mass transfer.

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CHAPTER 2

PREPARATION OF BIOCATALYSTS:

Agarose Beads & Functionalised Porous

2.1. PREPARATION OF BIOCATAYSTS

In the process of ethanol production using immobilized cells, mass transfer is an important phenomenon because the consumption of substrate by cells encapsulated depends on three steps: the diffusion of the substrate from bulk medium to the capsule and the diffusion within the capsule that follows the third step of the biochemical reaction (Lestari 2010). Immobilized cells' survival and their efficiency depend on mass transfer properties.

Immobilizing support material represents a multiphase system, in which the reaction substrate is transported from the bulk phase to the active site of the support materials. The mass transfer mechanism depends upon the porosity of the support. Convection and diffusion of the substrate are important factors controlling the reaction rate. Two kinds of polymers exist. one is slightly cross-linked making mass transfer only possible when the polymers swell. The second is made up of highly cross-linked polymers, developed only by polymerization. These polymers cannot swell, therefore, increasing the porosity. Highly crossed linked polymers are called as macroporous. The porosity and morphology of the support materials depend upon the chemical nature of the support (Svec and Gemeiner 1996).

2.2. SUPPORT SELECTION

A packed bed bioreactor and a stirred catalytic basket bioreactor is used in the study with immobilized cells. Selection of support for these bioreactors is critical, especially in packed bed bioreactor because of the rate of mass transfer and back pressure as the substrate solution is pumped through the column. The back pressure depends on the flow of the medium, size of the column, and immobilized particles of support inside. Support of uniform size and shape can reduce the backpressure in a packed bed bioreactor and the shear stress in a stirred bioreactor. The supports which are soft and easily deformed are not suitable to use in packed bed bioreactor but can be used in a stirred tank or basket bioreactor.

Internal stress can exist inside the carrier due to osmotic pressure which depends upon the pH of the medium around it. Swelling of the support results due to the ion exchange properties of a medium. To avoid internal stress, the pH ion-exchange properties of the medium should be monitored (Svec and Gemeiner 1996).

2.2.1. Re-usability

The loss of enzyme activity is a major problem in industries and can be resolved by immobilizing them on different support materials. If the activity of the enzyme is loosed due to incorrect operation, leaking of the enzyme, or bacterial contamination then the support materials can still

be reused for immobilization. If enzymes are immobilized by weak non-covalent bonds with the support materials, then these can be easily reused by freeing the enzymes. This is possible by changing the ionic strength, pH, or other conditions of the surrounding medium. Inorganic support materials are easy to reuse because these are stable even at high temperatures. These materials with an immobilized enzyme can be kept at high temperature to burn off the enzymes. Following, the carriers must only be washed and reactivated by using again (Svec and Gemeiner 1996).

2.2.2. Chemistry

The chemical properties of the support materials affect the reactivity and binding of the catalysts. The support materials can either be activated or reactive monomers can be copolymerized. For the first method, several reaction steps are needed to activate the inactive support. Many examples are available in the literature for such types of reactions like the reaction of 2-hydroxyethyl methacrylate copolymers with epichlorohydrin or the reaction of silica with 3-aminopropyltrimethoxysilane (Svec and Gemeiner 1996). For obtaining active support materials in a single step, it's only possible by copolymerization of reactive monomers like 4-fluoroethylene or 3 fluoromethacryamide, 2,3-epoxypropyl methacrylate, 4-iodobubutyl methacrylate, and many more (Svec and Gemeiner 1996). The selection of immobilizing support material is very important because its chemistry can affect the enzyme immobilization. The microenvironment developed by these carriers can help in enzyme stability and activity.

2.2.3. Prerequisites for catalyst

1. To choose suitable immobilizing supports, the following prerequisites should be satisfied (Kourkoutas, Bekatorou, et al. 2004); (Martin and Patel 1991); (Freeman and Lilly 1998); (Frechet and Svec 1995).
2. It must have a functional group for cell adherence.
3. It should be easy to handle and regeneratable.
4. It should retain the cell viability and operational stability for a long time.
5. The support should be porous as it allows the free exchange of substrate, product, or other metabolites, otherwise, these can inhibit the reaction by developing a concentration gradient.
6. It should be able to maintain chemical and mechanical stability if it is to be used in a bioreactor for a long period.
7. For industrial-scale bioreactors, it's necessary to control the size and porosity of support material.

8. The support should be stable at temperature and pH which is suitable for the microorganism immobilized.
9. The support should not be harmful to microorganisms or it should be inert.
10. If cells are immobilized in the growing phase, then the support should be able to hold the maximum ability of cells carrying capacity.
11. The shape of support material should be stable in the working medium.
12. The materials should have resistance to external and internal mechanical forces.

2.3 CALCIUM ALGINATE BEADS PREPARATION

A sterile sodium alginate solution (2.5% w/v, autoclaved at 121°C, for 15 min, was prepared in 50 mM phosphate buffer at pH 7). For yeast immobilization, a 3% final amount of the above-obtained cell suspension was mixed with the alginate solution. For the beads preparation, the alginate-yeast solution was allowed to dip using a 1 ml pipette tip into 200 ml, 180 mM $CaCl_2$. Beads were let to harden in this solution for 1 h. Beads were further rinsed three times with a sterile 2% NaCl solution and then with sterile water. The alginate beads with diameters between 3 and 4 mm were used in these experiments. For the preparation of alginate beads with chitosan coating, the above-prepared beads were dipped in a sterilized chitosan solution (3% chitosan, 0.1 N HCl, pH 5) for 10 min and later washed 3 times with sterile water.

Figure 2.1. Alginate beads preparation

2.3.1. Results

Many methods like adsorption, covalent binding, cross-linking, entrapment, and encapsulation are widely used for immobilization (Terada, Yuasa et al. 2006); (Borovikova, Scherbaka et al. 2014); (De Bari, De Canio et al. 2013); (Zhao and Delancey 2000). However, there has been an increasing interest in the research and development of advanced materials to obtain polymers

with well-defined structures and specific chemical, physicochemical, mechanical, and biological properties. (Hussain, Kangwa, et al. 2015).

Natural and synthetic polymers, such as cellulose, alginate, chitosan, agarose polyurethane, and polyacrylate are currently being used for cells (bacteria, yeast, fungi, and algae) immobilization for different bioprocesses (Gòdia, Casas et al. 1987); (Duarte, Rodrigues, et al. 2013) and have a potential application in bioethanol production due to their simplicity, affordable price, non-toxicity to the cell, and good mechanical properties. However, some drawbacks with their usage lie in gel degradation, severe mass transfer limitations, low mechanical strength as it can cause the release of cells from the support (Caşcaval, Galaction, et al. 2012); (Converti, Perego et al. 1985). Additionally, cell growth and gas production might rupture the carrier gel during fermentation (Rao, Pundle, et al. 1986).

To overcome this, a combination of chitosan, a polycationic polymer, and alginate is used. A polyanionic polymer is diffused into the alginate beads to provide a strong ionic interaction between chitosan amino groups and carboxyl groups of alginate, which forms a polyelectrolyte complex (PEC) that gives more mechanical support to cells.

Figure 2.1. (a) Cells immobilization in Alginate beads (b). Beads rupture during process.

2.3.2. Discussion

The immobilization of cells in alginate beads could be applied in various industries especially in industrial biotechnology. The formation of alginate beads is the result of gelation having a network in three dimensions. This gelation network will be formed when Ca+2 interacts with G-blocks of the alginate solution (Liouni, Drichoutis, et al. 2008). From the last two decades, this has been attracting attention because of the ease in product recovery and the continuous reuse of the biocatalyst. The use of Ca-alginate beads has however some disadvantages like its

instability or the release of cells during a continuous process operation and mass transfer resistances. The instability of beads might be due to the insufficient space provided to the cells when they grow resulting in their rupture and release out of the beads. To overcome this problem, single layer alginate beads are chemically treated with another natural polysaccharide like chitosan. This offers alginate beads another layer, which works as a coating. These two anions (alginate) and cation (chitosan) polymers form a polyelectrolyte complex that improves the stability and reduces the release of cells. On the other hand, the chitosan layer might affect the transfer or diffusion of the substrate into the cells. The pore size of the immobilized support is a major factor that controls the diffusion of molecules necessary for the survival of cells. The porosity of matrices (>90%) can provide a higher surface area and minimum diffusional limitations. Other key factors that affect diffusional properties are the size and the shape of immobilizing matrices, especially in the case of beads (alginate beads). Spherical matrices as compare to a flat shape provide more surface area for an efficient transfer of substrate or nutrients, as well as a significant attachment and growth of cells (Nedovic and Willaert 2013).

Although alginate beads have high cell viability and activity, several drawbacks have reduced its usage in the fermentation industry, such as diffusion limitations of nutrients and chemical/physical instability of the gel.

2.4. POLYURETHANE FOAMS

Various scientists made efforts to obtain polymers with specific chemical, physicochemical, mechanical, and biological properties. Polyurethane foams (PUF) are selected for work and prepared by polycondensation of isocyanates with polyols (diols, triols, polyether, or polyesters). The foams are very porous and have a low density and large solid surface area, but ‘‘reticulated or net’’ like foam.

These can be classified based on pore sizes as pores per inch (PPI). The support materials with a high PPI value are considered best because of their low weight, a high percentage of strength, and chemical resistance. The pore sizes of PUF are normally in the range of 4 to 120 PPI. These can be prepared on the base of void volume, (ratio of total voids to solid material) which can be up to98%. The PUF can be modified with organic reagents. The PUF is the best support material for the immobilization of yeast due to its easy availability, chemical and thermal resistance, high efficiency, and low cost.

2.4.1. Graft-Polymerization

The modification of support materials can be done with ‘‘Graft-polymerization’’ by ionizing radiation. Ionizing radiation can be of two kinds, either of corpuscular nature (alpha, beta, protons, neutrons or ions) or electromagnetic (gamma or X-rays). Modification of a polymer is possible by accelerated electrons and gamma rays. In this technique, gamma rays can modify the inner and outer surface of porous materials.

Surface grafting can change the physicochemical properties of a surface instead of changing the mechanical properties of the support material. With Gamma-ray irradiation radicals can be generated in the base materials and the monomers can be polymerized.

There are three methods of graft polymerization induced by radiation. The first method is ‘‘Pre-irradiating in vacuo’’. In vacuo, the sample is irradiated and then put in the monomer solution. With this method, it is possible to form homopolymers. The second method is ‘‘Pre-irradiation in air.’’ It is akin with the first method except for the pre- is done in the presence of air so the results are organic peroxides and hydroperoxides. Due to elevated temperature peroxides can release free radicals that can initiate the polymerization process. The third method of graft polymerization is the ‘‘simultaneous grafting polymerization’’ technique. In this technique, both steps are done simultaneously, and the grafting yield is higher as there are no free radicals. This method is considered most promising due to the low monomer concentration and low irradiation doses. With this method, homogeneous changes are possible in the whole material.

Some important parameters that can affect the grafting polymerization processes are the monomer’s concentration, the chemical composition of the base polymer, the solvents, the method of irradiation, and pH. 5.8. Polymerization process In this chemical process, several unsaturated monomers are covalently link.

2.4.2. Polymers

Polymers are formed by the repetition of covalently linked monomers. Macromolecules of polymers can be prepared by many repetitions of monomers. The molecule's characteristics will be the same as that of the monomers. Polymers can be classified as homo-polymers or as copolymers. In homopolymers, the monomers are chemically homogeneous while in co-polymers chemically different monomers are covalently attached.

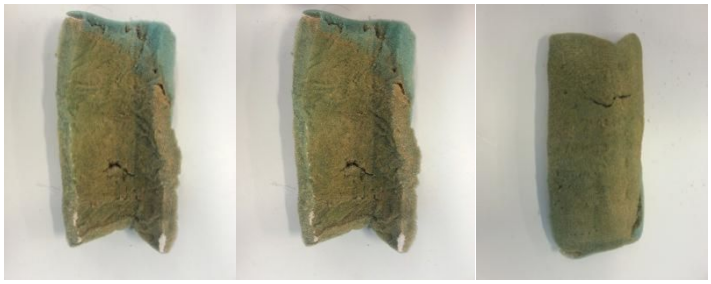
2.4.3. Polyvinyl Alcohol as Coating Polymers

Polyvinyl alcohol (PVA) is not prepared by polymerization as in other vinyl polymers and it is also a water-soluble synthetic polymer. It is only prepared by polymerizing of vinyl acetate that is later converted into PVA. PVA is adhesive, resistant to oil and grease, nontoxic, degradable, and has a high surface tension(Zeng, Aigner et al. 2005).

2.4.4. Glycidyl Methacrylate as a Monomer

Glycidyl methacrylate (GMA) is a monomer that possesses the functions of a vinyl group and an epoxy group within the same molecule. Using free radical polymerization the vinyl group can be polymerized while polycondensation can be used to polymerize the epoxy group.

Fig.2.2 Polyethylene sponges after adsorption of cells.



2.5. MATERIALS AND METHODS

2.5.1. Materials

Polyurethane foam was obtained from Eurofoam Deutschland GmbH with a pore size of 250 um. This foam was cut into small cylinders of 0.7 cm diameter and 2.8 cm high. Polyvinylalcohol (PVA) of 64 kDa and 72kDa, hydroxietilcellulose (HEC) 90 kDa, hydroxietilcellulose 720 kDa, glycidyl methacrylate (GMA) and dimethl acrylamide (DMAA) were obtained from biodynamics SRL. Acetone, sodium sulphite anhydrous, isopropanol, ethanol, and lysozyme were purchased from AppliChem GmbH.

2.5.2. Methods

2.5.2.1. Preparation of functional hydrogel

Polyurethane foam was prepared by three steps: foam coating, grafting/cross-linking, and ligand functionalization reaction. Reaction were performed immersing the grafted foils in in the 75% v/v EDA solutions for 48h at 45°C.

2.5.2.2. Foam Coating

Polyurethane cylinders were coated by with varying concentrations, pH, and temperatures. The coating was done by the deep immersion method, in which the foam was completely immersed in the polymer solution for ten seconds and then squeezed. Following, PUF was immersed in isopropanol at 85°C for ten seconds and squeezed before allowing it to dry under a vacuum at 55°C until its weight was constant. Coating degree (%C.G) was calculated as using the formula:

2.5.2.3. Grafting/cross-linking process: hydrogel activation

For the activation of the hydrogel, 0.4 g of coated and dried foam material was soaked in 100 ml monomer solution and kept in a sealed container. The container was irradiated by a 60°C irradiation source with a 10 kGy dose at a dose rate of 1kGy.h-1 at room temperature (PISI semi-industrial source, CNEA, Ezeiza, Argentina). A mixture of ethanol/water 1:1 v/v was used as an irradiation solvent. The solvent was degassed for 15 min by nitrogen gas bubbling. After irradiation, the material was washed with the solvent mixture and several times with 96% ethanol. The material was then dried at 55°C in a vacuum. The grafting degree (%G.D) was calculated as a percentage of increase in weight (Przybycien, Pujar, et al. 2004).

2.5.3. Functionalization

For the functionality of material, it was incubated overnight at 37°C in a mixture of sodium sulphite/isopropanol/water (10:15:75 w/w/w). After this material was washed with water and dried at 55°C. The remaining epoxy groups were then hydrolyzed to diols groups by incubation in a 0.5M solution of H_2SO_4 at 80°C (Yamagishi, Saito et al. 1991).

2.5.3.1. Physical characterization Swelling Degree

The polyurethane foam (0.4g) was incubated overnight and the weight gain was measured. After incubation, the material was removed from water and dried quickly with filter paper and weighed.

2.6. RESULTS AND DISCUSSION

To facilitate mass transfer monolith porous polymer materials were prepared. In this work, a novel material was prepared to overcome mass transfer limitations. PUF has different applications like in car industries as well as in biomedicines and nanocomposites (Kang, Kwon et al. 2013); (Li, Li et al. 2013);(Yeh, Liu et al. 2013) (Singhal, Small et al. 2014); (Hodlur and Rabinal 2014).

The functional hydrogel support was developed in three steps

I. Micro-hydrogel on PUF backbone
II. Micro-hydrogel crosslink and chemical activation
III. Micro-hydrogel functionalization

I. Preparation of micro-hydrogel on PUF

The coating process is the first step for the preparation of micro-hydrogel on polyurethane foam. It is done by the complete immersion of foam into a water-soluble polymer solution. Three different hydrophilic-polymers solutions were selected: a natural polymer (Agarose), a semi-synthetic (HEC), and a synthetic (PVA).

Foams were cut into a cylinder shape and coated with each polymer solution. Agarose showed low %CD as compare to HEC (40%). While PVA solution has the highest coating degree because it covers the whole PUF with hydrophilic film and can make inter or intramolecular hydrogen bonds the foam. It has been observed that a higher degree of the coating increases the adsorption properties. Furthermore, the adsorptive properties of PUF were increased and the PVA leaching was reduced with the coagulation process before the drying. This increased the degree of coating. For the coagulation of PVA, an aqueous solution of 2-propanol was used. The coated PUF was immersed in 2-propanol for 10 seconds and dried at 55^{o}C. The coagulation step increased %C.D. There are some other parameters like pH, temperature, and additives of coating solution that are known to affect the %CD.

II. Chemical activation and crosslinking of micro-hydrogel

Radiation is considered as the best technology for modification of a polymer. Radiation by electron beams and gamma rays can generate reactive radicals that allow for crosslinking, bond breaking, and polymerization..(Carbajal, Smolko, et al. 2003). Radical initiated polymerization (RIGP) is a common method for the addition of epoxy groups on materials. The glycidyl methacrylate (GMA) monomer has been used as a source of the epoxy group which allows

several functionalization reactions (Barrera, Zylstra et al. 1993); (Kiyohara, Sasaki et al. 1996); (Grasselli, del Cañizo et al. 1999); Saito, 2000; (Camperi, Grasselli et al. 2004).

III. Functionalization

There are some parameters which can affect the grafting yield and RIGP processes, such as the nature of the radiation, the monomer concentration, the type of solvent, and the dose rate. Nature of radiation. The nature of radiation is a very important parameter for the preparation of Polyurethane foam. Commercially available radiation sources are CO60, which is a source of gamma-rays and electron beams. The gamma rays are of utmost importance for grafting because of high penetration power. In this experiment, monomers were grafted by RIGP method with low dose rate gamma rays. The polymerization was of monomers used a total dose of 10kGy.

2.7. CONCLUSION

Polyurethane foam has been studied for many years as protein adsorptive materials, but not yet applied for cell. immobilization.. Three process were completed: PVA coating, GMA polymerization and ligand functionalization.

Polyurethane can be classified as inert making it a promising support.

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CHAPTER 3

LABORATORY SCALE PACKED BED REACTOR (PBR)

SUMMARY

Mass transfer is a major issue in the biochemical reaction of liquid-solid phases. In a heterogeneously catalyzed reaction, it is very important to improve or eliminate the mass transfer limitations (Salmon and Robertson 1987). The study of fluid-side and particle-side mass transfer influence on ethanol productivity as well as the performance of bioreactor is still needed. In this study, the packed bed bioreactor (PBR) is used.

It has several advantages over other bioreactors like low manufacturing and operating costs, automation process, and the ability to operate at low temperatures. This work focuses on the operational performance of the immobilized packed-bed bioreactor in the course of physiological and biochemical studies on the substrate uptake of immobilized yeast cells. The reactor was operated in a batch mode fermentation operation. The yeast physiology and mass transfer (external & internal) behaviors in PBR were monitored according to parameters such as glucose concentration, medium flow rate, and different support materials like alginate beads with and without chitosan coating, size of beads. However, this effect can be observed in the form of a variation in the lag phase at the start of the fermentation process, in the consumption time of glucose, and ethanol production.

Fluid-side mass transfer results show an improvement compared to some literature data, where it was observed that chitosan-covered alginate beads have a longer glucose conversion time when compared to alginate beads. Chitosan acts as a barrier in the transfer of substrate and products to/from the beads, respectively. At the initial time of fermentation, chitosan-coated beads show a longer lag phase than that of the non-coated ones. Moreover, it has been observed that the lag phase is directly depending on the glucose concentration as well as on the flow rate. The dependence of the lag phase on glucose concentration has been observed as a result of substrate diffusion and the increase in concentration gradients between the surface and the inner regions of the beads. When increasing the flow rate and glucose concentration, higher ethanol productivity was observed. This can be attributed to improved mass transfer properties when using higher flow rates and might be due to reduced substrate diffusional resistance. Furthermore, looking at the ethanol yield, the industrial strain Ethanol Red 11 showed higher yield than Baker's yeast at all flow rates and glucose concentrations. The ethanol yield for both strains has been observed to decrease when adding glucose

Moreover, particle-side mass transfer results show a longer lag phase was found at higher glucose concentrations, which disappear (i.e. no lag phase) using low glucose concentrations

in the medium. Internal mass transfer and glucose inhibition have been improved by using non-chitosan coated beads as compared to coated beads because of a shorter lag phase.

The time for glucose consumption can be reduced by reducing the size of beads and this might be due to the reduction of the concentration gradient between the two existing phases: the fluid medium and the center of alginate beads. At a higher flow rate, no significant difference in glucose consumption time has been noticed between chitosan-coated and non-coated alginate beads. This might be due to the removal of diffusional limitations in and around the beads. Higher ethanol productivity could be achieved by increasing the flow rate, glucose concentration, and varying the size of beads to improve mass transfer properties or reduce internal substrate diffusional resistance.

3.1. INTRODUCTION

As previously discussed, the immobilization technique offers numerous advantages over free cells in the ethanol industry. The mass transfer has a major role in all immobilized biological reactions. Two kinds of mass transfer steps are as follows

1) Fluid-side mass transfer

2) Particle-side mass transfer.

Fluid-side mass transfer properties and Particle-side mass transfer properties are discussed in this chapter using a packed bed bioreactor as a prototype plant in the experimental setup.

3.1.1. Fluid-Side Mass Transfer

Fluid-side mass transfer means nutrients transfer from the bulk fluid medium to the cells immobilizing material or carrier for the reaction to start. The mass transfer process occurs in two or multi phases having a concentration difference (concentration gradient) in between. The distance between reactants and the site of reaction also has a big influence on the substrate transfer and conversion (Doran 2012). As previously discussed in chapter 1, there are two types of reactions in the biochemical process: homogeneous and heterogeneous reactions.

Microorganisms play a vital role in waste water treatments, environmental bioremediation of land, yeast fermentation for producing ethanol and other fine chemicals, food fermentation processes, and many more. If the locality of the mass transfer resistance is known, different approaches for the elimination of that resistance could be developed. In heterogeneous reactions, the priority should be to eliminate the mass transfer resistance. The internal mass transfer also depends on the reaction inside the catalyst and its physical characteristics while the external mass transfer depends on the stagnant film thickness and the activity outside the catalyst or in the fluid medium (Klaewkla, Arend, et al. 2011).

Heterogeneous reactions (liquid-solid phase) in bioreactor undergo seven types of steps in which four steps represent external & internal mass transfer respectively as shown in figure 3.1 (Falconer 1998):

1. Convective/diffusive transfer of the substrate from liquid medium to the catalyst surface (cells pellets, alginate beads).

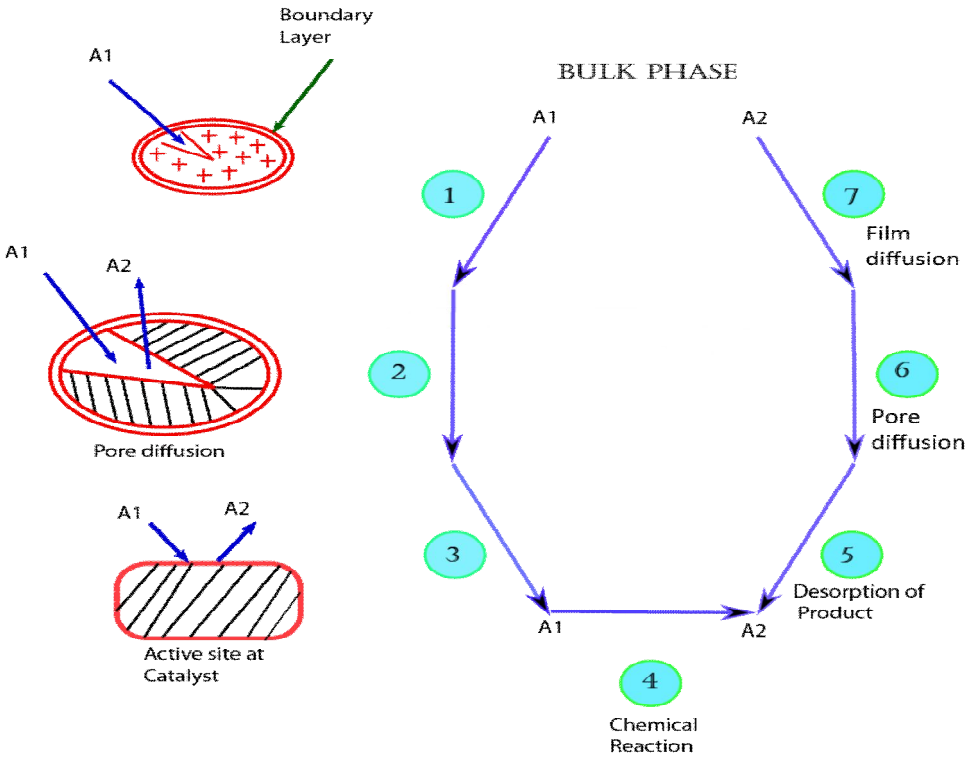
2. Adsorption of the substrate on the surface of the catalyst pores

3. Formation of the product takes place when the substrate or cells inside the pores of the catalyst contact each other.

4. Desorption of product from catalyst pores to the external surface of the catalyst.

When the product reaches the surface then it can diffuse out into the liquid medium by a gradient in concentration or pressure. The efficiency of these steps is affected by several factors: substrate concentration inside the fluid medium, fluid flow or mixing, heating, catalyst types, catalyst porosity, and substrate diffusion.

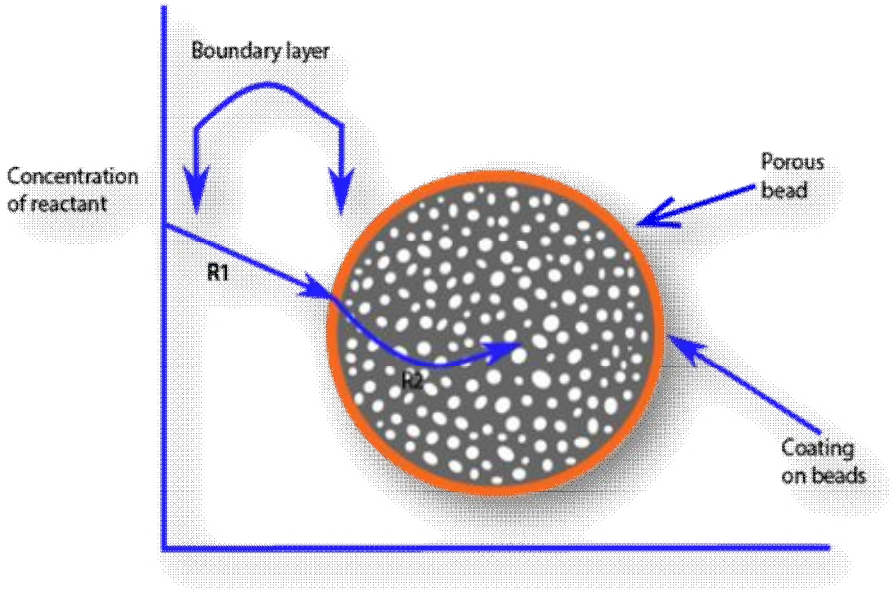
Figure 3.1 A1 represents the fluid-side mass transfer and A2 indicates particle-side mass transfer (Falconer 1998).



The most important factors that affect the first two steps (Fluid-side mass transfer) are discussed below:

1. Surface concentration of substrate or concentration gradient
2. Immobilizing Matrix
3. Types of bioreactor and conditions used

Figure 3.2. Profile of substrate diffusion into a porous bead (Davis and Davis 2012)



3.1.1.1. Surface concentration of substrate or concentration gradient

For an immobilized cell system, two more major factors that affect the transfer of the substrate from the bulk medium to the surface of the immobilized catalyst like one is the substrate concentration in the medium, and the other is a fluid film around the catalyst. Physical properties of the fluid medium used are the major factors for the thickness of film around the catalyst (Doran 2012); (Norton and D'Amore 1994). Liquid-solid mass transfer is so much important in an immobilized system having clumps, flocs, cells, or enzyme films. It refers to the transfer of the substrate from one phase (liquid in motion) to another phase (solid). Before the start of the reaction, nutrients in the liquid phase or medium should be transported to the site of reaction. This supply of nutrients can limit the biological conversion (Doran 2012); (Norton and D'Amore 1994).

Liquid-solid mass transfer has two parts.

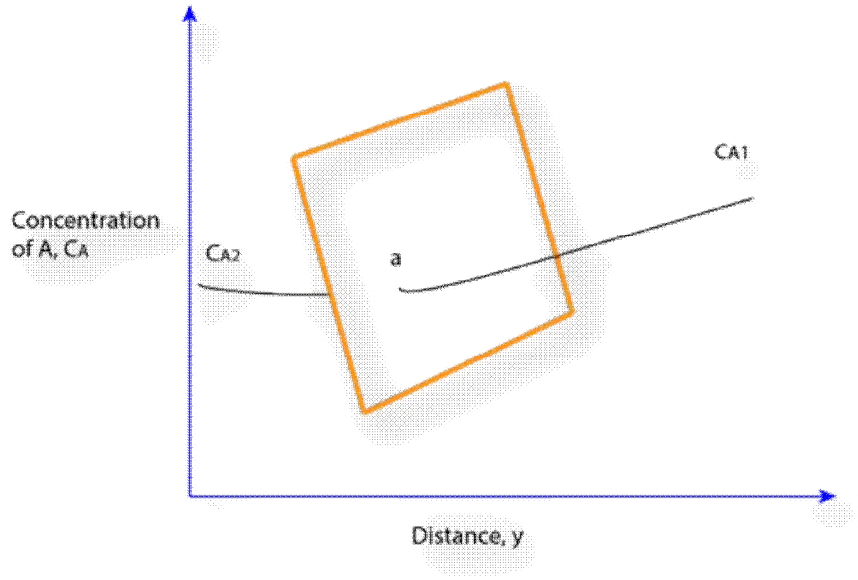
I. Convective mass transfer.
II. Interphase mass transfer

I. Convective Mass Transfer

A convective mass transfer occurs when favoring the bulk medium motion. The bulk fluid medium motion might be caused by a concentration gradient force or stirring of a fluid. As a result of a convective mass transfer, the overall rate of mass transfer will be faster. This convective mass transfer is much complicated than the diffusion mass transfer. However, understanding the convective mass transfer is important especially in multiphase- systems having a resistance of interfacial boundary layers. It can be expressed as follows:

Transfer rate = mass transfer coefficient x transfer area x driving force

Figure No. 3.3 Convective mass transfer in liquid-solid phases (Doran 2012).

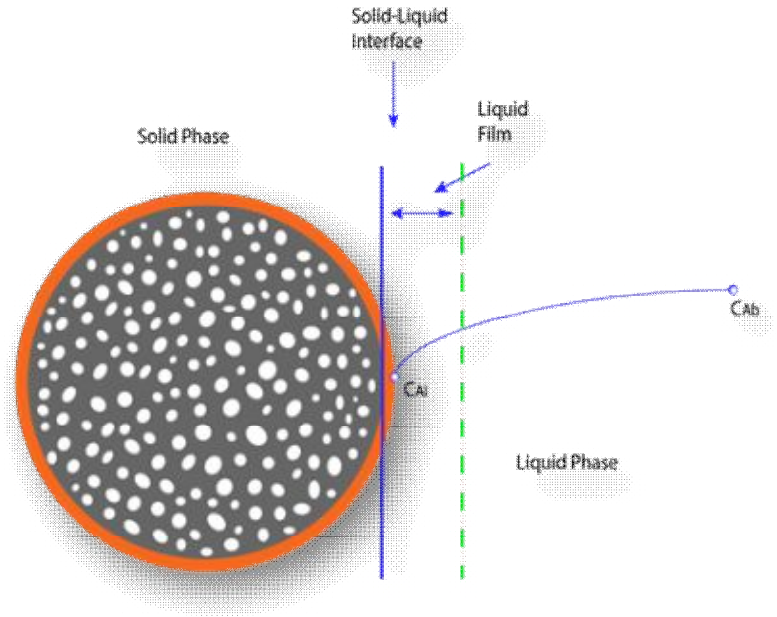


By enhancing the mass transfer coefficient, the rate of mass transfer will ultimately increase. This can be done by enhancing molecular diffusion, increasing the average velocity of the fluid around the catalyst, and reducing the film thickness around interphases.

II. Interphase Mass Transfer

Interphase mass transfer is the transfer of substrate or nutrients between the interfaces of two phases (Figure 3.4).

Figure. No.3.4 Interphase mass transfer on a porous catalyst surface. C_A and C_{Ai} are the concentrations of substrate on the interface of the liquid phase the solid phase, respectively (Doran 2012). The dotted line represents the film layer from the liquid side as well as the solid side.



The transfer of the substrate from the dotted line to the solid line in Figure 3.4 is only due to a diffusion process. This transfer is diffusion-controlled and depends on the presence of a concentration gradient (Houson 2011). The substrate concentration on the surface of the catalyst is important as it dictates whether the transfer of the substrate is efficient. The fluid-side mass transfer becomes faster in the presence of a higher substrate concentration in the medium. The reason behind this phenomenon is that a higher substrate concentration can exert a higher driving force, as described by many reviewers (Doran 2012) and (Davis and Davis 2012).

The concentration gradient develops between the fluid medium and the surface of a catalyst if the substrate concentration is higher in the medium, the film around the catalyst is thicker that can increase the difference in concentration between interphase & inside catalyst. If the catalyst has large pores, a higher concentration can induce pore diffusion.

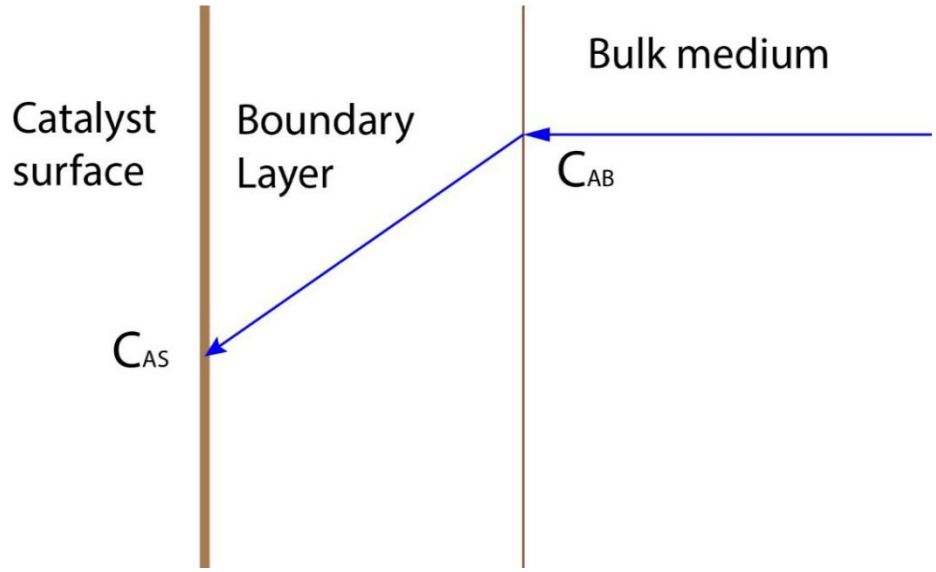
The concentration gradient has a direct relationship with the reaction inside the particle. The further reaction also depends on the availability of substrate concentration inside the catalyst pores.

The fluid-side mass transfer increases, if the demand of the substrate inside the catalyst decreases. In other words, by reducing the observed reaction rate, a better opportunity to supply substrate on the surface of catalyst pores can be achieved via a fluid-side mass transfer. This can be done by reducing the cells inside the catalyst. Fewer cells would have a fewer demand for substrate resulting in a lower observed reaction rate inside the catalyst that increases the efficiency of mass transfer. The interphase mass transfer can be explained by "Film Theory" and "Variation of substrate concentration in spherical catalyst".

a). Film Theory

Film theory helps to understand the external mass transfer phenomenon. In all multiphase biochemical reactions, the mass transfer occurs between phases come into contact and fluid velocity reduces at the point of interface. At this point, diffusion favors mass transfer across the interface of different phases. Mass transfer (substrate or product) between phases (having fluid film layer around) is the base of two phases of film theory. In other words, it is the transfer of the substrate from the interface of one phase (bulk medium) to the bulk of another phase (solid particle). We can assume that component A (substrate) is needed to transfer from the interfaces of two phases like liquid (medium) and solid (immobilizing particle) to immobilized cells. Initially, the bulk medium has a higher substrate concentration as compared to the concentration inside the particle. A hypothetical kind of stagnant film exists between the medium fluid and the particle surface. Higher resistance is observed at the point of the film layer as compared to the fluid medium. Component A will transfer from this liquid film only by molecular diffusion. The reaction starts when substrate A reaches the cells immobilized inside a solid catalyst via the flowing fluid. At the surface of the catalyst, the concentration of the substrate is expressed as C_{AS} as shown in Figure 3.5. The velocity of the fluid will be reduced at the interface that will eventually reduce the concentration of A, thus developing a concentration gradient between bulk fluid and inside the catalyst. If the solid catalyst is not porous, then substrate A does not penetrate inside the catalyst and stays at the interface. The concentration of the substrate at the bulk medium is expressed by C_{AB}.

Figure. 3.5 Film theory applied on a porous catalyst surface (Davis and Davis 2012)

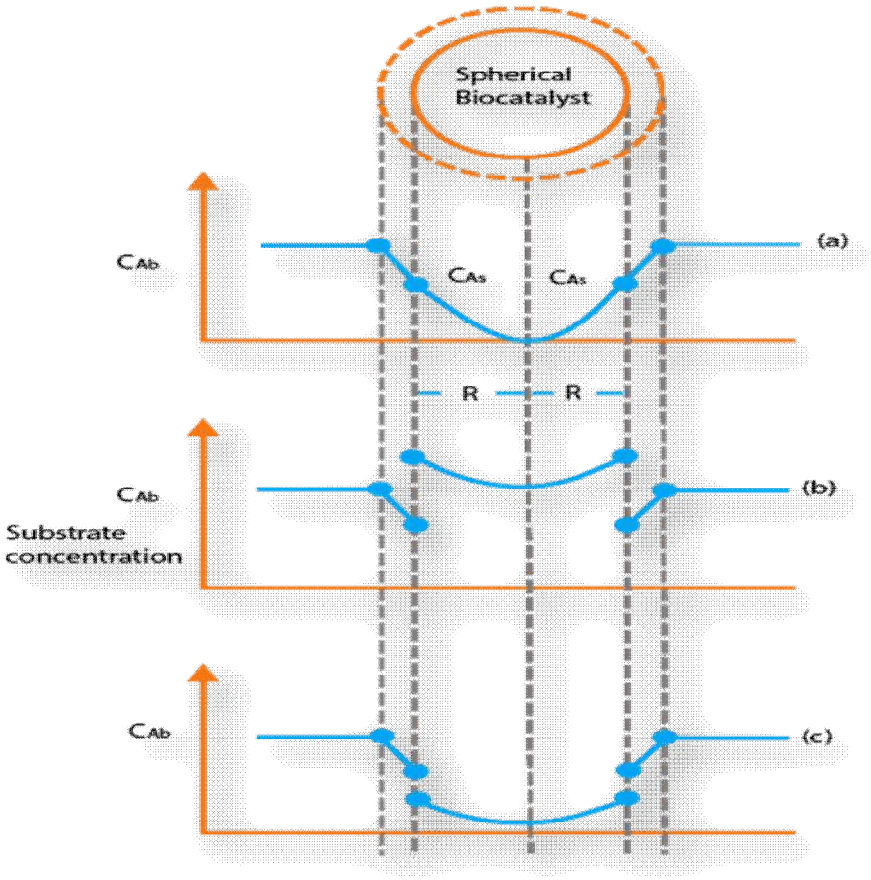


b). Variation of Substrate Concentration in Spherical Catalysts

If cells are immobilized by an entrapment method like in the case of alginate, agarose, and carrageenan, their soft and porous gel-like nature allows the substrate and products to diffuse in and out of the gel. If alginate beads have no cells dipped in a liquid medium of substrate concentration C_{AB}, after some time the concentration of substrate becomes equal to the concentration in the medium with time. On the other hand, if cells are present in the beads then the reaction will start after utilizing the substrate. As a result, the substrate concentration inside the beads will reduce. The resulted substrate concentration difference between the bead and the medium will drive more substrate to the site of reaction. The substrate concentration in the liquid medium is expressed as C_{AB} and on the liquid-solid interface as C_{As}. The concentration profile is represented in Figure 3.6 (Doran 2012). Figure 3.6a shows that the substrate concentration inside the beads is zero because all the substrate is consumed before reaching the central point of the beads. This happens only if the reaction rate is faster than the mass transfer rate. The lack of substrate at the central point of beads is called a dead zone. If cells are equally distributed, then the cells in the center will migrate to the surface of the beads or some cells can die due to substrate starvation if mass transfer is not enough or catalyst has lower porosity that can resist to diffuse substrate inside the catalyst. Figures 3.6b and 3.6c depict the hydrophobic interaction of substrate and liquid-solid phases when there is no reaction inside the cells. In this case, the substrate concentration might be higher or lower than that of the outside

liquid medium. In Figure 3.6b, the concentration on the liquid-solid interface is higher than that of the outside liquid medium and might be due to a higher attraction of the substrate to the solid phase. While Figure 3.6c shows a higher attraction of the substrate to the liquid medium phase.

Figure 3.6. Variation in the substrate concentration profile for spherical biocatalysts (Doran 2012).



3.1.1.2. Biological and Metabolic Change in Yeast

Numerous scientific discussions handled the issue of the biological and metabolic change in yeast activity and compared it to the case of free and immobilized cells. To date, the actual reasons for this change in activity still seem ambiguous. Several authors have reported observations that changing the physical and chemical environment in immobilized cells stimulate changes in the metabolic activity of yeast (Norton and D'Amore 1994). Limitations in mass transfer also have a severe effect on the physiology of immobilized yeast. This might be due to the reason that free cultivated yeast cells enjoy the natural environment, where

nutrients in a fluid medium can easily transfer into the yeast cells and products can easily come out.

While in the case of immobilized cells, the chance for transfer of nutrients is lower because of the closed system for cells cultivation. In this system, nutrients need to transfer from the liquid medium to a particle site where cells are attached or entrapped. Due to limitations in mass transfer to immobilized yeast cells some abnormal changes in microenvironment have been observed, such as an increase in storage of polysaccharides glycogen, glucan or trehalose, increase or static growth rate, longer lag phase, reduced glucose uptake rate, and many other abnormalities (Norton and D'Amore 1994). There is no strong evidence that proves whether cells change their activity by themselves or by induction via the changes in their microenvironment.

3.1.2. Particle Side Mass transfer Phenomena

The transfer of substrate within the particle or catalyst is called particle-side mass transfer or internal mass transfer. It can be defined as a transfer of substrate within the immobilizing support. It depends upon the size, texture, and porosity of the immobilizing materials.

The internal mass transfer is affected by several factors:

- Size of beads (immobilizing matrix)
- Surface concentration of substrate or concentration gradient
- Observed reaction rate
- Effective diffusivity
- Fluid dynamics

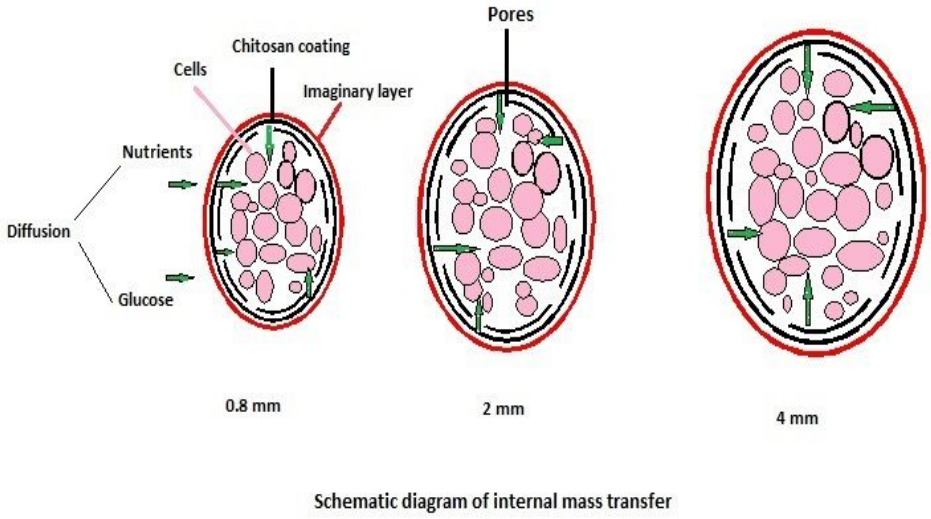
3.1.2.1. Size of the Beads

Internal mass transfer resistances can be eliminated by reducing the size of the beads. The choice of particle size is critical because using a sufficiently reduced size of particles efficiently is a difficult task. Using mini particles on the laboratory scale bioreactors could easily remove internal mass transfer limitations. On the other hand, on the industrial scale, these mini-particles create many problems, such as pressure drop, blockage of the air inlet, and separation from the medium reservoir. Some authors observed beads with a 4 mm diameter to have severe diffusion limitations. In this case, the concentration gradient developed within the beads and as a result, the cells biomass colony developed near to the outer surface of beads may enhance the diffusion limitations. On the other hand, beads with

a diameter of 2 mm or 1 mm have shown negligible mass transfer limitations (Norton and D'Amore 1994); (Klaewkla, Arend, et al. 2011); (Buchholz, Kasche et al. 2005).

During a biochemical reaction, as the substrate is consumed inside the particle, the concentration gradient develops inside and the surface of the beads. This concentration can drop to zero before the diffusion of the substrate from the outer surface to the inside takes place. This happens only when the distance of the substrate transport from the outer to the inner particle surface is big, in other words, if the immobilizing particle size is big. Thus, the internal flow of the substrate is controlled by the particle size. (Norton and D'Amore 1994); (Klaewkla, Arend, et al. 2011); (Buchholz, Kasche, et al. 2005).

Figure 3.7. Internal flow of substrate by varying particle size



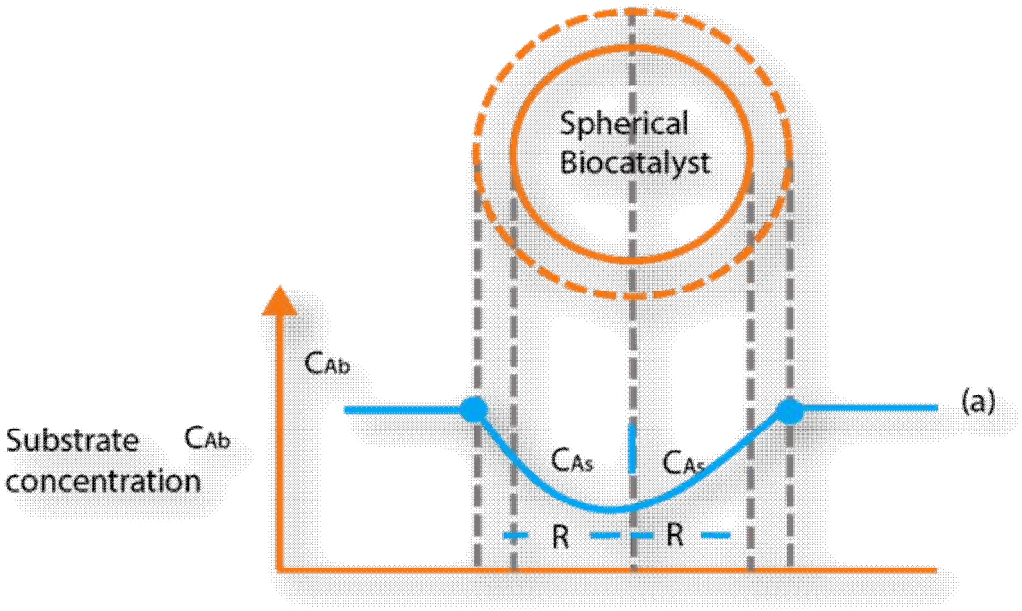
Schematic diagram of internal mass transfer

3.1.2.2. Surface Concentration Gradient

In a biochemical reaction, having more than one phase depends on the concentration gradient. An interface mass transfer is a mass transfer between two or three phases (gas, liquid, solid) that are separated by an interface. When the system is at its equilibrium with two or three phases then there is no mass transfer. Mass transfer only occurs when these phases have no equilibrium. In the immobilized cell system, a concentration gradient is caused due to substrate consumption and metabolites production. Interphase concentration gradients are commonly present in liquid–solid systems and can be decreased by reducing beads size and increasing the fluid flow rate (Perego and Peratello 1999). Figure 3.8 shows a

spherical catalyst or a particle when submerged in a fluid medium having a well-mixed substrate concentration. If the particle is dead or having non-active cells then the substrate concentration within particles becomes same as the outside concentration with time. On the other hand, if the particle contains active cells and as the reaction progresses, the substrate concentration will become lower inside the particle as compared to the outer bulk concentration. As a result of this action, the concentration difference or concentration gradient developed across the boundary layer or at the surface of the particle, forces the substrate to transfer into the porous particle.

Figure. 3.8 Interaction of mass transfer with reaction rate (Doran 2012)



3.1.2.3. Observed Reaction Rate Interaction

Inside the immobilized catalyst, the availability of all the reactant molecules on the site of reaction is necessary (Buchholz, Kasche et al. 2005). The rate of reaction of heterogeneous processes depends on the substrate available. This substrate availability depends on the diffusion or mass transfer of substrate from the surrounding layers of the fluid (External mass transfer) to inside the pores of solid catalysts (internal mass transfer) (Klaewkla, Arend et al. 2011). Therefore, the mass transfer from surface to pores of beads depends on the concentration gradient, which in turn depends on the consumption of substrate, as well on porosity and tortuosity of porous materials. The reaction inside the porous beads can speed up or enhance the mass transfer rate depending on the reaction conditions of pressure,

temperature, concentration gradient, and reaction kinetics. There are two possibilities regarding the reaction inside the solid catalyst and mass transfer (Parolini and Carcano 2010) as shown in figure 3.8.

If the reaction is slow inside the catalyst even if enough substrate is provided, the external mass transfer will be faster to fulfill the reaction substrate requirement. Such types of reactions are kinetically limited. If the reaction inside the catalyst is very fast than the mass transfer, the reaction will not have be supplied with enough substrate. Such types of reactions are called diffusion-limited or mass-transfer limited. In this scenario, there might be a possibility that all substrate on the particle surface are consumed before reaching to the center i.e. cells or enzymes in the center are starved.

3.1.2.4. Molecular Diffusion

It is the transfer of molecules under the pressure of concentration difference. Molecules move from higher concentration regions to lower concentration regions. The absence of both stirring in the fluid medium and a reaction inside the catalyst will result in a uniform composition in the whole system caused by molecular diffusion. The rate of internal diffusion is related to external mass transfer and depends on the pore structure of the particle. Internal mass transfer is governed by the Ficks law of diffusion that is related to mass flux by the concentration gradient.

$$J = -D_{AB}\left(\frac{dc}{dx}\right)$$

J = mass flux (mol/m^2 .s) , D_{AB} is mass diffusivity (m^2/s) , C concentration (mol/m^3)

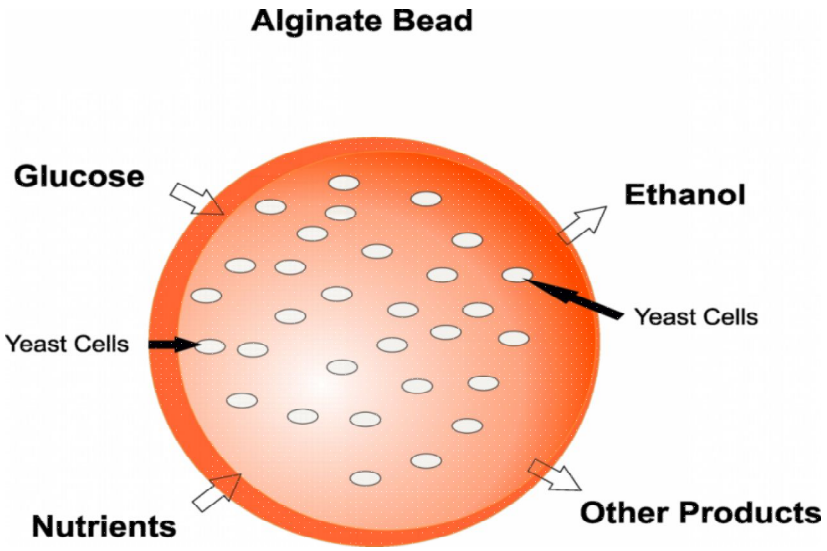
Molecular diffusivity depends on temperature and pressure but the rate of molecular diffusion inside the pores can be enhanced by sufficient stirring of the fluid medium around the catalyst that can minimize internal mass transfer resistances. This means, increasing the fluid side mass transfer reduces the solid side mass transfer limitations to a sufficient extent (Doran Paulin 2010; Buchholz K. 2005).

3.1.2.5. Effective Diffusivity

Molecular diffusion has a significant role to transfer the substrate inside the pores into the site of action and the product outside the particles. The effective diffusivity D_{Ae} describes how easily a substrate diffuses within the immobilizing particle and can be affected by its internal pores structure. Therefore, values of D_{Ae} are lower in porous solids as compared to

water due to the resistance present against diffusion inside. This resistance might be due to internal structure of pores and/or due to the concentration of cells or enzymes immobilized in it (Doran 2012); (Buchholz, Kasche et al. 2005).

Figure 3.9 Schematic representation of mass transfer of substances in and out of an alginate bead.



3.1.1.3 Types of Bioreactors and Conditions Used

The bioreactor is assimilated with the heart of biotechnological processes, being the equipment in which the substrate is converted to the desired product(s) via microbial cells or enzyme activity (Pilkington, Margaritis, et al. 1998); (Yu, Zhang et al. 2007); (Crespo, Badshah et al. 2012); (de Jong, Siewers et al. 2012); (Lee, Lee et al. 2012); (Mathew, Crook et al. 2014). In the past decades, researchers have focused on selecting the best favorable strains for bioconversion as well as on the design of bioreactors. In order to achieve a high, effective, and economically commercialized industrial production of bioethanol and other bio-products, there is a need to use an immobilized cell in a bioreactor having an enhanced flow regime that, in turn, will minimize external mass transfer limitations.

The study on the influence of external as well as internal mass transfer on productivity as well as the performance of bioreactor is still needed. These factors are severely affected by both external mass transfer limitations (transfer of reactants to and products from immobilized cell system) and internal mass transfer limitations (rate of transport inside the

system) (Saini and Vieth 1975); (Converti, Perego et al. 1985); (Anselme and Tedder 1987); (Galaction, Rotaru et al. 2011).

3.1.1.3.1. Bioreactor Design

Traditional setups like membrane, airlift, and stirrer tank bioreactors have been used in bio-ethanol production. These have some drawbacks of low product yield due to a low mass and heat transfer, inefficient conversion of the substrate, uneven mixing and distribution of flow, and shear stress on biocatalysts. Therefore, there is a need to utilize a reactor that can sustain an excellent hydrodynamic regime coupled to reduce the overall mass transfer limitations (Saini and Vieth 1975); (Pilkington, Margaritis et al. 1998).

Packed bed bioreactor (PBR) was most popular, especially for higher substrate conversion and productivity. Mass transfer is an important parameter for improving substrate consumption and productivity in PBR. To consider an external mass transfer, the choice of bioreactor and hydrodynamic conditions used are very important. On the industrial level, many problems regarding external mass transfer have been observed using PBR, especially channeling due to the use of different types of immobilizing materials, pressure drop due to different size of matrix or beads, and reactor plugging due to a difficulty in evacuating the Co2 produced during the fermentation process (Norton and D'Amore 1994).

In this chapter, the packed bed bioreactor (PBR) with one bed containing immobilized beads was used as a prototype to understand mass transfer or flow through alginate beads. It contains a vessel for culture medium, in which the culture medium is circulated from the vessel through the fixed bed and back (Figure 3.10). A medium enriched with glucose re-enters the packed bed where it can be re-utilized to convert glucose into ethanol. Toxic metabolites and other by-products are diluted; oxygen and pH can be adjusted to optimal levels. It has several advantages over other bioreactors like low manufacturing and operating costs, automation process, and the ability to operate at low temperatures.

This work focuses on the operational performance of the immobilized packed-bed bioreactor during physiological and biochemical studies on the substrate uptake of immobilized yeast cells. The reactor was operated in a batch mode fermentation operation. The yeast physiology and mass transfer behaviors in PBR were monitored according to parameters such as glucose concentration, medium flow rate, and different support materials like alginate beads with and without chitosan coating.

Figure 3.10. Schematic illustration of a packed bed reactor (PBR) of 100 ml media volume and a 20 ml capacity bead column.

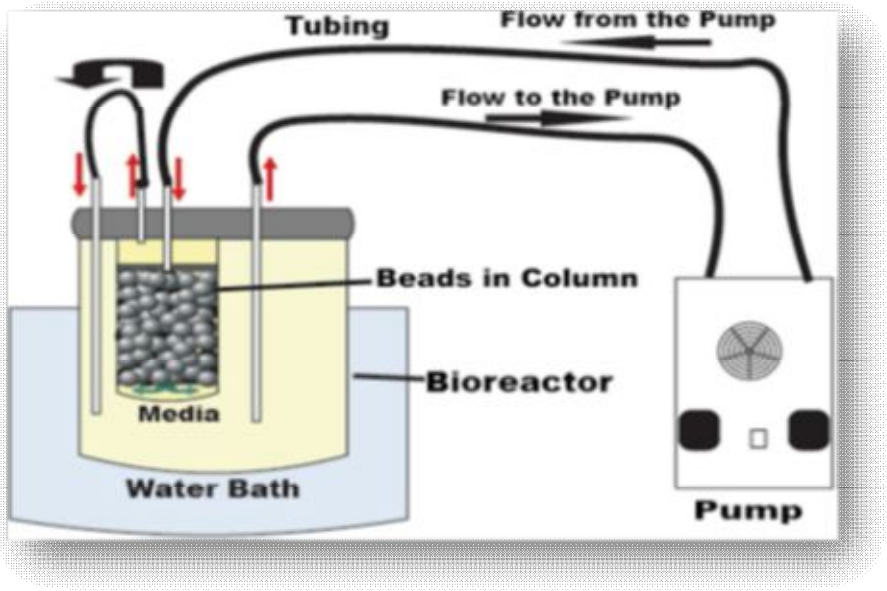


Figure 3.11 (a) Packed Bed Bioreactor from Medorex.e.K (b). Packing of beads in column

It has also been shown that the immobilization of yeast cells in alginate beads is one of the best strategies for improved industrial ethanol production and an easy scale-up of the bioreactor (Chen, Wang, et al. 2012); (Chien and Sofer 1985). The main problem in a bioreactor system is the inadequate transfer of the substrate, product, and other metabolites towards cells and out of the immobilizing matrix (Núñez and Lema 1987). Recently, scientists have shown great interest in the application of PBR for bio-ethanol

production due to its low manufacturing and operating costs (Galaction, Kloetzer, et al. 2012); (Rivaldi, Sarrouh et al. 2008).

A comprehensive understanding of mass transfer in an immobilized system using PBR as a prototype is required to achieve maximum productivity and bioreactor performance (Warnock, Bratch, et al. 2005); (de Jong, Siewers et al. 2012). There are two major steps involved in the substrate transfer (Figure.3.9) to immobilized alginate beads: (1) Transfer of substrate from a homogeneous bulk liquid to the external surface of beads passing through a hypothetically stagnant liquid film around the beads (external mass transfer). (2) Transfer of substrate to microorganisms through pores (internal mass transfer).

This study on PBR with immobilized *S. cerevisiae* cells in alginate beads shows that it is possible to have efficient external mass transfer without any loss of cell growth and physiology by selecting an optimum flow rate. It is possible to continue investigating the operational performance of the immobilized packed-bed bioreactor in the course of physiological and biochemical studies on the substrate uptake of immobilized yeast cells (Hussain, Kangwa, et al. 2015). The reactor was operated in a batch mode fermentation operation. The yeast physiology and mass transfer behaviors in PBR were monitored according to parameters such as glucose concentration, medium flow rate, and the different sizes of support materials. The use of PBR in the research is as a lab-scale reactor to understand how the system works in case of mass transfer.

3.2. MATERIAL AND METHODS

3.2.1. Microorganism

The yeast *S. cerevisiae* (baker yeast) was obtained from DHW Vital Gold, Nürnberg, Germany, while the *S. cerevisiae* Ethanol Red 11 strain was purchased from Fermentis Inc, Marcq-en-Baroeul, France and were both stored at 4 and −80 °C, respectively.

3.2.2. Fermentation medium and cultivation

A minimal media was prepared with 6.7 g/l yeast extract nitrogen base without amino acid, 1.7 g/l ammonium acetate and glucose (2, 4, 10, 20, and 40 g/l were prepared separately and mixed after sterilizing (121°C, 20 min). Amino acid mixture (100×) was prepared by mixing the following different amino acids; 200 mg l-arginine, 1,000 mg l-aspartic acid, 1,000 mg l-glutamic acid, 300 mg l-lysine, 500 mg l-phenylalanine, 4,000 mg l-serine, 2,000 mg l-threonine, 300 mg l-tyrosine, 1,500 mg l-valine, dissolved in water by adjusting pH to 10 with

0.1 N NaOH and using a 0.2 µm filter for sterilization. During culturing, 10 ml of amino acid solution was added to a final 1 l media.

Ethanol Red 11 strain was refreshed by streaked onto YPD agar plate (1% yeast extract, 2% peptone and 2% glucose, 2% agar), incubated for 2 days at 35 °C. The resulting single colonies were used to start a fresh culture. Twenty milliliters of YPD media (1% yeast extract, 2% peptone, and 10% D-glucose) in a 100 ml flask were inoculated with a single colony of Yeast Ethanol Red 11 grown overnight at 35°C with vigorous shaking at 250 rpm. One percent of the pre-culture was used to inoculate 2 L Erlenmeyer baffled flask containing 1,000 ml YPD media final volume. The inoculated flask was incubated on a rotary shaker at 200 rpm and 35°C for 24 h. Furthermore, the cells were collected by centrifugation at 4,000 rpm for 15 min, washed twice with sterile distilled water, centrifuged, and re-suspended in sterile water to obtain a dense cell suspension.

3.2.3 Calcium alginate beads preparation and yeast immobilization

The preparation of alginate beads is discussed in previous chapter 2.

3.2.4. Packed Bed Reactor and Beads Packaging

A packed bed bioreactor (100 ml) was purchase from Medorex GmbH, Noerden-Hardenberg, Germany. The bioreactor column has a 2 cm diameter glass vessel for the beads package, with one end close and the other closed by a rubber plug (Figure 3.10). The reactor was (2/3) filled with beads (shown in picture 3.11b) and the temperature was kept at 35 °C using a water bath. The immobilized yeast was grown on the minimal media using various conditions of glucose concentration (2, 4, 10, 20, and 40 g/l), flow rates (1, 4, 12, 30, and 90 ml/min), and supports (alginate bead with and without chitosan coating), while factors like initial cells amount (3%) and temperature (35°C) were kept constant. To understand the fluid-side and particle-side mass transfer only difference comes in alginate beads diameter. For understanding fluid-side mass transfer alginate beads with diameters, 4 mm were used. While for particle side mass transfer, alginate beads with diameters 1, 2, and 4 mm were used.

3.2.5. Glucose Consumption Measurements

The DNS method was used to measure the immobilized yeast glucose consumption. For each measurement, a 0.5 ml sample and a 0.5 ml DNS solution were mixed in a 1.5 ml Eppendorf tube, vortexed for 10 s, and incubated for 10 min at 90 °C. After incubation, 40% of 0.16 ml potassium sodium tartrate was added, mixed by vortex mixer, and placed on ice for 3 min.

Each sample with 200ul was measured at 575 nm. The obtained results were compared with a calibration curve of different glucose concentrations to get the actual concentration.

3.2.6. Ethanol Production Measurements

The concentration of ethanol produced in a fermentation broth as well as the calibration curve were determined as follows. The fermentation broth samples (each having a volume of 600 µl) were collected, transferred to an Eppendorf tube, and centrifuged at 9,000 rpm for 5 min to pellet the cells. Later, 500 µl of the clear supernatant were carefully transferred into a new tube without disturbing the cell pellet, and 5 µl of 1% *n*-butanol was added as an internal standard. After vortexing the samples for 30 s, 1 ml of 25% ethyl acetate was added with a further 5 min vortexing. The samples were centrifuged for phase separation at 5,000 rpm and the organic phase was used for gas chromatography (GC). The gas chromatograph equipped with a flame ionization detector (FID) was used for sample measurements. The columns used were the 30 mm and 0.25 mm CP WAX—57CB (Santa Clara, CA, USA). The column temperature was initially maintained at 120 °C for 2 min and later the oven temperature was increased at a rate of 10 °C/min until reaching 150 °C. The temperature of the injector and detector were kept at 150 °C and 200 °C, respectively. The flow rate for the carrier gas (Helium) was set at 30 ml/min. The injection sample volume was 2 µl. The reported value of each experiment is a mean average based on a triplicate.

3.3. RESULTS

3.3.1. Results for Fluid-Side Mass Transfer

Several fermentation experiments with two parameters; flow rate and glucose concentration were varied to understand the effect on lag phase and glucose consumption time (up to the level where $C/C_0 = 0.1$) in both chitosan and non-chitosan coated calcium alginate beads, where C_0 represents the initial glucose concentration at time zero, C is the concentration at a particular time and 0.1 (10%) is the remaining glucose in the media. Figures 3.12a–d show the flow rates used to determine the effect of chitosan coating on glucose consumption and from the curves, two phases were observed: a lag and an exponential phase. After the lag phase, no significant change was observed in both types of beads on glucose consumption within the same flow rate. Additionally, it was also observed that by increasing the flow rates, the lag phase and glucose consumption time decreased (Figure 3.12c, d).

The effect of flow rate and glucose concentration on the lag phase is shown in Figure 3.13. The results in Figure 3.13a, b shows two parameters: flow rate and glucose concentration, varied from 1 to 90 ml/ min with glucose 2 to 40 g/l, respectively, having a tremendous effect on the lag phase. In this study, it was observed that the lag phase of both types of beads decreases by increasing the flow rate. Moreover, a longer lag phase was found at higher glucose medium concentration. The maximum time of lag phase was found to be 290 min at a lower flow rate of 1 ml/min and 190 min at a higher flow rate of 90 ml/min when using 40 g/l of glucose. Further, it has been observed in figure 3.12 fermentation time of at least 300 minutes could be reduced in non-coated beads and 200 minutes in coated beads using a higher flow rate 90ml/min compared to a lower flow rate 1ml/min.

It was also observed that by decreasing the glucose concentration from 40 to 10 g/l, the lag phase decreased too. Furthermore, no lag phase was found at a glucose concentration of 4 and 2 g/l (Figures 3.12, 3.13). As shown in Figure 3.13, non-chitosan coated beads have a shorter lag phase as compared to coated beads, indicating an improved mass transfer effect observed at higher flow rates and a lower inhibition of glucose transfer. In order to support the data, the fermentation results of the Ethanol Red 11 yeast strain were compared with Baker's yeast using flow rates of 4 and 90 ml/min and glucose concentrations of 4 and 10 g/l. The results show that there is no significant difference in the lag phase of these two types of yeast.

Figure 3.12 Fermentation profile of immobilized *Saccharomyces cerevisiae* cells in PBR. Effect of flow rate,glucose conc. / Chitosan coating on lag phase, beads with (**a**, **c**) and non-chitosan (**b**, **d**).

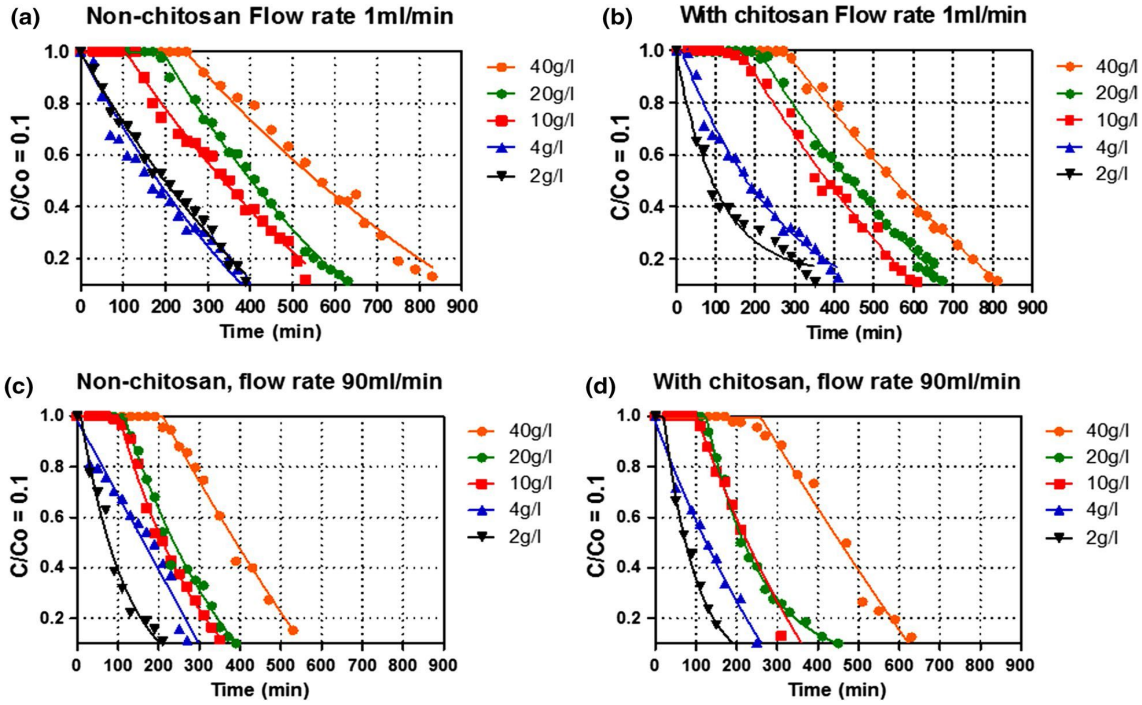
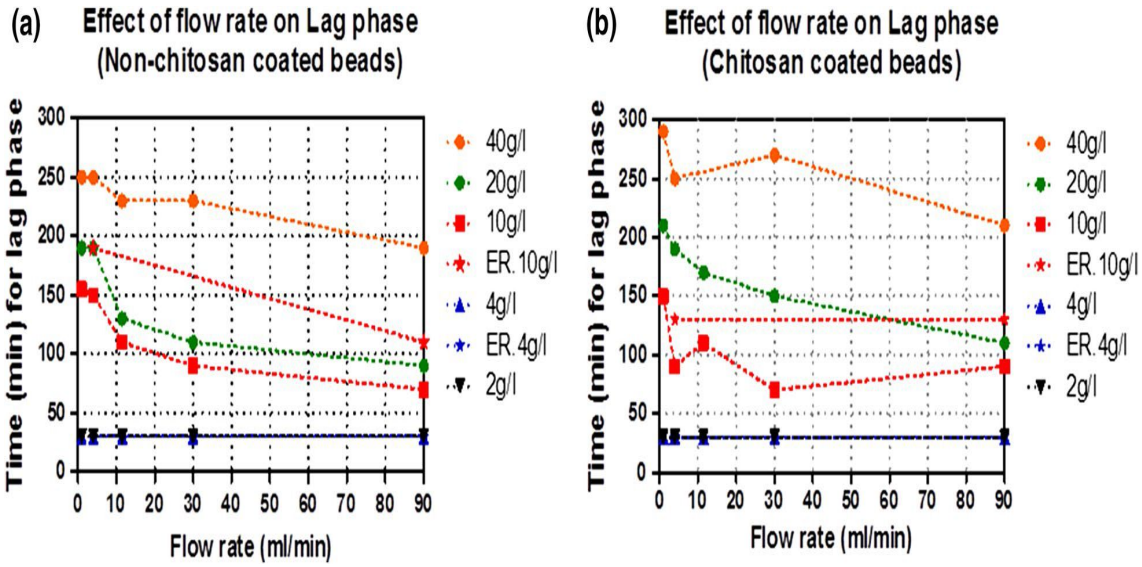


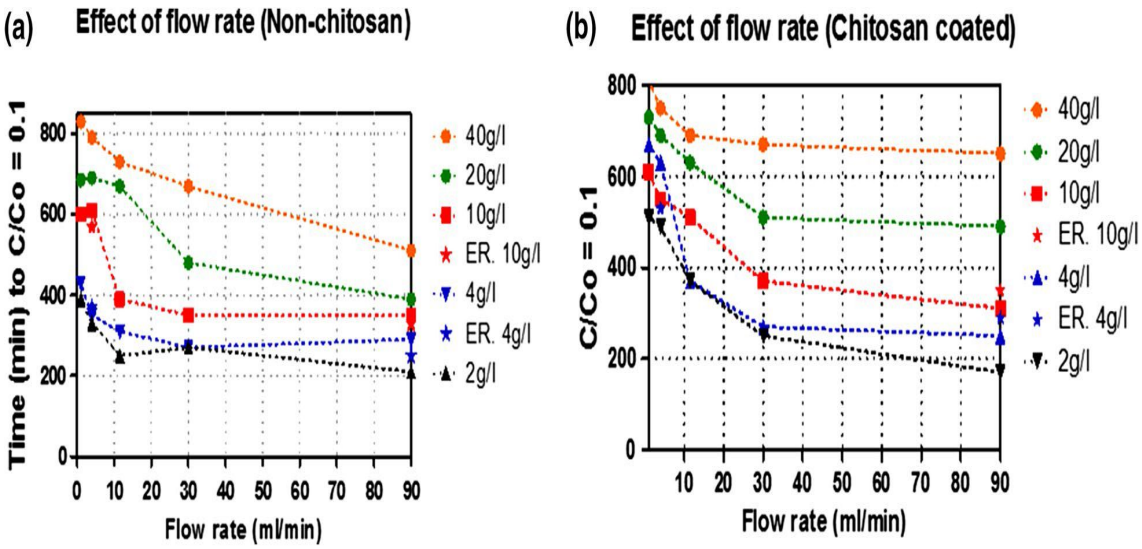
Figure 3.13 Fermentation profile of immobilized *Saccharomyces cerevisiae* cells in PBR. Effect of flow rate on lag phase using beads with (**a**) and without chitosan (**b**).



In this study, glucose consumption up to the level where C/C_0 = 0.1 was measured to understand the performance of the bioreactor and mass transfer properties regarding chitosan and non-chitosan coated beads. Figure 3.14 shows that by varying the flow rate from 1 to 90 ml/min, the time for glucose consumption decreases. The major difference in glucose consumption behavior was observed when using both types of beads at a higher flow rate (90 ml/min). The time for glucose consumption by chitosan coated beads at 30 and 90 ml/min is rather equal when using a higher glucose concentration i.e. 40 and 20 g/l as compared to lower glucose concentrations 10, 4 and 2 g/l. Moreover, with beads having no chitosan layer, the glucose consumption time tended to decrease by increasing the flow rate.

Further experiments have been performed to compare the *S. cerevisiae* Ethanol Red strain and wild type Baker's yeast using the glucose consumption time as a parameter. Both strains have relatively equal performance at 4 and 10 g/l glucose.

Figure 3.14. Fermentation profile of immobilized *Saccharomyces cerevisiae* cells in PBR. Effect of flow rate on glucose consumption time using beads (**a**) with and, (**b**) without chitosan.



The minimal medium was used in all experiments so that the yeast growth rate was at its minimal and the cells inside the beads were assumed to be uniform. Experiments were conducted using the above-mentioned yeast strains having initial glucose concentrations of 4 and 10 g/l and flow rate of 4 and 90 ml/min with a dilution rate (D) of 0.2 and 4.5 h−1, respectively. The effect of flow rate and dilution rate at different glucose concentrations on ethanol productivity as well as on ethanol yield is presented in Table 1. Ethanol productivity

means how much ethanol is produced by yeast inside the beads. It can be observed that at an initial glucose concentration of 4 and 10 g/l, the ethanol productivity increased linearly with the dilution rate from 0.2 to 4.5 h^{-1}. Optimal ethanol productivity of 21.9 g/(g.h) was obtained when using Ethanol Red strain at D of 4.5 h^{-1} with a glucose concentration of 10 g/l. It was also observed that there was no significant difference in ethanol productivity for both *S. cerevisiae* strains at lower flow rates i.e. 4 ml/min, while higher productivity was obtained at higher flow rates (90 ml/min). Further within the same dilution rate 0,2 or 4,5, if glucose concentration increased from 4-10g/l, reduction of ethanol yield is observed. Which is the pure. While higher yield at a lower dilution rate represents the higher residence time for the substrate to diffuse in.

Table 3.1. Ethanol productivity and yield (300 min) by yeast strains

Flow rate (ml/min)	Dilution rate	Glucose conc. (g/l)	Ethanol Productivity		Ethanol Yield	
			B.Yeast	ER. Yeast	B.Yeast	ER.Yeast
4	0,2	4	0,38	0,4	1,11	1,2
4	0,2	10	0,56	0,64	1,0	1,2
90	4,5	4	10,8	12,6	0,63	0,73
90	4,5	10	17,1	21,9	0,48	0,55

$$Dilution.rate(D) = \frac{FlowRate}{BedVolume}$$

P = Product concentration

$$Ethanol\ \mathrm{Pr}oductivity = D \times P = \left(\frac{g}{g.yeast.h}\right)$$

$$EthanolYield = Y\left(\frac{P}{S}\right) = \frac{PI - Po}{So - SI}$$

3.3.2. Results for Particle-Side Mass Transfer

Figures 3.15 a,b show the results obtained on glucose consumption 4 and 10 g/l, respectively, and ethanol production with time. The results show that when using a glucose concentration of 4 g/l, no lag phase was observed (Figure 3.15 a), while with 10 g/l a lag phase lasting for about 190 min is observed (Figure 3.15 b). Figure 3,16 represents the bar chart results obtained from four different parameters; flow rate (4, 30, and 90 ml/min), bead size (1, 2, and 4 mm), the glucose concentration of 10 g/l, and immobilizing matrix alginate beads (with chitosan & without chitosan). These parameters have a significant effect on internal mass transfer and can be observed in the form of a lag phase at the start of the fermentation process. Since there was no lag phase when using a glucose concentration of 4 g/l, the bar chart results were omitted. Figure 3.16 shows that the duration of a lag phase based on bead types decreases by increasing the flow rate and decreasing the size of beads. In figure 3.16 (a, b & c), between two types of beads a significant lag phase time difference approximately 30-40 minutes has been observed at bigger size 4 mm & 2 mm beads while in 1 mm beads difference is insignificant at a higher flow rate (30 ml/min & 90 ml/min). The results show that chitosan also influences the intra-particle transfer of the substrate.

This can be reduced by reducing the size of beads and increasing the flow medium around the beads which enhances the molecular diffusion in pores. Moreover, a longer lag phase was found at a higher glucose concentration (Figures 3.15). The maximum time of a lag phase was found to be 190 min at a lower flow rate of 4 ml/min and 90 min at a higher flow rate of 90 ml/min (10 g/l of glucose in medium with 4 mm sized beads) (Figs. 3.15, 3.16). By decreasing the size of beads to 2 and 1 mm, the duration of the lag phase decreased. The lowest time was 50 min when using 1 mm sized beads at a 10 g/l glucose concentrated medium (Figure 3.16). It was observed that by decreasing the glucose concentration from 10 to 4 g/l, the lag phase tends to decrease (Figure 3.15). No lag phase was found at a glucose concentration of 4 g/l (Figure 3.15) with specifically non-chitosan coated beads having a shorter lag phase as compared to the coated beads, indicating an improved internal mass transfer effect and lower inhibition by glucose. The major difference in lag phase time was observed when using both types of beads at higher flow rates like 90 ml/min. Moreover, reducing the size of beads from 4mm to 1mm reduces the lag phase time by approximately 140 min. The optimum parameters 90ml/min with the size of beads 1 mm have been observed that can reduce the time of fermentation.

Fig. 3.15 Effect of glucose concentration (4 g/l & 10 g/l) on lag phase.

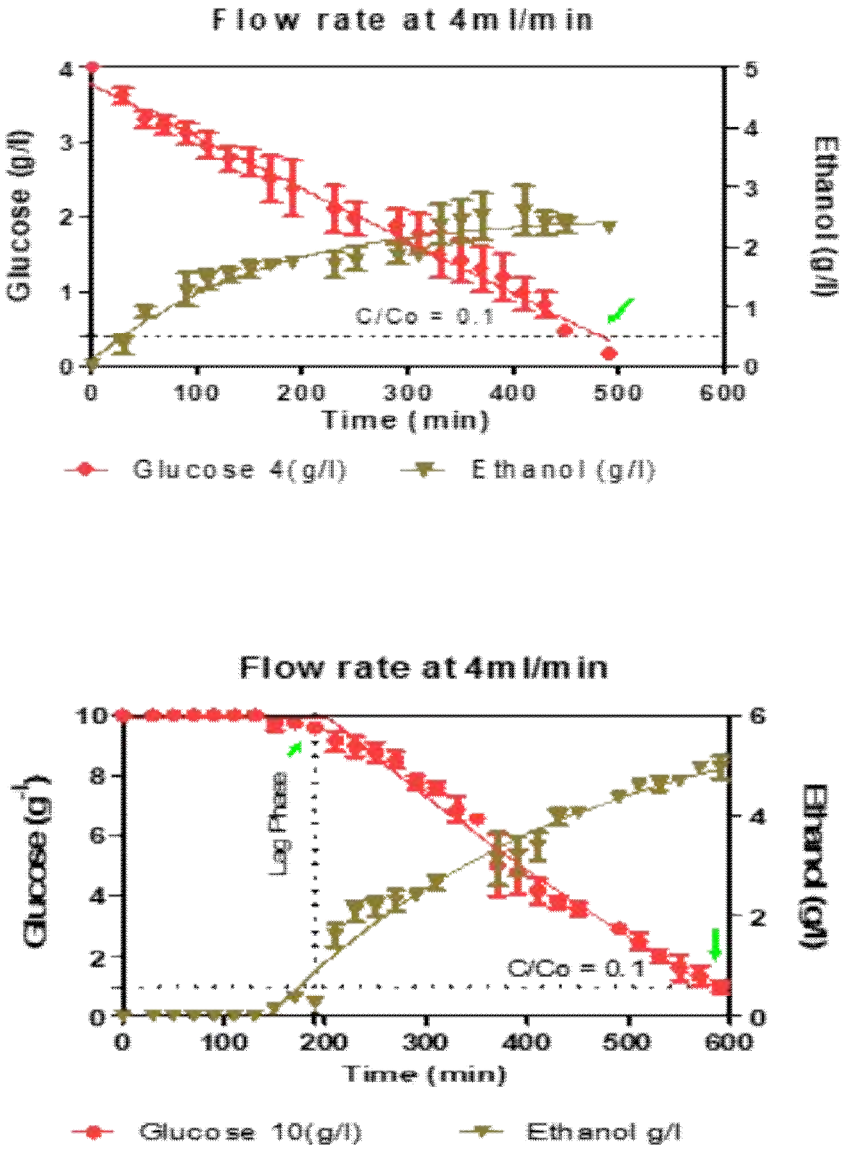
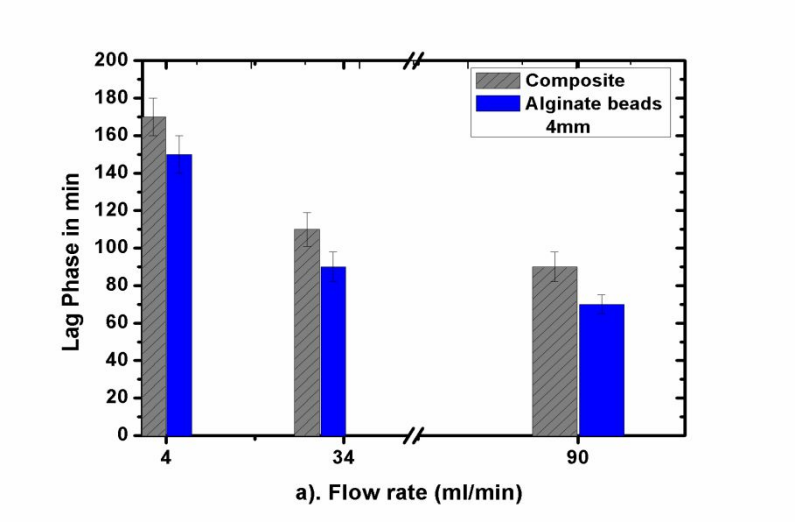
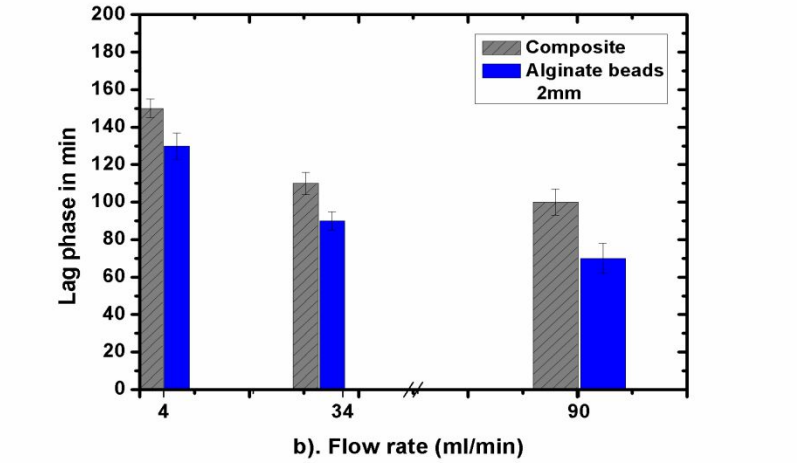
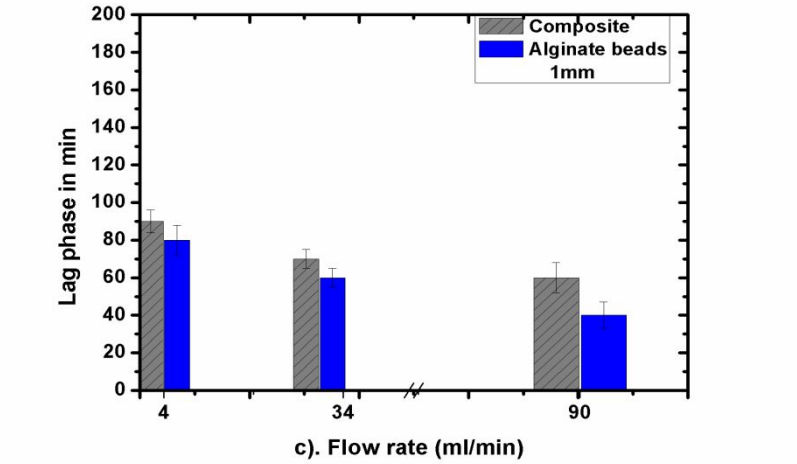


Fig. 3.16 (a,b,c). Effect of glucose conc. 10 g/l on lag phase with size of beads 4mm, 2mm and 1mm.



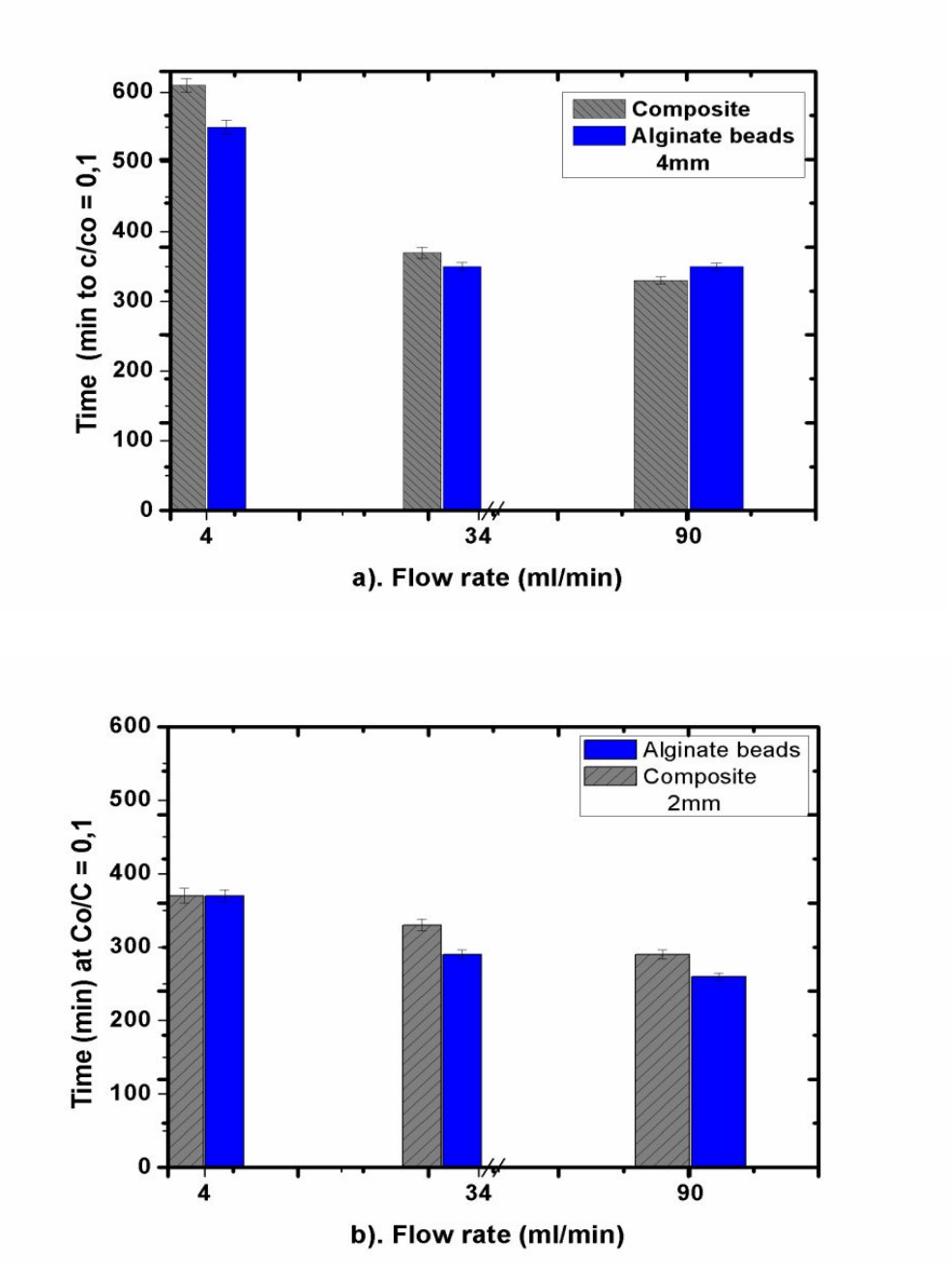


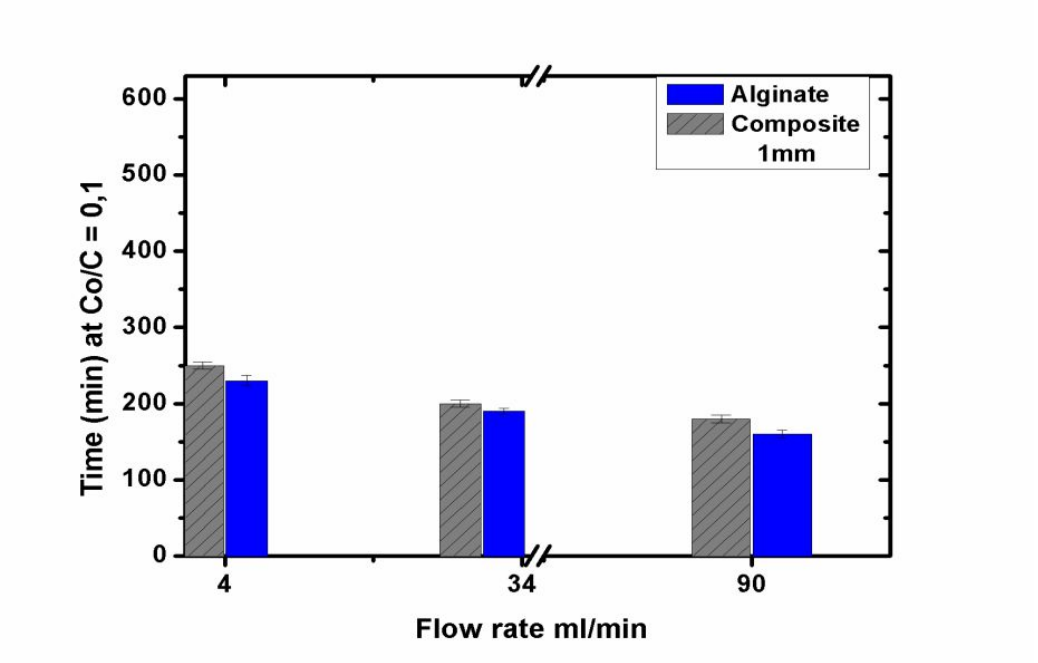


To understand the internal mass transfer properties of chitosan and non-chitosan coated beads, the glucose consumption up to the level where C/Co = 0.1 was measured. Where Co represents the initial glucose concentration at time zero and C is the concentration at a particular time where 0.1 (10%) is the remaining glucose in the media. By varying the flow rate 4, 30, and 90 ml/min (Figure 3.17), the time for glucose consumption fluctuated. The major difference in glucose consumption behavior was observed when using both types of beads at higher flow rates like 90 ml/min as compared to a low flow rate of 4 ml/min. The time for glucose consumption in the case of 4 and 2 mm sized beads at a flow rate of 30 and 90 ml/min was rather equal using 10 g/l glucose while at 4 g/l significant difference in consumption time is approximately 50 to 60 minutes. With beads having no layer of chitosan, the glucose consumption time tended to decrease by increasing the flow rate. In 2mm & 1mm sized beads time difference between chitosan & without chitosan-coated beads approximately 30-40 minutes has been observed that is reduced further using a higher flow rate of 90 ml/min. This higher flow rate could not work at a 4 mm size of beads. Figure 3.17 clearly shows that glucose consumption time sharply reduces by decreasing the size of beads. For beads size 4, 2, and 1 mm, at a glucose concentration of 4 and 10 g/l at a low flow rate (4 ml/min), the consumption time ranges from 350 to 600 min, 300 to 400 min, and 150 to 260 min, respectively. By increasing the flow rate (30 ml/min), it ranged from 250 to 350 min, 220 to 320 min, and 100 to 220 min. Further increasing the flow rate to 90 ml/min further reduces the consumption time by approximately 100 min for each bead size (Figure 3.17). The sharp reduction has been observed by a reduction in the size of beads from 2 mm to 1mm as compared to 4 mm. At glucose concentration of 4 g/l & 10 g/l fermentation time approximately 250 & 400 minutes respectively can be saved if the size of beads reduced from 4 mm to 1 mm.

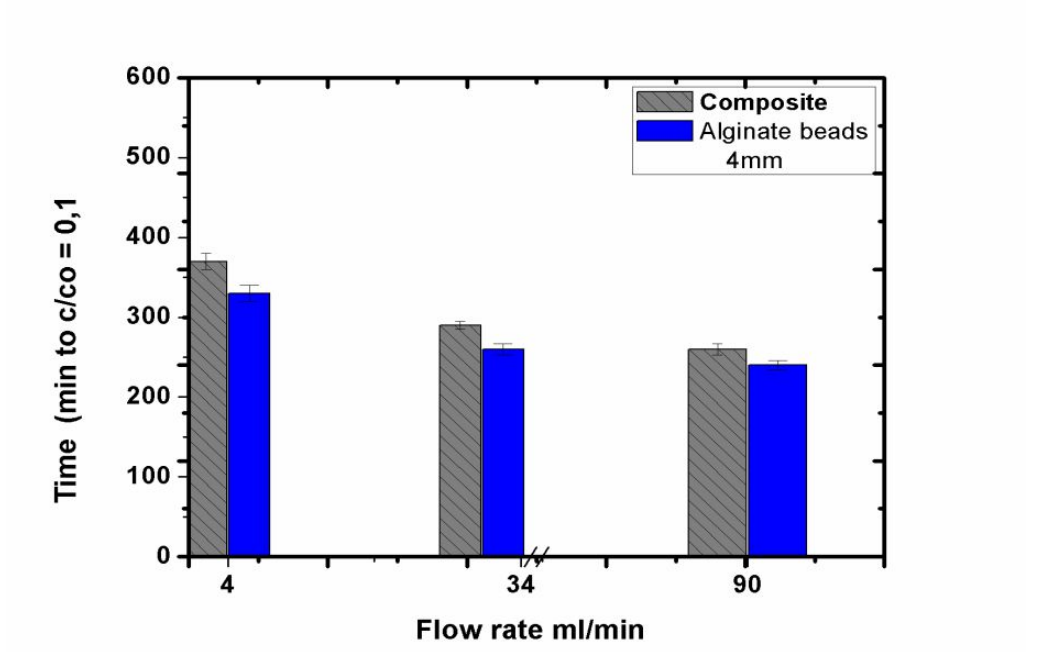
Fig. 3.17 Effect of flow rate, glucose concentration and beads size on glucose consumption. a–c and d–f represent data obtained from using a glucose concentration of 10 and 4 g/l, respectively.

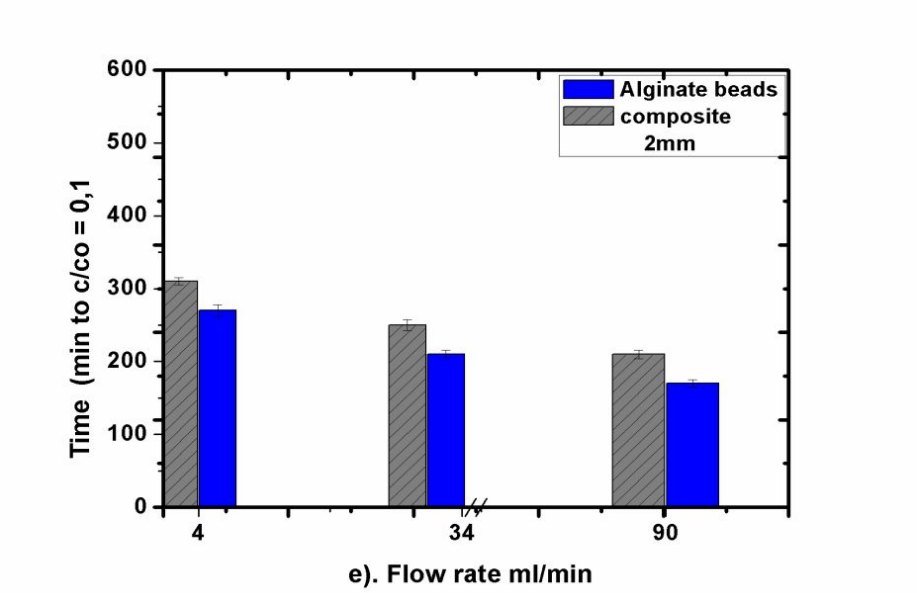
Glucose concentration 10g/l

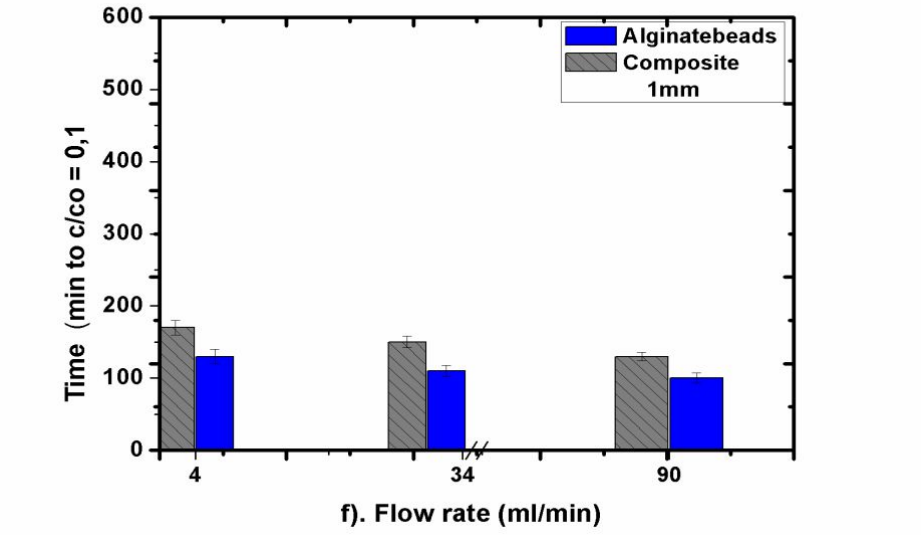




Glucose concentration 4g/l







The yeast cells inside the beads were maintained to be uniform by using the minimal medium where growth was at its minimal capacity. Using three types of beads (1, 2, and 4 mm), experiments were conducted having initial glucose concentrations of 4 and 10 g/l and flow rates of 4 and 90 ml/min with dilution rates D of 0.2 and 4.5 h^{-1}, respectively. The two important factors of fluid flow rate and dilution rate effect at different glucose concentrations on ethanol productivity and yield are presented in Table 2. Optimal ethanol productivity of 32.4 g/(g.h) was obtained when using 1 mm beads at D of 4.5 h^{-1} at a glucose concentration of 10 g/l. By using an initial glucose concentration of 4 and 10 g/l, ethanol productivity increases linearly with the dilution rate from 0.2 to 4.5 h^{-1}. Higher productivity at a glucose concentration of 10 g/l but on the other side ethanol yield is reduced at this

point when compared to 4 g/l glucose concentration which is an indication of substrate diffusion limitations.

Table 3.2. Ethanol productivity and yield by yeast strains.

Flow rate ($ml.min^{-1}$)	Dilution (-)	Glucose conc. (g/l)	Ethanol Productivity (g/g-h)			Ethanol Yield (g/g)		
			1mm	2mm	4mm	1mm	2mm	4mm
4	0, 2	4	0, 56	0, 50	0, 40	0, 90	0, 83	0, 80
4	0, 2	10	1, 18	1, 0	0, 56	0, 73	0, 62	0, 60
90	4, 5	4	15, 75	13, 90	10, 80	1, 16	1, 03	0, 63
90	4, 5	10	32, 40	27, 0	17, 10	0, 90	0, 75	0, 48

Flow rate = ($ml.min^{-1}$)

Beads size 1, 2 and 4 mm.

P = Product concentration

$$Dilution.rate(D) = \frac{FlowRate}{BedVolume}$$

$$Ethanol\ \mathrm{Pr}oductivity = D \times P = \left(\frac{g}{g.yeast.h}\right) \qquad EthanolYield = Y\left(\frac{P}{S}\right) = \frac{PI - Po}{So - SI}$$

3.4. DISCUSSION

3.4.1. Fluid-Side Mass Transfer

In recyclable biocatalysts, the mechanical strength of calcium alginate beads has not fully been found to effectively support entrapped cells. To solve this problem, Baker's yeast immobilized in chitosan-coated alginate beads of 4 mm in diameter was focused on to facilitate the needed mechanical support. However, the chitosan coating may cause resistance in external mass transfer. The results of Figure 3.12 indicate that coating has a significant effect on the lag phase duration, as it was observed with chitosan-coated beads being 30–40 min higher than that of the non-coated beads. While in non-coated beads, the reason for the reduction of the lag phase time is due to the lower external mass transfer

resistance as a result of the increase in flow rate as glucose is easily transported to and from the surface of the bead by diffusion as shown in figure 3.4 (Willaert 2009); (Caşcaval, Galaction et al. 2012); (Karagöz and Özkan 2014). Reducing the lag phase ultimately reduces the time for the fermentation process. The results show an improvement compared to some literature data, where it was observed that chitosan-covered alginate beads have a longer glucose conversion time when compared to alginate beads (Duarte, Rodrigues, et al. 2013).

Chitosan acts as a barrier in the transfer of substrate and products to/from the beads, respectively. At the initial time of fermentation, chitosan-coated beads show a longer lag phase than that of the non-coated ones. This study gives a significant understanding of both alginate beads with and without chitosan coating as indicated in the differences in their lag phases. Many researchers have been using chitosan coating on alginate beads to reduce cell and enzyme release, but it has a disadvantage on mass transfer and may have an impact on the metabolic activity of cells in beads due to limited substrate supply that ultimately may affect product formation. The lag phase is considered as the adaptation time of yeast within a new environment before the start of the fermentation process. A similar effect in Figure 3.13 was also observed by (Irfan, Nadeem, et al. 2014) indicating that the glucose concentration is critical in the fermentation process as it influences the physiological aspect of yeast, growth rate, production rate, and yield. The dependence of lage phase on glucose concentration (Figures 3.12, 3.13) might be due to substrate diffusion limitations and an increase in concentration gradient between the surface and the inner regions of beads (Caşcaval, Galaction, et al. 2012).

Also, the observed prolonged lag phase might be due to a higher accumulation of cAMP level stimulated by the effect of glucose on the cAMP synthesis, as the level of cAMP is higher during the initial fermentation time (Ma, Gonçalves, et al. 1997) i.e. the lag phase time decreased upon initiation of the exponential growth in yeast cells (Duarte, Rodrigues et al. 2013); (Djordjević, Gibson et al. 2015); (Mukherjee, Steensels et al. 2014).

This summarizes the fact that inter-particle diffusional resistance reduces by increasing the velocity around beads (Saini and Vieth 1975); (Zhao and Delancey 2000); (Caşcaval, Galaction et al. 2012). Consequently, chitosan-coated beads have more inter-particle diffusional resistance i.e. a longer lag phase at early fermentation times as compared to non-coated beads at lower flow rates. At this point, it can be concluded that the lag phase is not due to the physiology of yeast, but it may be due to the resistance in the internal diffusion of glucose. This could be due to the fact that the lag phase is directly dependent on the glucose

concentration as well as on the flow rate. Glucose is the most fundamental carbon source playing a central role in metabolic pathways providing energy to living organisms and for product synthesis. Yeast metabolizes glucose via the Embden–Mereyhof Parnas metabolic pathway - discussed in chapter 1- (Caşcaval, Galaction, et al. 2012) thereby producing the necessary energy for its survival. Furthermore, the efficiency of ethanol production could be affected by the glucose concentration and the flow rate.

From the ethanol production experiments, it was observed that the time for glucose consumption by chitosan coated beads, at 30 and 90 ml/min was rather equal when using a higher glucose concentration i.e. 40 and 20 g/l as compared to lower glucose concentrations 10, 4 and 2 g/l. This might be due to the glucose diffusion resistance that did not reduce even when using higher flow rates. However, this result indicates that chitosan coating characteristics influence glucose internal diffusion at higher glucose concentrations. In the literature, it was also observed that the magnitude of glucose diffusion resistance is directly related to the glucose concentration gradient created in and outside the beads (Galaction, Rotaru, et al. 2011), indicating a substrate inhibition phenomenon that affects the fermentation performance. In non-chitosan coated beads, glucose consumption time tended to decrease by increasing the flow rate. Such results have been observed in figure 3.12, fermentation time of at least 300 minutes could be reduced in non coated beads and 200 minutes in coated beads using a higher flow rate 90ml/min compared to a lower flow rate 1ml/min. The reason might be because these types of beads did not pose any significant barrier (Figure 3.14) for glucose diffusion to metabolically active cells.

This result was supported by Wang et al.'s observations that under scanning electron microscopy (SEM), the surface of chitosan-coated beads was rough and compact compared to the non-coated alginate beads, due to strong electrostatic interaction between chitosan and alginate (Chen, Wang, et al. 2012). The interpretation of these results indicates that the glucose consumption behavior was not due to the yeast strain, but rather to the mass transfer barrier that might have occurred by a layer of chitosan coating on alginate beads and by a glucose concentration inhibition phenomenon.

The reaction-diffusion model has been studied by investigating the influence of beads size, substrate concentration, and different flow velocities. As indicated in our results in Table 1, higher ethanol productivity was observed with increasing flow rates and glucose concentrations. Higher productivity can be attributed to the improved mass transfer properties when using higher flow rates that might be due to reduced substrate diffusional

resistance and reduced stagnant fluid film around beads (Anselme and Tedder 1987); (Yu, Zhang et al. 2007); (Matsushika, Inoue et al. 2009);(Pacheco); (Bangrak, Limtong et al. 2011); (Mathew, Crook et al. 2014).

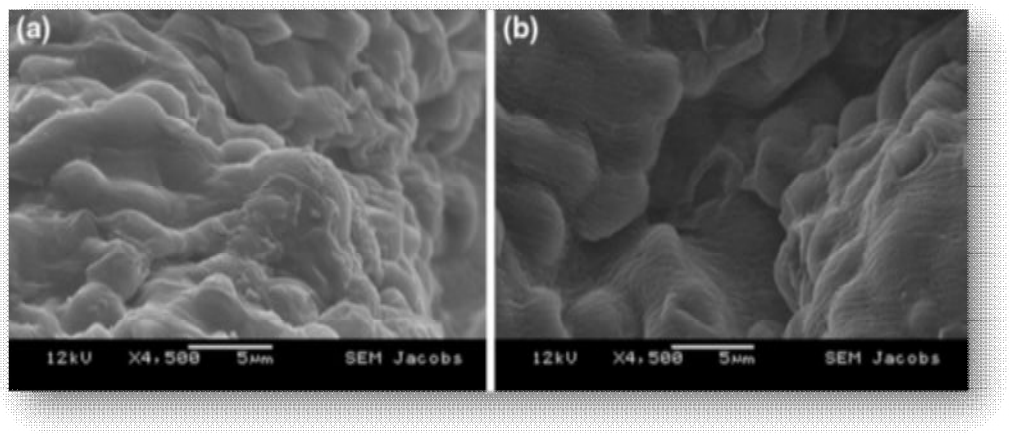
Although a higher glucose concentration can give higher productivity, it can also facilitate an increase in inter-particle diffusional resistance that enhances the lag phase as shown in Figure 3.14. It was also found that the enhancement of ethanol production on increasing liquid velocity decreases mass transfer resistances and substrate inhibitory effects (Bangrak, Limtong, et al. 2011)). (Duarte, Rodrigues, et al. 2013) found that the maximum ethanol production during fermentation was after 4 h for non-chitosan-coated alginate beads while for coated ones was after 6 h. It was also reported that hydrodynamics of the medium exhibits an important influence on glucose conversion and transfer processes which is briefly discussed in the next sections (Caşcaval, Galaction, et al. 2012); (Mathew, Crook, et al. 2014).

Furthermore, in the case of ethanol yield, the industrial strain Ethanol Red 11 strain had a higher yield than Baker's yeast at all flow rates and glucose concentrations. On the other hand at a 4 ml/min flow rate and a 10 g/l glucose concentration, the ethanol yield of both yeast strains was observed to be high as compared to a flow rate of 90 ml/min with the same glucose concentration. This result is due to a higher residence time increasing the efficiency of yeast immobilized (Singh, Srivastava, et al. 2009). The ethanol yield for both strains has been observed to decrease on the addition of glucose, which can be due to an increase in substrate diffusional resistances or we can say reaction inside the beads is mass transfer limited. It can be further explained that inside beads glucose concentration is too less due to an increase in reaction with the results demand of glucose is increased but the outside concentration is higher that cannot transport inside the pores of beads at once or it needs force to diffuse in.

The magnitude of resistance is directly related to the glucose concentration gradient between the inner and outer regions of beads, which induces substrate inhibition. It was found that there was a significant decrease in ethanol yield when adding sugar in a fermentation medium (Bangrak, Limtong, et al. 2011); (Caşcaval, Galaction et al. 2012);(Galaction, Kloetzer et al. 2012); (Galaction, Rotaru et al. 2011). It was also reported that in batch fermentation of *S. cerevisiae*, the ethanol yield significantly depended on the initial glucose concentration and the substrate inhibition was noticed at high initial glucose concentration (Wendhausen, Fregonesi, et al. 2001). Sequential experiments on varying flow rates and glucose in a PBR with immobilized *S. cerevisiae* cells shaded a significant

understanding of mass transfer. Moreover, glucose consumption at low flow rates was low compared to when higher flow rates were used. By means of the analysis of the influence of different glucose concentrations and varying flow rates; the optimum combination was found to be the use of high flow rates and a 10-20 g/l of glucose concentration. This combination leads to the optimum glucose consumption rate and maximum product formation. This selected system for mixing as well as glucose concentration will be used in further experiments to study internal mass transfer or active pharmaceutical ingredient production in this basket bioreactor.

Figure 3.18 Scanning electron microscopic (SEM) photographs of non-coated alginate beads (a), chitosan coated beads (b).



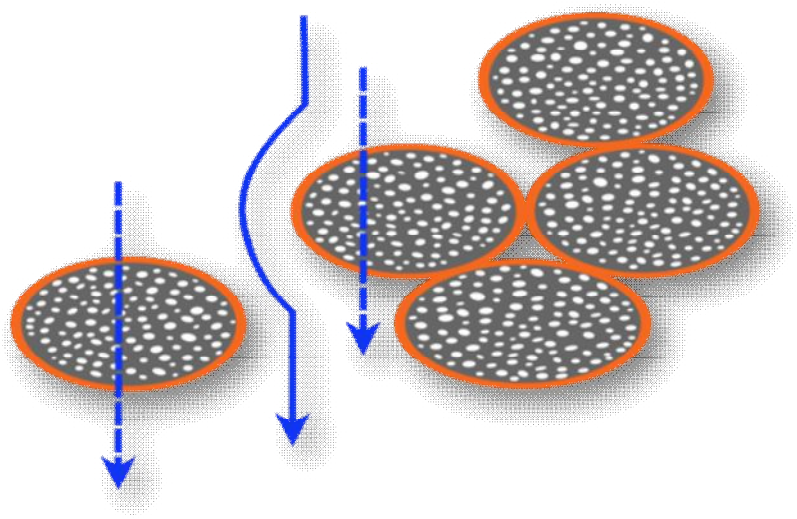
3.4.2. Particle-Side Mass Transfer

In a heterogeneously-catalyzed reaction, it is very important to improve or eliminate the mass transfer limitations (Salmon and Robertson 1987). In this study, the purpose was to understand the mechanism of internal mass transfer effects on the immobilized system used for ethanol production and the performance of PBR as a prototype reactor. Four operational parameters, glucose concentration, flow rate, the structure of alginate beads (chitosan and non-chitosan coated beads), and the size of beads have significant effects on the internal mass transfer. However, this effect can be observed in the form of a variation in the lag phase at the start of the fermentation process, in the consumption time of glucose, and ethanol production.

In PBRs with cells immobilized in alginate beads, the flow patterns of fluid can be classified into two types: firstly through interstitial spaces between beads and secondly through the pores of beads (i.e. intra-particle flow) (Figure 3.19). Such types of flow patterns are mainly

influenced by the flow rate and the size of beads. If beads are bigger in size, then the fluid medium will flow through interstitial spaces and the substrate is not easily reachable to the cells inside the beads. While intra-particle flow is only possible by reducing the size of beads and using an optimized flow rate. An optimized flow rate is more important because if the flow rate is too high, shear stress on cells inside the beads can be exerted. On the other hand, if the flow rate is too low, it could cause a dead zone inside the beads and as the formation of a bridge by cells aggregation with other carriers. In this case, cells can consume the substrate earlier before the diffusion of more substrate into the porous beads (Warnock, Bratch, et al. 2005).

Figure.3.19 Flow of medium through porous beads in a packed bed bioreactor (Doran 2012). Solid line indicates interstitial flow & dotted line represents intra-particle flow.



The effect of internal mass transfer resistance on the lag phase could be understood from Figures 3.17, which shows that the lag phase duration for both types of beads (chitosan and non-chitosan coated) sharply reduces by reducing the size of beads. Several literature are on the inhibition of yeast growth and metabolic activities by using higher initial substrate concentration (Galaction, Lupăşteanu, et al. 2010); (Lee, Lee, et al. 2012). It is depicted from the results that the substrate or product inhibition can limit the efficiency of ethanol production and lead to enhance the lag phase of the fermentation process. Talebnia and Taherzadeh (2007) also observed the limitation of substrate transfer into the center of beads and toxic metabolites out of it. Varying the size of beads, which may control internal mass transfer.

By reducing this factor, the distance for reactants to reach the active site of the catalyst, and the thickness of the fluid layer created around alginate beads is reduced. On the other hand, when beads are large, the substrate is depleted in the center and the core of beads are deprived of the substrate (Salmon and Robertson 1987). Moreover, internal mass-transfer limitations can be overcome with a high liquid flow rate that reduces the fluid layer around the beads and increases the surface concentration, which in return effectively increases its diffusivity.

The duration of a lag phase based on bead types decreases by increasing the flow rate. All these parameters were effective to reduce internal mass transfer limitations as we have observed in Figures 3.15,3.16. The flow rate of 90 ml/min is more effective than 4 ml/min in reducing the lag phase time to 100, 50, and 40 min for 4, 2, and 1 mm size of beads, respectively at a 10 g/l of glucose concentration in the medium. The hydrodynamics of the medium exhibits an important influence on glucose conversion and transfer processes briefly discussed in the next section (Hussain, Kangwa, et al. 2015). It was also observed that the lag phase is dependent on the glucose concentration and tends to decrease by reducing the concentration from 10 to 4 g/l. Even at a 4 g/l glucose concentration, no lag phase was observed indicating a less internal mass transfer limitation or steep concentration gradient effect by the substrate. The dependence of the lag phase on glucose concentration (Figure 3.15) might be the result of substrate diffusion and the concentration gradient between the surface and the inner regions of beads (Hussain, Kangwa et al. 2015); (Zhao and Delancey 2000).

Data analysis of the experiments shown in figure 3.17 that the time for glucose consumption at a flow rate of 30 or 90 ml/min was rather equal when using 4 mm sized beads. This time tended to decrease by further decreasing the size of beads from 2 to 1 mm due to the increase in the concentration gradient in and outside the beads (Galaction, Rotaru, et al. 2011). The concentration gradient is the difference of (substrate or product) concentration between the two phases present.

The external concentration gradient is the difference of concentrations between the bulk liquid and the external surface of the beads; it can never be observed without larger internal gradients within the beads (Salmon and Robertson 1987), with the results the product inside the beads diffuses out under the influence of concentration gradient. Further explanation is that in smaller sized beads substrate can be equally distributed and available for all cells immobilized. While in bigger sized beads substrate can be diminished before reaching in the

center of the beads and only cells near to the surface of the beads can survive and rest can die due to starvation. Bigger sized beads could have a diffusion-limited reaction.

An insignificant difference of the glucose consumption time was noticed between chitosan-coated and non-coated alginate beads at higher flow rates (30 and 90 ml/min) when compared to the lower flow rate (4 ml/min), especially for a smaller bead size (1 mm). Furthermore, in 2mm & 1mm sized beads time difference between chitosan & without chitosan-coated beads approximately 30-40 minutes has been observed that is reduced further using a higher flow rate of 90 ml/min. This higher flow rate could not work at a 4 mm size of beads.

This might be due to the removal of diffusional limitations in and around the beads especially in small-sized beads while in larger beads internal diffusion of the substrate (molecular diffusion inside the pores) could not be enhanced even at a higher flow rate. The fluid channels especially in bigger sized beads have been observed at a higher flow rate which is the major problem in packed be bioreactor. Further substrate does not have a chance to be equally distributed. In other case substrate needed to travel a higher distance from the outer surface of beads to the site of reaction as compared to smaller beads and fluid can flow through interstitial spaces with that the substrate is not easily reachable to the cells inside the beads. The further flow of fluid or hydrodynamic conditions is different around small and bigger beads, briefly discussed in the next section.

In the literature, it was found that substrate conversions yield is reduced by increasing the size of the biocatalyst particles and the glucose initial concentration due to higher resistance of the substrate internal diffusion. The time for glucose consumption in the case of 4 and 2 mm sized beads at a flow rate of 30 and 90 ml/min was rather equal using 10 g/l glucose while at 4 g/l significant difference in consumption time was approximately 50 to 60 minutes. It is depicted with high glucose concentration higher will be the concentration gradient that can enhance the mass transfer into the beads while this is not the case using 4 g/l. On the other hand at higher glucose concentration and lower flow rate, there will be substrate inhibition or diffusion limitations. This is the case of a diffusion-limited reaction. It was also concluded that the magnitude of resistance to the internal diffusion is directly related to the particle size and glucose concentration especially in case of alginate beads (Galaction, Lupăşteanu et al. 2010); (Engasser and Horvath 1973).

In figure 3.17 the results clearly show that the glucose consumption time sharply reduces by decreasing the size of beads. For beads size 4, 2, and 1 mm, at a glucose concentration of 4

and 10 g/l at a low flow rate of 4 ml/min, the consumption time ranges from 350 to 600 min, 300 to 400 min, and 150 to 260 min, respectively. By increasing the flow rate (30 ml/min), it ranged from 250 to 350 min, 220 to 320 min, and 100 to 220 min. Further increasing the flow rate to 90 ml/min reduces the consumption time by approximately 100 min for each bead size (Figure 3.17). The sharp reduction has been observed by the reduction in the size of beads from 2 mm to 1 mm as compared to 4 mm. At glucose concentration of 4 g/l & 10 g/l fermentation time approximately 250 & 400 minutes respectively can be saved if the size of beads reduced from 4 mm to 1 mm. It can be concluded that intra-particle mass transfer is enhanced by increasing the flow rate and reducing the size of beads. This might be the reason, these two factors can enhance the intra-particle flow.

It has been observed that higher ethanol productivity could be achieved by increasing the flow rate and glucose concentration because of improved mass transfer properties or reduced internal substrate diffusional resistances by varying the size of beads and increasing the flow rate (Chien and Sofer 1985); (Zhao and Delancey 2000). An insignificant difference was observed in the ethanol productivity for both types of beads at a lower flow rate i.e. 4 ml/min, which was higher at a higher flow rate (90 ml/min). By increasing liquid velocity, ethanol productivity was observed at its maximum because this enhances the surface substrate transfer (Bangrak, Limtong, et al. 2011). Higher glucose concentrations have a major role to achieve a maximum ethanol productivity (Converti, Perego, et al. 1985). But the internal mass transfer resistance has been observed at lower flow rates that induce glucose accumulation as a substrate inhibitory effect. Due to a low diffusion rate of ethanol from the inner region of beads to the medium, product inhibition might be also generated (Rotaru, Kloetzer et al. 2010); (Engasser and Horvath 1973); (Prasad and Mishra 1995).

Mass transfer increases with squire root of velocity ($Kl \propto U^{1/2}$). If the concentration is fixed the rate of reaction should vary with the factor of $U^{1/2}$, on the other hand, if velocity is continuously increased then can reach a point that reaction will no longer mass transfer limited and becomes independent on superficial velocity. Further work on the velocity distribution had been worked by Karman, Martinelli, Boelter as described by Lin (Lin, Moulton, et al. 1953).

If the rate of reaction inside the particle is slow then the mass transfer is dominated in the reaction and limits the overall rate of reaction. In this case, the reaction is not depending on the velocity of the fluid but can depend on the size of the particle. In 'Film theory' it is elaborated that mass transfer coefficients are directly proportional to mass diffusivity

(Potnis and Lenz 1996). Mass transfer boundary thickness increases if fluid past through the particle with low velocity and mass transfer will limit the rate of reaction. Further, if velocity is increased, the thickness of the layer around the particle will tends to reduce and mass transfer will not have a limiting effect on the rate of reaction.

Furthermore, in Table 2, beads with a 1 mm size have a higher yield than 2 mm at all flow rates and glucose concentration due to the reduction of intra-phase mass transfer limitations. Reduction in size of beads until no longer intra-phase mass transfer limitations exists, enhances the ethanol productivity which was also observed by (Duarte, Rodrigues et al. 2013); (Pilkington, Margaritis et al. 1998); (Boersma, Vellenga et al. 1979). Higher productivity at a glucose concentration of 10 g/l but on the other-side ethanol yield is reduced at this point when compared to 4 g/l glucose concentration. It can be depicted in both types of beads, the addition of glucose has also been observed to affect the ethanol yield that might be due to increased substrate diffusional resistance. Another reason might be the inhibition or reverse of reaction because of a higher rate of reaction upon increasing substrate concentration and also observed (Nikolić, Mojović, et al. 2009) a significant decrease in ethanol yield on the addition of sugar concentration in the fermentation medium. Furthermore, it was also found that intra-phase resistance is directly related to the substrate concentration which can induce substrate inhibition (Galaction, Lupăşteanu, et al. 2010).

3.5. CONCLUSION

3.5.1. Fluid-Side Mass Transfer

The operational performance of the immobilized packed-bed bioreactor in the course of immobilized yeast cells' physiological and biochemical changes (mass transfer properties) was monitored according to parameters such as glucose concentration, flow rate, and different immobilizing materials like alginate beads with and without chitosan coating.

This study gives a significant understanding of both alginate beads with and without chitosan coating as indicated in the differences of the resulted lag phases. As it was observed with chitosan-coated beads being 30–40 min higher than that of the non-coated beads. While in non-coated beads, the reason for the reduction of the lag phase time is due to the lower external mass transfer resistance. The maximum time of lag phase was found to be 290 min at a lower flow rate of 1 ml/min and 190 min at a higher flow rate of 90 ml/min when using 40 g/l of glucose. By decreasing the glucose concentration from 40 to 10 g/l, the lag phase

decreased too. Furthermore, no lag phase was found at a glucose concentration of 4 and 2 g/l. After the lag phase, no significant change was observed in both types of beads on glucose consumption with the same flow rate.

The dependence of lag phase on glucose concentration might be due to resistance in substrate diffusion or due to higher accumulation of cAMP level. It can be concluded that the lag phase is not due to the physiology of yeast, but it may be due to the resistance in the internal diffusion of glucose. Chitosan acts as a barrier in the transfer of substrate and products to/from the beads, respectively. Non-chitosan coated beads have a shorter lag phase as compared to coated beads, indicating an improved mass transfer effect observed at higher flow rates and a lower inhibition of glucose transfer. Moreover, the fermentation results of the Ethanol Red 11 yeast strain were compared with Baker's yeast using flow rates of 4 and 90 ml/min and glucose concentrations of 4 and 10 g/l. The results show that there is no significant difference in the lag phase of these two types of yeast. It has been observed that the lag phase is directly depending on the glucose concentration as well as on the flow rate.

By varying the flow rate from 1 to 90 ml/min, the time for glucose consumption decreases due to combined effect of convective mass transfer and molecular diffusion on the surface of beads.that enhance overall mass transfer. The major difference in glucose consumption behavior was observed when using both types of beads at a higher flow rate (90 ml/min). The time for glucose consumption by chitosan coated beads at 30 and 90 ml/min is rather equal when using a higher glucose concentration i.e. 40 and 20 g/l as compared to lower glucose concentrations 10, 4, and 2 g/l. While in non coated beads, time was further reduced by increasing flow rate from 30 to 90 ml/min.

When increasing the flow rate and glucose concentration, higher ethanol productivity was observed. This can be attributed to improved mass transfer properties when using higher flow rates and might be due to reduced substrate diffusional resistance. Furthermore, looking at the ethanol yield, the industrial strain Ethanol Red 11 showed higher yield than Baker's yeast at all flow rates and glucose concentrations. Optimal ethanol productivity of 21.9 g/(g.h) was obtained when using Ethanol Red strain at D of 4.5 h^{-1} with a glucose concentration of 10 g/l. It was also observed that there was no significant difference in ethanol productivity for both *S. cerevisiae* strains at lower flow rates i.e. 4 ml/min, while higher productivity was obtained at higher flow rates (90 ml/min). The ethanol yield for both strains has been observed to decrease when adding glucose.

3.5.2. Particle-Side Mass Transfer

The understanding of the mechanism of internal mass transfer effects on the immobilized system used for ethanol production and the performance of PBR as prototype was the first purpose of this study. Four operational parameters, glucose concentration, flow rate, alginate beads (chitosan and non-chitosan coated beads), and size of beads have significant effects on the internal mass transfer. These results depict that the substrate or product inhibition can limit the ethanol production efficiency and lead to enhance the lag phase of the fermentation process.

The results show that when using a glucose concentration of 4 g/l, no lag phase was observed, while with 10 g/l a lag phase lasting for about 190 min is observed. Further, the hydrodynamics has an important influence on substrate conversion time and mass transfer. The maximum time of a lag phase was found to be 190 min at a lower flow rate of 4 ml/min and 90 min at a higher flow rate of 90 ml/min. The chitosan also has an effect on intra-particle transfer of the substrate that can be reduced by reducing the size of beads and increasing the flow medium around the beads which enhances the molecular diffusion or eddies diffusion of solute in pores. Varying the size of beads is the best way to control internal mass transfer. By decreasing the size of beads to 2 and 1 mm, the duration of the lag phase decreased and the lowest time was 50 min when using 1 mm sized beads.

An insignificant difference of the glucose consumption time was noticed between chitosan-coated and non-coated alginate beads at higher flow rates (30 and 90 ml/min), especially for a smaller bead size (1 mm). This might be due to the reduction of solute diffusional distance from the surface to the site of reaction.

The time for glucose consumption in the case of 4 and 2 mm sized beads at a flow rate of 30 and 90 ml/min was rather equal using 10 g/l glucose while at 4 g/l significant difference in consumption time was approximately 50 to 60 minutes. It is depicted from results, with high glucose concentration higher will be the concentration gradient that can enhance the mass transfer into the beads while this is not the case using 4 g/l. Further increasing the flow rate to 90 ml/min reduces the consumption time by approximately 100 min for each bead size. The sharp reduction has been observed by the reduction in the size of beads from 2 mm to 1 mm as compared to 4 mm. At glucose concentration of 4 g/l & 10 g/l fermentation time approximately 250 & 400 minutes respectively can be saved if the size of beads reduced from 4 mm to 1 mm.

It has been observed that higher ethanol productivity could be achieved by increasing the flow rate and glucose concentration because of improved mass transfer properties. An insignificant difference was observed in the ethanol productivity for both types of beads at a lower flow rate i.e. 4 ml/min, which was higher at a higher flow rate (90 ml/min). Reduction in the size of beads until no longer intra-phase mass transfer limitations exist, enhances ethanol productivity. Beads with a 1 mm size have a higher yield than 2 mm type at all flow rate and glucose concentration due to the reduction of intra-phase mass transfer limitations. It was also concluded that the magnitude of resistance to the internal diffusion is directly related to the particle size and glucose concentration especially in case of alginate beads. A significant decrease in ethanol yield on the addition of sugar concentration in the fermentation medium has been observed. Furthermore, it was also found that intra-phase resistance is directly related to the glucose concentration which induces substrate inhibition.

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CHAPTER 4

BENCHTOP STIRRED BIOCATALYTIC BASKET REACTOR (SCBR)

SUMMARY

The performance of a bioreactor running a heterogeneous reaction depends on the rate of external and internal mass transfer. Mass transfer limitations can also be reduced to a certain extent by varying the stirrer speed, the medium glucose concentration, and the matrices utilized for immobilizing cells. Moreover, the impeller stirring speed is a major factor that helps to maintain the intra-particle flow by increasing the total flow rate, which can eliminate external mass transfer limitations.

In the PBR, a lag phase at the start of fermentation has been observed while in the SCBR no lag phase was observed even at higher substrate concentrations, which might be due to the well-mixing properties of this bioreactor. In order to understand the internal mass transfer, two types of matrices performance were compared: conventional alginate beads versus newly developed chemically grafted macro-porous DEAE sponges.

In the case of a highly concentrated glucose medium, a thick film layer develops around the alginate beads while this is not the case for sponges. Macroporous sponges have bigger pores that, facilitate faster glucose entry into the site of reaction consuming this sugar. Therefore, these sponges can eliminate external as well internal mass transfer limitations. The performance of both the Stirred Catalytic Basket Reactor (SCBR) with immobilized cells and the Stirred Tank Reactor (STR) with free cells were also evaluated and compared based on volumetric productivity, ethanol yield, and process completion time. The efficiency and physiology of free cells in STR were markedly affected by a higher glucose concentration inhibition and the shear effect of higher stirring speeds.

4.1. INTRODUCTION

4.1.1. Stirrer Catalytic Basket Reactor (SCBR)

The industrial production of ethanol or fine chemicals requires the development of new bio-catalytic reactors and cells immobilizing materials to achieve economically viable processes. As discussed in the previous chapters, traditional setups used in ethanol production like membrane bioreactor, airlift bioreactor, fixed bed bioreactors, and stirrer tank reactors have multiple drawbacks. To overcome these problems and improve the efficiency of a bioreactor, four most important factors are needed: choice of the fermentation process, the biocatalyst, the support for cell immobilization, and the bioreactor design.

As discussed in Chapter 1, three microbial fermentation processes are used for ethanol production: batch, fed-batch, and continuous. In this study, a batch fermentation process was selected due to the lower operational time needed (one single fermentation cycle). The third remaining aim of this study is to select a cultivation method (free cells or immobilized cells cultivation), support material for cells immobilization, and test them in a newly developed SCBR.

4.1.2. Cultivation Methods

Duarte, Rodrigues in. 2013 compared to the use of free cells, the immobilization technique offers higher productivity and efficiency in the ethanol industry. Some researchers Sendide et al reported that immobilized cells in Ca-alginate changed its morphological and metabolic activity. The cell immobilization has been common to improve the performance, cell physiology, and economics of most fermentation processes. Immobilized cells are not so much susceptible than free cells to the effect of substrate inhibition and pH variations (Hussain, Kangwa et al. 2015); (Corbo, Bevilacqua, et al. 2013) & (Pilkington, Margaritis et al. 1998); (Jamai, Sendide et al. 2001).

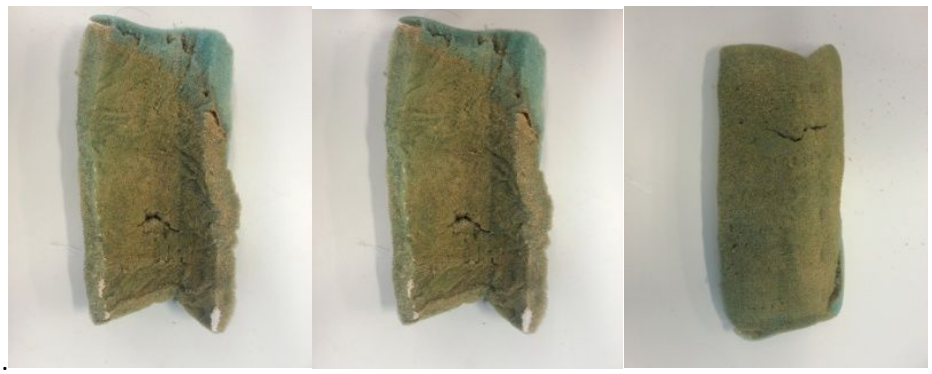
Several immobilization methods are available: physical entrapment, attachment or adsorption, self-aggregation by flocculation, etc. The choice of which method to use is mainly dictated by the nature of the application. The physical entrapment method is performed by entrapping the cells within a porous polymeric matrix, such as calcium alginate, carrageenan, chitosan, and other polymer beads. The attachment or adsorption method involves a reversible attachment of biomass to solid support mainly by electrostatic, ionic, and hydrogen bonding interactions (Pilkington, Margaritis, et al. 1998). This method is

usually used for different carriers like DEAE cellulose, porous glass, sponges, and wood blocks (Williams and Munnecke 1981).

The traditional support material, alginate beads, for the immobilization of cells is selected, where a simple entrapment method is followed. Alginate beads maintain good viability due to the mild environment of the gel network and provide a protective barrier towards physical stress (Lee and Mooney 2012). Although alginate beads have high cell viability and activity, several drawbacks have reduced its interest in the fermentation industry such as diffusion limitations of nutrients, chemical and physical instability of the gel and large scale production of these carriers require complex and sophisticated equipment making this immobilization type expensive (Verbele P J, 2006). It is important to note that despite the various advantages of immobilization, there is a possibility that it could reduce the productivity of the cell if significant diffusional limitations existed in the matrixes (Yabannavar and Wang 1991).

This is why various biocatalyst supports were developed and looked at newly produced polymers (biocatalyst support) use an attachment or adsorption method of immobilization due to the negative charge present on the surface of microbial cells, which can easily be adsorbed by electrostatic interaction on polymers having a cationic group i.e. positively charged (Nedovic, Kalusevic et al. 2011). These materials are reusable, have a lower cost, allow high cell densities, have a mechanical strength against share stress, and do not impair cell viability because it is prepared before immobilizing the cells. These are prepared by grafting a macro-porous polyethylene polymer (MPEP) with polyglycidyl methacrylate-ethylene diamine chains. In this grafted polymer, amino groups are attached, which help to interact with microbial cells.

Figure 4.1 Polyethylene sponges after adsorption of cells.



4.1.3. Design of a bioreactor

PBRs have been used for a long time for ethanol production due to higher productivities achieved using a continuous fermentation process. On the other hand, many disadvantages like low operational stability, the non-viability of yeast cells during the process, and the decrease of substrate surface contact with supports made this option not very attractive. Even on the industrial level, several engineering problems (mass transfer) have been seen, such as channeling in between carriers, drop in pressure, plugging of the bioreactor by a widespread growth, and higher production of product content or Co_2. The effect of pressure drop in packed bed bioreactors can be reduced by maintaining the size of the immobilized particle because the pressure drop is inversely proportional to the size of the particle (Norton and D'Amore 1994).

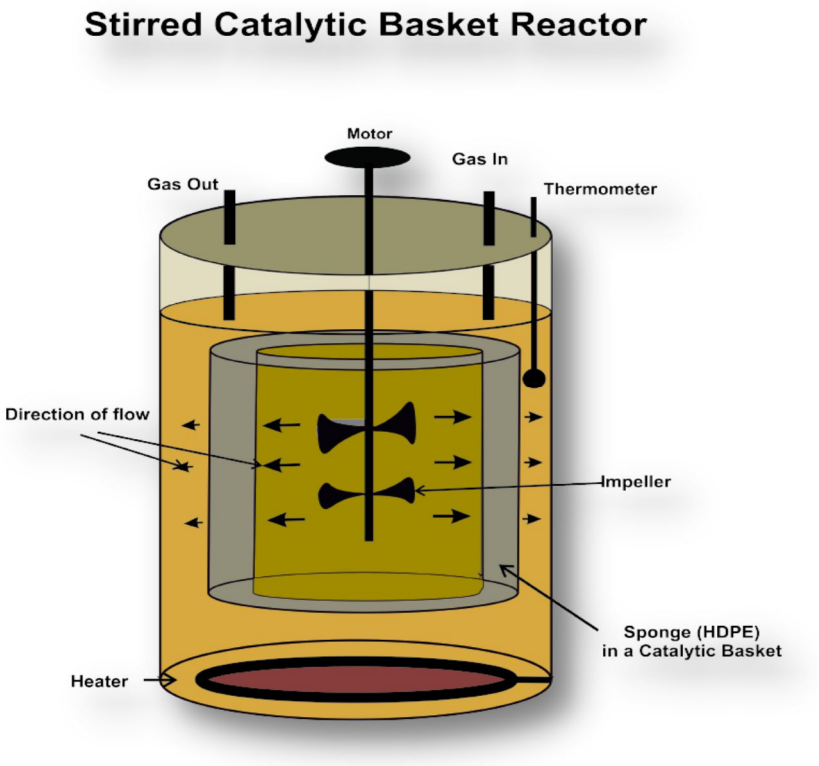
These disadvantages led to developing an SCBR. (Kourkoutas, Bekatorou, et al. 2004). It is important to evaluate and improve mass transfer properties in various bioreactors for ethanol production. The stirred tank reactor (STR) was also considered unfavorable (Norton and D'Amore 1994).

The Stirred Catalytic Basket Reactor (SCBR) (Figure 4.2) using immobilized cells was suggested to be suitable to support immobilizing particles and improve the surface contact between the substrate and immobilized cells. Therefore, the productivity of the bioreactor can be increased in shorter residence times. The objective of this chapter is to test the performance of SCBR using immobilized cells in different matrixes and compare its performance to free cells in the STR reactor with respect to the effect of mass transfer, cell physiology, and ethanol productivity.

The selection of the optimal laboratory reactor for intrinsic kinetics investigations is difficult due to the transport phenomena that could occur in liquid-solid interfaces. For the cultivation of free cells, STR is usually used due to its advantages of increasing mass transfer rates and a well-mixed environment. But for immobilized cells, care must be taken to ensure that the support is not damaged and the yeast cells do not suffer from shear stress. Many configurations are possible within the mentioned design concept, the basket (filled with immobilized catalyst) attached to the agitator shaft of the STR which gives efficient mixing, heat, and mass transfer to the immobilized cells (Gamarra, Cuevas et al. 1986). The basket has the advantage of permitting greater contact between reactants and biocatalysts, which in turn increases the reaction rate and efficiency of the bio-catalytic reaction.

The biocatalyst is separated from the reaction mixture simply by draining the circulating liquid (Baltaru, Galaction, et al. 2009). In SCBR, the pH and temperature are controlled by a well-mixed environment with the help of agitation. Consequently, the hydrodynamics of the broth in and around the basket show an important effect on the mass transfer processes involved in the substrate conversion.

Fig.4.2. Stirrer Catalytic Basket Bioreactor



4.2. MATERIALS AND METHODS

4.2.1. Reactors and chemicals

The STR and SCBR were bought from Bioengineering Inc, Germany. All other chemicals, including yeast extract nitrogen base without amino acid, ammonium acetate, amino acid mixture, sodium alginate, calcium chloride, sodium chloride, chitosan, hydrochloric acid, potassium sodium tartrate, dinitro salicylic acid (DNS), *n*-butanol, ethyl acetate, glucose, peptone, yeast extract, agar, sodium hydroxide were of analytical grades, are directly purchased from Sigma (USA) and Applichem (Germany).

4.2.2. Microorganism

The yeast strains *Saccharomyces cerevisiae* (baker yeast) was obtained from DHW Vital Gold, Nürnberg, Germany, while the *Saccharomyces cerevisiae* Ethanol Red11 strain was purchased from Fermentis Inc, Germany and were stored at 4 and −80 °C, respectively.

4.2.3. Strain preservation

For the strain preservation, the yeast strain was initially prepared in a sterile cultivation media. 1.0 ml of a late log or early stationary phase culture solution was mixed with an equal volume of 30% glycerol (w/v) solution in sterile 4 mL screw-cap vials mixed, freezed on dry ice, and stored at −80 °C. After that, the strain was scraped and streaked onto plates.

4.2.4. Inoculum Preparation

For the culture preparation, Ethanol Red 11 strain was refreshed by streaking onto YPD agar plate (1% yeast extract, 2% Peptone and 2% Glucose, 2% agar), incubated for 2 days at 35 °C. The resulting colonies were used to have a fresh culture. 20 ml of YPD media (1% yeast extract, 2% Peptone and 10% D-Glucose) in a 100 ml flask was inoculated with a single colony of Yeast Ethanol Red 11 grown overnight at 35 °C with vigorous shaking at 250 rpm.

One percent of the pre-culture was used to inoculate a 2 l Erlenmeyer baffled flask containing 1000 ml YPD media (final volume). The inoculated flask was incubated at 200 rpm and 35 °C for 24 h. Furthermore, the cells were collected by centrifugation at 4000 rpm for 15 min, washed twice with sterile distilled water, centrifuged, and re-suspended in sterile water to obtain a dense cell suspension.

4.2.5. Fermentation Medium and Cultivation

For this stage, minimal media was utilized in the cultivation process, prepared with 6.7 g/l yeast extract nitrogen base without amino acid, 1.7 g/l ammonium acetate and glucose (4 and 10 g/l) were prepared separately and mixed after sterilizing (121 °C, 20 min.). These different amino acids were mixed to prepare "amino acid mixture" (100×); 200 mg l-arginine, 1000 mg l-aspartic acid, 1000 mg l-glutamic acid, 300 mg l-lysine, 500 mg l- phenylalanine, 4000 mg l-serine, 2000 mg l-threonine, 300 mg l-tyrosine, 1500 mg l-valine. All components were dissolved in distilled water by adjusting pH to 10 with 0.1 N NaOH and filter using a 0.2 µm filter, and 10 ml of amino acid solution was further added to make a final 1 l media.

Fermentation procedure A 3.7 L stirred tank reactor (STR) (Bioengineering Co.) with a working volume of 2.5 L with vessel of diameter 12,5cm and height 15cm. was utilized free cells cultivation and SCBR having the same working volume was used for the cultivation of immobilized cells. The SCBR has basket made of stainless steel mesh with outer diameter 8,14cm and inner diameter of 6,64cm.

The "Minimal medium" composition as mentioned in section 'Fermentation medium and cultivation' was used, and yeast cells of 16 g/l were added in the fermenter in the case of free cell cultivation. Different glucose concentration (50, 100, and 200 g/l) and stirrer speed (200, 300, and 500 rpm) were chosen to analyse the effect of stirrer speed and glucose concentration parameters on the performance of SCBR respect to mass transfer properties and ethanol productivity.

4.2.6. Calcium alginate beads preparation and yeast

The Calcium alginate beads of 2.5% (w/v), was prepared in 50 mM phosphate buffer at pH 7. The cell suspension (1,6%) was mixed with alginate solution for immobilization of baker yeast. The full procedure is explained in section 3.2.

The alginate beads with diameters 1, 2 and 4 mm were used in experiments. For the preparation of chitosan-coated alginate beads, the above-prepared beads were dipped in sterilized chitosan solution (3% chitosan, 0.1 N HCl, pH 5) for 10 min and later washed 3 times with sterile water.

4.2.7. Polyethylene sponges immobilization and cultivation Conditions

For immobilizing yeast cells, MPEP sponges were initially autoclaved for 15 min at 121 °C and kept overnight at 4 °C to facilitate de-aeration. The MPEP surface was prepared according to the established protocol (Trelles et al. 2010) with some modification.

The SCBR basket was filled with polyethylene sponges, and then pre-cultured yeast cells (16 g/l) were aerobically fermented at 200 rpm and 35 °C for adsorption onto the support. After 2 days, the cell immobilized support was washed with sterile water and later used for an experiment using minimal media.

4.2.8. Glucose consumption measurements

The DNS method was used to measure the immobilized yeast glucose consumption. For each measurement, a 0.5 ml sample and a 0.5 ml DNS solution were mixed in a 1.5 ml Eppendorf tube, vortexed for 10 s, and incubated for 10 min at 90 °C.

After incubation, 40% of 0.16 ml potassium sodium tartrate was added, mixed by vortex mixer, and placed on ice for 3 min. 200ul of each sample was measured at 575 nm. The obtained results were compared with a calibration curve of different glucose concentrations to get the actual concentration.

4.2.9. Ethanol production measurements

The concentration of ethanol produced in a fermentation broth as well as the calibration curve were determined as discussed in previous chapter.

4.3. RESULTS AND DISCUSSION

4.3.1. Comparative dimensional Analysis

The initial results plotted in Figure 4.3 (a, b and c) showing free and immobilized yeast cells (alginate beads and sponges) suggest some information concerning mass transfer properties that are related to the stirring speed and the initial glucose concentration. For stirring speeds of 200, 300, and 500 rpm using a glucose concentration of 50g/l, 100g/l and 200 g/l the consumption of glucose up to the level where $C/C_0 = 0.1$ has been observed. In the free and immobilized cells, the time for glucose consumption decreased with increasing the stirring speed.

Using a speed of 200 rpm, the time of 50g/l glucose consumption was 5h, while at 300 rpm and 500 rpm, the time observed was significantly lower than 5h. The amplification of

consumption time difference in free and immobilized cells was observed by increasing the glucose concentration to 100 g/l and 200 g/l. Moreover, from Figure (4.3b) it can be observed that at 200 rpm with a 100 g/l glucose concentration, the sponges consume glucose in 9 h, while alginate beads and free cells consume it in 19 h and 20 h, respectively.

Increasing the agitation speed to 300 rpm and 500 rpm, decreased the glucose consumption time significantly in free cells (15 h and 10 h, respectively), alginate beads (12 h and 8 h, respectively), and in sponges (6,4h and 5 h, respectively). Therefore, the fluid velocity improves the mass transfer into the yeast cells immobilized in different matrixes. The magnitude of mass transfer resistance has an inverse relationship with the stirring speed. The reason behind it is that the stirring speed controls the internal diffusion of the substrate in the case of immobilized cells by enhancing eddies effect. The sponges showed a lower internal diffusion resistance as compared to alginate beads and a negligible effect when increasing the stirring speed.

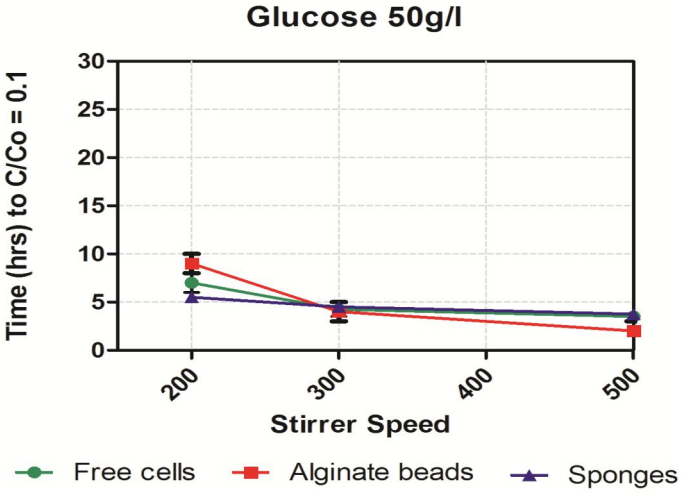
The results in figure 4.3c further support the above conclusions; the difference in time of glucose consumption was observed for free cells (30 h), alginate beads (24 h), and sponges (17 h) at a lower stirring speed of 200 rpm that tended to decrease (20, 7, and 8 h, respectively) at a higher stirring speed of 500 rpm. The higher consumption time in the case of the free cells might be due to the shear effects caused by stirring. Moreover, the time difference between sponges and alginate beads might be because of internal diffusion resistances that can be an accumulation of glucose concentration on the pore surface of alginate beads.

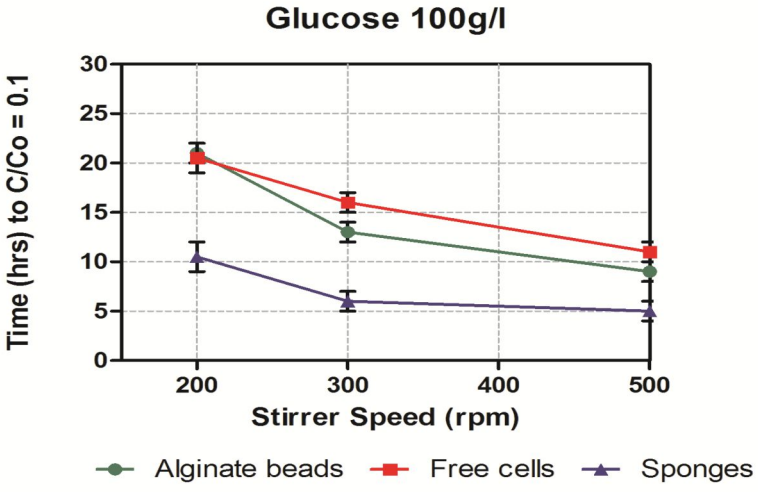
The resistance in internal diffusion is a major problem that can arise in immobilizing technology. It can be improved by using chemically grafted sponges and an optimized stirring speed.

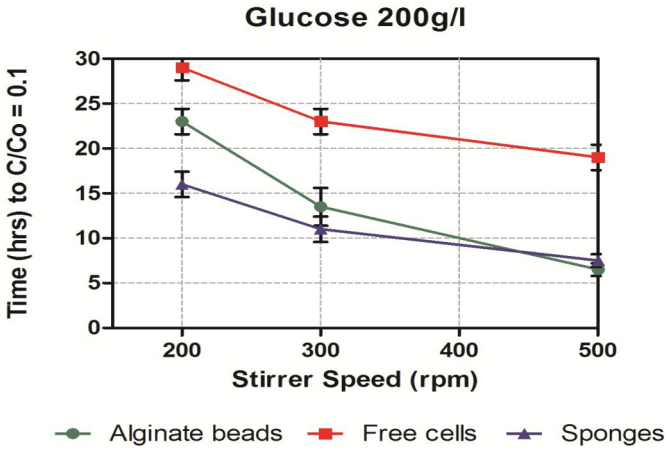
At higher stirrer speed 500rpm, time for glucose consumption is approximately the same (5h) at 50g/l, 100 g/l, or 200 g/l glucose. It is depicted that sponges have no external or internal mass transfer limitations or due to macropores' fluid flow is improved therefore there is no difference in consumption time at a higher speed. While in alginate beads and free cells, a significant difference comes out due to the increasing effect of substrate diffusion. Even at a lower stirrer speed 200rpm, cells consume 50 g/l in 5h and 100 g/l in 10h and 200 g/l in 15h. It means after 10h rate of reaction was so fast that cells consume 100g/l within 5h. While in the case of alginate beads consumption time difference is big 15h between 50g/l and 100g/l glucose concentration. It is evidence of substrate diffusion resistance in alginate beads.

Figure 4.3 (a, b, c) Effect of agitation speed and immobilizing matrices on glucose consumption.

a). Glucose 50g/l b). Glucose 100g/l c). Glucose 200g/l







The yeast cells inside the beads were maintained to be uniform by using the minimal medium where growth was at its minimal capacity. As growth takes place, the concentration of biomass inside the alginate beads increases, which can enhance the oxygen and nutritional diffusion limitations (Duff and Murray 1988). Using three methods for yeast cultivation, the experiments were conducted using free suspended cells in the STR, immobilized cells in alginate beads, and sponges in the SCBR.

Figures 4.4 and 4.5 show the effect of two important factors, stirring speed and immobilizing matrix, on ethanol production and yield. Figures 4.4a-c illustrate the graphs for initial comparative results of ethanol productivity between free and immobilized cells using a high glucose concentration of 200g/l and a stirring speed of 500 rpm to specifically be aware of the mass transfer properties in immobilized systems. The purpose of showing higher glucose concentration graphs (Figure 4.4) is to check whether a higher glucose concentration plays a role to achieve maximum ethanol productivity (Converti, Perego, et al. 1985).

At a glucose concentration of 200g/l, the optimal ethanol concentrations obtained when using sponges, alginate beads, and free cells were 85, 79, and 60g/l, respectively. It can be concluded that sponges could act as an optimal best support material for yeast immobilization because of the higher ethanol production achieved. Another essential part of these graphs is the complete conversion of glucose into ethanol. Higher performance in terms of glucose conversion time was observed in the SCBR as compare to the STR reactor. Free cells needed 20h to complete one batch process as compared to immobilized cells (alginate (7h) and sponges (8h) – the difference is more than double.

It can be concluded that immobilized cells could better preserve their activity than free cells. At lower stirring speed, the ethanol yield and the glucose conversion time are observed as limited by external diffusional resistances. The diffusivity of glucose through the boundary layer surrounding the biocatalyst particles has a major role to achieve a maximum ethanol yield that is directly controlled by the structure of the immobilizing matrix and the stirring speed, mode of operation, and design of a bioreactor.

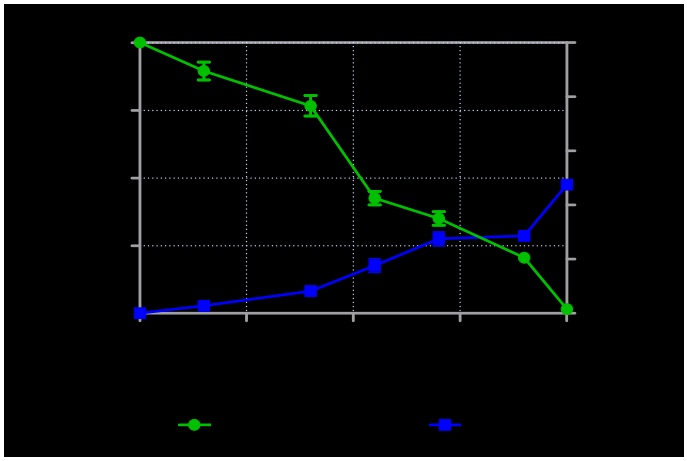
The medium hydrodynamics in bioreactors exhibit an important influence on glucose conversion and transfer processes (Galaction, Kloetzer et al. 2012). Figure 4.5 compares the ethanol yield and the time needed for one batch cycle to be completed up to the level where $C/C_0 = 0.1$ using low (200 rpm) and high (500 rpm) stirring speeds for comparison. At a lower stirring speed (200 rpm), the ethanol yield (0.15, 0.195, and 0.285) was achieved in

(30h, 24h, and 17h) for the free cells, alginate beads, and sponge, respectively. At a higher stirring speed (500 rpm), the increased ethanol yield (0.240, 0.35, and 0.405) was achieved in shorter times (20h, 7h, and 8h).

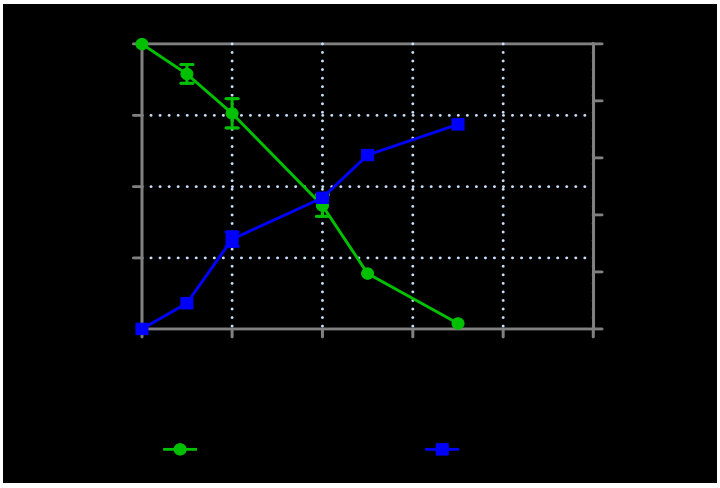
These results indicate that the immobilizing matrices characteristics also affect the internal diffusion of glucose and its consumption rate (Galaction, Lupăşteanu et al. 2010). This offers insightful information about the effect of immobilizing materials on ethanol production efficiency, mass transfer phenomena, and the overall performance of a bioreactor.

4.4 (a, b, c) Effect of immobilizing matrices on glucose consumption. Glucose 200g/l

Free Cells



Alginate beads



Sponges

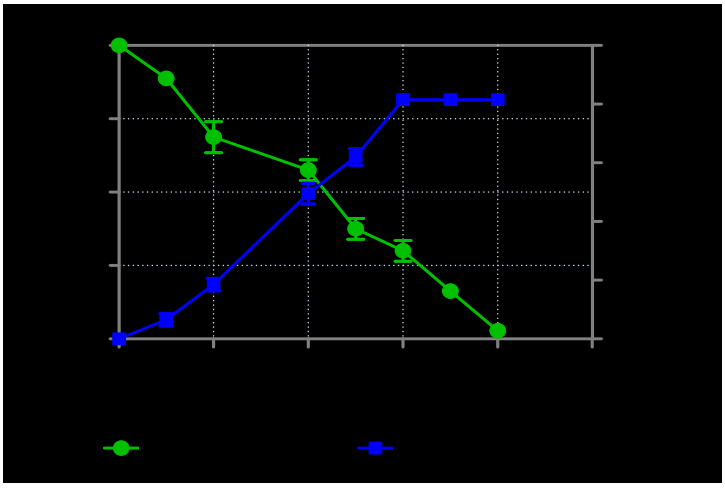
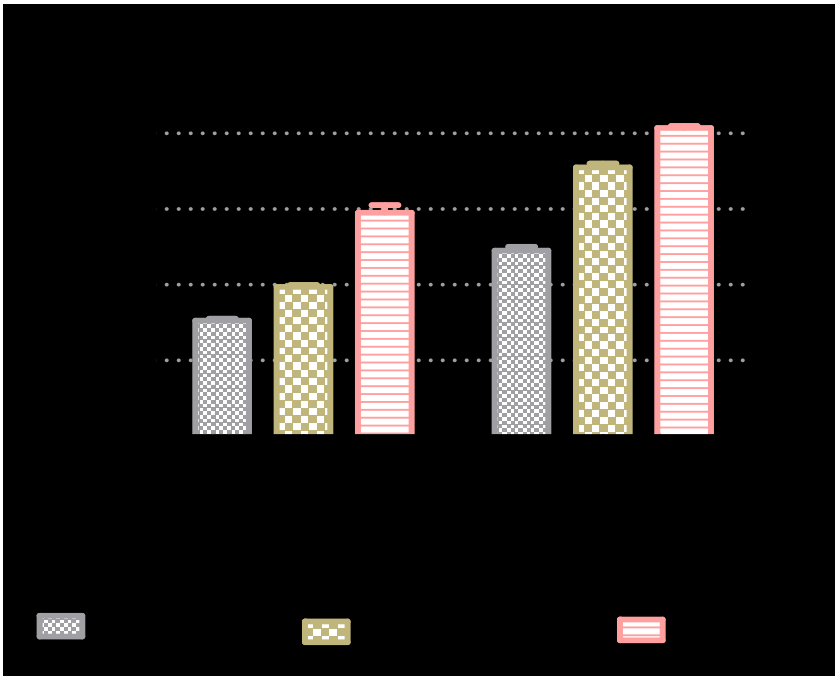


Figure 4.5. Effect of stirring speed (rpm) on ethanol yield and glucose consumption time at higher glucose concentration (200 g/l)



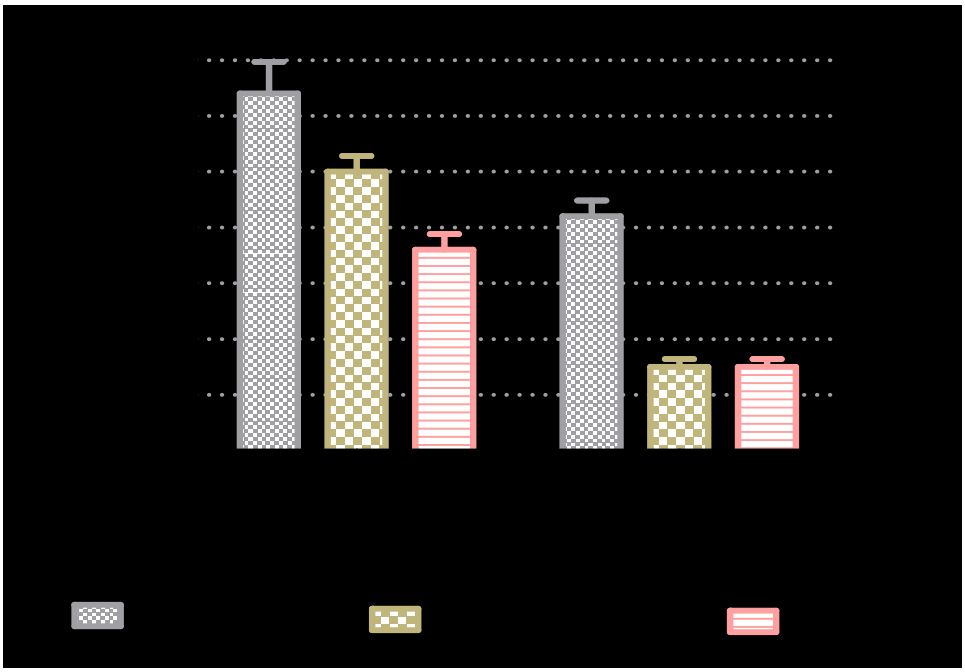


Table 4.1: Ethanol yield and Glucose consumption time by free cells and immobilized cells in Alginate bead

	Stirring speed (rpm)	Glucose conc. (g/l)	Ethanol Yield (g/g)	Glucose consumption time (h)
Free cells	200	200	0.15	30
Alginate	200	200	0.195	24
Sponges	200	200	0.285	17
Free cells	500	200	0.24	20
Alginate	500	200	0.35	7
Sponges	500	200	0.405	8

Ethanol Yield

$$EthanolYield = Y\left(\frac{P}{S}\right) = \frac{PI - Po}{So - SI}$$

Sl= Substrate concentration at a specific time C/Co = 0,1

Pl = product concentration at a specific time

Po=Product concentration at time zero

So=Substrate concentration at time zero

4.3.2. Discussion (Dimensional Analysis)

As a reminder, inhomogenous reactions (as in the case of free cells), mass transfer effects are negligible. However, for the heterogeneous case (alginate beads/sponges), the reaction only takes place when reactants are transferred to the catalytic reaction site by diffusing across the external fluid layer around the catalyst (external mass transfer) into the pores within the catalyst (internal mass transfer).

The performance of a bioreactor is strongly dependent on the stirring speed, glucose concentration in the medium, and matrices for immobilizing cells. In other words, the performance of a bioreactor depends on the rate of external/internal mass transfer, which is dictated by these factors. This was seen when the effect of varying the above-mentioned factors on the performance of the SCBR and the transport process (i.e. consumption behavior of glucose) and behavior of fluid dynamics was studied in the next section. The external mass transfer involves the transport of a substrate from the bulk medium to the surface of immobilized matrices.

In this case, the first resistance issue is the fluid film around the matrix surface and its thickness, which depends on various physical properties of the fluid i.e substrate concentration and velocity of fluid "MODES OF MASS TRANSFER - CHERIC." higher substrate concentration on the surface of beads possibly causing the substrate to be inhibited. Warnock stated that in immobilized cell systems, a concentration gradient between bulk and an intra-particle medium develops when glucose is consumed while metabolites are produced (Warnock, Bratch, et al. 2005). Talebnia and Taherzadeh in 2007 discussed the limitations of substrate transfer into the center of immobilizing matrices and toxic metabolites out of it.

The optimized stirring speed in a bioreactor plays a major role in immobilized cell systems to ensure insignificant temperature and concentration gradients at the catalyst surface. In fact, the effect of stirring speed on metabolic activities of *Saccharomyces cerevisiae* was found to be a major factor in affecting the product yield (Ghigliazza, Lodi, et al. 1996); (Converti, Casagrande, et al. 1996). As shown in Chapter no.3, a lag phase at the start of fermentation in PBR using an immobilized system (entrapped cells in alginate beads) appeared because of poor mixing or mass transfer resistances. The time for the lag phase was inversely proportional to the flow rate.

The presence of the lag phase is an indication of a concentration gradient around the surface and within the beads that can be controlled by the optimized flow of the fluid medium. In SCBR, no lag phase was observed even at higher substrate concentrations because of the well-mixed environment of this bioreactor (lower concentration gradients). The influence of mass transfer limitations on reactors' performance using immobilized cells is a critical topic (Willaert 2009). Thus, the improvement of mass transfer limitations is desired to enhance the efficiency of a bioreactor (Salmon and Robertson 1987).

The internal mass transfer involves the transport of substrate from the surface of immobilized matrices to the site of reaction and this depends strongly on the properties of the matrix used. In this study, two types of matrices performance are compared.

Figure 4.3 shows that the glucose consumption time was rather equal at different stirring speeds 200, 300, and 500 rpm at a lower glucose concentration (50g/l) using 4 mm sized beads. This indicates that if the medium glucose concentration is low, a thin film around the matrices develops; there might be a linear substrate gradient across the thin film. The difference of time in glucose consumption between free and immobilized cells (alginate and sponges) tended to increase by increasing the glucose concentration (100 and 200 g/l) in the

medium. In this case, a thick layer around the particle developed, which can increase the steep concentration gradient in and outside the particle due to resistance in substrate diffusion. As the substrate progress to be consumed, the substrate may be available around the whole particle (matrices). The substrate here is not equally available because it could not reach the middle of the immobilized particle, which becomes deprived of substrate consumption and thus affecting the whole productivity of the system. This happens especially in the case of alginate beads and not sponges because the latter takes less time to consume glucose and might have no external film developed due to their bigger pore size that enhances the intra-particle flow (Warnock, Bratch et al. 2005).

It can be concluded that using sponges, external, and internal mass transfer limitations of substrate concentration can be eliminated by improving fluid flow through pores which are explained in the next part of this study. Galactic et al. found the same behavior using alginate beads in SCBR at a higher glucose concentration (150 g/l). The glucose concentration on the surface of the beads increased while the glucose consumption rate was reduced due to the higher substrate concentration gradient. It is very important to diffuse out the toxic metabolites from the particle, which can be possible by improving the internal mass transfer phenomenon as in macro-porous catalysts (Galaction, Kloetzer, et al. 2012).

There is one major factor, which can eliminate mass transfer limitations i.e. stirring speed that ultimately enhances the fluid dynamics around the catalyst This parameter helps maintaining the intra-particle flow by increasing the total flow rate and should be optimized in a way to avoid shear effects on the performance of cells as observed in STR. The cells could suffer from internal damage by higher stirring speeds in the case of a weaker cell membrane.

The poor velocity however can also create problems of substrate inhibition, bridging, and channeling problems especially using alginate beads. The ideal flow pattern in any reactor is not possible, but we can improve and avoid these problems by recognizing optimum factors. Figure 4.3 a-c shows the effect of stirring speed on free and immobilized cells. The glucose consumption time is higher at lower stirring speeds (200 rpm) both for free and immobilized cells due to inefficient mass transfer. To increase its efficiency, the substrate should reach the yeast cells by diffusion and convection through the external liquid film, liquid-solid interface, and resistance caused by liquid and micro-colonies of yeast within the particle (Hussain, Kangwa, et al. 2015).

The stirring speed induces the diffusion and convection process of the substrate. If the stirring speed is not enough, the substrate concentration on the surface and within the

matrix will be lower than the concentration in the bulk medium. In this case, a dead necrotic zone in the center of the matrix can develop and only cells biomass around the matrix periphery can consume the substrate. These reasons support the result that at lower stirring speeds, cells take a longer time to consume glucose. While increasing the stirring speed to 300 rpm and 500 rpm, the glucose consumption time tended to decrease at all glucose concentrations (50 g/l, 100 g/l, and 200 g/l). This clearly indicates that the internal diffusion resistance due to higher substrate concentration is not dominantly present: the substrate can travel easily to the reaction site of yeast cells and be consumed as faster as allowed by the diffusion process by the production of eddies with lower pressure gradient.

On the other hand, shear effects at higher stirring speeds (even at 500 rpm) have been observed in Figure 4.3c indicating that the time for glucose consumption is higher in the case of free cells as compared to alginate beads and sponges. Bleoanca et al. described multiple yeast stress factors that can directly affect cellular activity and the overall fermentation performance. Mechanical stress (shear stress) was one of the factors having an impact on the yeast cell wall and its functionality (reduction of viability and vitality) (Bleoanca, Silva, et al. 2013).

In order to achieve higher volumetric productivities, cell density in the fermentation system is essential and that is only attainable by immobilizing the cells as free cells cannot maintain a specific growth rate. The performance of SCBR was evaluated based on its volumetric productivity, ethanol yield, and process completion time. The SCBR ethanol productivity was higher than that of the STR used as a control. Moreover, the fluid velocity is another factor that improves the mass transfer into yeast cells immobilized in different matrices.

Therefore, the distance for reactants to reach the active site of the catalyst, the film diffusion barrier around the catalyst, and the substrate gradient effect inside the immobilizing matrix are progressively reduced, while in contrast, more surface area could be exposed that enhances the surface substrate transfer (Bangrak, Limtong, et al. 2011), (Gamarra, Cuevas, et al. 1986). Some other researchers (Chien and Sofer 1985; Zhao and Delancey 2000) found higher ethanol productivity by optimizing the stirring speed and glucose concentration because of improved mass transfer properties or reduction of internal substrate diffusional resistances using immobilizing materials.

It could be possible only by understanding the behavior of fluid Do (1984) used a continuous stirred basket reactor for the deactivation of immobilized enzymes and discussed that this kind of reactor was mostly used to study the chemical and kinetics to find out that external and internal mass transfer limitations are eliminated using small sizes of catalysts (Do 1984).

Figures 4.4 a-c indicate that immobilized cells preserved their activity than free cells and are more resistant to heat and shear effect. Free cells needed 20h to complete one batch process as compared to immobilized cells (alginate (7h) and sponges (8h)) – the difference is more than double.

The effect of a higher glucose concentration on ethanol productivity can be observed in Figures 4.4a-c that indicate the reduction of ethanol productivity in alginate beads as compared to sponges. This might be due to the inhibition caused by a higher rate of reaction upon increasing the substrate concentration. Also (Nikolić, Mojović, et al. 2009) observed a significant decrease in ethanol yield once adding further sugar in the fermentation medium and (Galaction, Lupăşteanu, et al. 2010) found that the intra-phase resistance induces the substrate inhibition, which is directly related to the glucose concentration.

The lower ethanol yield using free cells (Figures 4.4 and 4.5) might be due to the effect of glucose inhibition that tends to increase upon using a higher glucose concentration. The efficiency and physiology of free cells are markedly affected by a higher glucose concentration inhibition and shear effects caused by a higher stirring speed. Le (2008) discussed the inhibition of yeast growth and metabolic activities by high initial substrate concentrations. While the results of (Galaction, Lupăşteanu, et al. 2010) depict that the substrate or product inhibition phenomenon can limit the efficiency of ethanol production. Moreover, the productivity difference between sponges and alginate beads shows the presence of internal diffusional resistances in alginate beads.

Figure 4.5 shows that there is an insignificant difference in the glucose consumption time between sponges and alginate beads at high stirring speed (500 rpm). The reason might be due to the removal of diffusional limitations in and around the supporting materials. Consequently, it can be excluded that the magnitude of resistance to the internal diffusion is directly related to the types of immobilizing matrixes and glucose concentration (Galaction, Lupăşteanu, et al. 2010); (Engasser and Horvath 1973). The time for glucose consumption or in other words the transfer of the substrate from the outer surface into the matrix depends on the (1) kinetics (2) reactor design (i.e. mode of operation) (3) stirring (4) type of catalyst (5) Stirring (6) reaction conditions (Satterfield 1970). The results in Figure 4.5 indicate that sponges take less time to complete one batch process than alginate beads and free cells. This might be because cells immobilized in sponges have lower mass transfer limitations and no diffusional barriers by the immobilizing reagent as compared to alginate beads. Although alginate beads are porous, a further internal mass transfer related disadvantage is that they

don't have a convective flow inside so nutrients travel to the cells by diffusion only (Najafpour 2015).

4.4. CONCLUSION

In heterogeneous, the reaction only takes place when reactants are transferred to the catalytic reaction site by diffusing across the external fluid layer into the pores. The performance of a bioreactor is strongly dependent on the stirring speed, glucose concentration in the medium, and matrices for immobilizing cells. The purpose of this study is to select a cultivation method (free cells or immobilized cells cultivation), support material for cells immobilization, and test them in a newly developed SCBR. The immobilization technique offers higher productivity and efficiency in the ethanol industry. It is important to note that despite the various advantages of immobilization, there is a possibility that it could reduce the productivity of the cell if significant diffusional limitations existed in the matrixes (Yabannavar and Wang 1991). This is why various biocatalyst supports have developed a use for an attachment or adsorption method of immobilization due to the negative charge present on the surface of microbial cells.

A higher substrate concentration can raise the resistance in substrate diffusion between the bulk liquid and the interior surface of beads possibly causing the substrate to be inhibited. This indicates that if the medium glucose concentration is low, a thin film around the matrices develops. The difference of time in glucose consumption between free and immobilized cells (alginate and sponges) tended to increase by increasing the glucose concentration (100 and 200 g/l) in the medium. In this case, a thick layer around the particle developed, which can enhance the resistance in substrate diffusion into the pores of alginate beads.

There is one major factor, which can eliminate mass transfer limitations i.e. stirring speed that ultimately enhances the fluid dynamics around the catalyst This parameter helps maintaining the intra-particle flow by increasing the total flow rate and should be optimized in a way to avoid shear effects on the performance of cells as observed in STR.

Fluid velocity improves the mass transfer into the yeast cells immobilized in different matrices. The magnitude of mass transfer resistance has an inverse relationship with the stirring speed. The reason behind it is that the stirring speed controls the internal diffusion of the substrate in the case of immobilized cells. The sponge showed a lower internal diffusion resistance as compared to alginate beads and a negligible effect when increasing

the stirring speed. In fact, the effect of stirring speed on metabolic activities of *Saccharomyces cerevisiae* was found to be a major factor in affecting the product yield.

The time for the lag phase was inversely proportional to the flow rate. The presence of the lag phase is an indication of an internal diffusion resistance of the substrate around the surface and within the beads and can be controlled by the optimized flow of the fluid medium. In SCBR, no lag phase was observed even at higher substrate concentrations because of the well-mixed environment of this bioreactor.

The higher consumption time in the case of the free cells might be due to the shear effects caused by stirring. Moreover, the time difference between sponges and alginate beads might be due to the effect of internal diffusion resistances that could develop steep concentration gradients inside and outside the surface of the immobilized matrices. The resistance in internal diffusion is a major problem that can arise in immobilizing technology. It can be improved by using chemically grafted sponges and an optimized stirring speed that is further briefed in fluid dynamics.

It can be concluded that by using sponges, external, and internal mass transfer limitations of substrate concentration gradients can be eliminated by improving fluid flow through pores. At higher stirrer speed 500rpm, time for glucose consumption is approximately the same (5h) at 50g/l, 100 g/l, or 200 g/l glucose. It is depicted that sponges have no external or internal mass transfer limitations or due to macropores' fluid flow is improved therefore there is no difference in consumption time at a higher speed.

It can be concluded that immobilized cells could better preserve their activity than free cells. At lower stirring speed, the ethanol yield and the glucose conversion time are observed as limited by external diffusional resistances. The diffusivity of glucose through the boundary layer surrounding the biocatalyst particle has a major role to achieve a maximum ethanol yield that is directly controlled by the structure of the immobilizing matrix and the stirring speed, mode of operation, and design of a bioreactor. Although alginate beads are porous, a further internal mass transfer related disadvantage is that they don't have a convective flow inside so nutrients travel to the cells by diffusion only (Najafpour 2015).

It is concluded that by using sponges, external, and internal mass transfer limitations of substrate concentration can be eliminated by improving fluid flow through pores. At a glucose concentration of 200 g/l, the optimal ethanol concentrations obtained when using sponges, alginate beads, and free cells were 85, 79, and 60g/l, respectively. It can be concluded that sponges could act as an optimal best support material for yeast immobilization because of the higher ethanol production achieved.

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CHAPTER 5

ROLE OF FLUID DYNAMICS IN REACTOR PERFORMANCE:

SUMMARY

In a heterogeneously-catalyzed reaction, it is very important to improve or eliminate the mass transfer limitations (Salmon and Robertson 1987). In this study, the purpose was to understand the mechanism of fluid flow by dimensionless mass transfer effects in the immobilized system used for ethanol production and the performance of PBR. The performance of a bioreactor depends on the rate of external and internal mass transfer. Mass transfer limitations can also be reduced to a certain extent. Liquid phase transport properties play a significant role to understand the trend of mass transfer coefficients and rate of reaction on the particle surface and it helps in maintaining the intra-particle flow by increasing the total flow rate, which can eliminate external mass transfer limitations. This chapter has two parts: the first part is about fluid dynamics in a packed bed which is used as a prototype reactor & the second part is about fluid dynamics in (SCBR) Stirred Catalytic Basket reactor.

Four operational parameters, glucose concentration, flow rate, coating alginate beads (chitosan and non-chitosan coated beads), and size of beads have significant effects on the internal mass transfer. In the first part of this chapter, mass transfer data in packed bed is analyzed in terms of fluid flow pattern over particle (fluid hydrodynamics) and entropy generation or energy dissipation. When viscous flow passes through over the spherical particles in a packed bed, the flow will not be laminar anymore but in a steady-state where viscous stresses and velocity gradients are established. which are the main factors of momentum transfer:

If Rep is smaller, the rate of change of momentum is observed smaller as compare to viscous and pressure forces. The streamlines are also in asymmetry. As Rep increases, the flow pattern and forces over the particle surface will also change. In order to enhance the mass transfer size of the particle should reduce, the reaction rate per unit volume of the particle will increase because mass is transferred in the center of the particle within a short time as compared to larger particles. If fluid flows over a particle, boundary layers separate from the surface due to adverse pressure gradient in the boundary layers and flow has high pressure at the stagnant and rare point with lower pressure on the top and bottom of the particle. Due to velocity and pressure gradient, a large wake is developed behind the particle (Doran 2013). Higher pressure diffusion moves to the direction of small pores and depends on the value of the Knudsen diffusion coefficient and binary diffusion coefficient. The binary diffusion coefficient is a function of temperature, pressure, and concentration (Satterfield

1970; Eigenberger 1992). Mass transfer resistance and diffusion have a direct relationship with the boundary layer thickness on the interface of liquid and solid that affect the rate of mass transfer as well on the mass transfer coefficient (Potnis and Lenz 1996). There are some parameters on which the thickness of the boundary layer depends like fluid mass transfer properties, size of particle as catalysts, and the velocity with which fluid reaches to the surface of the particle, velocity gradient, and pressure gradient develops on the surface (Lal, Kumar, et al. 1988).

In the PBR, a lag phase at the start of fermentation has been observed while in the SCBR no lag phase was observed even at higher substrate concentrations, which might be due to differences in flow behavior in both reactors. In order to understand the internal mass transfer, fluid dynamics in two types of matrices performance are compared: alginate beads and sponges. The formation of the boundary layer is important to understand the flow characteristics and its effect on mass and heat transfer between phases. A thick film layer develops around the alginate beads while this is not the case for sponges because of improved internal flow. Sponges have bigger pores that, facilitate faster glucose entry into the site of reaction consuming this sugar. Therefore, these sponges can eliminate external and internal mass transfer limitations caused by substrate concentration gradients. Fluid flow through porous materials is due to external forces expressed as the pressure in the form of the pressure gradient. At low pressures, the diffusional transfer is expected to happen.

The flow below a critical value of Re_p is laminar while the above critical value is turbulent flow. In the turbulent region due to eddy movements, mixing occurs called eddy diffusion. In turbulent boundary layers, eddies of different lengths and time scales move randomly and transfer energy near to the surface of the particle. As fluid comes close to the surface of particle by fluid motion, the fluid velocity is reduced with the consequence molecular diffusion becomes an important step of mass transfer, and turbulence of fluid goes to end. Turbulent flow intensity depends on the components of velocity fluctuation and is expressed by energy dissipation. As kinetic energy carried out by larger eddies depends on the fluctuating velocity. Further a correlation of mass transfer dimensionless numbers with hydrodynamic and properties of sponges. It enhances the mass transfer due to its properties of short contact time with high reaction rate and low-pressure drop, high surface area, and radial mixing in tortuous pathways (Garrido, Patcas, et al. 2008), Collins 1976). The efficient mixing of a reactor could be determined by the dissipation kinetic energy. As the laminar flow change into transition or turbulent flow, such kind of effect also existed due to the

reason that eddy currents increase the molecular exchange and momentum flux in all directions (Doran 1995).

5.1. INTRODUCTION

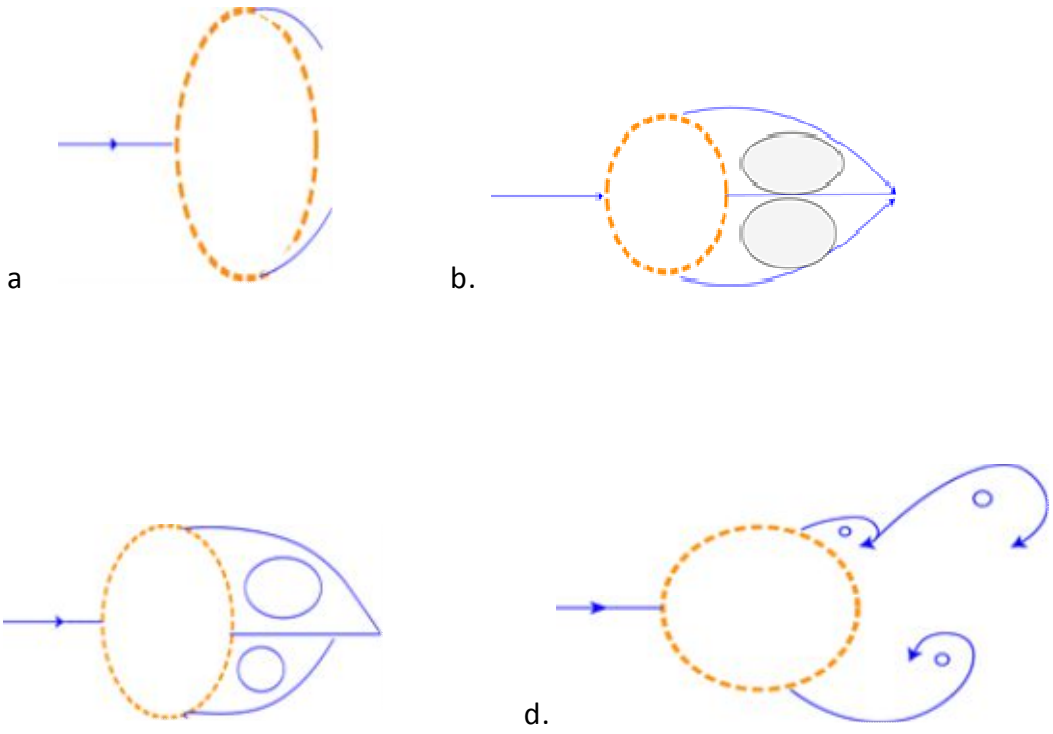
5.1.1. Role of fluid dynamics in mass transfer through alginate beads in PBR

In industrial mass transfer operations like fermentation with immobilized cells in a packed bed or fluidized bed, bioreactors are used. Numerous investigators have measured mass transfer coefficients but the correlation of experimental mass transfer data with fluid hydrodynamics and thermodynamics is still needed to explore (McCabe 1976). In this study, mass transfer data in packed bed is analyzed in terms of fluid flow pattern over particle (fluid hydrodynamics) and entropy generation or energy dissipation (Fluid thermodynamics). (Lin, Moulton et al. 1953), (Pal 2019).

In a packed bed bioreactor as fluid starts to flow, particles remain in the rest position. As fluid velocity increases, with time flow lift the particles bed when fluid forces on the bed equal to the weight of particles. Particles that go in suspended and fluidized state and whole bed come in the uniform condition of temperature and concentration (Eigenberger 1992) (Potnis and Lenz 1996). In laminar flow, the streamlines do not cross each other or no convective mass transfer between adjacent layers occurs. The only diffusion allows the mass transfer in the direction of flow and viscous forces (intramolecular forces) to become dominant over the inertial forces. When viscous flow passes through over the spherical particles in a packed bed, the flow will not be laminar anymore but in a steady-state where viscous stresses and velocity gradients are established. which are the main factors of momentum transfer.

When viscous fluid past through a spherical particle, flow is in steady-state with low Reynolds numbers. If $Re_p < 1$, the flow pattern is symmetrical from the front to the back of the particle. On the front side flow lines are straight and uniform but deflected as passed around the particle.

Figure 5.1 (a, b,c & d) Creeping flow and vortex formation (Sumer 2006)



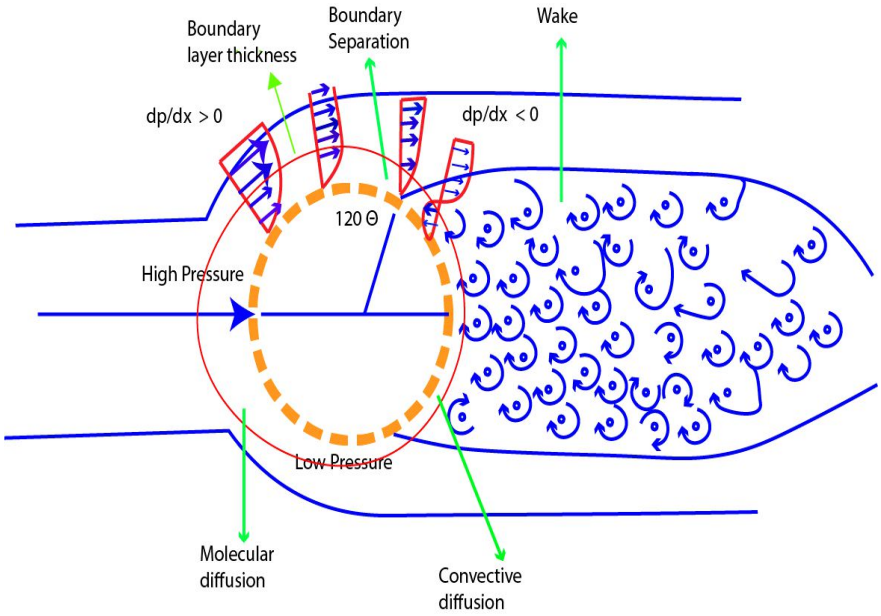
If Re_p > 4 then two small steady eddies appear behind the particle and form a separation zone. Wake in this point laminar and vortex rotate consistently concerning outer flow. As Re_p increases eddies grow in length and width (Leal 1992). At Re_p < 20 the flow is steady, laminar and flow pattern will not change with time. Vorticity was developed on the surface of the particle due to the no-slip boundary condition.

In this condition, there is no movement of the fluid layer within the boundary surface. As Re_p = 20 the flow disturbance increases and vortices behind the cylinder appear that increase in size and detached periodically (Sumer 2006). If Re_p is smaller, the rate of change of momentum is observed smaller as compare to viscous and pressure forces. The streamlines are also in asymmetry. As Re_p increases, the flow pattern and forces over the particle surface will also change. The asymmetry of streamline also increases and steady wakes become unsteady if Re_p increases further (Sumer 2006). The flow pass over the surface of the sphere exerted two forces; pressure force and friction force as shown in figure 5.2.

If fluid flows over a particle, boundary layers separate from the surface due to adverse pressure gradient in the boundary layers and flow has high pressure at the stagnant and rare point and lower pressure on the top and bottom of the particle. Due to velocity and pressure gradient, a large wake is developed behind the particle (Doran 2013). At Re_p = 40 boundary layer separation occur at θ = 82° due to pressure gradient. In this flow drag is mainly due to

friction and flow will lose its symmetry. If Re_p = 40 vortex formation in the wake region is an important phenomenon, vortex shedding undergoes a periodic change of forces on the surface. As Re_p increases Re_p > 40 separation point moves downstream at θ > 82° and eddies formed from vortices on the particle surface (Sumer 2006).

Figure. 5.2. Flow over spherical particle and vortex producing (Summer 2006)



One upper vortex if becoming bigger than the lower one and the vorticity of the bigger vortex will cut off it from the boundary layer. After this, a new vortex will be formed on the upper side. The same procedure will be repeated by the lower side and vortex shedding occurs on the lower side. In this way, vortex shedding occurs from the upper and lower side of the particle. Further vortex shedding occurs when there is an interaction between two shear layers.

Figures 5.1 and 5.2 show the shear layer over the particle surface and vorticity after separation of the boundary layer from the particle surface. As shown in figure 5.1 pair of vortex developed, after the separation of boundary layers, are unstable if there is a small increase in Re_p > 40 like 50 to 100 (Sumer 2006). As Re_p from 50 to 100, flow is not in steady-state, and due to detachment of vortices, new vortices come and replace the old vortices. As is shown in figure 5.2 fluid is chaotically mixed between particles. If Cs surface concentration value becomes different as compare to bulk concentration Cb, it means there is an exchange of mass between bulk fluid and the surface of the particle. As reactants are consumed inside the particle, the concentration gradient works as the driving force. To enhance the mass transfer size of the particle should reduce, the reaction rate per unit volume of the particle

will increase because mass is transferred in the center of the particle within a short time as compared to larger particles.

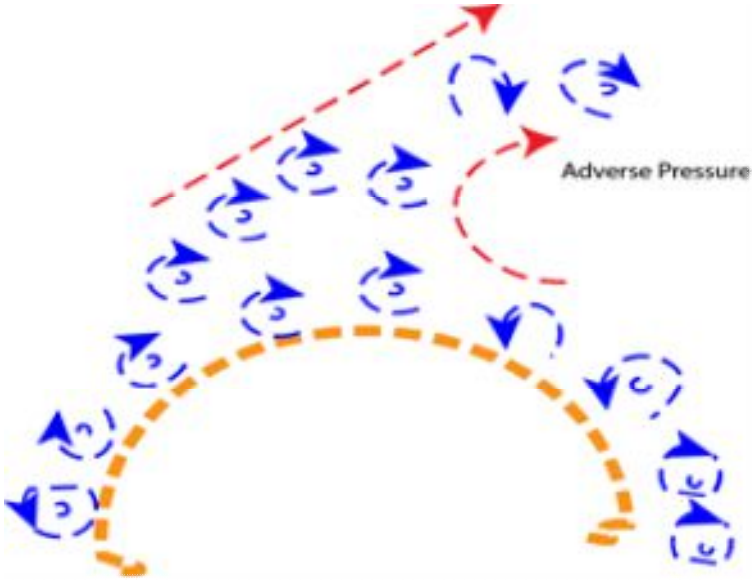
For all this process porous catalyst is desirable whose internal surface should be so effective that liquid carrying reactants can easily diffuse into the particle surface. The geometry of pores in a particle is important because the internal surface of the particle will be effective if there is effective surface diffusion. Diffusion in the catalysts is due to bulk diffusion, Knudsen diffusion, or by surface diffusion. If pores are large and filled with liquid then diffusion will be due to bulk diffusion in which collisions of liquid molecules with walls of pores are less important as compared to the collision between molecules (Satterfield 1970; Eigenberger 1992). In some catalysts, pore size is smaller in a way that diffusing molecules collide with the wall of pores more frequently as compared to collision with each other and is a function of effective diffusivity and diffusion is by Knudsen diffusion. Knudsen diffusion is proportional to pore diameter. As the pressure is reduced bulk diffusion is changed into Knudsen diffusion because the mean free path of molecules becomes equal to the radius of pores. It means at lower pressure Knudsen diffusion is predominant and mass flux increases with an increase in pressure. While in bulk diffusion mass flux reaches a constant value at higher pressure where concentration difference is direct in relation. At higher pressure, diffusion moves to the direction of small pores and depends on the value of the Knudsen diffusion coefficient and binary diffusion coefficient. The binary diffusion coefficient is a function of temperature, pressure, and concentration (Satterfield 1970; Eigenberger 1992).

Further when fluid past through a spherical particle as shown in figure 5.2, fluid velocity will vary at different positions of the sphere. The fluid layer next to the surface of the particle takes the velocity of the particle, there might be the condition of no-slip condition. Due to molecular motion momentum diffuses through the fluid layer. With the results shear stress developed which is directly proportional to the velocity gradient. This layer exerts shear stress to the next layer and accelerates or decelerates them. As the process proceeds momentum diffuses through layers. Such momentum diffusion is more prominent in viscous fluids.

The boundary layer over the surface of the particle developed due to momentum diffusion. If the fluid has higher viscosity there will be high momentum diffusion but less shear stress. On the other hand if the fluid has lower viscosity there will be lower momentum diffusion with higher shear stress. In this boundary layer viscous forces dominant over inertial forces while viscous forces become negligible outside the boundary layers. On the front stagnation

pressure gradient is favorable and in the direction of flow and the velocity gradient is steep while on the back stagnation point pressure gradient is adverse that pushes the fluid on the surface in opposite direction with the consequence velocity gradient become less steep (Kundu, Cohen et al. 2008);(Apsley 2007).

Figure 5.3. Boundary layer separation (Sumer 2006)



Two kinds of boundary layers are assumed. One is the hydrodynamic boundary layer from the surface of the particle to bulk fluid where fluid velocity is maximum. Second is mass transfer boundary layer thickness "δ“ from the surface of the particle to bulk fluid where reactant concentration is maximum. The change in reactant concentration takes place in this layer and all the mass transfer resistance also exists. Further properties of the outer side of this layer are like the bulk fluid. Mass transfer resistance and diffusion have a direct relationship with the boundary layer thickness on the interface of liquid and solid that affect the rate of mass transfer as well on the mass transfer coefficient (Potnis and Lenz 1996). There are some parameters on which the thickness of the boundary layer depends like fluid mass transfer properties, size of particle as catalysts, and the velocity with which fluid reaches to the surface of the particle, velocity gradient, and pressure gradient develops on the surface (Lal, Kumar, et al. 1988).

Fluid thermodynamics states that entropy production and energy dissipation occur in all viscous fluids. When fluid flow over the surface of particles viscous stresses opposes its motion with the results dissipation of mechanical energy into internal energy occurs

recognized in term of entropy generation (Pal 2019). Entropy generation is a measure of disorders of a system as well as the efficiency and feasibility of any process. Greater is the disorders of the process from the steady condition, the higher will be the entropy of the system and higher will be the feasibility and possibility of the process. Higher entropy means a higher loss of power or energy due to disorders and measures the distribution of thermal energy and mechanical energy. It is indicated that the change in entropy is related to the change in pressure (Pal 2019).

5.1.2. Role of fluid dynamics in mass transfer through alginate beads in SCBR Basket

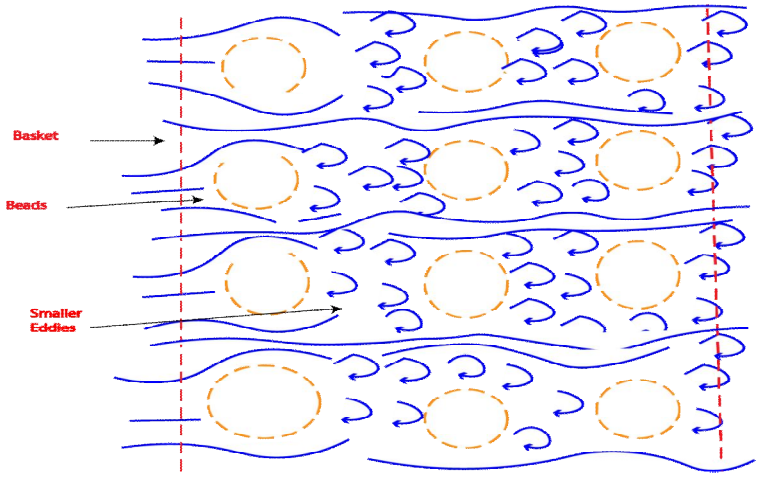
The first objective of this section is to correlate with mass transfer dimensionless numbers with hydrodynamic and properties of alginate beads in the basket of SCBR. Flow in SCBR may be laminar or turbulent represented as a function of Re_p. Liquid phase transport properties played a significant role to understand the trend of mass transfer coefficients and rate of reaction on the particle surface (Potnis and Lenz 1996). Hydrodynamic factors have a significant influence on the convective mass transfer or mass transfer by force. These factors depend on the relation between momentum and concentration gradient in the boundary layer. Hydrodynamic variables and conditions of boundary produced by fluid flow have a significant influence on the convective mass flux from bulk liquid to particle surface (Vary 1970). When fluid flowing through a surface of the particle, three kinds of layers has been proposed by Karman because the hydrodynamic boundary layer plays a major role in convective mass transfer (Lin, Moulton et al. 1953), (Welty, Wilson, et al. 2008). It has been postulated that a very thin layer near to the wall of the particle called the sublaminar layer, second is the turbulent core and the third one is the buffer region between these two layers.

The convective mass transfer has importance in the case of flow inside a stirred catalytic basket bioreactor which involves the transfer of mass between the boundary surface and circulating fluid (Welty, Wilson, et al. 2008). Kolmogorov has proposed such a theory of isotropic turbulence that gives information about turbulence intensity around the particle. Further shows that mass transfer rate depends on the particle diameter and physical properties (Lal, Kumar, et al. 1988).

If the fluid motion is in random and chaotic condition with three-dimensional vorticity called ‘Turbulence’. With the result there is an increase in energy dissipation, mixing, mass transfer, and drag. Turbulent flow can generate new vortices from old vortices. In turbulent flow,

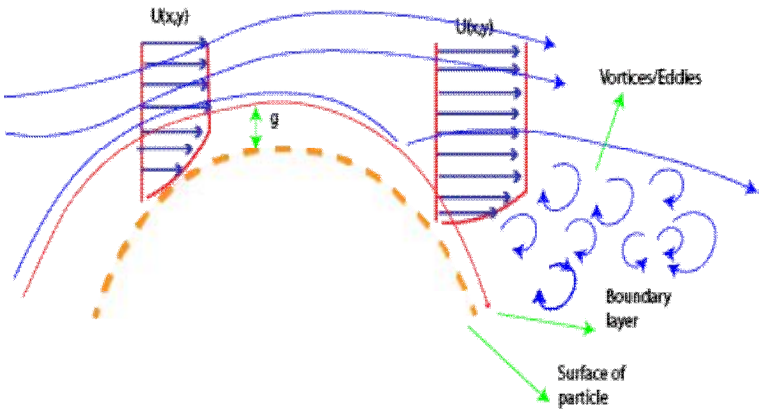
molecular interactions are the cause of momentum transfer as shown in figure 5.4 (Apsley 2007).

Figure.5.4. Radial flow over beads in basket.



Viscosity has a tremendous effect on the fluid motion, mixing, and mass transfer and can be determined by relating the velocity gradient to shear force (Doran 1995). The ratio of shear stress to shear rate depends on the shear force exerted on fluid. The formation of the boundary layer is important to understand the flow characteristics and its effect on mass and heat transfer between phases. The turbulent boundary layer has a steep velocity gradient on the surface of the particle or a higher tangential velocity (du/dy) gradient with higher wall shear stress and depends on flow speed or Reynolds Number as shown in figure 5.5.

Figure.5.5. Velocity distribution as fluid flows over particle surface (Summer 2006).



With the result surface has more shear stress that causes drag on the surface consequently, a high mass transfer rate occurs. As fluid moves through particles then exert drag in the

direction of parallel to fluid velocity. On the other hand, lift forces exerted that is perpendicular to the liquid velocity. Momentum is expressed by (Mv) where M is mass and v is the fluid velocity. Shear stress is the flux of x-momentum in the y-direction. Flux can be defined as a rate per unit area and flux of momentum that is directly proportional to the velocity gradient. Momentum is transferred from a high-velocity region to a lower velocity region and perpendicular to the direction of flow. The magnitude of the velocity gradient is same as the magnitude of momentum flux and is the driving force for momentum transfer.

Non-Newtonian or in-compressible fluid depends on shear stress and the density of fluid remains nearly constant. The rate of deformation or fluid motion depends on velocity distribution. In steady flow, velocity is independent of time while in unsteady flow or turbulent flow velocity distribution varies with time (Green and Perry 2019). The viscosity of the fluid is the ratio of shear stress to shear rate. Force over the area is called shear stress while force is directly proportional to area. Shear rate is the gradient in velocity (Green and Perry 2019).

As fluid flows over the surface of the particle, the upper surface will come into rest due to viscous drag that is transmitted to the upper layer and results in the velocity gradient. The velocity gradient is the change in velocity with distance from the surface. Further, as fluid flow over beads, separation of the boundary layer happens due to the opposing pressure gradient. Fluid flow has high pressure on the front and back of the sphere and low pressure on the top and bottom. This difference in pressure drives the inner flow in the opposite direction of fluid flow and lifts the boundary layer away from the surface that makes the wake behind the particle. In this wake region, smaller eddies are produced that enhance the eddy diffusion as well as vortex shedding occurs.

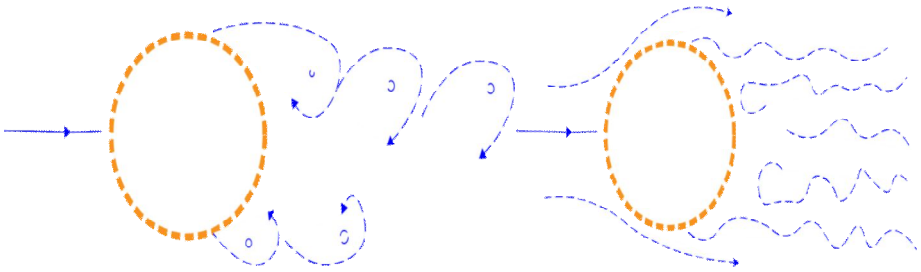
Flow which has less viscous effects is an inviscid flow or ideal flow. Two kinds of flows are laminar flow and turbulent flow. In laminar flow velocity components and streamline are smooth while if the flow is unsteady velocity components vary in a chaotic manner concerning position and time (Green and Perry 2019). The flow below a critical value of Re_p is laminar while the above critical value is turbulent flow. At $Re_p = 30$ a vortex in a ring form develops at the rear stagnation point of the particle.

As shown in figure 5.5, further increase in Re_p can affect the separation point like upon increasing $Re_p > 100$ to 300 the angle of separation will change from 130° to 115°. At this point, the vortex becomes unstable and begins to oscillate but remains to attach with particle. If $Re_p > 500$, vortices start to shed if $Re_p > 2000$ flow near to wake again becomes in

steady motion, and separation of flow over the surface will be at an angle of 80° (Sumer 2006). Perpendicular to the direction of flow, vortex shedding exerts lateral forces on the particle that cause resonance frequency (Green and Perry 2019).

Figure. 5.6. Flow over spherical beads and vortex or eddies formation (Sumer 2006)

a). vortex formation at smaller Re_p **b).** vortex formation at higher Re_p



a) b)

In turbulent flow spinning or swirling kind of flow develop called eddies. In eddies, fluid velocities are changing in magnitude and direction. Eddies with different size appeared in a turbulent flow. In the case of higher Re_p, large eddies turbulence has most of the kinetic energy and is not affected by viscosity but is the main reason for momentum transport.

As shown in figure 5.6, large eddies are unstable and divided into smaller eddies which further goes on to divide into smaller in size as the Re_p increases. Large eddies carried out a lot of energy produced by the impeller and transferred to smaller eddies. Vortex stretching is the main mechanism of transferring energy from larger to smaller eddies. Smaller eddies further dissipate this energy into heat with the effect of viscosity and friction due to fluid. With the result of this, the eddy structure and velocity gradient produced in fluid motion is destroyed (Doran 1995).

As shown in figure 5.6 b, due to eddy movements mixing occurs in the turbulent region called eddy diffusion. Eddy mass diffusivity will be higher as molecular diffusivity in this turbulent core. The motion of the eddy has the main role in any fluctuation of fluid velocity. Eddy momentum diffusivity (EM) is analogous to the (V) molecular momentum diffusivity or kinematic diffusivity. Mass diffusivity of the vortex is an analogous mechanism as molecular diffusion of particles. As smaller vortex or eddies are produced on the surface of the particle and have their specific direction of rotation and strength. Vortex induces the velocity on the surface of the boundary which cancel the existing velocity and is the condition of no-slip

velocity. Further recreation and strength of vortexes satisfy the no-slip condition. Surface shear stress (that enhance the skin friction force) is directly proportional to the surface vorticity (Bird, Steward, et al. 2007).

As fluid flows over a particle, the surface of the particle will have shear stress (viscous drag) and pressure difference (pressure drag). As fluid turbulence increases pressure drag is reduced as compared to viscous drag. Eddy motion in turbulent flow sufficiently transfers momentum as compared to molecular momentum transfer called turbulent viscosity. In turbulent boundary layers, eddies of different lengths and time scales move randomly and transfer energy near to the surface of the particle. Therefore, turbulent boundary layers have enough momentum to overcome the adverse pressure gradient consequently boundary layer separation is delayed. Such a boundary layer has more kinetic energy that is converted into potential energy. Mixing smaller the scale of Kolmogorov is micromixing or molecular diffusion and is the final step of the mixing process.

Molecular diffusion is the movement of molecules under the influence of concentration gradient and its direction is from the higher concentration to lower gradient (Doran 1995). According to Fick's law of diffusion, mass flux is directly proportional to the concentration gradient where mass flux can be explained by the rate of mass transfer per unit area and is perpendicular to the direction of the fluid. As fluid comes close to the surface of a particle by fluid motion, the fluid velocity is reduced with the consequence molecular diffusion becomes an important step of mass transfer, and turbulence of fluid goes to end. Diffusion on the interface could be better explained by film theory described in chapter 1.

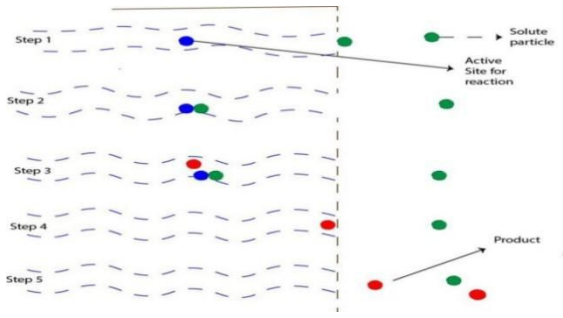
In a turbulent motion mass transfer data can be correlated with agitation power per unit volume. According to this theory, kinetic energy from large eddies are transferred to smaller eddies by inertial forces. As large eddies interact with each other to produce smaller eddies so directional nature of large eddies is lost. Turbulent motion of smaller eddies is an-isotropic and universal equilibrium range where these become independent on the impeller and dependent on the energy dissipation rate by the eddies (Brian, Hales, et al. 1969). Turbulent flow intensity depends on the components of velocity fluctuation and is expressed by energy dissipation. As kinetic energy carried out by larger eddies depends on the fluctuating velocity. Fluid thermodynamics states that entropy production and energy dissipation occur in all viscous fluids. When fluid flow over the surface of particles viscous stresses opposes its motion with the results dissipation of mechanical energy into internal energy occurs recognized in term of entropy generation (Pal 2019). Entropy generation is a

measure of disorders of a system. Greater is the disorders of the process from the steady condition, the higher will be the entropy of the system. Higher entropy means a higher loss of power or energy due to disorders and measures the distribution of thermal energy and mechanical energy. Viscous shear stress sustains the turbulent flow by the continuous supply of energy. Large scale vortices are a source of kinetic energy as shown in figure 5.6a. As kinetic energy is converted into internal energy by inertial forces, the turbulence of fluid dissipates fastly (Cussler 2009). This process continues until small scale eddies develop. These eddies are so small that molecular diffusion and dissipation of energy take place.

5.1.3. Role of fluid dynamics in mass transfer through macro-porous sponges in SCBR Basket

In the last decade, polyurethane foam as catalysts has gained great interest in industrial applications of the liquid-solid catalytic process. It enhances the mass transfer due to its properties of short contact time with high reaction rate and low-pressure drop, high surface area, and radial mixing in tortuous pathways (Collins 1976). Many authors have published their studies on mass transfer in porous media but very few studies on the dimensionless correlation of momentum and mass transfer with fluid mechanics and properties of sponges have been reported. Macro-porous foams have excellent properties of higher porosity and a three-dimensional pore network for higher radial mixing, lower pressure drop that enhance heat and mass transfer (Inayat 2013).

Figure. 5.7 Steps for mass transport and reaction into pores of porous material (Inayat 2013)



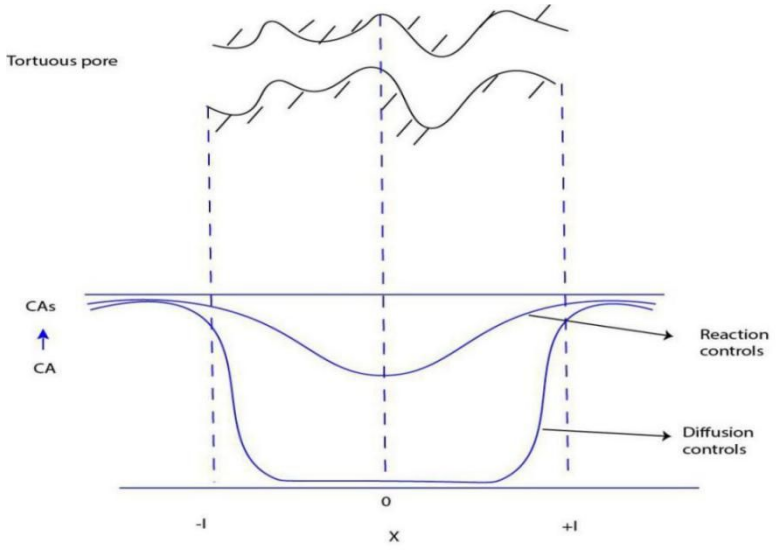
Step 1. Transfer of solute from bulk to surface of catalyst.
Step 2. Diffusion of solute into pores
Step 3. Heterogeneous reaction undergoes inside pores
Step 4. Resulting product diffuse out of pores
Step 5. Product moves out into bulk liquid

By increasing surface area per mass of catalyst step 3 can be faster. It means the reaction rate is proportional or function of available surface area. By slowing down steps 2 & 4, step 3 becomes independent of the flow-through bed but depends on catalyst pore size. If the size of the pore is smaller Step 3 is affected because steps 2 & 4 involve the diffusion of solute into active sites of pores and diffusion of product out of the pore. While in the case of large-size pores, diffusion is the same as outside the catalyst (Cussler 2009).

An additional most important aim of this study to prepare macro-porous support material for cell immobilization and further use in newly developed stirred catalytic basket bioreactor (SCBR). So, the objective of this section is to correlate with mass transfer dimensionless numbers with hydrodynamic and properties of sponges. In a heterogeneous system, mass transfer is the motion of molecules from one point to another point by driving force. This driving force can be in the form of concentration gradient, pressure gradient, or velocity gradients in the molecular diffusion or convection process.

The flow pattern in an agitated bioreactor is quite complex because of the difference in fluid velocity in the vessel. Fluid velocity in the vessel can be divided into three parts. In the first part, fluid velocity can be radial and direction will be perpendicular to the impeller shaft. The second part can be longitudinal and acts along the shaft of the impeller. The third would be tangential or rotational and acts in the tangential direction to the circular path of the impeller or shaft.

Figure.5.8. Concentration profile for porous sponge (Inayat 2013).



5.1.3.1. Transport and Concentration gradient

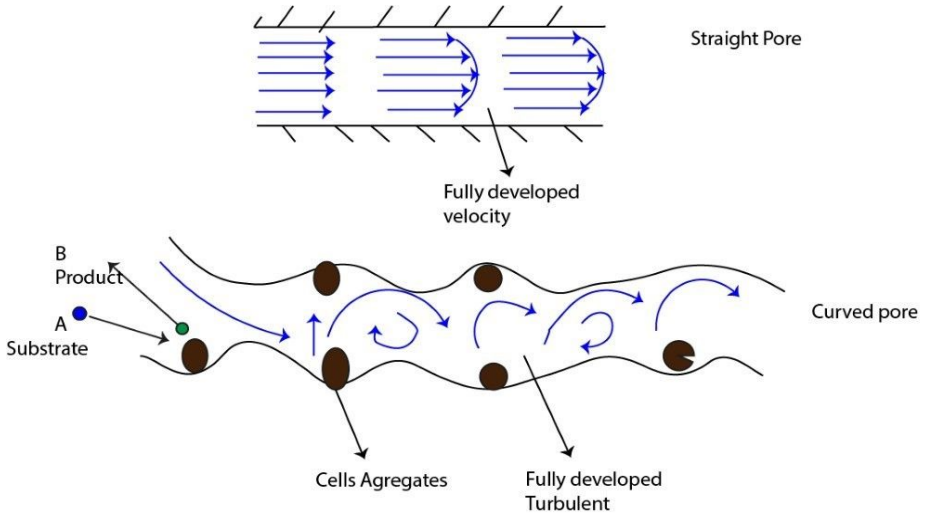
An inevitable concentration gradient occurs as the catalytic reaction starts within pores of the sponge. For step 2 there must be the difference of substrate concentration in the bulk fluid and on the surface of the pore or within the pore. For step 4, there must be the same difference in product concentration as in substrate concentration. The concentration gradient is the function of x of the pore. Step 3 is proportional to the substrate concentration in bulk fluid (CA). If the mass transfer coefficient is large, such a system is called reaction limited and the concentration gradient will be higher that drive the solute into the pore or site of action. While in case of a large rate coefficient, the system will be mass transfer limited and the concentration gradient will be less.

5.1.3.2. Hydrodynamic factors affecting mass transfer

As fluid flows through a phase boundary of pores in porous materials, local velocity becomes zero due to resistance inflow near to the surface. Due to this, a stagnant fluid film over the surface is expected and the thickness of this film depends on fluid flow condition, as it can be either laminar or turbulent. Fluid flow in porous materials is usually a complex process due to its tortuous structure. Therefore, the film thickness can be replaced by a characteristic length 'L (characteristics) that can be the width of the sponge sheet or hydraulic diameter of pores. Mass transfer between phase boundaries is only by molecular diffusion if there is laminar flow in the system (Garrido 2008).

Two kinds of fluid flow have been observed inside the pores of sponges: mass transfer and hydrodynamic. The flow is considered to be in mass transfer developing region if the boundary layer of the wall is changing due to change in the length of pores. On the other hand, the flow is in the hydrodynamic developing region when local velocity depends on its position inside the pore (De Lathouder 2007). At the entrance of the pore, the velocity profile is flat and as it develops it becomes laminar. Therefore, developing at the entrance of pore has a direct influence on mass transfer. The further characteristic length of developing mass and hydrodynamic boundary layer is the function of Re_p. In the case of hydrodynamic characteristic length, the velocity profile can change due to the irregular structure of pores in the sponge. With the results, eddies and turbulent flow developed. Furthermore, the velocity profile inside the pores can vary due to cell density and Re_p number (Pangarkar, Yawalkar, et al. 2002)

Figure 5.9 Flow through pores(Pangarkar, Yawalkar, et al. 2002)



Flow through pores can be studied in laminar and turbulent state. Due to presence of pores sizes distribution, flow from laminar to turbulent state will not be at once but in between is transitional state which occurs in gradual with the effect of inertial flow or turbulence at higher Reynolds numbers (Collins 1976).

5.1.3.3. Geometric factors affecting mass transfer

5.1.3.3. 1. Porosity and pressure drop

In engineering processes, it is important to investigate the relation of fluid flow through porous materials with pressure drop because it plays an important economic role (Garrido 2008). Flow in pipes is simple as compare to pores in porous materials due to its tortuous structure. So, velocity and pressure distribution in this system is complex but can be easily determined by dimensionless pressure drop or called 'friction factor (f, ζ)' (Garrido 2008). It represents the energy loss due to frictional or drag forces.

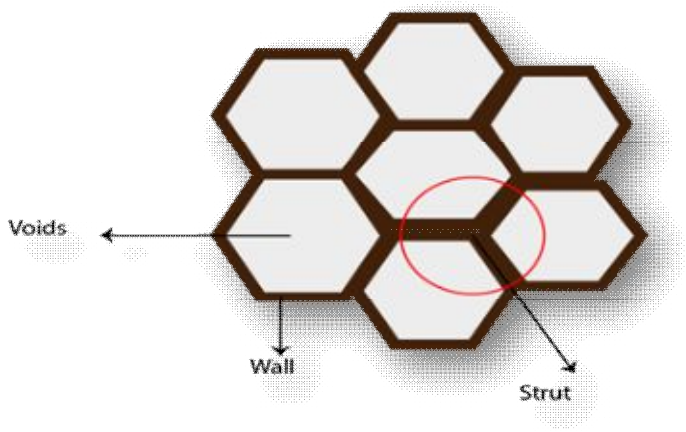
$$\text{Friction factor}(f) = \frac{0.87 + 13.56}{\text{Re}\,s}$$

Re_s= Reynolds number concerning the specific surface area.

Fluid flow through porous materials is due to external forces expressed as the pressure in the form of the pressure gradient. At low pressures, the diffusional transfer is expected to

happen. In porous materials relation of fluid flow and pressure drop is important especially in Biochemical Engineering where a mass transfer is a big issue to solve. The porosity and geometry of foam as catalysts have a direct relation with pressure drop. It has been explained by literature that increasing PPI value of foams, pore size (window size), and cell size decreases due to increasing number of pores per inch and specific surface area (Breitkopf, Galinsky, et al. 2011).

Figure. 5.10 Structure of wall or struts in macro-porous sponge(Breitkopf, Galinsky, et al. 2011)



5.1.3.4. Turbulent Kinetic energy

Turbulent kinetic energy is identified as the rate of dissipation of kinetic energy per unit mass of fluid (k) that is related to fluctuating velocity components. It is done by fluctuating pressure gradients and viscous stresses. This dissipation occurs in the smallest turbulence scale called Kolmogorov microscale and it is an important parameter use to identify the most effective zone of turbulent mixing (Pal 2019).

For turbulent flow in porous materials, two approaches; time averaging and volume have been developed by Pedras and de Lemos (Pedras and de Lemos 2000). They have proposed a similar equation for mean flow in porous materials and a different equation for turbulent kinetic energy. Further two-equation for turbulence model has been proposed for turbulent kinetic energy and dissipation rate that quantify the effect of porous materials morphology (Pedras and de Lemos 2001). The main objective of this study is to observe the performance of SCBR with immobilized cells in DEAE matrices by analyzing; (i) the effect of foam geometry on the hydrodynamic behavior of the system, (ii) understanding the mechanism of external and internal mass transfer effect on the immobilized system in conjunction with the performance of SCBR.

In liquid-solid phases the experimental results are expressed in terms of dimensionless groups. The results show the variation because of complex particle to liquid mass transfer characteristics like shape factor, the specific surface and diameter distribution. Most commonly used dimensionless groups are Sherwood number "Sh" (ratio of concentration gradient on surface to overall concentration gradient or ratio of molecular mass transport resistance to convective mass transfer resistance), Schmidt number "Sc" (ratio of molecular diffusivity of momentum and mass), and particle Reynolds number "Re_p" (momentum transfer). These three parameters are normally used for the analysis of two kind of mass transfer processes, mass transfer in kind of stream flow under forced convection and second is mass transfer between phases under natural convection (Welty, Wilson et al. 2008).

5.2. MATERIALS AND METHODS

5.2.1. METHODOLOGY FOR PBR

5.2.1.1 Correlation of experimental data in PBR with alginate beads

The molar flux of reactants is the combined effect of molecular diffusion flux due to the concentration gradient and the flux due to the bulk motion of the fluid. The PBR column is packed with alginate beads, the boundary layer near the liquid-solid interface is laminar because of molecular diffusion (Potnis and Lenz 1996). If the reaction is instantaneously on the surface of the particle, reactants concentration on the surface will be as $C_{As} = 0$. At this point rate of diffusion through a film equals to rate of reaction on the particle surface. In the first case of mass transfer limited reaction, reactants concentration C_{As} on the surface are neglected concerning CA in the bulk fluid so $C_A > C_{As}$.

5.2.1.1.1. Mass transfer coefficient (Kl)

$$Kl = Kr(obs) \times \left(\frac{Vl}{Am} \right)$$

$Kr_{(obs)}$ = Observed rate of reaction

V_l = Volume of liquid in reactor

A_m = Total area of beads

5.2.1.1.2. Schmidt Number

Schmidt number is the ratio of momentum diffusivity to mass diffusivity and is dimensionless. In boundary layer theory, it has been postulated that the concentration

boundary layer is related to the hydrodynamic boundary layer (Welty, Wilson, et al. 2008). The exponent of Sc represents that mass transfer is controlled by slip velocity condition. In laminar flow, a no-slip velocity mechanism existed because of more viscosity in fluid.

$$Sc^{\frac{1}{3}} = \frac{\delta h}{\delta c}$$

The "δ" symbol represents the thickness of hydrodynamic boundary layer and "δc" as thickness of concentration boundary layer. In the asymptotic suction profile the value of "δc" is defined as.

$$\delta c = \frac{v}{V}$$

Where v is the kinematic viscosity and V is the velocity of the fluid.

There is a need to establish a convenient method for the applying correlation of different analogies regarding the mass transfer. In the present study, results of mass transfer are correlated by three dimensionless groups analyses like particle Reynold number (**Re_p**), Sherwood number (Sh), and Schmidt number (Sc).

$$\text{Schmidt Number}\left(\text{Sc}\right) = \frac{\text{v}}{\text{De}}$$

v is kinematic viscosity,

De is the diffusivity of solute in fluid

5.2.1.1.3. Sherwood Number (Sh)

$$Sh = C.\text{Re}^{a}.Sc^{b}$$

Sherwood, Gilliland and Linton correlated the results as Sherwood Number

$$Sh = Kl \times \left(\frac{dp}{De}\right) \qquad \textbf{(Calculated)}$$

$$Sh = \varepsilon JD \times \text{Re}_{\text{p}} \times Sc^{\frac{1}{2}} \qquad \textbf{(Correlation equation)}$$

Ranz Marsh$_a$l correlation equation is valid when 5 **<** Re_p < 200 and 1000 < Sc < 6000. Such a correlation is the function of particle diameter and fluid velocity or Reynolds number.

In the mass transfer mechanism, it is considered that transfer of mass and momentum are analogous processes. Further, if the value of Schmidt number (Sc) is 1 then flowing fluid has resistance because of the skin or structure of particle and number greater than this is due to pressure drag (Welty, Wilson, et al. 2008).

5.2.1.1.4. Reynolds Analogy

Reynolds analogy proposed a relationship between heat or mass transfer with fluid friction. It postulates that the ratio of momentum lost by friction to the total momentum of the fluid will be the same. The ratio of mass transfer is expressed not only by the change in concentration but also in terms of mass transfer coefficient (Chilton and Colburn 1934).

$$\mathrm{Re_p} = \left(\frac{dp \times u}{v} \right)$$

d_p is the particle diameter, U is the fluid velocity and v is the kinematic viscosity.

5.2.1.1.5. Chilton Colburn theory

It is assumed that the best method of comparing data about mass, momentum, and heat transfer is to compare Chilton Colburn mass transfer factor "**εJD**" with " $\mathbf{Re_p}$ " Reynold Number. Chilton-Colburn factor "**εJD**" for mass transfer correlation equation has a relation of Sherwood number, Reynolds number, and Schmidt number (represent the thickness of the boundary layer) and it is equal to $\mathbf{C_f/2}$

$$\varepsilon JD = \frac{Sh}{\mathrm{Re_p} \times Sc^{1/3}} \qquad \textbf{(Calculated)}$$

The correlation of experimental data for the laminar in a single straight line that is expressed in a single line (McCabe 1976).

$$\varepsilon JD = 0.453 \times \mathrm{Rep}^{0.453} \qquad \textbf{(Correlated)} \qquad \textbf{Where } 5 < \mathbf{Re_p} < 200$$

$$\mathrm{E} = \frac{\mathrm{P} \times gc}{Vl \times \pi}$$

E = Energy Dissipation in Packed Bed Bioreactor, P= Power applied by liquid, gc = gravitational force, Vl = Fluid volume m^3

$$P = U \times \pi \times Vl$$

P = Power applied by fluid , U = Fluid velocity, m/sec Vl = Volume of liquid m^3

5.2.2. METHODOLOGY FOR SCBR

5.2.2.1. Correlation of experimental data for Alginate beads

The data has been obtained in liquid-solid convective mass transfer by measuring the rate of reaction, diffusion (De), and mass transfer coefficient (Kl). Impeller speed was varied as 200rpm, 300rpm, and 500rpm by using a variable speed controller.

5.2.2.1.1. Mass transfer coefficient was obtained by a glucose diffusion-controlled fermentation process under different conditions using alginate beads and macro-porous sponges inside the catalytic basket. The rate of mass transfer from or to the solid particles inside liquid depends on the mass transfer coefficient (Kl), interfacial area (A), and concentration gradient as a driving force (Pangarkar, Yawalkar, et al. 2002). Mass transfer coefficient, Kl defined as a transfer of mass per unit time, unit area, and the unit difference in pressure due to concentration difference between bulk fluid and solid interface (Colburn 1930)

$$Kl = \left(\frac{Vl}{At}\right) \times \ln\left(\frac{Co}{C}\right)$$

Where C is the concentration in liquid at time t after starting reaction and Co is the concentration at the start of the reaction. VL is the volume of liquid. In the present work, mass transfer coefficients in (SCBR) were experimentally measured by diffusion of glucose and the observed rate of reaction depends on liquid velocity as well on particle diameter. KL is necessary to quantify the rate of mass transfer from liquid to solid and vise versa.

$$Kl = Kr_{(obs)} \times \left(\frac{Vl}{dp}\right)$$

From the plot between lnCo/C vs t plot, the slope of lnCo/C vs t observed reaction coefficient Kr(obs) was calculated. The mass transfer coefficient (Kl) was determined for different glucose concentrations under different rotation speeds. If the solid-liquid mass transfer is by diffusion and forced convection then many variables can affect mass transfer coefficient KL, molecular diffusivity De, fluid density, fluid viscosity u, particle diameter (dp), and velocity of fluid U. These variables are grouped into the following dimensionless groups. Grouping of these variables can simplify the process development and data correlation to understand the behavior of a system.

5.2.2.1.2. Particle Reynolds Number

The effect of flow behavior on mass transfer has been presented as flow effect is presented in terms of impeller Reynolds number based on impeller rotation speed or fluid velocity. Reynolds number can be used when transport process is completely due to forced convection.

With dimensionless group involving agitation power as suitable form of Reynolds number.

$$\mathrm{Re_p} = \left(\frac{dp \times u}{v} \right)$$

dp is the particle diameter, U is the fluid velocity and v is the kinematic viscosity.

5.2.2.1.3. Colburn Chilton JD Mass transfer factor.

Colburn chilton analogy represent the mass transfer coefficient in turbulent flow and it not applicable for mass transfer rate (Venkatesan and Fogler 2004).

$$\varepsilon JD = \frac{Sh}{\mathrm{Re_p} \times Sc^{1/3}}$$ (Calculated equation)

$$\varepsilon JD = 0.453 \times \mathrm{Re_p}^{\wedge 0.453}$$ (Correlation)

5.2.2.1.4. Sherwood Number

In two phase (solid-liquid), dimensionless group sherwood number is calculated as.

$$Sh = Kl \times \left(\frac{dp}{De} \right)$$

which depends on experimental calculated mass transfer coefficient, diffusion of glucose and as well size of particle.

Overall correlation data was calculated by the equation.

$$\frac{Sh - 2}{Sc^{1/3}}$$

The data for a Stirred Catalytic Basket Bioreactor fit the equation.

$$Sh = \varepsilon JD \times \mathrm{Re\,p} \times Sc^{\frac{1}{3}}$$

The exponent of Sc is obtained in previous studies on mass transfer.

Further calculated values can be correlated by adoptibility of the Frössling type equation

$$Sh = 2 + 0.012 \times \left(\frac{\mathrm{E}dp^4}{V^3} \right)^{0,41} \times Sc^{1/3}$$

5.2.2.1.5. Schmidt Number

It is the ratio of momentum diffusivity to mass diffusivity and it is the relative effectiveness of momentum as well as mass. This correlation shows Sherwood number not only depends on the diffusion of glucose in the form of Shmidt number (Sc) but also on the geometry and rotation of the impeller in the form of energy dissipation.

$$\text{Schmidt Number}\left(\mathrm{Sc}\right) = \frac{\mathrm{v}}{\mathrm{De}}$$

v is kinematic viscosity, and De is the diffusivity of solute in fluid.

The energy dissipation rate was calculated from the relation.

$$\mathrm{E} = \frac{N\mathrm{p} \times Ni^3 \times Di^5}{Vl}$$

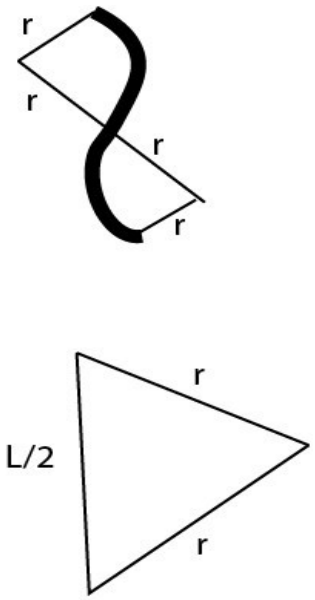
Where N_p is the power number, Ni is impeller rotation speed, Di is the diameter of impeller and V is the fluid volume in tank. These figures show that mass transfer coefficient of catalyst in SCBR is well correlated by the parameter of **Log (Edp^4/v^3)** due to flow behavior of fluid produced by different impeller rotation speed.

5.2.2.2. Correlation of experimental data for Macro-porous sponges

5.2.2.2.1. Fluid velocity

Figure.5.11. Description of Characteristic length and radius of tortuos pores (Jakobsson 2002)

In porous media, S shaped channels or holes covered 35% of total area.



$$Le = \pi r \quad while\ L = 2\sqrt{2r}$$

Effective velocity in channel $Ve = \left(\frac{\tau}{\varepsilon}\right) \times V$

where v is apparent velocity.

$$Tortuosity \quad \tau = \left(\frac{Le}{L}\right) = \frac{(\pi.r)}{\left(\sqrt[2]{2r}\right)}$$

Porosity(ε)=V (flow channel) /V (total) = $0.35 \times \frac{(\pi.r)}{\left(\sqrt[2]{2r}\right)}$

How to calculate linear velocity from impeller rotation speed.

V = Linear Velocity

$V = \omega \times R$ = 50 * 0,128 = 6,4 m/sec

$\omega = 2\pi N$ = 2*3,14*500 = 50 per sec

R = Radius = 0,128 m

5.2.2.2.2. Mass transfer coefficient

It was obtained by a glucose diffusion-controlled fermentation process under different conditions using DEAE sponges inside the catalytic basket. The rate of mass transfer from or to the pores of porous sponges inside liquid depends on the mass transfer coefficient (Kl), interfacial area (A), and concentration as a driving force (Pangarkar, Yawalkar, et al. 2002). Mass transfer coefficient, Kl defined as a transfer of mass per unit time, unit area, and the unit difference in pressure due to concentration difference between bulk fluid and solid interface (Colburn 1930).

$$Kr_{(obs)} = \ln\left(\frac{Co}{C}\right) \; vs \; \mathrm{t}$$

Where C is the concentration in liquid at time t after starting reaction and Co is the concentration at the start of the reaction. From the plot between ln Co/C vs t plot, the slope of ln Co/C vs t observed rate of reaction (**$Kr_{(obs)}$**) was calculated.

In the present work, mass transfer coefficients in (SCBR) were experimentally measured by diffusion of glucose and the observed rate of reaction depends on liquid velocity as well on the geometry of porous materials. KL is necessary to quantify the rate of mass transfer from liquid to solid and vice versa

. $$Kl = Kr_{(obs)} \times \left(\frac{Vl}{dp}\right)$$

Mass transfer coefficient was determined for different glucose concentration under different rotation speed. V_L is the volume of liquid. Dimensionless Groups If the solid-liquid mass transfer is by diffusion and forced convection then many variables can affect like mass transfer coefficient KL, molecular diffusivity De, fluid density p, fluid viscosity u, pores diameter, and velocity of fluid U. These variables are grouped into the following dimensionless groups. Grouping of these variables can simplify the process development and data correlation to understand the behavior of a system.

5.2.2.2.3 Reynolds Number

The effect of flow behavior on mass transfer has been presented as flow effect is presented in terms of particle Reynolds number based on pores diameter, porosity or tortuosity and interstitial fluid velocity.

$$\mathrm{Re\,p} = \left(\frac{dp \times u}{v} \right)$$

dp is the pore diameter, which is explained by kelvin cell model. v is the kinematic viscosity, τ is Tortuosity, ε is porosity and Ve is effective velocity.

$$Ve = \left(\frac{\tau}{\varepsilon} \right) \times V$$

If $Re_p < 1$ then flow is laminar and on the other hand if $Re_p > 1000$ flow will be in turbulence.

5.2.2.2.4. Sherwood Number

In two phase (solid-liquid), dimensionless group sherwood number is calculated as.

$$Sh = Kl \times \left(\frac{dp}{De} \right)$$

which depends on experimental calculated mass transfer coefficient, diffusion of glucose and as well size of particle. The data for a Stirred Catalytic Basket Bioreactor fit the equation.

$$Sh = \varepsilon JD \times \mathrm{Re}_p \times Sc^{\frac{1}{3}}$$

The exponent of Sc is obtained in previous studies on mass transfer.

Further calculated values can be correlated by adoptability of the Frössling type equation in case kinetic energy dissipation.

$$Sh = 2 + 0.012 \times \left(\frac{\mathrm{E}dp^4}{V^3} \right)^{0,41} \times Sc^{1/3}$$

5.2.2.2.5. Schmidt Number

Schmidt number is the ratio of momentum diffusivity to mass diffusivity and it is the relative effectiveness of momentum as well as mass. This correlation shows Sherwood number not only depends on the diffusion of glucose in the form of Shmidt number (Sc) but also on the geometry and rotation of the impeller in the form of energy dissipation.

$$\text{Schmidt Number}\left(\text{Sc}\right)=\frac{\text{v}}{\text{De}}$$

v is kinematic viscosity, and D_e is the effective diffusivity of solute in fluid.

5.2.2.2.6. Colburn Chilton εJD Mass transfer factor

Colburn Chilton analogy represent the mass transfer coefficient in turbulent flow and it not applicable for mass transfer rate (Venkatesan and Fogler 2004).

$$\varepsilon JD = 0.453 \times \text{Re}\, p\text{^}0.453$$

The energy dissipation rate was calculated from the relation.

$$\text{E} = \frac{Np \times Ni^3 \times Di^5}{Vl}$$

The mass transfer coefficient of catalyst in SCBR is well correlated by the parameter of **Log (εdp4/v3)** due to flow behavior of fluid produced by different impeller rotation speed. Which can be denoted as **Reε**.

5.2.2.2.7. Kelvin cell model

According to kelvin cell model, pore diameter has been calculated as characteristics length (Lc) as 'External pore size'that can be defined as internal pore size (dp) plus strut size (ds) (Lucci, Della Torre et al. 2014).

$$Lc = dp + ds$$

Here dp is the cross sectional area of pore. ds is the strut diameter that can be calculated as

$$ds = \left(\sqrt{\frac{4}{3}} \times \pi \times (1-\varepsilon)\right) \times a$$

a). Porosity

$$\varepsilon = 0.35 \times \frac{(\pi . r)}{\left(\sqrt[2]{2r}\right)}$$

b). Pressure drop

The pressure gradient is given by the total drag force on the cell divided by the volume of fluid. Further pressure drop of fluid flowing through porous materials can be described by Forchheimer equation.

$$\frac{dp}{L} = f1 \times Uo \times f2 \times Uo^2$$

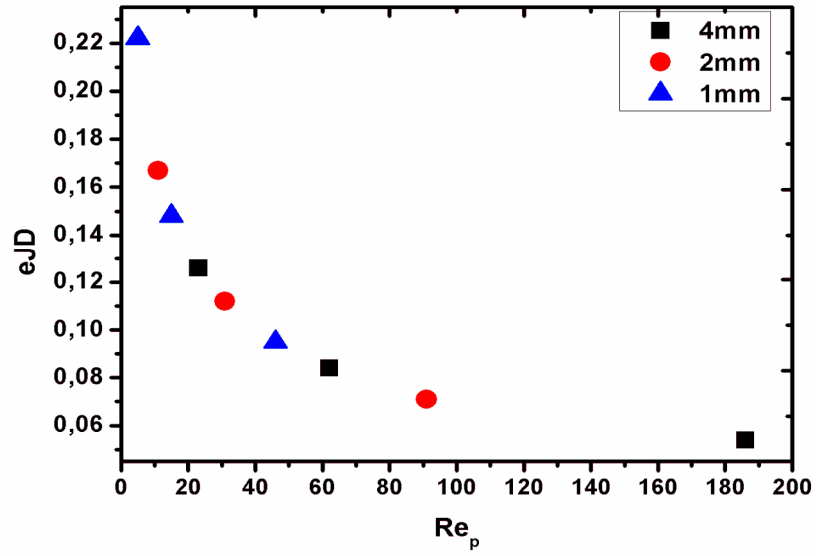
$$\frac{dp}{L} = \left(\frac{\mu}{k_1}\right) \times U_o + \left(\frac{\rho}{k_2}\right) \times U_o^2$$

Here f1 and f2 are the viscous and inertial constants of sponges and depends on fluid properties

5.3. RESULTS

5.3.1. PBR with Alginate Beads

Figure 5.12 Effect of Re_p on εJD factor for Mass transfer



Tremendous changes have been observed in the fluid flow over spherical particles with a certain Re_p value such flow has inertial forces and viscous forces. Reynolds number is the ratio of inertial forces and viscous forces. Inertial forces are forces that are exerted on the wall of the column and particle due to the motion of fluid over and viscous forces mean forces between intra-molecules of fluid. Mass transfer is a function of particle Reynold Number as shown in figure 5.12.

Chilton Colburn (εJD) mass transfer factor could be used to predict the relation of viscous momentum transfer to convective mass transfer and can be defined as the relation of convective mass flux with mass flux due to bulk fluid. The Colburn factor εJD is used for the correlations of mass transfer factor as a function of particle Reynolds number (Re_p) (alginate beads) with three different sizes (1mm, 2mm & 4mm). Results show that the mass transfer coefficient factor is independent of particle size at higher Re_p. Slope of a data line for all particles is the same. The results are interesting because of showing similarities in the trend of mass transfer coefficient factor for different size of the particles (Re_p) that means all particles have same trend of relation between viscous momentum transfer and convective mass transfer. Such trend has been expressed by increasing Re_p.

At 5 **<** Re_p < 50, εJD values for all particles are higher which indicates the higher concentration gradient and higher boundary thickness even on smaller particles. Because viscosity or viscous forces are dominant over inertial force. At this point viscous momentum transfer is higher as compare to convective mass transfer and the mass transfer is controlled by no-slip velocity mechanism, flow maldistribution (flow in channels), pore diffusion, concentration gradient, and pressure gradient. It is depicted in figure 5.12, as Re_p increases 50 **<** Re_p < 200, εJD value tends to decrease which shows that at this point inertial forces become dominant over the viscous forces. That means viscosity or viscous forces are reduced. At a higher Re_p value a point comes where the viscous momentum transfer is reduced to a minimum value and mass transfer coefficient shows independency on Schmidt number or molecular diffusion. The Mass transfer coefficient is inversely proportional to $\mathbf{Sc^{1/3}}$

Vortices in the wake region remain laminar at Re_p < 200 and after that wake becomes irregular and vortices become turbulent. Total drag forces on the surface of the particle will be the combination of pressure forces and viscous shear stress forces. Due to high total drag by increasing velocity the laminar boundary layer become unstable and separates from the surface viscous layers. Further, it turned into a turbulent boundary layer Doran, P. M. (2013). Further, increase in Re_p, the flow will be transition flow where vortices detach in the irregular manner and viscosity of fluid tends to decrease even become negligible and inertial forces become dominant due to bulk fluid movement.

Figure 5.13a. Effect of Re_p on Sherwood Number with 4g/l glucose concentration

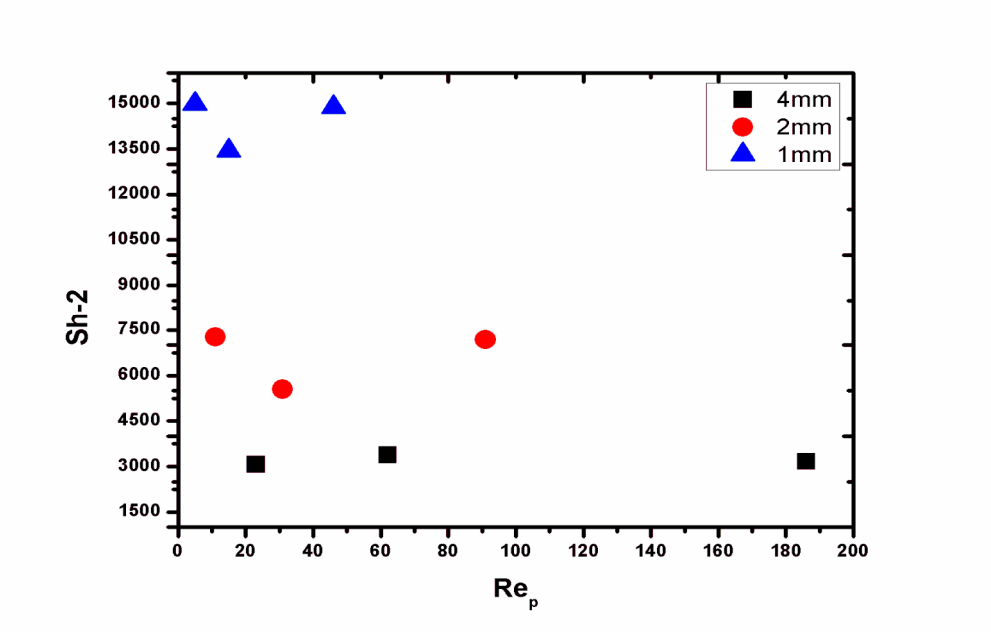
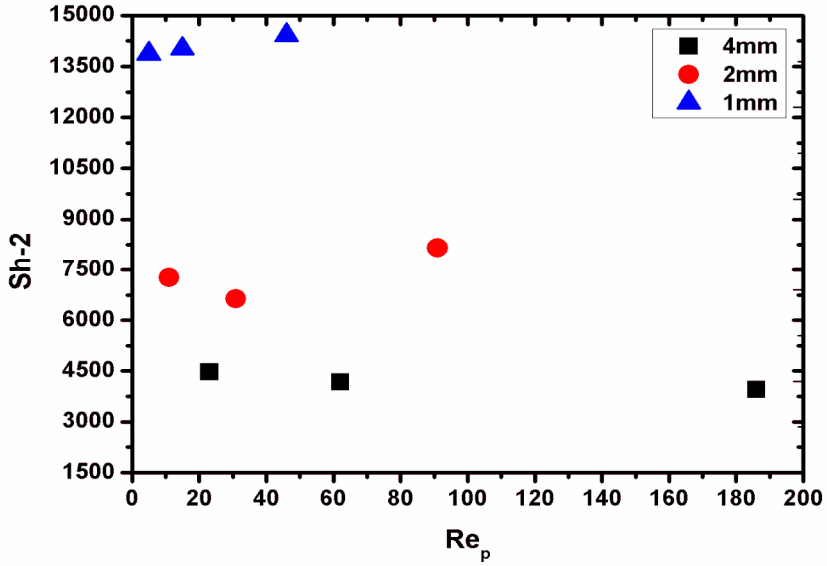


Figure 5.13b. Effect of Re_p on Sherwood Number with 10g/l glucose concentration



The relationship between particle Reynolds number and Sherwood number using different sizes of the particle is presented in figures 5.13 a & b. Ranz Marshal correlation for Sherwood number is valid below the point of 1.6 x 10^4 and before particle Reynolds number of 100 for glucose concentration of 4g/l & 10 g/l. Further, calculated value of Sherwood number is valid for smaller particles of size 1mm but not for big size particle of 2mm or 4mm at both glucose concentration. It is observed from results in figure 5.13 a & b, that agitation speed, catalyst size, and velocity

around particle has a tremendous effect on Sh. In the case of the size of the particle, the mass transfer coefficient correlated well with solid-liquid dispersion. On the other hand, the validity of (Sh) equation for smaller beads (1mm & 2mm) and not for larger beads (4mm). In a packed bed bioreactor bigger size of particle can be the reason for higher pressure drop (Bird, Stewart, et al. 2006). Mass transfer is strongly dependent on pressure drop. For better mass transfer there must be a low-pressure drop. As higher pressure or higher force is needed to drive fluid inside the pores of beads as explained in figure 5.2. Therefore, a low-pressure drop is required for the fluid flowing in a porous system. Higher flow resistance at lower Re_p can increase the pressure drop. The reason behind is as fluid flows over spherical particles in a column or packed bed reactor, boundary layers are formed and molecules of liquid colloids each other in the wake region. With the results, kinetic energy is converted into potential energy or internal energy that is further useful for convection-diffusion of solutes. To overcome the loss of kinetic energy, pressure energy is converted into kinetic energy. So we feel a drop in pressure due to some work done. Further pressure drop is directly proportional to (v) velocity and (v2) at a laminar flow and turbulent flow respectively.

The same is the case with drag force that can be defined as forces acting in the opposite direction of the motion of a particle. At lower Re_p flow over particle without flow separation, flow is not turbulent. In this case drag force (Fd) is directly proportional to v. On the other hand, if flow over particle is turbulent (higher Re_p) with boundary layer separation, drag forces will be directly proportional to v^2. At higher velocity around the sphere particle, mass transfer increases without the strong influence of bed voidage and observed rate of reaction as well intrinsic rate of reaction increases with the consequence resistance to mass transfer between fluid and surface of the catalyst is reduced. The diffusion process involves the pattern of fluid flow and other factors like velocity distribution, concentration gradient, and pressure gradient on the surface of particle that affects directly on diffusion or internal mass transfer.

Figure 5.14a. Overall mass transfer correlation with respect to Re_p at 4g/l

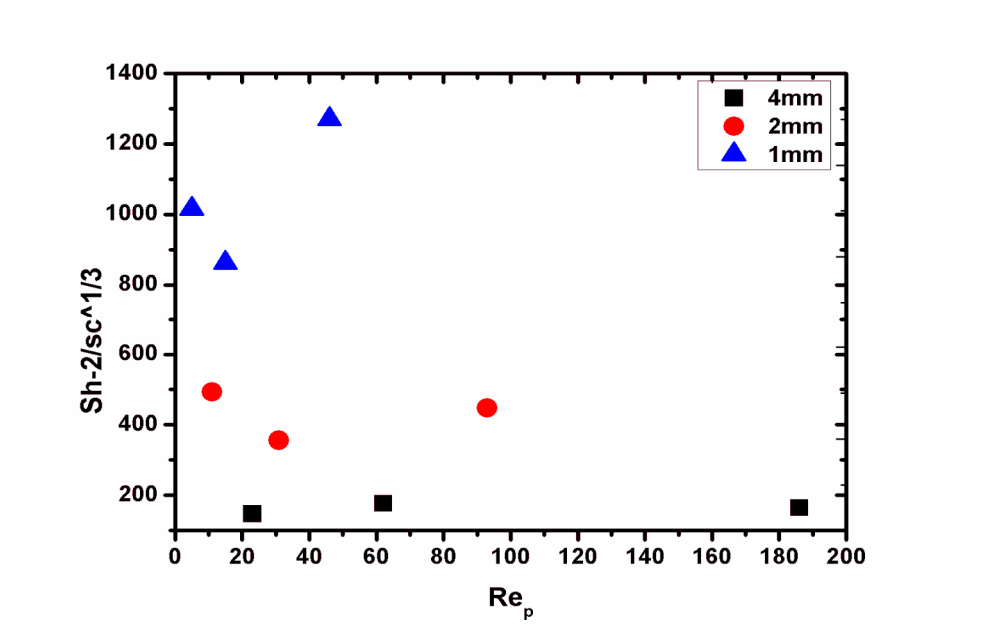
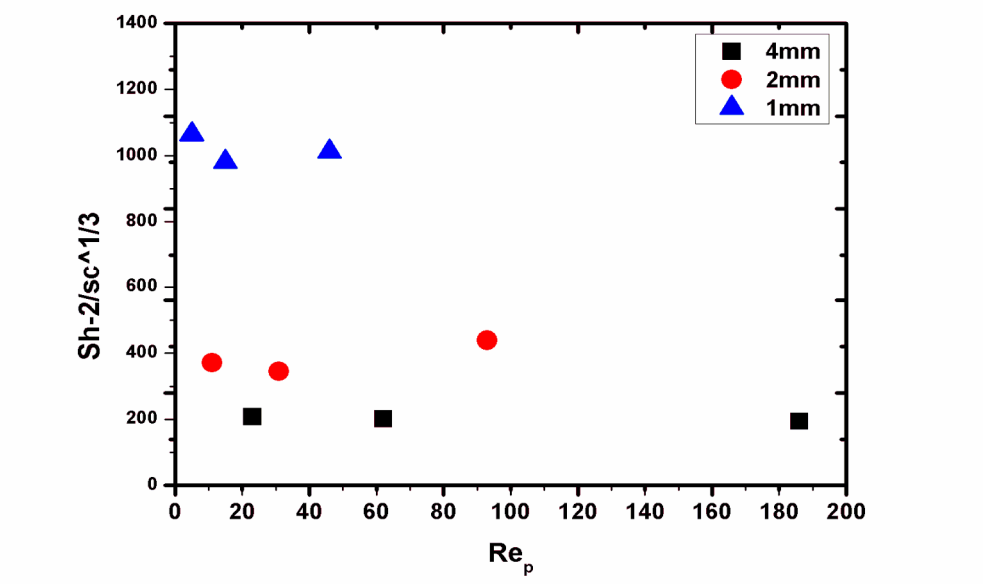


Figure 5.14b. Overall mass transfer correlation with respect to Re_p at 10g/l



These figures 5.14 a & b show the effect of Re_p on the overall mass transfer (ratio of Sherwood number to boundary layer thickness which is defined as ratio of Hydrodynamic boundary layer thickness to concentration boundary layer thickness). The exponent of Schmidt number represents the effect of the 'No-slip velocity' mechanism on mass transfer. This experimental data graph depicted that if molecular diffusivity decreases the laminar type of diffusion film becomes thinner near to the surface of the particle (Lin, Moulton, et al. 1953).

In figures 5.14 a & b at Re_p < 50 smaller particles (1mm) enhance the overall rate of reaction for mass transfer limited reactions as compared to 2mm particle size. Consequently, entropy generation in laminar flow through packed bed changed. While larger particle size can increase the bed porosity resulting in a bed with more open spaces for the flow of fluid, less fluid resistance, and less fluid-solid contact area per unit volume of bed. It means due to bigger-sized particles steep reduction in energy dissipation and entropy generation occurs. In a packed bed by using smaller particles, intra particle flow will increase due to the reduction of channel diameter consequently mass transfer is increased. In the dimensionless groups, the turbulent Schmidt group "v/De" is the ratio of momentum transfer coefficient to the eddy diffusion coefficient. At higher velocity or higher Re_p coefficient of mass transfer is greater than the coefficient of momentum transfer. At a certain point, the Schmidt group becomes independent of Re_p (Sherwood 1950). The exponent on Schmidt number shows that mass transfer is effected by a no-slip mechanism due to higher viscous forces in laminar flow. In other words when adhesive forces (attraction between fluid particles and solid particles) are higher than cohesive forces (attraction between fluid particles).

From the boundary layer theory, it is explained that the fluid boundary layer near to the surface of the particle will have the same velocity as the particle surface. Therefore velocity of fluid over the surface of the particle is zero. This is due to the viscous forces that reduce the velocity of the boundary layer near to the surface. It is concluded from the theoretical analysis of different researchers that the mass transfer coefficient is dependent on the physical properties of the fluid (velocity, kinematic viscosity, diffusion coefficient) (Sherwood 1950). If the Schmidt number is smaller then molecular diffusivity will be higher that will be the result of the laminar diffusion layer.

All the theories of mass transfer are associated directly with the molecular and eddy diffusion across the diameter of the particle. The solute binary diffusion coefficient is higher if molecular interaction is small between mobile phase molecules and solute molecules that is inversely proportional to pressure. Lower pressure in the wake behind the particle means a higher value of the binary diffusion coefficient that enhance inter-phase mass transfer. An increase in substrate concentration enhances the concentration gradients between bulk fluid and particles consequently mass flux is increased. In smaller particles, mass flux is high due to smaller diffusion pathways which are large in larger particles so smaller particles have less mass transfer hindrance than larger particles (Gutenwik, Nilsson, et al. 2002). Therefore, smaller particles have large overall mass transfer even at lower Re_p number.

Figure 5.15a. Effect of Energy dissipation on Sherwood Number at 4g/l glucose concentration

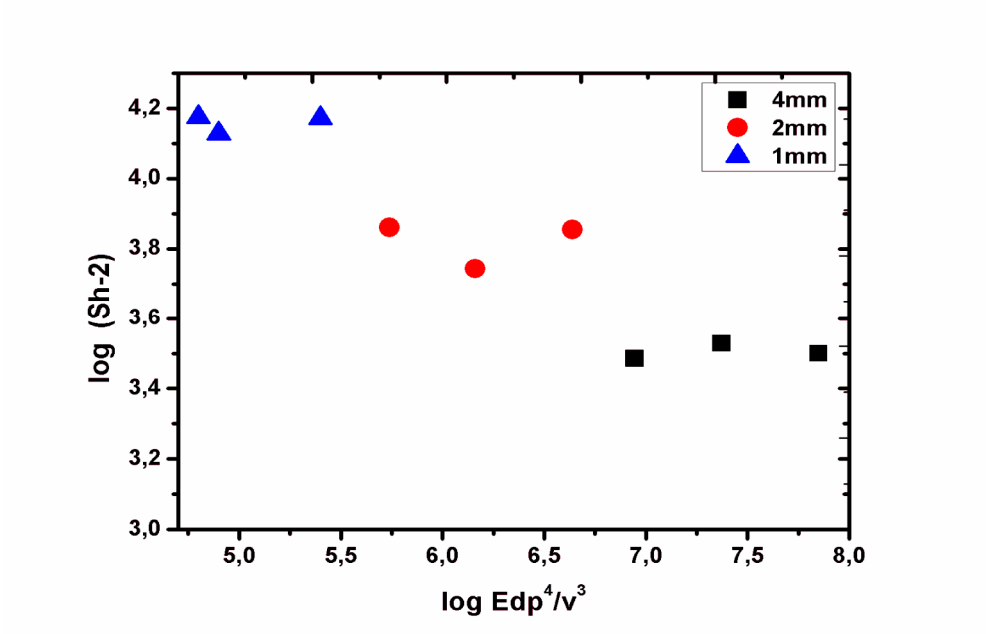


Figure 5.15b. Effect of Energy dissipation on Sherwood Number **at 10g/l glucose concentration**

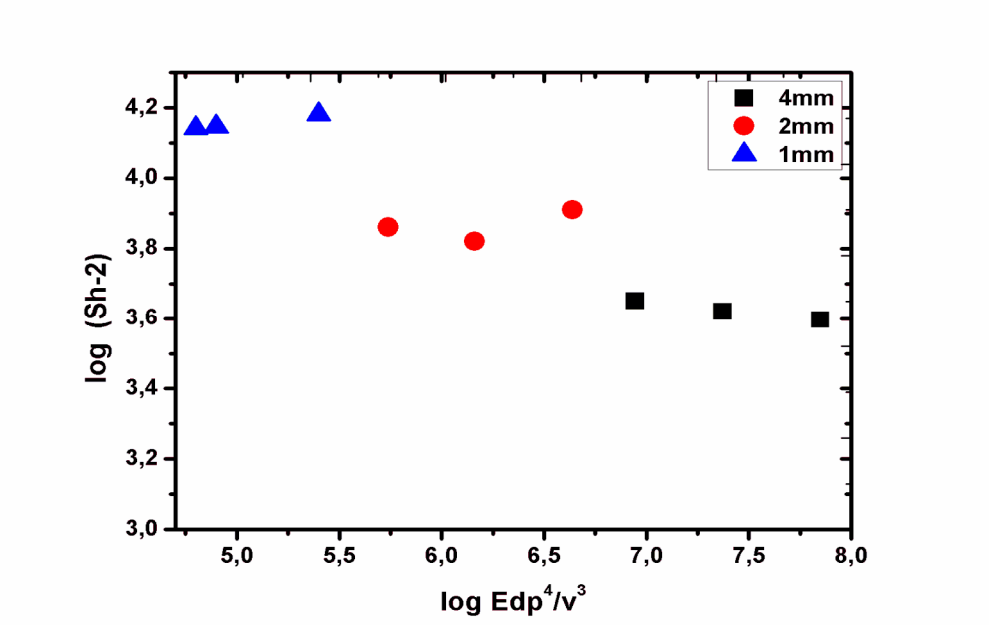


Figure 5.15 a & b show some of mass transfer data in form of log Sh. It exhibits the hindered flow and unhindered flow at smaller and large dissipation energy respectively. The reason behind is as the higher fluid velocity power increase the liquid-solid interfacial area will increases. Pressure drop and frictional drag can be reduced by reducing the cross-section area of the particle over which fluid flows which is indicated in figure 5.15 a & b results. Further, it could be concluded that higher energy dissipation means the higher mass transfer is by convective diffusion of

eddies. Several previous workers have reported that particle diameter is a function of the mass transfer coefficient and has an inverse relation to each other (Lal, Kumar, et al. 1988). The result shows that to reduce the mass transfer resistance there is a need to decrease the diameter of a particle because fluid flows over in asymmetry and there is less chance of boundary layer separation. Due to boundary separation, a lot of energy of the system can lose. To understand the drag force on spherical particles, there is a need to apply mechanical energy balance. These principle states work done by fluid flow over a sphere that is equal to viscous dissipation as shown in figures 5.15 a & b. As the flow passes through passages in particles packed in a bioreactor, such elongation and stretching of flow will result in pressure drop and additional dissipation of energy Pal, R. (2019). Frictional factor in packed bed can determine the pressure loss in a packed bed that is described as a function of Reynold number. As the rate of energy dissipation is due to the friction of fluid. Mass transfer on the particle surface will be due to molecular motion because of slow or zero velocity. As Re_p increased fluid velocity increased that enhance turbulence in fluid streamlines as shown in figure **5**.2 Such unsteady eddies motion is the main source of mass diffusivity. Further, it is considered that momentum, mass, and energy are transferred due to eddy diffusion. At lower Re_p number or higher wave number, the net effect of energy transfer is too smaller scales due to higher viscosity or higher fluctuating velocity gradient. While at higher Re_p or lower wavenumber, the fluctuating velocity gradient is relatively smaller, large eddies are developed that are the reasons for higher energy transfer.

5.3.2. SCBR with Alginate beads

Solid-liquid mass transfer is important in many processing as it may be the rate-limiting in heterogeneous processes. In a stirred vessel it can be assumed that the solution inside is perfectly mixed with less viscosity. Figure 5.16 shows that rotation speed has a significant effect on the mass transfer coefficient. The data for the εJD factor of mass transfer with three different rotation speed in terms of particle Reynolds number (Re_p) was obtained in the range of $6 \times 10^3 \leq Re_p \leq 2.0 \times 10^4$.

Figure 5.16. Effect of Re_p on the mass transfer factor (εJD).

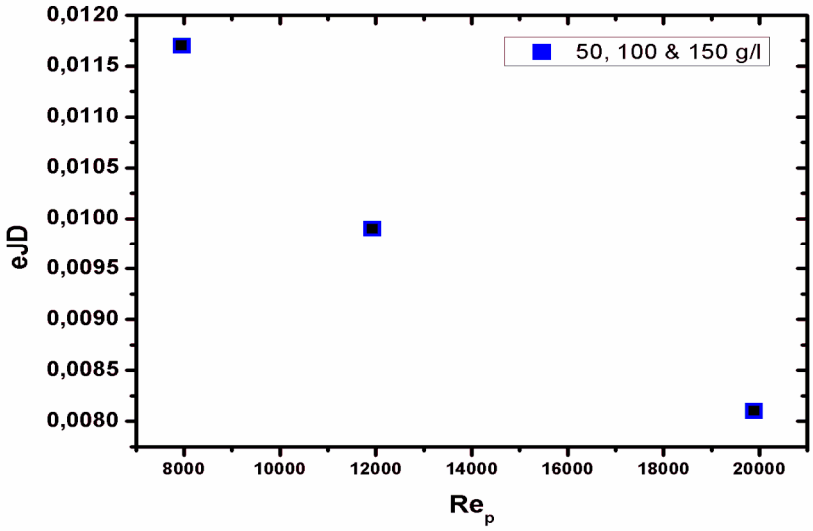


Figure 5.16 shows a relationship between Colburn factor εJD of mass transfer coefficient and Reynolds number (Re_p). It shows the relation of mass transfer with momentum transfer in terms of Colburn factor (εJD) and Reynold (Re_p) analogy which is the ratio of inertial forces to viscous forces. In turbulent flow at high Re_p, inertial forces become dominant over viscous forces that produce vorticity and instabilities in flow. The result shows εJD factor falls consistently at $Re_p > 6 \times 10^3$. As Re_p increases $6 \times 10^3 < Re_p < 2 \times 10^4$ εJD factor appear nearly flat. Mass transfer factor analogy by considering the mass transfer and momentum transfer as analogous processes that are the exchange of mass, momentum, and energy at a bigger scale. At this point, mixing is not only by molecular diffusion in the form of eddy diffusion but also with convective diffusion

Normally at $Re_p > 300$ vortex shedding occurs and wake is completely in turbulent condition. At $300 < Re_p < 3 \times 10^5$ flow regime wake is turbulent while boundary layer separation is in laminar. Wake vortices depend on the pressure gradient. At $Re_p = 3.5 \times 10^5$ boundary layer will be turbulent on both sides of the particle at separation points that move downward as compared to laminar flow. At this point, vortices interact with each other faster that results in vortex shedding. Even at higher Re_p, vortex shedding will be regular and in order. Further increase in Re_p boundary layer separation becomes turbulent on one stagnation point of particle and the other side remains laminar. At $3 \times 10^5 < Re_p < 1.5 \times 10^6$ boundary layer separation on both stagnation points of particle become turbulent (Sumer 2006) Figure 5.16. At $Re_p > 7 \times 10^3$, non-separated flow can reappear as in our case and at the value of $Re_p > 10^5$ vortex formation becomes independent of Re_p value. Therefore in figure 5.16, εJD value may not undergo the largest changes by a further increase in Re_p.

Figure 5.17. Effect of glucose concentration on overall mass transfer at different Re_p

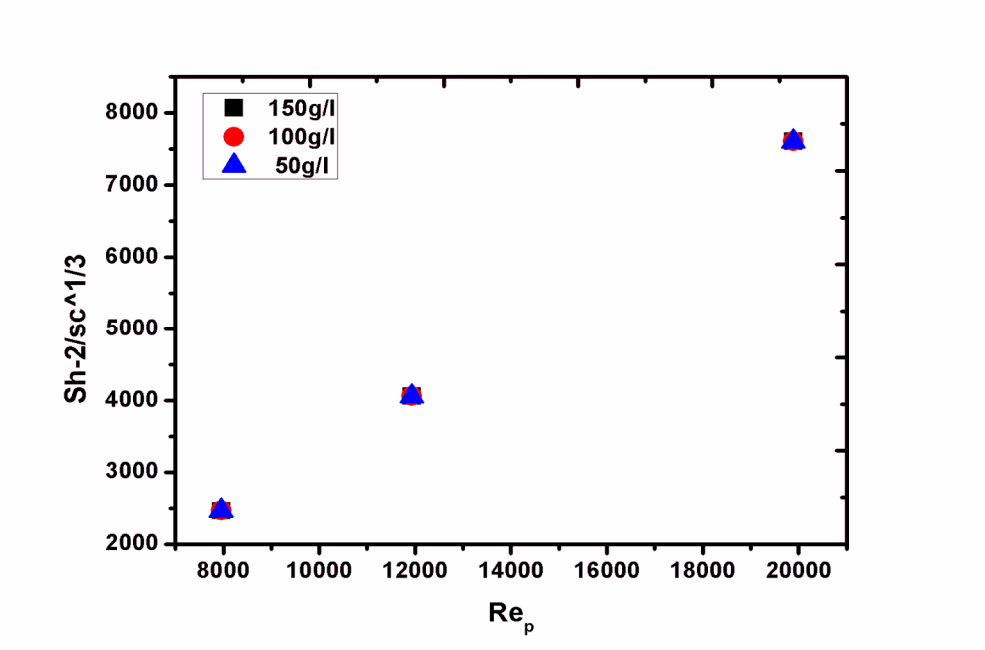
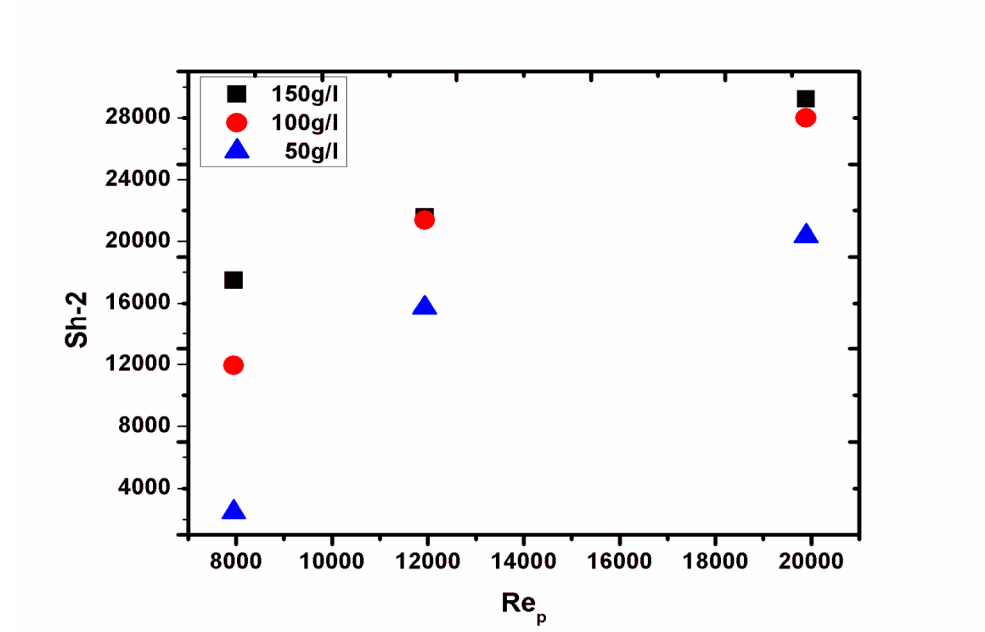


Figure 5.18. Effect of glucose concentration on Sherwood Number (Sh) at different Re_p.



In figure 5.17, the overall correlation of mass transfer can be understood under the condition of $7 \times 10^3 < Re_p < 2 \times 10^4$; $15 < Sc < 360$. It illustrates that the overall mass transfer correlation that is the ratio of Sherwood number (Sh-2) to film thickness ($Sc^{\wedge 1/3}$) observed direct proportional to Re_p and glucose concentration. This correlation illustrates the effect of flow behavior over the solid-liquid interface that ultimately effect on overall mass transfer. The exponent of the Schmidt number (Sc) represents that mass transfer is controlled by slip velocity when turbulent flow

existed. Under the present condition of Re_p, turbulence flow is induced by the effect of impeller rotation. Figure 5.18 depicts the effect of particle Reynolds number on Sherwood number (Sh), higher Re_p number means high turbulence in flow that enhances the role of convective mass transfer. As the agitation power increased the liquid-solid interfacial area increases.

Figures 5.17 & 5.18 demonstrate the effect of fluid flow behavior in terms of Re_p and different glucose concentrations on the overall correlation of mass transfer and convective mass transfer. It seems that the rate of mass transfer controlled by diffusion of glucose concentration is not only affected by the turbulence generated by different impeller rotation speed but also on the overall flow pattern inside the basket as packed bed under the agitated vessel. Figure 5.18 demonstrates the direct relation of the effect of glucose diffusion, due to concentration gradient, on Sherwood Number (Sh) at different Re_p. It is concluded from data that glucose concentration or concentration gradient as the driving force has a negligible influence on Sh. In a turbulent condition or higher Re_p value boundary layer separation is formed. The induced turbulence has a tremendous effect on the formation of eddies and eddy diffusivity that enhance the overall mass transfer effect. The above results indicate that higher Sherwood number or improvement of the rate of the mass transfer due to the reduction of boundary layer thickness and improving the diffusion coefficient.

Figure 5.19. Overall correlation of mass transfer with respect to Energy dissipation

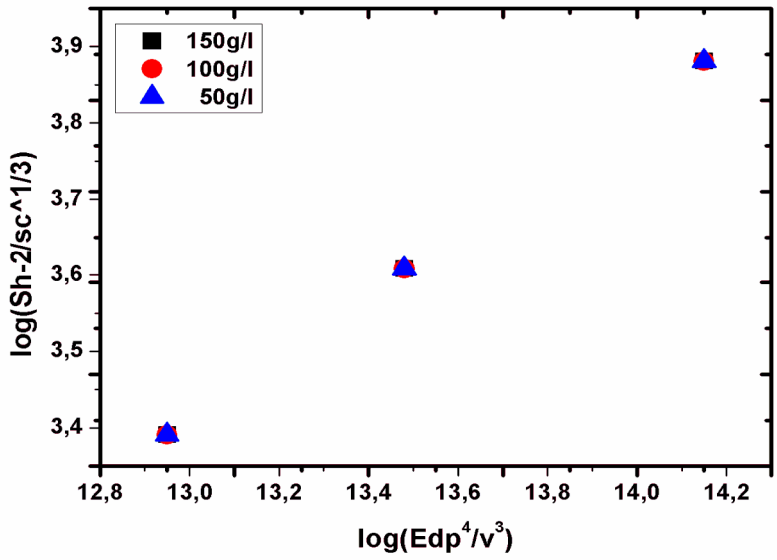
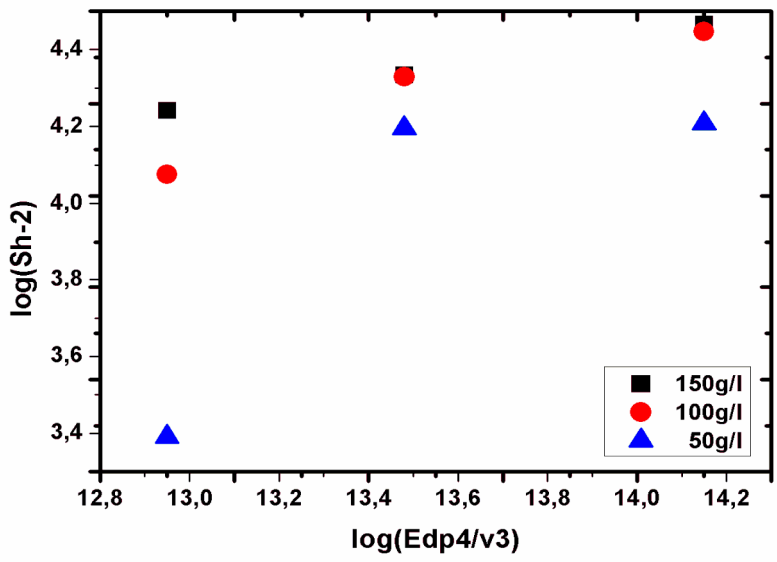


Figure 5.20. Effect of Energy dissipation on Sherwood Number (Sh)



In a stirred vessel, rheological properties of fluid have an influence on energy dissipation or power consumption and ultimately on mass transfer rate (Lal, Kumar, et al. 1988). Several investigators have correlated the mass transfer data with specific agitation power to assess the applicability of Kolmogoroff theory in a stirred tank reactor. Figures 5.19 & 5.20 show the effect of energy dissipation on overall mass transfer and Sherwood Number (Mass transfer coefficient) in stirred catalytic basket bioreactor.

The mass transfer coefficient represents the effect of mass transfer in a system of the boundary layer. This is shown in figure 5.19 that mass is transferred inside the bed by turbulent flow and (Sh-2/Sc^$^{1/3}$) is an overall mass transfer coefficient, due to turbulence inflow, over the surface of the particle. Further, the correlation results have been observed almost the same in the case of different glucose concentrations in the whole range of log (Edp4/v^3). While this is not the case in figure 5.20 with the correlation of Sherwood Number (Sh-2) & Log (Edp4/v^3). Figure 5.20 shows a higher value of log(Sh-2) at higher glucose concentration and a higher value of log (Edp4/v3). It can be concluded that Sherwood Number not only the function of energy dissipation but also on glucose concentration.

The entropy generation rate per unit volume in turbulent flow is directly proportional to the cube of superficial flow velocity. On increasing Re, the rate of mechanical energy dissipation into internal energy increases. Entropy generation is directly proportional to energy dissipation, as it increases when the internal energy of the system increases (Pal 2019). Further entropy generation is independent of fluid viscosity at higher Re due to less resistance in a fluid motion.

5.3.3. SCBR with DEAE sponges

Figure.5.21. Relationship between (εJD) and Re_p

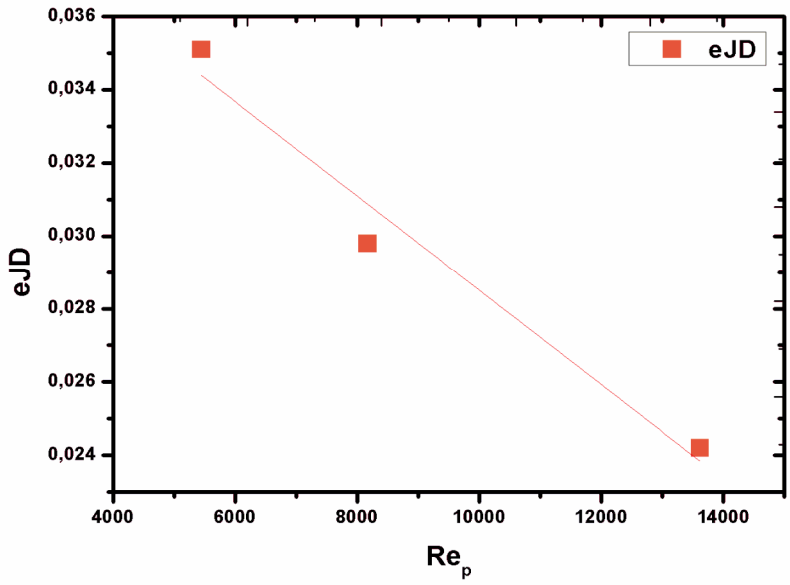


Figure 5.21 shows the Chilton Colburn analogy and represents the reduction of (εJD) mass transfer factor with increasing the particle Reynolds number from 10^3 to 10^5 with the results of an increase in reaction rate and reduction of concentration, temperature, or pH gradient in the system due to well-mixed vessel of SCBR. Further, the Chilton Colburn analogy gives an inspired guess about liquid-solid interface mass transfer limitations. Reynolds analogy in liquid gives an expression of two levels of turbulent mixing: Macroscopic (dominant eddies) and Microscopic (dominant diffusion, conduction, and viscosity). It means the conversion of substrate into the product at the maximum rate with minimum mass transfer limitations. Higher Re_p or interstitial velocity in pores and reactor configuration has a significant relation in reducing mass transfer limitations. It is interesting to see the effect of increasing Reynolds number because the reaction rate over the sponges is not constant. Further increasing Re_p would lead to a decrease εJD factor that means lower convective or molecular mass transfer limitations that decrease boundary layer thickness. Also, convective mass transfer is not further influenced by viscous transport of momentum. It is concluded that the sponges are the best choice to decrease the external and internal diffusion limitations.

Figure.5.22. Relation of Sh with Re_p

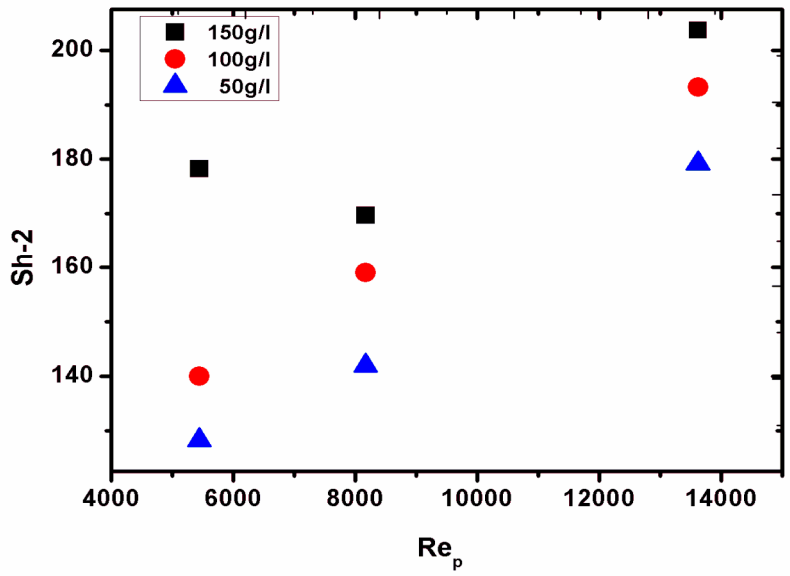


Fig.5.22 displays the Sherwood number of DEAE sponge with pore densities 10 and 45 PPI and porosity of 85% as a function of Reynolds Number (Re_p). The results exhibit very smaller difference in Sherwood numbers over the whole glucose range. Sherwood number is the dimensionless form of mass transfer coefficient and Reynolds number expressed hydrodynamic properties. Sherwood number increases with an increase in Re_p due to higher interstitial velocity in pores.

Velocity distribution inside the pore becomes independent of the magnitude of velocity if the flow is laminar (Collins 1976). While in the case of turbulence or higher Re_p if external conditions and system properties are constant, the flow becomes steady or stationary. External forces due to fluid viscosity, pressure, or gravity, acting on liquid within pore represent pressure. Due to the tortuous path of porous materials, the velocity of flow change from one point to another, and forces that are responsible for such change will also vary. But such variation in the magnitude of fluid velocity along the tortuous path will be uniformly distributed with a mean of zero. The mean of all lateral forces responsible for microscopic velocity variation will also be zero. On the other hand, as fluid flow with low Re_p inertial forces will not be zero but can be neglected (Collins 1976). The further diffusional process becomes dominant at very low pressure or lower Re_p. As Sh increases on increasing Re_p but Re_p = 6000 is approximately minimum above which Sh increase rapidly but there is no big difference of Sh value between Re_p = 6000 and 14000.

As mass transfer properties of DEAE foam depend on hydrodynamic properties like superficial or interstitial velocity (density, viscosity, and diffusion coefficient), it also depends

on the geometric properties of the sponge (Green and Perry 2019). Pore count influences the porosity as well on the fluid flow through voids of foam. Further, it has been observed by many investigators that mass transfer coefficient dependency on interstitial velocity increases by increasing the size of the pore (Green and Perry 2019). A good correlation of Sh with Re_p mainly with large pore size foam has been found as mass transfer increases by decreasing pore count (PPI).

Figure.5.23. Relation of Overall correlation of mass transfer with (Re_p)

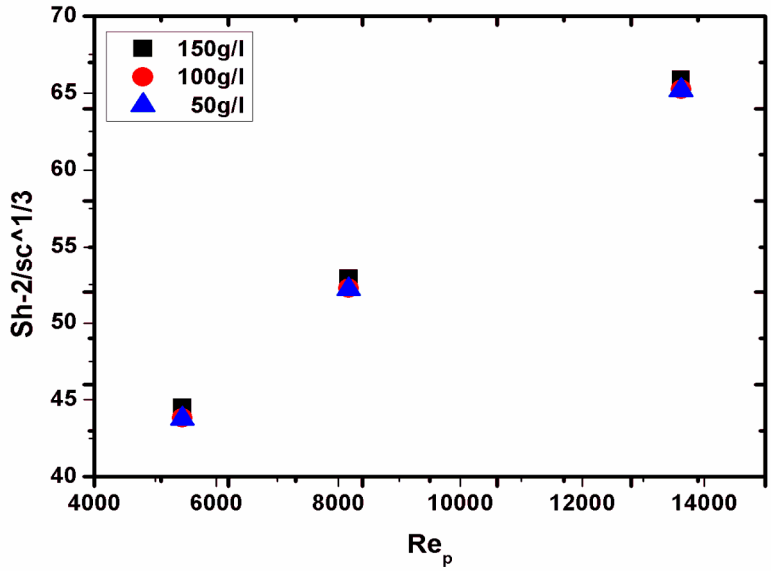


Figure 5.23 describes that mass transfer properties of foam can be correlated by Reynolds number (Re_p) with Schmidt number (Sc). Schmidt number is the ratio of the hydrodynamic boundary layer and mass transfer boundary layer. The exponent of $Sc^{1/3}$ depicts the boundary layer. In figure 5.23, the results for Sh and Sc as a function of Re_p are presented, and seems experimental data are in good agreement with the model expected for sponges. (Bakker, Groendijk, et al. 2007) (De Lathouder, Lozano-Castelló, et al. 2007). Further geometric foam features have also influence on mass transfer. A parameter 'characteristic length' in the form of pore diameter and density has a direct influence on the direction of fluid flow (Green and Perry 2019).

However, figure 5.23 is based on film thickness and showed improved mass transfer through stagnant film. Further, it has been observed that convective mass transfer is directly related to different variables like fluid velocity or impeller rotation speed, concentration driving force, and size of the particle. Further by using DEAE sponges as catalysts support at higher Re_p, Sh becomes independent of substrate concentration as shown in figure 5.23. It can be

explained in a sense that convective mass transfer becomes dominant due to pressure gradient not because of the concentration gradient.

Figure.5.24. Relation of dimensionless pressure drop and Sh

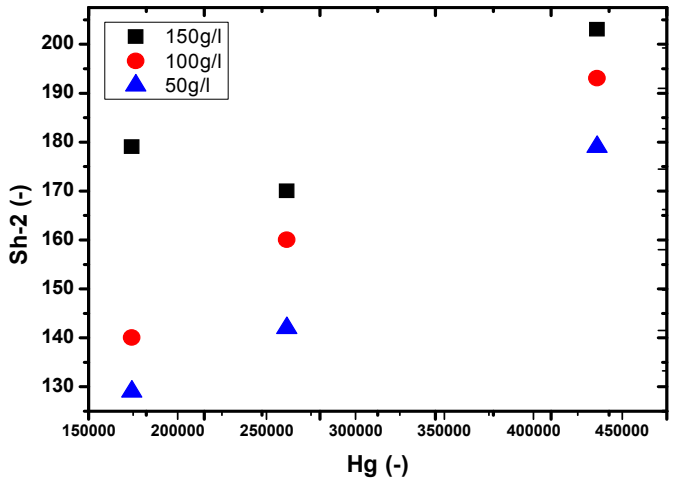


Figure 5.24 depicts the relation of dimensionless pressure drop and dimensionless mass transfer coefficient or Sherwood number. The further analogy has been observed between pressure drop and mass transfer coefficient in the case of sponges that have irregular cellular structure and represents qualitatively hydrodynamic mass transfer behavior of sponges. Further, it is depicted that mass transfer coefficients of sponges are proportional to the cubic root of the pressure gradient. Immobilized cells in tortuous paths of pores can enhance the limitations in internal diffusion, but the convective transfer can easily remove these limitations at even a lower pressure drop.

Figure.5.25. Relation between pressure drop and Re_p

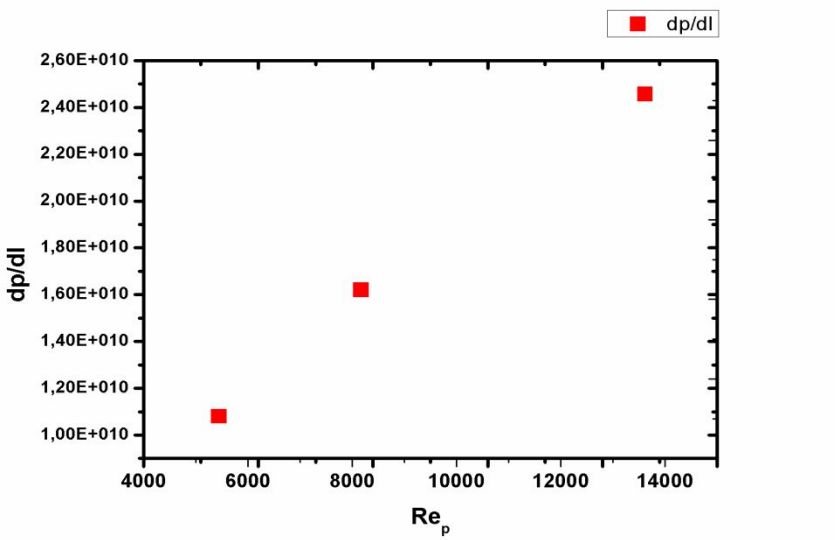


Figure.5.26. Relation between friction factor (f) and Re_p

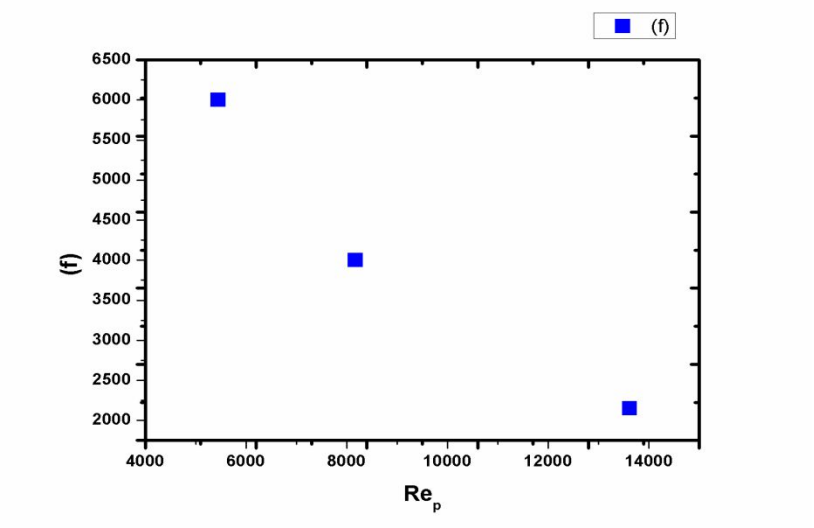
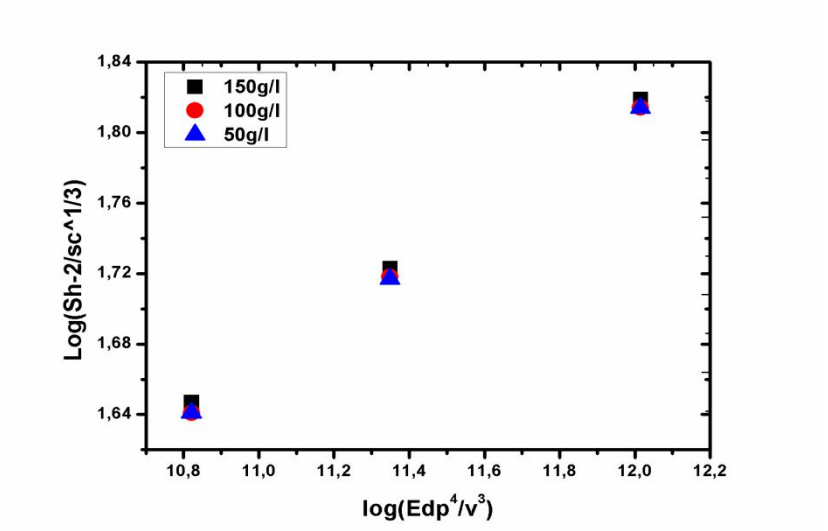


Figure 5.26 shows that the friction factor (f) of macro-porous sponges reduces with an increase of Re_p that means fluid is well mixed at different boundary layers and the thickness of the sublayer is reduced. At higher Re_p number or higher inertial forces, fluid particles packed tightly, and higher pressure is required to overcome fluid intermolecular forces and no-slip velocity condition at the pore wall. A higher drag coefficient CD can develop because some sponges have closed pores and irregularities that have a strong effect on pressure drop and mass transfer properties. Therefore, the presence of closed pores can be determined by pressure drop behavior. As pore size decrease pressure drop increases that mean pressure drop has an inverse relation with pore size and pressure drop increases with decreasing void fraction.

Figure.5.27. Relation between overall mass transfer and Reϵ



Mass transfer coefficient as Sherwood number obtained at agitated power input causing complete mixing or dispersion of liquid in the reactor vessel. Figure 5.27 shows the overall

trend of the Kolmogoroff theory and shows the relationship between power input at higher Reϵ. Overall mass transfer is improved because higher turbulent motion develops large primary eddies that have large velocity fluctuations as well contain the bulk of the kinetic energy. By interaction with the moving stream, primary eddies produce smaller eddies of higher frequency that are dissipated into heat by viscous forces (Levicky 2013). Due to the dissipation of energy, the main flow stream can lose the directional element. It is concluded that this data comes into satisfactory agreement with the Kolmogoroff theory of isotropic eddies. Where mass transfer becomes dependent on the rate of dissipation instead of substrate concentration. Further, if the turbulent intensity is high, its effect on mass transfer cannot be ignored. It can be concluded that Mass transfer through macro-porous sponges has an analogy with total power input or average energy dissipation rate (ε) because of no external or internal limitations.

Figure.5.28. Relation of Sh and Reϵ

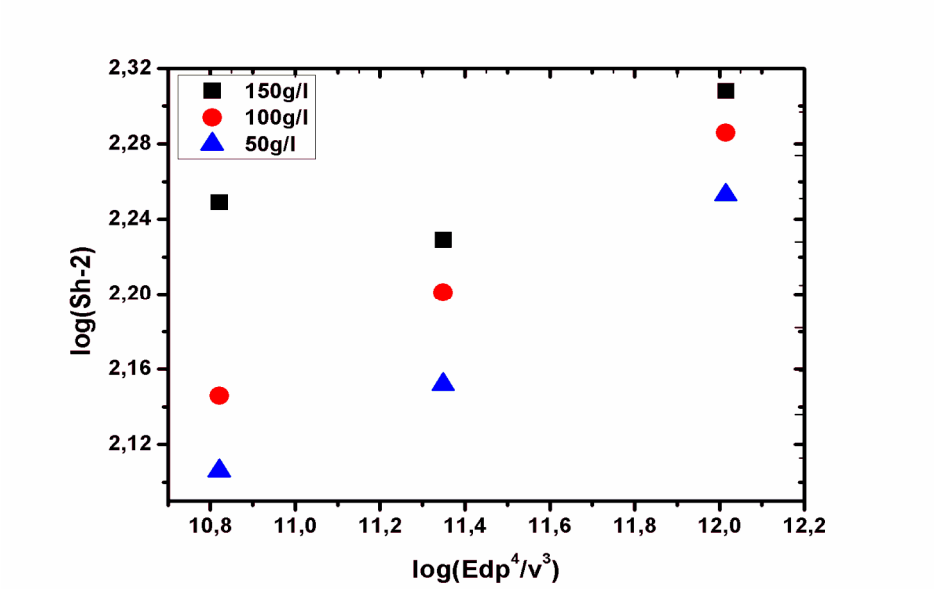


Figure 5.28 shows a direct relation of (sh-2) with (ε dp^4 /v^3). This is like a relation of Sh and Reϵ as in figure 5.22 but in this graph Reynolds number (Reϵ) defined where relative velocity depends on two parameters dp and ε (energy dissipation per unit mass). It is shown Sh varies by varying ε for different Reϵ (Levicky 2013). Further, it is depicted from the figure that liquid-solid mass transfer is a function of energy input per unit mass which is the application of Kolmogoroff theory of isotropic turbulence to mass transfer coefficient in SCBR. At higher Reϵ, Sh becomes independent of substrate concentration because the mixing of solute inside the reactor is fast due to the convection process with the results

solute concentration become uniform. At higher Reϵ, a small dissipative scale of motion become isotropic that is independent of direction.

5.4. DISCUSSION

5.4.1. PBR with Alginate Beads

As Reynolds analogy (1874) suggests that in a flowing fluid mass transfer, heat transfer, and momentum transfer at the same rate. In this analogy diffusion coefficient, thermal conductivity, and viscosity is involved. Tremendous changes have been observed in the fluid flow with certain Re_p value and such flow has inertial forces and viscous forces. Reynolds number is the ratio of inertial forces and viscous forces. Further, he argued that mass and heat transfer involved two processes; One is natural internal molecular diffusion when the fluid has a smaller velocity to flow, and the second forced diffusion caused by eddies when the fluid has turbulent velocity.

If fluid cross over a porous particle with higher velocity, vortices or eddies are produced behind and carry solute particles to diffuse into the surface of the porous particle. This analogy further suggests that as fluid velocity increases and eddies dominant then all transport is by eddy mixing and independent of diffusion coefficient or viscosity. This is the case explained in figure 5.2 at $50 < Re_p < 200$. A serious deviation in the JD factor has been observed due to the concentration gradient that affects the thickness of the laminar boundary layer. Such kind of deviation in Colburn analogy has been observed by Venkatesan and Fogler (Venkatesan and Fogler 2004). Figure 5.3 depicts the explanation that Re_p increase means velocity or inertia of fluid increase. With the consequence velocity gradient decreases, 'No-slip velocity' condition also decrease and slip velocity of fluid increase. It is concluded that slip velocity is direct concerning velocity gradient and mass transfer is directly controlled by slip velocity.

Figure 5.12 data also support several scientists' results that if the Re_p value is 5 then boundary layer separation occurs. Some vortices formed in the wake region of flow over particle If $5 < Re_p < 40$. Upon increasing Re_p vortex length increases and wake become unstable with the results vortex shedding occurs. At this point, inertial forces tend to dominant over viscous forces. These results supported Reynolds's argument about cross flow over spherical particles where forced diffusion is caused by eddies. If Reynolds numbers are $40 < Re_p < 200$ wakes of fluid flow will be in the form of laminar vortex street (Sumer 2006). It means in figure 5.2 wake region is in the form of laminar and at $Re_p = 200$

transitional stage appears. After this Re_p value, the transition to turbulence flow in the wake region occurs, and the viscosity of fluid decreases and viscous momentum transfer also decreases and become steady at one point of Re_p. .

Further, if the particle is very close to the wall of the packed bed bioreactor column some changes have been observed in the flow. Like vortex shedding is suppressed, stagnation points move to the lower angular position, and separation points change (Sumer 2006). If vortex shedding is suppressed due to wall the interaction of two vortexes produced upper and downstream of the particle is affected that ultimately effect on vortex shedding. At higher Re_p fluid dispersion in the column of packed bed bio-reactor is normally by fluid convection and mixing but as laminar with the orderly flow in a steady-state or streamlines. Fluid flows axially and mass is transported in a convective way due to higher porosity between particles near to the wall that effect effective mixing between particles (Eigenberger 1992).

Flow behavior between particle and wake regions has a tremendous effect on Intra-particle mass transfer. When fluid flows over the particle carried the solutes inside the wake region produced by vortexes and outside the wake region due to vortex shedding. Solutes that are carried inside the wake region are mixed and pushed to the base of the particle where eddy diffusion plays a major role in the diffusion of solutes inside the particle. While vortex shedding carrying solutes enhance the convective diffusion as a result of potential flow (Sumer 2006). As the flow regime is laminar at lower Re_p, momentum transfer is by viscosity and velocity fluctuation while the mass transfer is through molecular diffusion and concentration fluctuation. As flow regime change from laminar to the transitional or turbulent core at higher Re_p, viscous transport of momentum has less influence on convective transport (R. Levicky Introduction to Turbulent flow),(Lin, Moulton, et al. 1953).

Viscosity plays an important role in the smaller eddies produced if the flow is in transitional to turbulent conditions. In the smaller scale of eddies, higher shear stress developed. Further shear stress in laminar flow is less as compared to the turbulent flow. Shear stress due to the viscosity of fluid influences the vortex shedding. In a sphere particle, shear stress is in the cross-flow direction as in figure 5.2.

Further the results in figure 5.12, could be explained by understanding the effect of higher velocity or higher Re_p value on the production of eddies that enhance the diffusion on the particle surface as well as on the forces affecting shear stress, and pressure gradient. In laminar flow shear stress also existed, like in turbulent flow, due to the molecular interchange between adjacent layers and cohesive forces between liquid molecules. As the

laminar flow change into transition or turbulent flow, such shear effect is due to the reason that eddy currents increase the molecular exchange and momentum flux in all directions. It indicates that higher shear stress is produced at higher Re_p as compare to lower Re_p number. On the surface of a particle, there are two kinds of drag forces one is by the particle to fluid and equivalently by fluid to the particle surface. This is also called skin friction drag that will be higher if the fluid has a higher viscosity and decreases as the velocity of fluid increases. As Re_p increase, frictional drag is reduced even at higher Re_p frictional drag become negligible. Further frictional drag is higher as the size particles increases because it is directly proportional to the area of contact to flowing fluid.

The front and back points of a particle are called stagnation points where velocity is also assumed to zero. The pressure on the surface is also distributed symmetrically but is inverse of velocity variation. Higher pressure is assumed on the stagnation point while is the uniform pressure away from the surface. Due to boundary layer separation, the wake behind the particle developed, with the consequence large pressure difference between stagnation points of particle surface occur called pressure drag or form drag as it depends on the shape and size of the particle. Pressure forces on the surface of particles can create a large difference or gradient of pressure (Smith 2008). It indicates a bigger particle will have higher form drag or higher pressure gradient that cause boundary layer separation. Boundary layer separation can be reduced by changing the shape of the particle or reducing the size of the particle.

Reynolds number indicates the flow regions (wake and boundary layers thickness) produced when fluid flows over the surface of alginate beads as a particle. These flow regions are directly proportional to the diameter of the particle as wake extends and boundary layer thickness increases as diameter increase (Sumer 2006). The influence of Reynolds number and fluid mechanics as boundary layer theory and film theory on mass transfer in the form of Sherwood Number is observed. Sh is the ratio of convective mass transfer to diffusive mass transfer. Data is separated into two groups as fluid flows over small and large-sized particles in packed bed particles. The results show the influence of particle Reynold Number to understand the effect of flow behavior on mass transfer; as increasing Re_p fluid flow over particle enhance the convective mass transfer by eddy circulation with the consequence intra-particle mass transfer increases due to higher availability of substrate concentration on the surface of the particle (Bird, Steward, et al. 2007).

In the case of system properties, the size of particles has a big impact on the mass transfer coefficient. Further, sufficiently small particles alter the mass transfer regime into a reaction

rate limited regime (Bird, Stewart, et al. 2006). According to previous workers, the Re_p value higher than the critical point does not show a steep change in Sh. A further effect of particle size can be explained in terms of turbulent eddies. An increase in boundary shear stress increases the turbulence in the vicinity of wake near to particle surface (Lal, Kumar, et al. 1988). Turbulent eddies carry solute particles, mixed them by vertical velocity fluctuation. At this point, convective diffusion has a major role to transfer solute from liquid to the surface of the particle. From the surface to the pores of particle molecular diffusion has the role of transportation. The further drag coefficient has an inverse relation while pressure drop has a direct relation with Reynolds number. This correlation of particle in dispersed phase is that mass transfer between the phases is retarded as in boundary layer theory and unhindered flow is responsible for higher transfer coefficients. Such results agree with those reported by several previous workers (Calderbank and Moo-Young 1995).

The particles with the size of the millimeter have shearing stress due to slip velocity around the particle surface that controls mass transfer (Lal, Kumar, et al. 1988). The results in figure 5.1 could be explained in a way that fluid slip velocity influences the thickness of diffusion film. Larger particles have more film thickness. At lower Re_p, the stagnant film model is applicable for the calculating mass transfer coefficient across the film. At the lower Re_p effect of the film, the thickness is more pronounced due to the reason the dissipating eddies become smaller than the size of the particle.

While on the other side at higher Re_p the scale of eddies produced by turbulent flow is bigger than the size of the particle therefore slip velocity become independent of particle size and the influence of particle size on the mass transfer coefficient will be completely diminished (Lal, Kumar et al. 1988). As Re_p increases better mixing of liquid occurs on the point of contact in the film over the surface of the particle consequently increases the mass transfer coefficient or sh. The boundary layer or film thickness is the distance from the surface to that point up to that viscous forces affect. As Re_p increases, the thickness of the boundary layer decrease or in other words Inertial forces are dominant over viscous forces. By decreasing the thickness of the viscous force of the boundary layer will also reduce.

These results are supported by explaining the effect of flow at low or higher Re_p. At low Re_p mass transfer mostly depends on it. As the Re_p increase dependency of mass transfer decrease and until a level come that mass transfer does not depend on the Re_p. Further higher inertial forces produced has a diminishing influence on the mass transfer rate (Lal, Kumar, et al. 1988). When particles as catalysts are in contact with flowing liquid in the presence of reactants that diffuse to the surface of the particle and from surface into the

active site of the particle for the reaction. After reaction products are diffused out to the surface of particles. By increasing the Re_p boundary layer separation point moves gradually to downstream with $\phi > 90°$ and flowing liquid remains in contact with the surface of the particle. By the further increase in velocity, the wake region will also reduce. Because of diffusion (molecular and convective diffusion) mass transfer coefficient is increased.

Uniform flow distribution is difficult to obtain as in the case of larger particles. The pressure drop also strongly depends on the void fraction of the particles in the packed bed column. Therefore, the packing of particles should be careful to avoid the bypass flow between particles (Eigenberger 1992). In incompressible laminar flow pressure drop is proportional to fluid viscosity and superficial velocity while at higher Re_p it is directly proportional to fluid density and square of superficial velocity (Green and Perry 2019). In viscous flow as Reynolds number increases, turbulence in flow has been observed due to small disturbance in the flow and carrying potential energy from laminar flow. With the results of this, surface begins to exhibit waves and grow further by taking energy from primary flow (Norberg 1987). Such waves or instabilities enhance surface tension, fluctuation of pressure gradient, and fluctuating viscous stresses superimposed on the laminar flow.

A smaller Reynolds number represents the smaller amplitude of the disturbance called linear disturbance. As fluctuation increases due to an increase in velocity, the amplitude will no longer as laminar flow and flow become unstable in the state of transition to turbulent (Norberg 1987). The properties of eddies depend on the rate of energy supplied by larger eddies and the rate of energy dissipation by viscosity. A further dimension of the smaller eddies indicates that the scale of turbulence in the system could be reduced in size by decreasing the viscosity or increasing the energy dissipation rate (Doran 1995).

The results correlate the mass transfer data using different particle size with the energy dissipation. It seems that the average transport coefficient correlates with the average energy dissipation rate even though the local dissipation rate is smaller in the laminar flow of fluid. In another way, we can say higher flux in the pores of particles by reducing the size of particles apparent activation energy increases consequently diffusivity increases. In the wake region there is higher temperature and lower pressure due to smaller eddies, the reaction inside particle is controlled by mass transfer while in mild condition controlled by diffusion. Diffusion is directly proportional to temperature and inversely proportional to fluid viscosity. The binary diffusion coefficient of liquid increases with temperature while Knudsen diffusion increases as the square root of the temperature.

Entropy generation has a relation with the dissipation of energy. As the rate of dissipation means the rate at which kinetic energy is converted into internal energy. In figure 5.15 a & b, as the Re_p increases viscous stress opposes the fluid motion with the results highly ordered energy is dissipated into disordered internal energy that enhances the (Sh) or convective mass transfer as well as the entropy generation in the system. As the entropy generation is directly proportional to the loss of potential work in the form of energy dissipation in the system. At higher Re_p when eddies are produced these attenuate and dissipate the energy that heats the fluid and pressure in the eddy region reduce. Due to the pressure difference in the front and back stagnation, drag force is applied over the particle. Drag force depends on the shape and cross-section area of the particle. The higher the dissipation energy means the higher is work done by eddies and the higher is pressure drag on the object. It is expressed by the value of the Reynolds number. Smaller Re_p presents higher frictional drag as compare to pressure drag while at higher Re_p pressure drag dominant over frictional drag (Satterfield 1970).

Small mixing occurs on the surface of the particle, but maximum mixing occurs away from the surface and in the region of wake or vorticity. On the surface of particle mass transfer is by molecular diffusion (Satterfield 1970). In a packed bed with a lower Re_p number overall mass transfer process is proportional to molecular diffusion. If the Schmidt number is high then the concentration gradient becomes narrow and very close to the surface. It can be explained in other words that molecular diffusion starts to influence mass transfer if eddy diffusion is considerably less than molecular diffusion. If molecular diffusion decreases, then the layer of laminar diffusion become narrow.

5.4.2. SCBR with Alginate Beads

In turbulent flow, momentum transfer is by viscosity and velocity fluctuation while the mass transfer is using molecular diffusion and concentration fluctuation. As discussed before that in turbulent flow regime or turbulent core viscous transport of momentum has less influence on convective transport (Levicky 2013),(Lin, Moulton, et al. 1953). Further, it can be explained that mass transfer from bulk fluid in a turbulent regime to the film surface is by convection or eddy motion and through the film is by molecular diffusion and both processes are analogous to the mechanism of momentum transfer in both bulk fluid and film.

On the other hand, the results in the form of Re_p show the flow behavior and effect of the boundary layer around the sphere as shown in figure 5.4 & 5.6 because flow behavior over the sphere is strongly dependent on Reynolds's number. As fluid flow radially with higher

Re_p from the impeller into beads inside the basket show the flow is in a turbulent regime shown in figure 5.4. When Reynolds's number is small, there is a small separation of boundary layers and high viscous drag friction, with the resulting flow, is in the laminar regime. When the Reynolds number is high as shown in data the viscous drag friction is less which results in higher separation of boundary layers. With the consequence, vortices, and eddies formed within wake as shown in figure 5.6 b. The eddy movements enhance the mixing throughout the turbulent regime called eddy diffusion and result in rapid transport in the turbulent region. Further, this can reduce the composition (temperature, pressure, or concentration) gradient around the sphere surface. At this point, transport is only by molecular diffusion because turbulence around the sphere is reduced and disappears (Green and Perry 2019). As $Re_p > 300$ vortex shedding occurs and wake is completely in turbulent condition.

It is depicted from the result that with higher Re_p, the effect of momentum transfer on the whole mass transfer is minimized by convection as presented by the εJD factor. It means that at $Re_p > 7 \times 10^3$ convection mass transfer is not further influenced by momentum mass transfer. In the vicinity of the surface turbulent viscosity is reduced because the size of vortices decreased and even vanished with time. With the consequence viscous momentum transfer become again dominant. As $Re_p > 4 \times 10^3$, non-separated flow can reappear and at value of $Re_p > 10^5$ then vortex formation become independent of Re_p value. Therefore in figure 5.16, εJD value may not undergo largest changes by increasing Re_p. The result shows εJD factor falls consistently at $Re_p > 7 \times 10^3$. As Rep increases $7 \times 10^3 < Re_p < 2.0 \times 10^4$ εJD factor appear nearly flat.

Further, an increase in Re_p $3 \times 10^5 < Re_p < 1{,}5 \times 10^6$ boundary layer separation on both stagnation points of the particle becomes turbulent with the results convection is followed by molecular diffusion that reduces the boundary layer thickness over the particle surface (Sumer 2006).

In the vortex regime, the vortices curve will bend down and enhance the lifting frequency that increases turbulence. Once turbulence is established in flow over the particle surface, no separation occurs but the flow will no longer viscous. The further particle will oscillate with a frequency equal to the frequency of lift. Colburn analogy has a reasoning relationship with Reynolds's analogy between fluid friction and mass transfer. Further, it can be explained that the ratio of the momentum is lost by friction between two sections to the total momentum of fluid. This reasoning is the same as the ratio of material actual supplied to film to a whole fluid which would have been supplied. In a turbulent regime, the

mechanism of mass transfer will be by convection or motion of eddy from fluid turbulent regime to film boundary and by diffusion process across the film to liquid (Colburn 1930).

As the value of $Re_p > 10^5$ then vortex formation becomes independent of Re_p value. The results in figure 5.16 could be explained by understanding the effect of higher rotation speed or higher Re_p value on the production of eddies as well as on the forces affecting. At this higher Re_p, inertial forces are dominant over the viscous forces. In a turbulent flow with higher Re_p, a point comes where the mass transfer coefficient becomes independent on the Schmidt number or molecular diffusion into the boundary layer.

Eddy diffusivity also has a direct relation with velocity. Such relation can be explained that at the lower velocity the eddy diffusivity reduced. Eddy diffusivity is maximum at a higher velocity and it becomes independent on the distance between particles (Barker and Treybal 1960). Kolmogorov's theory of isotropic turbulence also supports our results as in figure 5.16. According to this condition, some eddies do not involve significantly in turbulent energy dissipation and develop a universal structure. Further, these eddies become independent on viscosity and external constraints as well as on mean flow. This supports our results that at a critical point of Re_p, εJD become independent Re_p. Such eddies are called inertial subrange in which the inertial mechanism of vortex stretching is responsible for the transfer of energy. Such kinds of eddies are in between the larger and smaller eddies. The energy that is transferred from larger eddies to smaller eddies should cross though this inertial subrange zone. If the fluid is in a turbulent regime then momentum is transferred by viscosity and velocity fluctuation. Velocity fluctuation can be defined as the mixing length or free path for fluid turbulence. In the third buffer layer mass transfer is due to the action of both molecular motion and eddy diffusion (Lin, Moulton, et al. 1953). Microscale eddies in the vicinity of liquid-solid phases have a major effect on the intra-particle mass transfer. This is the verification of reasoning why the εJD factor of mass transfer is decreasing by increasing the Reynolds number as in figure 5.16.

In figure 5.17, the overall correlation of mass transfer can be understood by the ratio of Sherwood number to film thickness (**Sc^1/3**) which has a purely direct relation with Re_p and illustrate the flow behavior on the solid-liquid interface. Under the present condition of Re_p, turbulent flow is induced by the effect of impeller rotation. Further glucose concentration does not have a significant role. Reynolds Number (Re_p) represents the impeller rotation speed. So overall mass transfer effect shows very weak dependency on the lower number of Re_p. After this it increases more rapidly with the increase of Re_p numbers. Further increase in Re_p, overall mass transfer effect will not be pronounced as in figure 5.17. Such observations

have been noticed by other researchers and reported that shear rate in a stirred vessel is directly proportional to the Re_p number (Lal, Kumar, et al. 1988). The turbulent boundary layer has a steep velocity gradient on the surface of the particle or a higher tangential velocity (du/dy) gradient with higher wall shear stress and depends on flow speed or Reynold Number as shown in figure 5.5 With the result surface has more shear stress that causes drag on the surface as a consequence high mass transfer rate occurs. Total shear stress in turbulent flow will be the combination of momentum transfer in laminar flow and turbulent shear stress due to the reason of velocity fluctuation and eddy motion. Further turbulent mass transfer coefficient becomes equal to the mean fluctuating velocity. Turbulent has a tremendous effect on vortex shedding.

Figure 5.18 illustrates the increasing trend in Sh value against Re_p may be attributed to the bioreactor ability to induce axial flow along with radial and swirl flow. Axial flow has a significant effect on the mixing of fluid that can enhance the rate of mass transfer by convection while radial flow enhances the molecular diffusion by the formation of eddies. On the other hand swirl and the radial flow inside the basket can reduce the slip velocity between rotating fluid and alginate beads (Sedahmed, Farag, et al. 1998).

The enhancing effect of Re_p on Sh as in figure 5.18, is attributed to the flow pattern produced by the impeller in the presence of a basket near to the wall of the vessel. Such behavior can be explained that in the case of axial flow fluid moves axially downward to the bottom of the vessel, then radially and upward through the basket (Mowena, Zaatout, et al. 2013). In this case, there is a breakdown of fluid swirl motion that can enhance the good mixing in-vessel as well inside the basket. The increasing trend in Sh against Re_p due to a particular relationship of turbulent motion and its diffusing power (Pangarkar, Yawalkar, et al. 2002). Convection of fluid on the surface of a particle is induced by pressure gradient with the consequence solutes are available to diffuse into the pores of the particle. The diffusion of solutes depends on the gradients of concentration between bulk fluid and the site of action in pores of particles. As the solutes approach inside the particle, depends on the rate of mass transfer, the reaction takes place. The effect of solute concentration on diffusion can be estimated by diffusion-reaction theory.

Figure 5.18 depicts that a higher Re_p number means high turbulence in flow that enhances the overall mass transfer and increases the liquid-solid interfacial area. Figures 5.17 & 5.18 demonstrate the effect of fluid flow behavior and different glucose concentration. Further rate of mass transfer is not only controlled by diffusion of glucose concentration but also by the turbulence generated by different impeller rotation speed.

Figure 5.18 demonstrates the effect of glucose concentration on Sh. Glucose concentration has a negligible influence on Sh probably due to the concentration gradient between two phases (liquid-solid). For a given set of condition, figure 5.18 shows that increase in the diffusion-controlled mass transfer depends on higher glucose concentration and higher value of Re_p. Higher value of Re_p may be explained by the higher degree of turbulence that contributes to enhancing the mass transfer effect by convective mass transfer in bulk fluid and molecular diffusion into boundary layer on the surface of the particle. Further turbulence in fluid motion can breakdown the laminar flow inside the basket and enhance the diffusion of glucose inside the particle. Due to turbulence catalyst particles can have a colloidal motion that can enhance the micro-convection by eddy motion with a consequent increase in mass transfer (Sedahmed, Farag, et al. 1998). Different researchers show the result of an increase in the rate of mass transfer due to suspended particles (Hajar and Vahabzadeh 2016). In convective mass transfer, the transfer of mass into boundary layers is effected by molecular diffusivity and eddy diffusivity. It means boundary layers are associated with the mass transfer as well as with the momentum transfer as shown in figure 5.5 & 5.18. In the laminar layer, no turbulence of fluid or eddy diffusion is assumed but the mass transfer is only by molecular motion. On the other hand in a turbulent regime, mass transfer is controlled by eddy diffusion of momentum and eddy diffusion of mass.

According to Ficks equation in boundary layer mass flux is directly proportional to the concentration gradient, diffusion coefficient, and density of the fluid. In the case of turbulent boundary layers, eddy diffusion is dominated over molecular diffusion. Further eddy diffusion is the function of the eddy length for mixing and molecular diffusion is the function of the concentration gradient (Vary 1970). Mass transfer in turbulent flow is due to eddies and fluctuations of velocity and concentration. Eddy diffusion has the main role in the transport of solute to the site of reaction in particle and enhances the transport in turbulent core by reducing the composition gradient. This reduction is necessary because a higher composition gradient near to the surface of the particle enhances the resistance of mass transport in the boundary layer. At the surface of the particle, turbulence reduces and disappears in the immediate to surface of the particle and molecular diffusion will increase the transport.

In a stirred vessel, rheological properties of fluid have an influence on energy dissipation or power consumption and ultimately on mass transfer rate (Lal, Kumar, et al. 1988). Several investigators have correlated the mass transfer data with specific agitation power to assess the applicability of Kolmogoroff theory in a stirred tank reactor. Figures 5.19 & 5.20 show

the effect of energy dissipation on overall mass transfer and Sherwood Number (Mass transfer coefficient) in stirred catalytic basket bioreactor. This shows that mass is transferred inside the bed by the convective flow. It is noted in figure 5.19 that (Sh-2/Sc^$^{1/3}$) is an overall mass transfer coefficient, due to turbulence in flow, over the surface of the particle. These correlation results have been observed almost the same in the case of different glucose concentrations in the whole range of log (Ed_p^4/v^3). Our results support the theory as both mass and momentum transfer have a direct influence on the boundary layer thickness and the transfer properties of the fluid (Vary 1970). In a turbulent condition or higher Reϵ value boundary layer separation is formed. The induced turbulence has a tremendous effect on the formation of eddies and eddy diffusivity. While this is not the case in figure 5.20 with the correlation of Sherwood number log (Sh-2) & log (Ed_p^4/v^3). Figure 5.20 shows a higher value of log(Sh-2) at higher glucose concentration and a higher value of log (Ed_p^4/v^3). It can be concluded that Sherwood Number not only directly related to the energy supplied but also on glucose concentration.

The entropy generation rate per unit volume in turbulent flow is directly proportional to the cube of superficial flow velocity. On increasing Reϵ, the rate of mechanical energy dissipation into internal energy increases. Entropy generation is directly proportional to energy dissipation, as it increases when the internal energy of the system increases (Benamara, Assoua, et al. 2018). Further entropy generation is independent of fluid viscosity at higher Reϵ due to less resistance in a fluid motion. In the case when particles are packed in a bed submerged in a mixing vessel, the density difference between phases (fluid and solid surface) can induce the dispersion that can determine the rates of mass transfer (Calderbank and Moo-Young 1995). In Kolmogoroff theory **"ϵ"** is the total agitation power per unit mass of fluid which assumed that all of the energy dissipation is due to the turbulent motion of the fluid. Such an assumption only could be applied when Reynolds Number is high.

Energy dissipation is directly related to Reϵ, if Reϵ is higher then larger vortices or eddies are produced that contained most of the kinetic energy and disintegrated to smaller eddies as shown in figure 5.6 a & b. Larger vortices are turbulence generating features and are not affected by viscosity. If the scale of the main flow is large, then there are intermediate eddies that can dissipate some part of the energy to smaller eddies as described by Calderbank and Moo-Young in 1995. These smaller eddies are independent of the larger vortices but depend on the local energy dissipation rate per unit mass of fluid as explained by Kolmogoroff (Kaneda, Ishihara, et al. 2012). Smaller eddies are smaller enough to be

controlled by viscosity. The rate of energy dissipation depends on two parameters like viscosity and velocity gradient produced by the turbulent fluid as shown in figure 5.19 & 5.20 when energy dissipation increased to a limit needed then a small increase in mass transfer coefficient, such correlation has been observed by Calderbank in 1995. This energy dissipation findings allow a correlation of mass transfer coefficients for fluids in a turbulent situation that is induced by mechanical manner or impeller (Calderbank 1995). Such findings depict the interrelation of energy dissipation and specific turbulent transport phenomena which is a general law of isotropic turbulence and support the Kolmogorov theory using agitation power (Brian, Hales, et al. 1969).

Figure 5.19 correlation data of energy dissipation with overall mass transfer based on Kolmogoroffs theory of isotropic turbulence. In this kind of turbulence, the flow of fluid is independent of the direction and does not have a mean velocity gradient as shown in figure 5.4. All the fluctuating velocities having positive or negative values, their average will be zero. In larger eddies, produced in turbulent flow, such isotropic conditions existed because of the overall flow direction. At higher Reϵ value due to the presence of the energy cascade process, the directional elements of the main flow are lost. The reason behind this is that smaller eddies dont have a direct influence on the mean flow therefore flow is assumed in anisotropy condition (Doran 1995).

Turbulent velocities are the function of two parameters; one is energy dissipation and the second is kinematic viscosity. The second law of thermodynamics is involved in the entropy generation of the system. Entropy generation is directly related to the energy dissipation rate that is a direct indication of the feasibility and efficiency of the process. By increasing the Reϵ number molecules kinetic energy will increase that enhances the temperature of the fluid. The net result is a decrease in mechanical energy with an equal increase in thermal energy. This increase in disorder or distribution between thermal and mechanical energy is measured by the change in entropy. Lower entropy at lower Reϵ means a higher proportion of mechanical energy and flow has great potential to do useful work (Pangarkar, Yawalkar et al. 2002).

5.4.3. SCBR with DEAE Sponges

The dependence of the εJD factor on Re_p has been evaluated for a long time. But the results were not in agreement because of different hydrodynamic conditions and characteristics lengths used (Garrido, Patcas, et al. 2008). A correlation as suggested by Chilton Colburn and Dwivedi and Upadhyay has been established whose results come in acceptable agreement.

At higher Re_p number, as fluid flows through pores of porous material it slows down near to the walls of solid porous material, and far away its velocity is high with a profile in slightly parabolic shape as shown in figure 5.9. At this point, mass transfer is influenced by the stagnant fluid film on the walls of pores. Film thickness has a great influence on the retardation of velocity. At higher film thickness, the motion of fluid stops (Khan, Gul, et al. 2017). With an increase in Re_p number film thickness reduces and steps 1 & 2 are enhanced as described in figure 5.7. At higher Re_p convective transportation enhanced with the results solute quickly come close to the surface of pores and film thickness reduced so solute doesn't need more time to diffuse through stagnant liquid film. At this point (step 2) diffusion is facilitated because upon reaching into the pores of the sponge, fluid with higher turbulence develop eddies as shown in 5.10. With higher Re_p, the range of eddy size due to turbulence is inertial subrange where the transfer of mass or energy is by the inertial mechanism. The eddies at this stage become independent of mean flow or viscosity. In other words, convective mass transfer is not further influenced by viscous transport of momentum. Isotropy of eddies is assumed at the point of nodes in pores of sponges where turbulence becomes intense (Doran 2013), (Doran 1995).

The liquid-solid mass transfer in sponges in the form of dimensionless analysis has been studied extensively in the literature. Sherwood number is important for the dimensionless correlation of mass transfer. It describes the ratio of molecular momentum diffusivity to molecular mass diffusivity or ratio between actual mass transfer and diffusion (De Lathouder 2007). The value of Sherwood number does not vary greatly at smaller variation in Reynold number and glucose concentration but a higher Sh value is observed in figure 5.22 if there is a big variation in Re_p. Velocity distribution in the pores become independent of velocity magnitude but dv/dz (directional of velocity) is proportional to volumetric flow rate over the area (q/A) (Collins and Gorton 1976).

Many authors come on this point that as hydrodynamic and fluid properties, geometric and materials properties also have a major role to describe mass transfer properties of sponges. Sherwood also depends greatly on sponges' characteristics like pore size, geometric surface area because all these factors affect on fluid dynamics that ultimately effect on mass transfer. It has been reported in the literature that foam with lower PPI is performing better than higher PPI due to the reason of big pore diameter in case of lower PPI. While in the case of higher PPI pore size can be reduced and struts of foam will not be uniformed. With the results, more materials can accumulate on the strut nodes that can affect on the specific surface area. A higher specific surface area of foam is also the reason for the higher value of

Sh number because of the availability of more area for reaction. Further, the limitations of diffusion as in micropores can also affect on the Sh number (Garrido 2009; Inayat 2013).

The dependence of mass transfer coefficient on superficial velocity is higher in the case of large pores (10-20 PPI) as compared to smaller pores. It is reported in the literature that 5-folds of fluid velocity can increase the mass transfer coefficient about 50% which indicates that flow is in the Taylor flow regime. The effect of higher Re_p or turbulence on overall mass transfer is more pronounced because open pores of sponges are filled with liquid carrying substrate that results in increases of reaction. Under the condition of reaction, mass is transferred from bulk flow to the pores of sponges and thin catalyst stagnant layer on the pore wall. Open pores of macro-porous sponges optimize the contact area and minimize the internal diffusion limitations. This novel DEAE sponge prevents plugging and allows higher flow inside the pores due to the action of pressure drop created at higher stirring effect or turbulence. It can be concluded that convection of reactants through pores of the sponge is directly proportional to Re_p and pressure drop (De Lathouder 2007). Concerning porosity, it has been observed that the mass transfer coefficient value is higher in lower porosity sponges. A slope of Sh-Re_p values of 10 PPI (big pore diameter) sponges was higher as compare to beads due to higher interstitial velocity inside the pores of the sponge (Garrido, Patcas, et al. 2008). In a dimensionless analysis of sphere beads and sponges, it has been observed that Sherwood's number of 20 and 45 PPI sponges have a slightly lower value as compare to beads. On the other hand, the mass transfer coefficient can be reduced due to higher cell density that can reduce the pore diameter by plugging. Many authors have evaluated that dependence of overall mass transfer is not great on reactor geometry (Garrido, Patcas, et al. 2008).

As shown in figure 5.24 & 5.25 pressure has a great role in fluid motion or mass transport in porous materials. At higher stagnant film thickness of pores walls, higher pressure or higher force is needed to drive fluid inside the pores. Therefore, a low-pressure drop is required for the fluid flowing in the porous system. Higher flow resistance in smaller pore size can increase the pressure drop. On the other hand, Incera Garrido found a lower pressure drop with 10PPI of another foam due to the larger diameter of its window or effective pore size (Garrido, Patcas et al. 2008) (Inayat 2013). Foam with 20-30 PPI have higher porosity and expected lower pressure drop but in some experiments of different authors, maximum pressure drop has been observed due to closed or smaller size window of foams. Darcy Forchheimer's law proposed the relation of pressure drop and fluid velocity. Further work has been done by extending this law in considering the longitudinal flows and pressure drop

due to channel wall friction (Dean 1978; Vafai and Kim 1990; Chandesris, Serre, et al. 2006). Our results indicate the correlation of pressure drop with mass transfer and momentum transfer.

The correlation of pressure drop and mass transfer published in the literature have great variability due to differences in experimental conditions and sponges physical parameters, porosity, specific surface area, pores diameter. Some authors have sponges with closed pores or no regularity that can affect the flow of fluid through pores and the accumulation of materials at the cell nodes (Lucci, Della Torre, et al. 2014). Pressure strain rate exchange energy between different components of turbulence. It is expressed as it steals kinetic energy from the rich and gives to the poor. Even at higher Reε dissipation of kinetic energy in every component is equal. The pressure strain rate act as a process in which all components receive energy even energy is produced in one component. While the effect of pressure strain rate vanishes in the isotropic state of turbulence (Bakker 2002). A new type of analogy has been proposed by Martin, between momentum transfer that can be due to friction or pressure drop and mass or heat transfer (Garrido, Patcas, et al. 2008). Such a correlation is observed in figure 5.24, an analogy is observed between the mass transfer coefficient and dimensionless pressure drop or Hagen number where Sh is an increasing function of pressure drop.

There is a need to consider the turbulent kinetic energy inside the interstices or channels of porous materials. For this one approach has been proposed for the longitudinal flow and microscopic transverse flow through porous materials (Chandesris, Serre, et al. 2006). The production of kinetic energy means interchange of energy between the mean flow and fluctuations. The overall exchange of energy can be understood by 'Reynolds stress' and 'viscous stress'. Reynolds stress can transfer kinetic energy from mean motion to the fluctuating motion and is against the mean velocity gradient and referred to as the rate of turbulence energy production. While viscous stress resists deformation by instantaneous velocity gradient and removes energy from the flow (Bakker 2002). Transfer of kinetic energy from a region of higher kinetic energy to lower kinetic energy. Further diffusion of kinetic energy is proportional to the gradients of kinetic energy. The production of kinetic energy in sphere porous materials could not be the same as porous material with channels.

For fully developed flow in porous materials, the internal production of kinetic energy becomes balanced by the dissipation of energy because all kinetic energy is converted into heat called viscous dissipation. The production is directly proportional to the power of wall

frictional forces due to the direct conversion of turbulent kinetic energy into heat (Chandesris, Serre, et al. 2006).

Kolmogorov argued that at higher Re_p smaller eddies become isotropic and independent of bulk motion. Such eddies have properties of energy dissipation rate (ϵ). At higher Re_p, turbulence come on equilibrium due to the rate of energy dissipation (ϵ) and kinematic viscosity (v) (Bakker 2002).

$$\eta = (v^3 \epsilon)^{1/4}$$

η = is the length scale of eddy dissipation energy by viscous dissipation.

A velocity scale can be defined as $V = (v \epsilon)^{1/4}$

Figure 5.28 represents the results of the analogy of mass transfer with energy dissipation per unit mass which is the application of Kolmogoroff theory of isotropic turbulence.

If the Reϵ of the mainstream is higher there is the existence of an inertial subrange in which viscous dissipation becomes unimportant. A broad spectrum of eddy sizes is generated in which larger eddies have large kinetic energy and small eddies dissipate it. Smaller eddies are independent of the boundary of the system. At this point, eddies depend on the rate of energy supplied by larger eddies and the rate of energy dissipation by viscosity.

Further by using DEAE sponges as catalysts support at higher Reϵ, Sh becomes independent of substrate concentration as shown in figure 5.28 It can be explained in a sense that convective mass transfer becomes dominant due to pressure gradient not because of a concentration gradient. In literature mass transfer coefficient KL has been observed directly proportional to $\epsilon^{\wedge 1/10 \text{ to } 1/6}$. In the model of K-ϵ, the dissipation rate is directly proportional to the production of kinetic energy. This model was further improved by introducing a time scale model. As we have observed from results in figures 5.27 & 5.28, this model depends on the cascade of energy in which larger eddies carry the energy and dissipate it to smaller eddies (Chen and Kim 1987).

5.5. CONCLUSION

5.5.1. Packed Bed with alginate beads

Flow behavior between particle and wake regions has a tremendous effect on Intra-particle mass transfer. When fluid flows over the particle carried the solutes inside the wake region produced by vortexes and outside the wake region due to vortex shedding. Solutes that are carried inside the wake region are mixed and pushed to the base of the particle where eddy diffusion plays a major role in the diffusion of solutes inside the particle. While vortex shedding carrying solutes enhance the convective diffusion as a result of potential flow. As the flow regime is laminar at lower Re_p, momentum transfer is by viscosity and velocity fluctuation while the mass transfer is through molecular diffusion and concentration fluctuation. As flow regime change from laminar to the transitional or turbulent core at higher Re_p, viscous transport of momentum has less influence on convective transport (R. Levicky Introduction to Turbulent flow),(Lin, Moulton, et al. 1953).

For better mass transfer there must be a low-pressure drop. As higher pressure or higher force is needed to drive fluid inside the pores of beads. Therefore, a low-pressure drop is required for the fluid flowing in the porous system. Higher flow resistance at lower Re_p can increase the pressure drop. In a packed bed with a lower Re_p number overall mass transfer process is proportional to molecular diffusion. If the Schmidt number is high then the concentration gradient becomes narrow and very close to the surface. It can be explained in other words that molecular diffusion starts to influence mass transfer if eddy diffusion is considerably less than molecular diffusion. In a packed bed with a lower Re_p number overall mass transfer process is proportional to molecular diffusion. If the Schmidt number is high then the concentration gradient becomes narrow and very close to the surface. It can be explained in other words that molecular diffusion starts to influence mass transfer if eddy diffusion is considerably less than molecular diffusion (Sumer 2006).

Agitation speed, catalyst size, and velocity around the particle have a tremendous effect on Sh. In the case of the size of the particle, the mass transfer coefficient correlated well with solid-liquid dispersion.

Further pressure drop is directly proportional to (v) velocity and (v2) at the laminar flow and turbulent flow respectively. The diffusion process involves the pattern of fluid flow and other factors like velocity distribution, concentration gradient, and pressure gradient on the surface of particles that affect directly diffusion or internal mass transfer.

At lower Re_p flow over particle without flow separation, flow is not turbulent. In this case drag force (Fd) is directly proportional to v. On the other hand, if flow over particle is turbulent (higher Re_p) with boundary layer separation, drag forces will be directly proportional to v^2.

Pressure drop and frictional drag can be reduced by reducing the cross-sectional area of the particle over which fluid flows which is indicated in figure 5.14 a & b results. Further, it could be concluded that higher energy dissipation means the higher mass transfer is by convective diffusion of eddies. If fluid cross over a porous particle with higher velocity, vortices or eddies are produced behind and carry solute particles to diffuse into the surface of the porous particle. This analogy further suggests that as fluid velocity increases and eddies dominant then all transport is by eddy mixing and independent of diffusion coefficient or viscosity.

While larger particle size can increase the bed porosity resulting in a bed with more open spaces for the flow of fluid, less fluid resistance, and less fluid-solid contact area per unit volume of bed. It means due to bigger-sized particles steep reduction in energy dissipation and entropy generation occurs. In a packed bed by using smaller particles, interstitial velocity will increase due to the reduction of channel diameter consequently mass transfer is increased. Reynolds number indicates the flow regions (wake and boundary layers thickness) produced when fluid flows over the surface of alginate beads as a particle. These flow regions are directly proportional to the diameter of the particle as wake extends and boundary layer thickness increases as diameter increases. As Re_p increase, frictional drag is reduced even at higher Re_p frictional drag become negligible. Further frictional drag is higher as size particles increases because it is directly proportional to the area of contact to flowing fluid.

5.5.2. SCBR with Beads & Sponges

In turbulent flow, momentum transfer is by viscosity and velocity fluctuation while the mass transfer is through molecular diffusion and concentration fluctuation. Mass transfer from bulk fluid in turbulent regime to the film surface is by convection or eddy motion and through the film is by molecular diffusion and both processes are analogous to the mechanism of momentum transfer in both bulk fluid and film. The eddy movements enhance the mixing throughout the turbulent regime called eddy diffusion and result in rapid transport in the turbulent region. Colburn analogy has a reasoning relationship with Reynolds's analogy between fluid friction and mass transfer. Further, it can be explained that

the ratio of the momentum is lost by friction between two sections to the total momentum of fluid. As the value of Re_p > 105 then vortex formation becomes independent of Re_p value. At this higher Re_p, inertial forces are dominant over the viscous forces. In a turbulent flow with higher Re_p, a point comes where the mass transfer coefficient becomes independent on the Schmidt number or molecular diffusion into the boundary layer.

The increasing trend in Sh value against Re_p may be attributed to the bioreactor ability to induce axial flow along with radial and swirl flow. Axial flow has a significant effect on the mixing of fluid that can enhance the rate of mass transfer by convection while radial flow enhances the molecular diffusion by the formation of eddies. These both mass and momentum transfer have a direct influence on the boundary layer thickness and the transfer properties of the fluid (Vary 1970). In a turbulent condition or higher Re_p value boundary layer separation is formed. The induced turbulence has a tremendous effect on the formation of eddies and eddy diffusivity.

A higher value of log(Sh-2) at higher glucose concentration and higher value of log (Edp^4/v^3). It can be concluded that the Sherwood number not only directly related to the energy supplied but also on glucose concentration. Our correlation data of energy dissipation with the overall mass transfer is based on Kolmogorov's theory of isotropic turbulence. In this kind of turbulence, the flow of fluid is independent of the direction and doesn't have a mean velocity gradient.

Chilton Colburn analogy gives an inspired guess about liquid-solid interface mass transfer limitations. Reynolds analogy in liquid gives an expression of two levels of turbulent mixing: Macroscopic (dominant eddies) and Microscopic (dominant diffusion, conduction, and viscosity). It means the conversion of substrate into the product at the maximum rate with minimum mass transfer limitations. Further increasing Re_p would lead to a decrease εJD factor, it means lower convective or molecular mass transfer limitations that decrease boundary layer thickness. It is concluded that sponges are the best choice to decrease external and internal diffusion limitations.

At higher Re_p convective transportation enhanced with the results solute quickly come close to the surface of pores and film thickness reduced so solute doesn't need more time to diffuse through stagnant liquid film. At this point (step 2) diffusion is facilitated because upon reaching into the pores of the sponge, fluid with higher turbulence develops eddies. With higher Re_p, the range of eddy size due to turbulence is inertial subrange where the transfer of mass or energy is by the inertial mechanism. The eddies at this stage become independent of mean flow or viscosity. Sherwood number is important for the

dimensionless correlation of mass transfer. It describes the ratio of molecular momentum diffusivity to molecular mass diffusivity or ratio between actual mass transfer and diffusion (De Lathouder 2007). The value of Sherwood number does not vary greatly at smaller variation in Reynold number and glucose concentration but a higher Sh value is observed if there is a big variation in Re_p. Higher specific surface area of foam is also the reason for the higher value of Sh number because of availability of more area for reaction. Further, the limitations of diffusion as in micropores can also affect on the Sh number.

Our results for Sh and Sc as a function of Re_p are presented and seems experimental data are in good agreement with the model expected for sponges. Further by using DEAE sponges as catalysts support at higher Re_p, Sh becomes independent of substrate concentration. It can be explained in the sense that convective mass transfer becomes dominant due to pressure gradient not because of the concentration gradient. The effect of higher Re_p or turbulence on overall mass transfer is more pronounced because open pores of sponges are filled with liquid carrying substrate that results in increases of reaction. Under the condition of reaction, mass is transferred from bulk flow to the pores of sponges and thin catalyst stagnant layer on the pore wall. Open pores of macro-porous sponges optimize the contact area and minimize the internal diffusion limitations.

Pressure has a great role in fluid motion or mass transport in porous materials. At higher stagnant film thickness of pores walls, higher pressure or higher force is needed to drive fluid inside the pores. Therefore, a low-pressure drop is required for the fluid flowing in the porous system. Higher flow resistance in smaller pore size can increase the pressure drop. Darcy Forchheimer's law proposed the relation of pressure drop and fluid velocity. Our results indicate the correlation of pressure drop with mass transfer and momentum transfer.

Further, it is depicted that mass transfer coefficients of sponges are proportional to the cubic root of the pressure gradient. Immobilized cells in tortuous paths of pores can enhance the limitations in internal diffusion but the convective transfer can easily remove these limitations at even a lower pressure drop. The friction factor (f) of macro-porous sponges reduces with an increase of Rep that means fluid is well mixed at different boundary layers and the thickness of the sublayer is reduced.

At higher Re_p number or higher inertial forces, fluid particles packed tightly, and higher pressure is required to overcome fluid intermolecular forces and no-slip velocity condition at the pore wall. As pore size decreases pressure drop increases that mean pressure drop has an inverse relation with pore size.

For fully developed flow in porous materials, the internal production of kinetic energy becomes balanced by the dissipation of energy because all kinetic energy is converted into heat called viscous dissipation. Our results of the analogy of mass transfer with energy dissipation per unit mass which is the application of Kolmogoroff theory of isotropic turbulence. The overall trend of Kolmogoroff theory and shows the relationship between power input shows that at higher Reϵ overall mass transfer is improved because higher turbulent motion develops large primary eddies that have large velocity fluctuations as well contain the bulk of the kinetic energy. By interaction with the moving stream, primary eddies produce smaller eddies of higher frequency that are dissipated into heat by viscous forces.

Further by using DEAE sponges as catalysts support at higher Reϵ, Sh becomes independent of substrate concentration. It can be explained in the sense that convective mass transfer becomes dominant due to pressure gradient not because of the concentration gradient. Liquid-solid mass transfer is a function of energy input per unit mass which is the application of Kolmogoroff theory of isotropic turbulence to mass transfer coefficient in SCBR. At higher Reϵ, Sh become independent of substrate concentration because the mixing of solute inside the reactor is fast due to the convection process with the results solute concentration become uniform.

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CHAPTER 6

CONCLUDING REMARKS

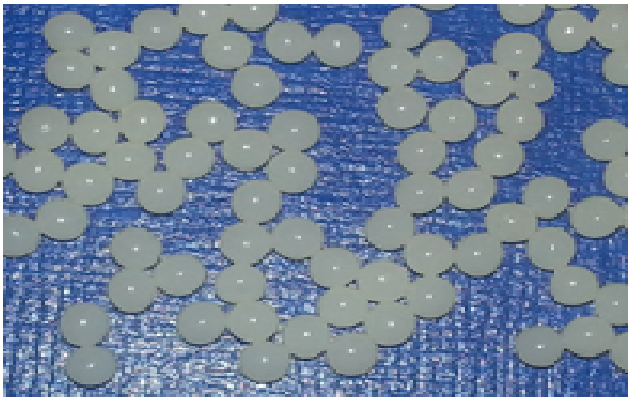
6. CONCLUDING REMARKS

6.1. GENERAL DISCUSSION

In biochemical industries, cell immobilization has been used as an important tool to improve the performance of fermentation processes. Immobilized cells are so much advantageous over free cell cultivation especially to enhance productivity (Hussain A, 2015; Corbo M R, 2013 & Pilkington PH, 1998). Different immobilization methods like physical entrapment, attachment or adsorptions, self-aggregation by flocculation are currently in use. The commonly used support materials like calcium alginate, chitosan, and other polymer beads are examples of physical entrapment methods which means entrapment of cells within a porous matrix. While on the other hand when biomass is attached to the solid support by electrostatic, ionic and hydrogen bonding interaction such immobilization method is called the attachment or adsorption method.

Such types of immobilization methods are most commonly used for different carriers like DEAE cellulose, porous glass, sponges, and wood blocks (Pilkington PH, 1998 & Williams D, 1981). The immobilizing material like alginate beads has been used commonly in the fermentative ethanol production process where cells are immobilized by simple entrapment method. The use of alginate beads especially on the large-scale fermentation industry is reduced due to several drawbacks such as gel degradation due to chemical and physical instability of the gel and mass transfer limitations of nutrients. In the results of diffusional limitations, such type of immobilization might reduce the productivity of the cell. Furthermore, the preparation of such support materials needs complex and expensive equipment especially for large scale production (Verbele P J, 2006, Yabannavar V. M. and Wang D. 1. C,1990).

Figure 6.1. Encapsulated yeast (Alginate beads).

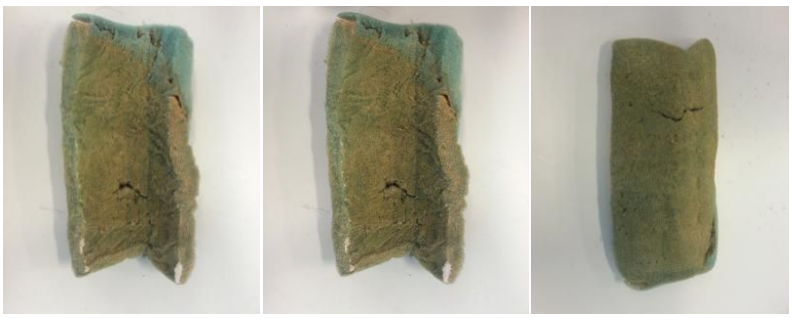


This issue has enhanced the interest in developing advanced immobilizing support materials with characteristics of long term stability, inert, insoluble, large surface area, easily available, low price and non-biodegradable polymers to enhance the productivity by reducing limitations in mass transfer. (Y. Kourkoutas. et al.2004; Terada et al. 2006; Duarte et al. 2013).

The **new biocatalyst support** was developed and used in our research with well-defined structures. The attachment or adsorption method of immobilization was used for this polymer (biocatalyst support). The microbial cells have negative charges on the surfaces so they can be easily adsorbed on a polymer having a cationic group (Nedovic V, 2004).

Such polymers are low in cost, allow high cell densities, mechanical strong against share stress through agitation of the stirred reactor, and nontoxic to the cells. Furthermore, cell viability will not be impaired because these are prepared before immobilization of cells, suitable for mass transfer, and are reusable. These are prepared by grafting of macroporous polyethylene polymer with chains of polyglycidyl methacrylate-ethylene diamine. In this grafted polymer, amino groups are attached which helps in the interaction with microbial cells.

Fig.6.2. Polyethylene sponges after cells adsorption.



In recent most studies about immobilizing materials, alginate beads were compared with other support materials based on their operational stability, rheological properties, and its productivity. While in our study the mass transfer properties of alginate beads were compared with newly grafted polymers.

6.2. GENERAL CONCLUSION

The immobilization technique offers numerous advantages over free cells in the ethanol industry. Further, Packed bed reactors have been used for a long time in ethanol production due to higher productivity achieved using a continuous fermentation process. On the other hand, many disadvantages like the low operational stability and the non-viability of yeast cells during the process have been observed. Even on the industrial level, several engineering problems (mass

transfer) have been seen. **Therefore, a Packed bed reactor as a prototype reactor with different size of alginate beads was tested to understand the external & internal mass transfer limitations.** In the course of physiological and biochemical studies on the substrate uptake of immobilized yeast cells, there was need to continue investigating the operational performance of the immobilized packed-bed bioreactor (Hussain, Kangwa, et al. 2015).

6.2.1. Packed Bed Reactor (PBR) with Alginate beads (Dimensional Correlation)

6.2.1.1. Fluid-Side Mass Transfer

The operational performance of the immobilized packed-bed bioreactor in the course of immobilized yeast cells' physiological and biochemical changes (mass transfer properties) was monitored according to parameters such as substrate concentration, fluid flow rate, and different immobilizing materials like alginate beads with and without chitosan coating. This study gives a significant understanding of both alginate beads with and without chitosan coating. As it was observed with chitosan-coated beads being 30–40 min higher than that of the non-coated beads. The maximum time of lag phase was found to be 290 min at a lower flow rate of 1 ml/min and 190 min at a higher flow rate of 90 ml/min when using 40 g/l of glucose. By decreasing the glucose concentration from 40 to 10 g/l, the lag phase decreased too. Furthermore, no lag phase was found at a glucose concentration of 4 and 2 g/l. After the lag phase, no significant change was observed in both types of beads on glucose consumption within the same flow rate. The dependence of lag phase on glucose concentration might be due to substrate diffusion limitations between the surface and the inner pores regions of beads or due to higher accumulation of cAMP level. It can be concluded that the lag phase is not due to the physiology of yeast, but it may be due to the resistance in the internal diffusion of glucose. Moreover, the fermentation results of the Ethanol Red 11 yeast strain was compared with Baker's yeast using flow rates of 4 and 90 ml/min and glucose concentrations of 4 and 10 g/l.

The results show that there is no significant difference in the lag phase of these two types of yeast. It has been observed that the lag phase is directly depending on the glucose concentration as well as on the flow rate.

By varying the flow rate from 1 to 90 ml/min, the time for glucose consumption decreases. The major difference in glucose consumption behavior was observed when using both types of beads at a higher flow rate (90 ml/min). The time for glucose consumption by chitosan coated beads at 30 and 90 ml/min is rather equal when using a higher glucose concentration i.e. 40 and 20 g/l. At higher stirrer speed 500rpm, time for glucose consumption is approximately the same (5h) at 50g/l, 100 g/l, or 200 g/l glucose. It is depicted that sponges have no external or internal mass

transfer limitations or due to macro-pores fluid flow is improved therefore there is no difference in consumption time at a higher speed. While in alginate beads and free cells, a significant difference comes out due to the effect of increasing substrate concentration.

Upon increasing the flow rate and glucose concentration, higher ethanol productivity was observed. This can be attributed to improved convective mass transfer properties when using higher flow rates and reduced substrate diffusional resistance. Furthermore, looking at the ethanol yield, the industrial strain Ethanol Red 11 showed higher yield than Baker's yeast at all flow rates and glucose concentrations. Optimal ethanol productivity of 21.9 g/(g h) was obtained when using Ethanol Red strain at D of 4.5 h−1 with a glucose concentration of 10 g/l. It was also observed that there was no significant difference in ethanol productivity for both *S. cerevisiae* strains at lower flow rates i.e. 4 ml/min, while higher productivity was obtained at higher flow rates (90 ml/min). While ethanol yield for both strains has been observed to decrease when adding glucose.

6.2.1.2. Particle-Side Mass Transfer

The understanding of the mechanism of internal mass transfer effects on the immobilized system used for ethanol production was the purpose of this study. Four operational parameters, glucose concentration, flow rate, alginate beads (chitosan and non-chitosan coated beads), and size of beads have significant effects on the internal mass transfer. The results show that when using a glucose concentration of 4 g/l, no lag phase was observed. The maximum time of a lag phase was found to be 190 min at a lower flow rate of 4 ml/min and 90 min at a higher flow rate of 90 ml/min. Varying the size of beads is an additional factor, which may control internal mass transfer. By decreasing the size of beads to 2 and 1 mm, the duration of the lag phase decreased and the lowest time was 50 min.

An insignificant difference of the glucose consumption time was noticed between chitosan-coated and non-coated alginate beads at higher flow rates (30 and 90 ml/min), especially for a smaller bead size (1mm). This might be due to the reduction of solute diffusional distance from the surface to the site of reaction. The sharp reduction has been observed by the reduction in the size of beads from 2 mm to 1mm as compared to 4 mm. At glucose concentration of 4 g/l & 10 g/l fermentation time approximately 250 & 400 minutes respectively can be saved if the size of beads reduced from 4 mm to 1 mm.

It has been observed that higher ethanol productivity could be achieved by increasing the flow rate and glucose concentration. An insignificant difference was observed in the ethanol productivity for both types of beads at a lower flow rate i.e. 4 ml/min, which was higher at a higher flow rate (90 ml/min). Reduction in the size of beads until no longer intra-phase mass

transfer limitations exist, enhances ethanol productivity. Beads with a 1 mm size have a higher yield than 2 mm type at all flow rate and glucose concentration due to the reduction of intra-phase mass transfer limitations. A significant decrease in ethanol yield on the addition of sugar concentration in the fermentation medium has been observed. Furthermore, it was also found that intra-phase resistance is directly related to the blockage of pores due to higher glucose concentration which induces substrate inhibition.

6.2.2. Stirrer Catalytic Basket Reactor (SCBR) with Alginate beads and DEAE Polymer (Dimensional Correlation)

The first aim of the study was to select a cultivation method (free cells or immobilized cells cultivation) and support material for cell immobilization and test them in a newly developed SCBR. The lag phase time was inversely proportional to the flow rate. The presence of the lag phase is an indication of a concentration gradient around the surface and within the beads that can be controlled by the optimized flow of the fluid medium. In SCBR, no lag phase was observed even at higher substrate concentrations because of the well-mixed environment of this bioreactor (lower concentration gradients).

If the medium glucose concentration is low, a thin film around the matrices develops; there might be a linear substrate gradient across the thin film. At higher stirrer speed 500rpm, time for glucose consumption is approximately the same (5h) at 50g/l, 100 g/l, or 200 g/l glucose. It is depicted that sponges have no external or internal mass transfer limitations or due to macro-pores fluid flow is improved therefore there is no difference in consumption time at a higher speed. While in alginate beads and free cells, a significant difference comes out due to the effect of increasing substrate concentration. Even at a lower stirrer speed 200rpm, cells consume 50 g/l in 5h and 100 g/l in 10h and 200 g/l in 15h. It means after 10h rate of reaction was so fast that cells consume 100g/l within 5h. While in the case of alginate beads consumption time difference is big between 50g/l and 100g/l glucose concentration. It is evidence of substrate inhibition effect on alginate beads. Further, higher substrate concentration can raise the pore's diffusion resistance that possibly causing the substrate to be inhibited.

Fluid velocity improves the mass transfer into the yeast cells immobilized in different matrices. The magnitude of mass transfer resistance has an inverse relationship with the stirring speed. The reason behind it is that the stirring speed controls the internal diffusion of the substrate in the case of immobilized cells. The sponge showed a lower internal diffusion resistance as compared to alginate beads and a negligible effect when increasing the stirring speed. The effect of stirring speed on metabolic activities of *Saccharomyces cerevisiae* was found to be a major factor in affecting the product yield.

The higher consumption time in the case of the free cells might be due to the shear effects caused by stirring. Moreover, the time difference between sponges and alginate beads might be due to the effect of internal diffusion resistances that could develop concentration gradients inside and outside the surface of the immobilized matrices.

The resistance in internal diffusion is a major problem that can arise in immobilizing technology. It can be improved by using chemically grafted sponges and an optimized stirring speed that was briefed in fluid dynamics. It can be concluded that by using sponges, external, and internal mass transfer limitations of substrate concentration gradients can be eliminated by improving fluid flow through pores. At a glucose concentration of 200g/l, the optimal ethanol concentrations obtained when using sponges, alginate beads, and free cells were 85, 79, and 60g/l, respectively. It can be concluded that sponges could act as an optimal best support material for yeast immobilization because of the higher ethanol production achieved.

It can be concluded that immobilized cells could better preserve their activity than free cells. At lower stirring speed, the ethanol yield and the glucose conversion time are observed as limited by external diffusional resistances. The diffusivity of glucose through the boundary layer surrounding the biocatalyst particle has a major role to achieve a maximum ethanol yield that is directly controlled by the structure of the immobilizing matrix and the stirring speed, mode of operation, and design of a bioreactor. Although alginate beads are porous, a further internal mass transfer related disadvantage is that they don't have a convective flow inside so nutrients travel to the cells by diffusion only (Najafpour 2015).

6.2.3. Role of Fluid Dynamics on Mass Transfer (Dimensionless Correlation)

6.2.3.1. Fluid dynamics in PBR with alginate beads

Flow behavior between particle and wake regions has a tremendous effect on Intra-particle mass transfer. When fluid flows over the particle carried the solutes inside the wake region produced by vortexes and outside the wake region due to vortex shedding. Solutes that are carried inside the wake region are mixed and pushed to the base of the particle where eddy diffusion plays a major role in the diffusion of solutes inside the particle. While vortex shedding carrying solutes enhances the convective diffusion as a result of potential flow (Sumer 2006). As the flow regime is laminar at lower Re_p, momentum transfer is by viscosity and velocity fluctuation while the mass transfer is through molecular diffusion and concentration fluctuation. As flow regime change from laminar to the transitional or turbulent core at higher Re_p, viscous transport of momentum has less influence on convective transport (R. Levicky Introduction to Turbulent flow),(Lin, Moulton, et al. 1953).

For better mass transfer there must be a low-pressure drop. As higher pressure or higher force is needed to drive fluid inside the pores of beads. Therefore, a low-pressure drop is required for the fluid flowing in the porous system. In a packed bed with a lower Re_p number overall mass transfer process is proportional to molecular diffusion. If the Schmidt number is high then the concentration gradient becomes narrow and very close to the surface. It can be explained in other words that molecular diffusion starts to influence mass transfer if eddy diffusion is considerably less than molecular diffusion. In a packed bed with a lower Rep number overall mass transfer process is proportional to molecular diffusion. If the Schmidt number is high then the concentration gradient becomes narrow and very close to the surface. It can be explained in other words that molecular diffusion starts to influence mass transfer if eddy diffusion is considerably less than molecular diffusion. Further pressure drop is directly proportional to (v) velocity and (v^2) at the laminar flow and turbulent flow respectively. The diffusion process involves the pattern of fluid flow and other factors like velocity distribution, concentration gradient, and pressure gradient on the surface of particles that affect directly diffusion or internal mass transfer. At lower Re_p flow over particle without flow separation, flow is not turbulent. In this case drag force (Fd) is directly proportional to (v) velocity. On the other hand, if flow over particle is turbulent (higher Re_p) with boundary layer separation, drag forces will be directly proportional to v^2.

Pressure drop and frictional drag can be reduced by reducing the cross-sectional area of the particle over which fluid flows which is indicated in figure 5.14 a & b results. Further, it could be concluded that higher energy dissipation means the higher mass transfer is by convective diffusion of eddies. If fluid cross over a porous particle with higher velocity, vortices or eddies are produced behind and carry solute particles to diffuse into the surface of the porous particle. This analogy further suggests that as fluid velocity increases and eddies dominant then all transport is by eddy mixing and independent of diffusion coefficient or viscosity.

While larger particle size can increase the bed porosity resulting in a bed with more open spaces for the flow of fluid, less fluid resistance, and less fluid-solid contact area per unit volume of bed. It means due to bigger-sized particles steep reduction in energy dissipation and entropy generation occurs. In a packed bed by using smaller particles, interstitial velocity will increase due to the reduction of channel diameter consequently mass transfer is increased.

Reynolds number indicates the flow regions (wake and boundary layers thickness) produced when fluid flows over the surface of alginate beads as a particle. These flow regions are directly proportional to the diameter of the particle as wake extends and boundary layer thickness increases as diameter increases. As Re_p increase, frictional drag is reduced even at higher Re_p

frictional drag become negligible. Further frictional drag is higher as size particles increases because it is directly proportional to the area of contact to flowing fluid.

6.2.3.2. Fluid dynamics in SCBR with Alginate beads & DEAE Sponges

In the turbulent regime of SCBR, mass transfer from the bulk fluid to the film surface is by convection or eddy motion. While within the boundary layer mass is transferred by molecular diffusion and both processes are analogous to the mechanism of momentum transfer. The eddy movements enhance the mixing throughout the turbulent regime called eddy diffusion and result in rapid transport in the turbulent region. Colburn analogy has a reasoning relationship with Reynolds's analogy between fluid friction and mass transfer. As the value of Re_p > 105 then vortex formation becomes independent of Re_p value. At this higher Re_p, inertial forces are dominant over the viscous forces.

In a turbulent flow with higher Re_p, a point comes where the mass transfer coefficient becomes independent on the Schmidt number or molecular diffusion in the boundary layer. The increasing trend in Sh value against Re_p may be attributed to the bioreactor ability to induce axial flow along with radial and swirl flow. Axial flow has a significant effect on the mixing of fluid that can enhance the rate of mass transfer by convection while radial flow enhances the molecular diffusion by the formation of eddies. These both mass and momentum transfer have a direct influence on the boundary layer thickness and the transfer properties of the fluid (Vary 1970).

In a turbulent condition or higher Re_p value boundary layer separation is formed. The induced turbulence has a tremendous effect on the formation of eddies and eddy diffusivity. The higher value of log(Sh-2) at higher glucose concentration and higher value of log (Edp^4/v^3). It can be concluded that the Sherwood number not only directly related to the energy supplied but also on glucose concentration. Our correlation data of energy dissipation with the overall mass transfer is based on Kolmogorov's theory of isotropic turbulence. In this kind of turbulence, the flow of fluid is independent of the direction and doesn't have a mean velocity gradient. While at higher Rep or lower wavenumber, the fluctuating velocity gradient is relatively smaller, large eddies are developed that are the reasons for higher energy transfer Chilton Colburn analogy gives an inspired guess about liquid-solid interface mass transfer limitations. Reynolds analogy in liquid gives an expression of two levels of turbulent mixing: Macroscopic (dominant eddies) and Microscopic (dominant diffusion, conduction, and viscosity). It means the conversion of substrate into the product at the maximum rate with minimum mass transfer limitations. Further increasing Re_p would lead to a decrease εJD factor, it means lower convective or molecular mass transfer limitations that decrease boundary layer thickness. It is concluded that sponges are the best choice to decrease external and internal diffusion limitations. At higher Re_p convective transportation enhanced, with the resulting solute quickly come close to the surface of pores and

film thickness reduced so solute doesn't need more time to diffuse through stagnant liquid film. At this point (step 2 in figure 5.7) eddy diffusion is facilitated because upon reaching into the pores of the sponge, fluid with higher turbulence develops eddies. With higher Re_p, the range of eddy size due to turbulence is inertial subrange where the transfer of mass or energy is by the inertial mechanism. The eddies at this stage become independent of mean flow or viscosity. Sherwood number is important for the dimensionless correlation of mass transfer. It describes the ratio of molecular momentum diffusivity to molecular mass diffusivity or ratio between actual mass transfer and diffusion (De Lathouder 2007). The value of Sherwood number does not vary greatly at smaller variation in Reynolds number and glucose concentration but a higher Sh value is observed if there is a big variation in Re_p. Higher specific surface area of foam is also the reason for the higher value of Sh number because of availability of more area for reaction. Further, the limitations of diffusion as in micropores can also affect on the Sh number.

Our results for Sh and Sc as a function of Re_p are presented and seems experimental data are in good agreement with the model expected for sponges. Further by using DEAE sponges as catalysts support at higher Re_p, Sh becomes independent of substrate concentration. It can be explained in the sense that convective mass transfer and molecular momentum diffusivity become dominant due to pressure gradient not because of the concentration gradient.

The effect of higher Re_p or turbulence on overall mass transfer is more pronounced because open pores of sponges are filled with liquid carrying substrate that results in increases of reaction. Under the condition of reaction, mass is transferred from bulk flow to the pores of sponges and thin catalyst stagnant layer on the pore wall. Open pores of macro-porous sponges optimize the contact area and minimize the internal diffusion limitations.

Pressure has a great role in fluid motion or mass transport in porous materials. At higher stagnant film thickness of pores walls, higher pressure or higher force is needed to drive fluid inside the pores. Therefore, a low-pressure drop is required for the fluid flowing in the porous system. Higher flow resistance in smaller pore size can increase the pressure drop. Darcy Forchheimer's law proposed the relation of pressure drop and fluid velocity. **Our results indicate the correlation of pressure drop with mass transfer and momentum transfer.**

Further, it is depicted that mass transfer coefficients of sponges are proportional to the cubic root of the pressure gradient. Immobilized cells in tortuous paths of pores can enhance the limitations in internal diffusion but the convective transfer can easily remove these limitations at lower pressure drop. The friction factor (f) of macro-porous sponges reduces with an increase of Re_p that means fluid is well mixed at different boundary layers and the thickness of the sub-layer is reduced. At higher Re_p number or higher inertial forces, fluid particles packed tightly, and

higher pressure is required to overcome fluid inter-molecular forces and no-slip velocity condition at the pore wall.

For fully developed flow in porous materials, the internal production of kinetic energy becomes balanced by the dissipation of energy because all kinetic energy is converted into heat called viscous dissipation. Our results of the analogy of mass transfer with energy dissipation per unit mass which is the application of Kolmogoroff theory of isotropic turbulence. The overall trend of Kolmogoroff theory shows the relationship between power input that means at higher Reϵ overall mass transfer is improved because higher turbulent motion develops large primary eddies that have large velocity fluctuations as well contain the bulk of the kinetic energy. Further by using DEAE sponges as catalysts support at higher Reϵ, Sh becomes independent of substrate concentration. It can be explained in the sense that convective mass transfer becomes dominant due to pressure gradient not because of the concentration gradient. Liquid-solid mass transfer is a function of energy input per unit mass which is the application of Kolmogoroff theory of isotropic turbulence to mass transfer coefficient in SCBR. At higher Reϵ, Sh become independent of substrate concentration because the mixing of solute inside the reactor is fast due to the convection process with the results solute concentration become uniform.

6.3. PROBLEM STATEMENT RESOLUTION

6.3.1. DEAE sponges are better than Alginate beads

6.3.1.1. Glucose consumption time and Ethanol production

With the parameter of glucose consumption behavior, fluid-side or external mass transfer through immobilizing materials (alginate beads) has been evaluated. An imaginary fluid film is developed by substrate and fluid around support that causes resistance for more substrate to enter. Higher substrate concentration increases the substrate concentration on the entrance of pores of the beads causing inhibition of substrate represented in the form of prolongation of the lag phase. Because cells near to the surface of beads can be consumed easily but due to pore diffusion resistance cells in the center of beads can not survive due to a shortage of substrate. Further, the resistance by the imaginary film was observed only around alginate beads, not in polymers. It is shown in experiments that at a lower concentration of glucose, there is not so much difference in consumption time between alginate beads and polymers. As the glucose concentration in the medium is increased, there is a big difference in the consumption time between both. The amplification of consumption time difference in free and immobilized cells was also observed by increasing the glucose concentration to 100g/l and 200 g/l. From results, it has been observed that at even 200 rpm with a 100 g/l glucose concentration, the sponges consume glucose in 9 h, while alginate beads and free cells consume it in 19 h and 20 h, respectively.

Alginate beads take a longer time to consume than polymers. This might be due to the increase of concentration gradient because of the thick layer around beads. As concentration gradient drive mass transfer but higher concentration gradient needs more convective flow or intra-particle flow to diffuse substrate inside pores of beads. But this might not be the case of DEAE polymer because polymers have a larger pore size that can enhance the intra-particle flow. In this case, the concentration gradient cannot be developed and with the result external and internal mass transfer limitations are eliminated or at least reduced to such an extent that substrate can be easily available for microorganisms. This intra-particle flow can be compensated by optimizing the speed of the stirrer in (SCBR) bioreactor or fluid velocity in a packed bed bioreactor.

Because stirrer speed can enhance the availability of substrate on the surface of the catalyst that further increases the observed rate of reaction. The magnitude of mass transfer resistance has an inverse relationship with the stirring speed. The sponges showed a lower internal diffusion resistance upon increasing the stirring speed as compare to alginate beads.

In SCBR, at a higher glucose concentration of 200 g/l, the volumetric productivity of polymer was higher as compare to alginate beads. These polymers are presumed as best immobilizing materials than alginate beads with higher ethanol productivity and lower glucose consumption time. This might be due to a lower difference in concentration between bulk and inside polymer or lower film diffusion barrier in sponges. The time for consumption or in other words the transfer of the substrate from the outer surface to matrix depends on the (1) kinetics (2) reactor design (3) stirring (4) type of catalysts (immobilizing matrices) (5) reaction conditions.

In other words lower ethanol productivity in alginate beads might be due to slow intra-particle flow, as compared to sponges, with the result substrate inhibition is induced because of high inter-phase diffusion resistance. This indicates that the efficiency and physiology of cells immobilized in alginate beads were effected. It means cells exhibit different metabolism if immobilized in alginate beads and grafted polymers.

Ethanol productivity is affected by the diffusion or traveling of the substrate from the outer surface to inside the support material, depends on the speed of the stirrer for mixing liquid medium and the texture of the support material used for immobilizing cells. In the results, it's indicated that sponges take less time to complete one batch cycle as compare to alginate beads. This might be due to no diffusional barrier existing in sponges while on the other hand alginate beads have no convective flow inside and the substrate only transfer inside by diffusion which is a slow process. The effect of a higher glucose concentration on ethanol productivity was observed that indicates the reduction of ethanol productivity in alginate beads as compared to

sponges. This might be due to inhibition of substrate caused by a higher rate of reaction inside alginate beads upon increasing the substrate concentration.

Further alginate beads and sponges results were evaluated by correlating mass transfer with dimensionless numbers. From results it has been observed that newly developed sponges enhance the mass transfer due to its properties of short contact time with high reaction rate and low-pressure drop, high surface area, and radial mixing in tortuous pathways, Collins 1976).

6.3.1.2. Hydrodynamic properties of Alginate beads within SCBR Basket

Hydrodynamic factors have a significant influence on the convective mass transfer or mass transfer by force. These depend on the relation between momentum and concentration gradient in the boundary layer. In laminar flow velocity components and streamline are smooth while if the flow is unsteady velocity components vary chaotically concerning position and time (Tilton 2007).

The results of Rep show the flow behavior and effect of the boundary layer around the sphere. It is depicted from the results that with higher Re_p, the effect of momentum transfer on the whole mass transfer is minimized by convection as presented by the εJD factor. It means that at higher Re_p > 6.5 x 103 convective mass transfer is not further influenced by momentum mass transfer. In the vicinity of the surface, turbulent viscosity (rate of turbulent mixing) is reduced because the size of vortices decreased and even vanished with time. With the consequence viscous momentum transfer become again dominant on the surface. The mass transfer coefficient factor (εJD) value may not undergo larges changes by increasing Re_p and falls consistently even appear nearly flat. In a turbulent regime, the mechanism of mass transfer will be by convection or motion of eddy from fluid turbulent regime to film boundary and by diffusion process across the film to liquid (Colburn 1930).

Overall mass transfer effect shows very weak dependency on the lower number of Re_p. After this dependency increases more rapidly with the increase of Re_p numbers. By the further increase in Re_p, the effect of inertial forces on the overall mass transfer effect was not pronounced.

The increasing trend in Sh against Re_p is due to a particular relationship between turbulent motion and its diffusing power (Benamara, Assoua, et al. 2018). Due to turbulence spherical alginate beads can have a colloidal motion that can enhance the micro-convection by eddy motion with a consequent increase in mass transfer (Sedahmed, Farag, et al. 1998). The larger the Re_p, the smaller are the size of eddies. It is observed by Kolmogorov that if eddy size is 2/3 or 1/3 of particle size, it has a detrimental effect on cells in particle.

Mass transfer correlation data with specific agitation power were used to assess the applicability of Kolmogorov theory in this system. Energy dissipation is directly related to Reϵ, if Reϵ is higher,

larger vortices or eddies are produced that contained most of the kinetic energy and disintegrated into smaller eddies. It is noticed in the results that (Sh-2/Sc^$^{1/3}$) is an overall mass transfer coefficient, due to turbulence inflow over the surface of the particle. These correlation results have been observed almost the same at different glucose concentrations within the whole range of log (Edp^4/v^3). For correlation of Sherwood number log (Sh-2) & log (Edp^4/v^3) at higher glucose concentration and higher value of log (Reϵ).

It can be concluded that Sherwood Number depends directly on energy supplied but less on glucose concentration. The rate of energy dissipation depends on two parameters like viscosity and velocity gradient produced by turbulent fluid. Our results indicate that as energy dissipation increased to a limit needed then a small increase in mass transfer coefficient, has been observed as observed by Calderbank in 1995.

6.3.1. 3. Hydrodynamic properties of DEAE sponges within SCBR Basket

In the last decade, polyurethane foam as catalysts has gained great interest in industrial applications of the liquid-solid catalytic process. Macro-porous foams have excellent properties of higher porosity and a three-dimensional pore network for higher radial mixing, lower pressure drop that enhance heat and mass transfer as compared to alginate beads (Inayat 2013). So the main objective was to correlate with mass transfer dimensionless numbers with hydrodynamic properties of sponges. In a heterogeneous system, mass transfer is the motion of molecules from one point to another point by driving force. This driving force can be in the form of concentration gradient, pressure gradient, or velocity gradients in the molecular diffusion or convection process. Macro-porous foams have excellent properties of higher porosity and a three-dimensional pore network for higher radial mixing, lower pressure drop that enhance heat and mass transfer (Inayat 2013).

As fluid flows through a phase boundary of pores in porous materials local velocity becomes zero due to resistance inflow near to surface. With the results, a stagnant fluid film over the surface has been expected and the thickness of this film depends on flow condition either fluid is flowing in laminar or turbulent condition. In turbulent condition stagnant film reduced due to the motion of eddies inside the pores. At the entrance of the pore, the velocity profile is flat and as it developed it becomes laminar. Therefore, developing at the entrance of pore has a direct influence on mass transfer.

Chilton Colburn analogy has a relation to Reynolds analogy. Reduction of (εJD) mass transfer factor with increasing the particle Reynolds number from 10^^{3} to 10^^{5} with the results of an increase in reaction rate and reduction of concentration, temperature, or pH gradient in the system due to well-mixed vessel of SCBR. Higher Rep or interstitial velocity in pores and reactor

configuration has significant relationships in reducing mass transfer limitations. Further increasing Re_p would lead to a decrease εJD factor that means lower convective or molecular mass transfer limitations that decrease boundary layer thickness. It is concluded that the sponges are the best choice to decrease the external and internal diffusion limitations due to larger pores that enhance the internal fluid flow.

Sherwood number increases with an increase in Re_p due to higher interstitial velocity in pores. Further, it has been observed by many investigators that mass transfer coefficient dependency on interstitial velocity increases by increasing the size of the pore (Garrido, Patcas, et al. 2008). A good correlation of Sh with Re_p mainly with large pore size foam.

The effect of higher fluid velocity or turbulence on overall mass transfer is more pronounced because open pores of sponges are filled with liquid carrying substrate that results in increases of reaction. Under the condition of reaction, mass is transferred from bulk flow to the pores of sponges and thin catalyst stagnant layer on the pore wall. Open pores of macro-porous sponges optimize the contact area and minimize the internal diffusion limitations. This novel DEAE sponge prevents plugging and allows higher flow inside the pores due to the action of pressure drop created at higher stirring effect or turbulence. It can be concluded that convection of reactants through pores of the sponge is directly proportional to Re_p and pressure drop as it was observed by De Lathouder in 2007.

A new type of analogy has been proposed by Martin, between momentum transfer that can be due to friction or pressure drop and mass or heat transfer (Garrido, Patcas, et al. 2008). From the results, the analogy is observed between the mass transfer coefficient and dimensionless pressure drop (Hagen number) where Sh is an increasing function of pressure drop. The further analogy has been observed between pressure drop and mass transfer coefficient in the case of sponges that have irregular cellular structure and represents qualitatively hydrodynamic mass transfer behavior of sponges. Further, it is depicted that mass transfer coefficients of sponges are proportional to the cubic root of the pressure gradient.

It is interesting to see the effect of increasing Reynolds number because the reaction rate over the sponges is not constant. Further increasing Re_p would lead to a decrease εJD factor that means lower convective or molecular mass transfer limitations that decrease boundary layer thickness. It is concluded that sponges are the best choice to decrease external and internal diffusion limitations. At higher Re_p convective transportation speed up and solute quickly come close to the surface of pores and film thickness reduced so solute doesn't need more time to diffuse through stagnant liquid film. At this point (step 2 in figure 5.7) diffusion is facilitated because upon reaching into the pores of the sponge, fluid with higher turbulence develops eddies.

With higher Re_p, the range of eddy size due to turbulence is inertial subrange where the transfer of mass or energy is by the inertial mechanism. The eddies at this stage become independent of mean flow or viscosity. Isotropy of eddies is assumed at the point of nodes in pores of sponges where turbulence becomes intense. Further by using DEAE sponges as catalysts support at higher $Re\epsilon$, Sh becomes independent of substrate concentration due to no concentration gradient. It can be explained in the sense that convective mass transfer becomes dominant due to pressure gradient not because of the concentration gradient. In literature mass transfer coefficient KL has been observed indirectly proportional to $\epsilon^{\wedge 1/10 \text{ to } 1/6}$

It is concluded that this data comes into satisfactory agreement with the Kolmogorov theory of isotropic eddies. Where mass transfer depends on the rate of dissipation instead of the substrate concentration gradient. Further, if the turbulent intensity is high, its effect on mass transfer can not be ignored. It can be concluded that Mass transfer through macro-porous sponges has an analogy with total power input or average energy dissipation rate (ε) because of no external or internal limitations. Further, it is depicted from results that liquid-solid mass transfer is a function of energy input per unit mass which is the application of Kolmogorov theory of isotropic turbulence to mass transfer coefficient in stirred tank reactor.

6.3.2. SCBR is better in performance than PBR

Further, a summary of the SCBR bioreactor with immobilized cells in alginate beads and sponges is presented. We used glucose with different concentrations as substrate and the system has been working at 35 ^{0}C and the starting pH of the medium was 7. We have assumed 0.45 g ethanol produced per 10 g of glucose consumed. In SCBR complete conversion of 200g/l glucose, dissolved in medium, has been observed in a short time (5h) as compare to Packed Bed. In SCBR, no lag phase was observed even at higher substrate concentrations because of the well-mixed environment of this bioreactor (lower concentration gradients). At higher stirrer speed 500rpm, the time for glucose consumption (5h) was approximately the same for 50g/l, 100 g/l, or 200 g/l glucose. It is depicted that sponges have no external or internal mass transfer limitations or due to macro-pores fluid flow is improved therefore there is no difference in consumption time at a higher speed. The significant improvement in the ethanol production of SCBR was obtained by using sponges for immobilizing cells instead of alginate beads. Further SCBR can be operated continuously for a long time or at least 400h with necessary maintenance. For a small scale, ethanol production SCBR with alginate beads would be possible to use. On the other hand for large-scale economical production, sponges could be most important due to low in cost and ease in availability and their stability especially at higher stirring or stress effect.

The cost of the SCBR with alginate beads as compare to sponges is compared. The SCBR with alginate beads is not more attractive in the large scale industries because of the higher cost of alginate beads preparation as well as their instability and also alginate beads take longer time to complete one fermentation cycle due to complex hydrodynamic behavior and limitations in mass transfer. Therefore now a day's most of the companies are planning to use different types of sponges or polymers for cell immobilization.

The SCBR with cell immobilized in sponges is more attractive and economical, especially on an industrial scale. As if the cells are immobilized in sponges, they take less time to consume the whole substrate or for completion of one fermentation cycle. It indicates that SCBR with sponges does not require higher energy for consumption therefore use of sponges for cell immobilization purposes significantly reduces energy consumption.

Major issues in the packed bed bioreactor system are mass transfer resistance. This has been observed by the delay in the consumption time of the substrate or completion of one batch fermentation cycle and the appearance of the lag phase. Thus the economic comparison of traditional and newly developed bioreactor systems shows that Packed Bed Bioreactor is less favorable to the Stirred Catalytic Basket Bioreactor. For this purpose, we support our ''Stirred Catalytic Basket Bioreactor'' and compared it with traditionally used ''Packed Bed Bioreactor''. This ''SCBR'' bio-reactor will deliver a low mass transfer resistance and intensified unit operation that could accommodate a large number of transformations of current industrial interest. Based on the experimental data and review of other researchers we have assessed the feasibility of utilizing the bioreactor as well as biocatalysts especially for the production of ethanol as well as for fine chemicals.

In the turbulent regime of SCBR, mass transfer from the bulk fluid to the film surface is by convection or eddy motion. While within the boundary layer mass is transferred by molecular diffusion and both processes are analogous to the mechanism of momentum transfer. The eddy movements enhance the mixing throughout the turbulent regime called eddy diffusion and result in rapid transport in the turbulent region. At higher Rep or lower wavenumber, the fluctuating velocity gradient is relatively smaller, large eddies are developed that are the reasons for higher energy transfer.

A preliminary economic evaluation of ''Stirred Catalytic Basket Bioreactor'' is generally more economically feasible for ethanol production by using a newly developed grafted polymer as compared to conventionally immobilizing support material like alginate beads in ''Packed Bed Bioreactor''. The grafted polymer can improve the productivity of a bioreactor.

6.3.3. Conclusion

1. The system of SCBR allows the complete consumption of substrate in less time as compared to traditionally one. The complete conversion of 200g/l glucose has been observed in a short time (5h).
2. The SCBR can be operated in a continuous mode as well as on large scale production of ethanol with 0.405 g/l ethanol produced per 1.0 g/l of glucose.
3. However if sponges are used in SCBR, for cell immobilization, the process would be most economical and feasible. Because sponge showed a lower internal diffusion resistance as compared to alginate beads.
4. SCBR provides an efficient radial & axial mixing system and if there is a combination of macro-porous sponges, the process time can be reduced by reducing mass transfer limitations.
5. Our results indicate the analogy of pressure drop with mass transfer and momentum transfer.

6.3.4. Economic Evaluation of Stirred Catlytic Basket Reactor (SCBR)

The current importance of ethanol has been recognized especially for the safe environment. A mainly global environmental crisis comes from the burning of fossil fuel with that carbon dioxide, methane and other harmful gases are way out to the environment. Such harmful gases are generated by the incomplete burning of commonly used fossil fuel while ethanol has 35% oxygen which helps for the complete burning process therefore toxicity of gases is not so much harmful as compared to the gases from petroleum sources. By blending ethanol into diesel (E10 or E30) the number of acetone in the fuel is reduced and the complete burning of fuel is facilitated and further greenhouse gas emission can be reduced.

Many countries especially the US and Brazil welcome the production of ethanol as fuel. The main issue is the economical production of bio-ethanol on a commercial scale. For this purpose, so many advancements in ethanol production technology have been developed from the last many decades. Still, further research methods are needed to produce economical and environmentally sustainable fuel. It is very difficult to produce ethanol by fermentation in an economical way. Many factors can affect the cost of bio-ethanol production like the use of low-cost feedstock, efficient use of enzymes or cells, scale-up of production level, and new development in immobilizing support materials and designing of bioreactors.

Low-cost feedstock for ethanol production are sunflower stalks and hulls, alfalfa fibers, damaged or crushed wheat, rice and sorghum grains, fruits and vegetable wastes and many other agro wastes are the best examples. (Chandel AK. 2007)

For the production of ethanol, the successful use of enzyme and microbial cells as low-cost biocatalysts have proved an efficient and applicable approach. The traditional manufacturing of pharmaceuticals involves toxic reagents and an enormous amount of energy. Such biocatalysts offer great potential for reducing the cost of the fermentation process. Scale-up of the ethanol production plant is another strategy to reduce the cost of production and also invest per unit output will be reduced. The choice of fermentation mode can also affect the economy of ethanol production like the use of continuous fermentation with immobilized cells in terms of higher yield as compared to the batch process.

Furthermore, our project has delivered an intelligent combination of the two most important strategies likes the development of immobilizing support materials (Macro-porous DEAE Polymer) and designing of the catalytic bioreactor (SCBR). The economical ethanol production can be improved with these strategies by continuous operation and maintaining the cell density. The major issues in the economical large scale ethanol production like instability of immobilizing support material, a decrease of cells or enzyme activity, inhibition of substrate or product that ultimately effect on the ethanol productivity because of low mass transfer into the immobilizing matrix. To shorten the cost of the fermentation process, it's necessary to reduce the external and internal mass transfer limitations. If such limitations are removed then a bioreactor with higher productivity in a short time, reduced energy requirement, and with small capital and operating cost can be developed. Mass transfer limitations can be reduced by improving the external and internal flow of fluid which results in the improved accessibility of substrate by immobilized cells and higher mass transfer rate (Roy et al, 2016).

The economic feasibility of SCBR was not discussed in the earlier studies. The main aim is that bioreactor should be economically attractive. The economic feasibility of SCBR was compared with the traditionally packed bed bioreactor (Shuler et al 1991).

6.4. FUTURE SCALE UP STRATEGY/MODELING

The SCBR has never been utilized as a large scale processing unit in the biochemical process industries. However, Domenech et al (2000) in Cuba reports producing ethanol with a 2000 liters SCBR system with yeast immobilized on wood chips with good results. Since 2000 liters already is a representative level, it can be confident that there will not appear problems at larger scales. The scale-up process influenced the production capacity and efficiency. The bio-economy also offers a huge potential for social innovation in the area of health, diet, education, and rural and

coastal development. Bio-economy social innovation could include increasing energy and resource efficiency through attitudinal and organizational changes, ways to re-use and recycle bio-based products, treatment of end-use level waste, and the development of local networks for the production and distribution of fine chemical products requiring new forms of production. In the future scale-up process, a large-scale bioreactor (50 L – 500 L) will be done and its performance will also be evaluated. For this purpose, it is necessary to keep many parameters (height to diameter ratio) constant as in a laboratory-scale bioreactor.

The development of the SCBR system requires the study of a large number of topics. The scale-up criteria will be proposed and reactor performance will be evaluated, as we did on the pilot plant, on the characteristics of agitation or shear effect on the productivity as well as on physiology or metabolism of cells and maintenance of mass transfer coefficient.

6.4.1. Installation of the set-up and repeated batch culture behavior

In SCBR, a hollow cylindrical bed is placed inside any ordinary stirred tank reactor. We can try to work at the highest possible scale, in such a way that the results will be representative of those who meet in industrial conditions. To carry out this, the system must be provided with equipment to measure and control pH, agitation rate, power consumption, ethanol production, substrate consumption, and temperature. At this stage, the best development strategy is to work in repeated batch culture because the operation in continuous flow is very expensive in terms of both time and money and most conventional ethanol production plants work in this mode. Therefore batch processing provides the best benchmark for comparison of newer process alternatives.

As in the older report ‘‘Fuel from Farms, a Guide to Small-Scale Ethanol Production’’ of the Department of energy-DOE of the United States; there are strong indications that a farm-based fermentation ethanol industry can provide a decentralized system of fuel production. Also, on-farm production would provide energy self-sufficiency for the farm community. It has been also demonstrated that, in the case of on-farm fermentation ethanol production, certain economics of scale are present for small-scale production which may balance the economic advantage of large-scale plants. As a result, the report deduces, small-scale production of ethanol may be achieved with product costs comparable to those from larger plants. Therefore, there will be a good market for small, very productive, and easy to operate reactors, as SCBR.

6.4.2. Optimization of the main parameters that control the productivity of the system

The behavior and the productivity of the SCBR depend primarily on the following parameters: agitation speed, substrate concentration, bead size, cell density inside the beads, type of strain like yeast or bacteria utilized. Since these factors are complementary, the best strategy is to optimize them simultaneously by a simple trial and error empirical procedure. The goal is to maximize productivity and to minimize power consumption.

6.4.2.1. Development and Scale up of the system

The first catalytic basket reactor has been used in heterogeneous catalytic reactions (especially kinetic of gas-solid reaction). After that, a lot of studies have been done on liquid-solid reactions. Gamara et al has published his work on SCBR as a biochemical reactor but on a lab-scale and reports the production of ethanol with immobilized yeast cells. Camera reports the production of ethanol high as 12 g/liter hour with low fermentation time as 4 hours. These results were 400 to 600% higher in productivity than classic industrial reactors. SCBR system has versatility in the mode of operation. For the lab-scale production, the batch mode of production has been used because the system can complete one cell recycle. Domenech reports the feasibility of SCBR on an industrial scale especially 2.000 liters reactors with good production rates.

6.4.2.2. Development of Kinetic Model

Gamara has demonstrated the performance of SCBR regarding mass transfer rate and also modeled mathematically by pseudo-homogeneous, steady-state, asymptotical model. The model depicts the basics of the behavior of the system, SCBR behaves as a plug flow reactor. The plug flow behavior is a result of the internal recycling of the broth created by the stirring of the reactor.

The blades of the agitator work as a centrifugal pump that directed the flow of liquid through the hollow bed. After meeting the wall of the fermenter, the stream returns to the center of the hollow cylinder bed and recircled internally many times. When five or more reactors ensemble in series behaves as plug flow but in SCBR reactor such plug flow is best from a kinetic and economic point of view in product inhibition.

Taking a differential mass balance about a cylindrical layer of the catalyst, we can obtain the

following nonlinear partial differential equation for the reactor:

$$\frac{\partial c}{\partial t}+\left\{Ur\frac{\partial c}{\partial r}+U\Theta\frac{1}{r}\frac{\partial c}{\partial \Theta}+Uz\frac{\partial c}{\partial z}\right\}-Dr\left\{\frac{1}{r}\frac{\partial c}{\partial r}\left(r\frac{\partial c}{\partial r}\right)+\frac{1}{r^2}\frac{\partial^2 C}{\partial \Theta^2}+\frac{\partial^2 C}{\partial^2 Z}\right\}-R=0$$

Where: C = C (r) = concentration field in the annulus; r = current radius; Θ = angular coordinate; Z= axial coordinate; Ur = average radial flow velocity component; Dr = radial dispersion coefficient;R = reaction rate; t = time.

As it stands, the equation of the reactor is very difficult to integrate. To obtain an asymptotic, analytical solution, it is necessary to make a number of simplifying assumptions: first order kinetics, steady state and isothermal conditions, boundary conditions of the first kind and perfect symmetry of the cylindrical hollow bed.

Under these circumstances, the partial differential equation

(1) transforms into the following two points, boundary value, and ordinary differential equation:

$$Ur\frac{\partial C}{\partial r}=-Kc$$

Where: K is a first order reaction kinetic constant.

Moreover, for the scale-up of the SCBR system, we need to obtain kinetic data and kinetic model of the system. Because the behavior of isothermal heterogeneous reactor depends on the flow pattern, mass transfer rate, and chemical kinetics of the reaction. In SCBR, for obtaining kinetic data free from mass transfer effects, the consequence of the stirring will eliminates the external diffusive layer; with this, intrinsic kinetics of the process will be established with help of an algebraic equation.

$$\frac{M}{f}=\frac{Xf-Xi}{Rp}$$

Simplified integral equation is as follows.

$$\frac{M}{F}=\int_{Xi}^{Xf}\frac{dX}{Rp}$$

Where

R_p = Global reaction rate per unit weight of catalyst

F = Feed rate of the broth to the reactor

Xf and Xi = final and initial concentration respectively of the main reagent.

For the purpose of obtaining reference data at this stage pure reagents should utilize. In order to simulate the conditions, the system with real life, it is necessary to work with low priced industrial media.

6.4.2.3.Assessment of a scale-up criterion

At this stage, the problems in the scale up of the system must be investigated. Two scale up criteria can be proposed based on the maintenance of a constant mass transfer coefficient:

$$Sh = 2 + a\left(\frac{\varepsilon d^4}{v}\right)^m Sc$$

Where:

Sh = Sherwood number

d = particle diameter

m and a = dimensional constants

$$\varepsilon = \frac{Pg}{V\rho}$$

P = power supplied to the stirrer

g = gravitational conversion factor

ρ= density of the liquid

The other based on the constancy of the overall heat transfer coefficient that is proposed to test the correlation of Poggemann:

$$Nu = 0{,}46\,\mathrm{Re}^{2/3}\mathrm{Pr}^{1/3}\left(\frac{\eta}{\eta_w}\right)^{0{,}14}$$

Where:

Nu = Nusselt number

Pr = prandtl number

Re = Reynolds number

η and ηw = dynamic viscosity of the liquid broth and water respectively.

6.4.2.4. Testing of system in continuous flow conditions

Exclusive use of continuous process is being used in modern heavy chemical industry. Therefor, we must ascertain the conversion per pass and the behavior of the system under various conditions, such as: dilution rate, bed width, agitation rate and different raw materials.

6.4.2.5. Mathematical model and simulation runs of system

After validation, the mathematical model can be used to simulate the behavior of the reactor under all possible conditions.

$$C(r) = A.e^{\frac{-K.r}{Ur}}$$

Where A is an integration constant. If we assume complete mixing in the liquid that surrounds the annular bed, it results:

$$C(Ri) = C_{bulk}$$

and equation (3) becomes:

$$C(r) = C_{bulk}.e^{-kr/Ur}$$

Ri = inner radius of the annular bed,

Re = outer radius of the annular bed,

From this equation we can obtain an expression for the unconverted fraction per pass: fp, a parameter that correlates with agitation rate, bed width and kinetics;

$$\ln fp = e^{-\left(k/UR\right)W}$$

W = width of the hollow cylinder bed

By taking fixed values of K and Ur, if the model is correct and different bed widths, a plot of Infp against bed width, must be a straight line throughout the origin.

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