CHAPTER II

BACKGROUND AND LITERATURE REVIEW

2.1 Ethanol fermentation

Ethanol fermentation is the biological process by which sugars are converted into ethanol and carbon dioxide. Ethanol fermentation is responsible for the rising of bread dough, the production of ethanol in alcoholic beverages, and for much of the production of ethanol for use as fuel [7].

Due to the growing demand for energy and diminishing supply of fossil fuels, increasing attention has been given to alternative sources of energy. Alternative fuels need to be renewable, sustainable, efficient, cost-effective and safe. In addition these fuels should reduce harmful pollutants and exhaust emissions. Ethanol is one of the alternative fuels being considered to replace some of the oil based fuels and fuels additives. When ethanol is burned it produces carbon dioxide and water. The carbon dioxide produced from burning ethanol is much less harmful than other gasses produced from burning gasoline decreasing the damage to the environment [8].

2.1.1 Raw materials

Raw materials used in the manufacture of ethanol via fermentation are conveniently classified under three types of agricultural raw materials: sugar, starches, and cellulose materials.

1. Sugars (from sugar cane, sugar beets, molasses and fruits) can be converted to ethanol directly.
2. Starches (from grains, potatoes and root crops) must first be hydrolyzed to fermentable sugars by the action of enzymes from malt or molds.
3. Cellulose (from wood, agricultural residues, waste sulfite liquor from pulp and paper mills) must likewise be converted to sugars, generally by the action of

mineral acids. Once simple sugars are formed, enzymes from yeast can readily ferment them to ethanol [9].

2.1.2 Microorganisms

There are many kind of microorganism that can produce ethanol. However, yeast (*Saccharomyces cerevisiae*) is the most common type of fermentation because it provides high production rate and efficiency. Under anaerobic condition, yeast metabolizes glucose to ethanol primarily by Embden-Meyerhof pathway (EMP), as illustrated in Figure 2.1 The overall net reaction produce 2 mol each of ethanol, carbon dioxide and ATP per mol of fermented glucose. The metabolic reactions involved are as follows:

$$Glucose \longrightarrow 2\,Pyruvate + 2\,ATP + 2\,NADH$$
$$Pyruvate \longrightarrow Acetaldehyde + CO_2$$
$$Acetaldehyde \longrightarrow Ethanol$$

Theoretically, the yield is 0.511 for ethanol and 0.489 for carbon dioxide on a mass basis of glucose metabolized.

In recent year, *Zymomonas mobilis* has been study for ethanol production because this strain exhibits higher ethanol yield and productivity than *S. cerevisiae*. *Z. mobilis* is an anaerobic, gram-negative bacterium which produces ethanol from glucose via the Entner–Doudoroff pathway (ED), as illustrated in Figure 2.2. The overall net reaction produce 2 mol of ethanol, 2 mol of carbon dioxide and 1 mol of ATP per mol of fermented d-glucose [10].

Compared to *S. cerevisiae*, *Z. mobilis* produces one mole ATP/glucose via the ED pathway instead of two moles of ATP/glucose via the EMP pathway. Thus, less energy is available for growth and consequently less substrate is diverted to biomass production with higher ethanol yield [11]. It was reported that the ethanol yield of *Z. mobilis* could be as high as 97% of the theoretical yield of ethanol to glucose, while only 90–93% can be achieved for *S. cerevisiae*. Although these advantages, *Z. mobilis* is not suitable for the industrial ethanol production, because the undesirability of its biomass to be used as animal feed and it's specific substrate spectrum including only three sugars: D-glucose,

D-fructose, and sucrose. The ethanol fermentation industry cannot use pure glucose as its raw material like many researchers did in their laboratory studies. Thus, this species cannot readily replace *S. cerevisiae* in ethanol production [10].

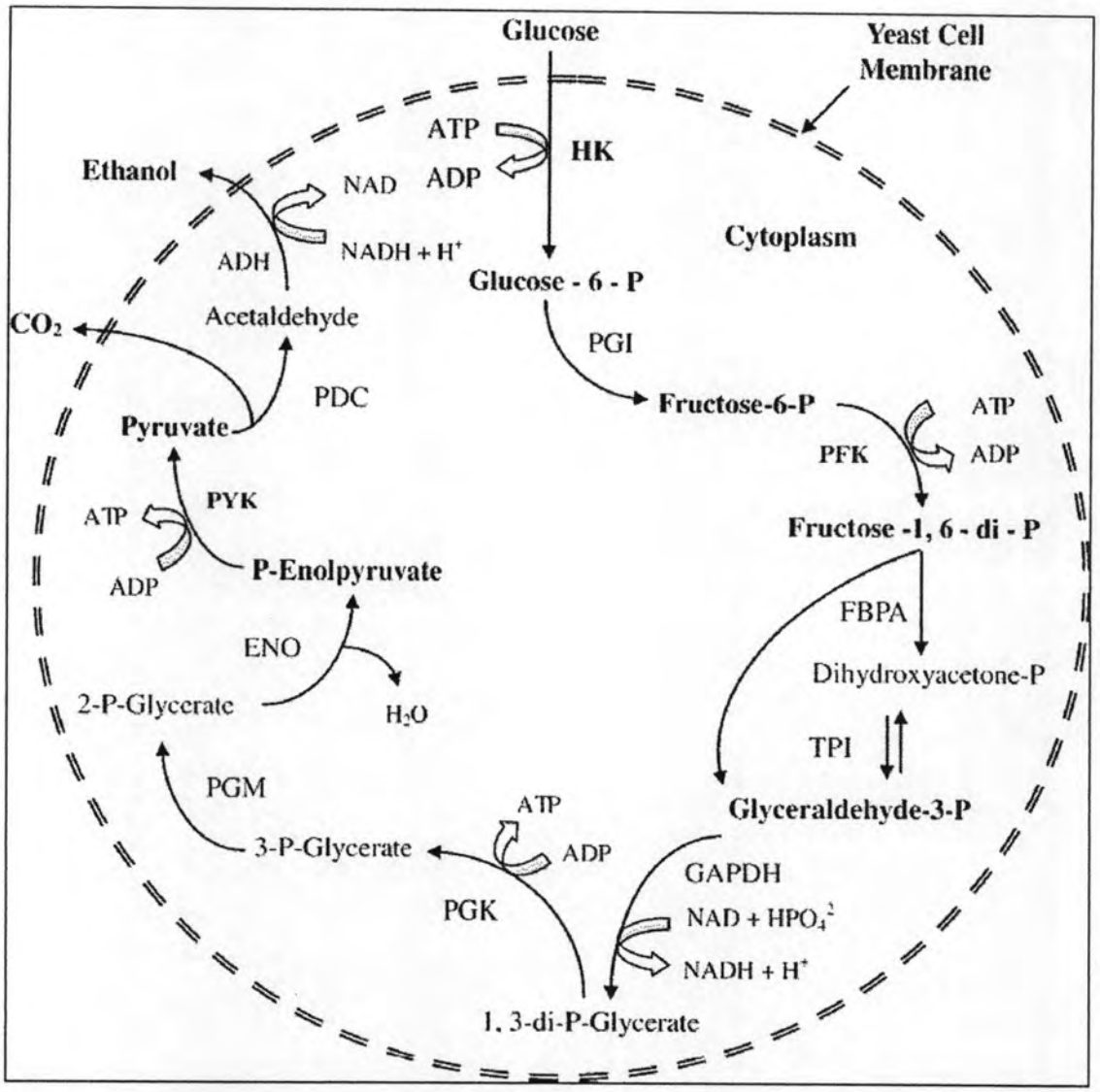

Figure 2.1 Embden-Meyerhof pathway [10]

Abbreviations:
HK: hexokinase, PGI:phosphoglucoisomerase, PFK: phosphofructokinase,
FBPA: fructose bisphosphate aldolase, TPI: triose phosphate isomerase,
GAPDH: glyceraldehydes-3 phosphate dehydrogenase, PGM: phosphoglyceromutase,
PGK: phosphoglycerate kinase, ENO: enolase, PYK: pyruvate kinase,
PDC: pyruvate decarboxylase, ADH: alcohol dehydrogenase

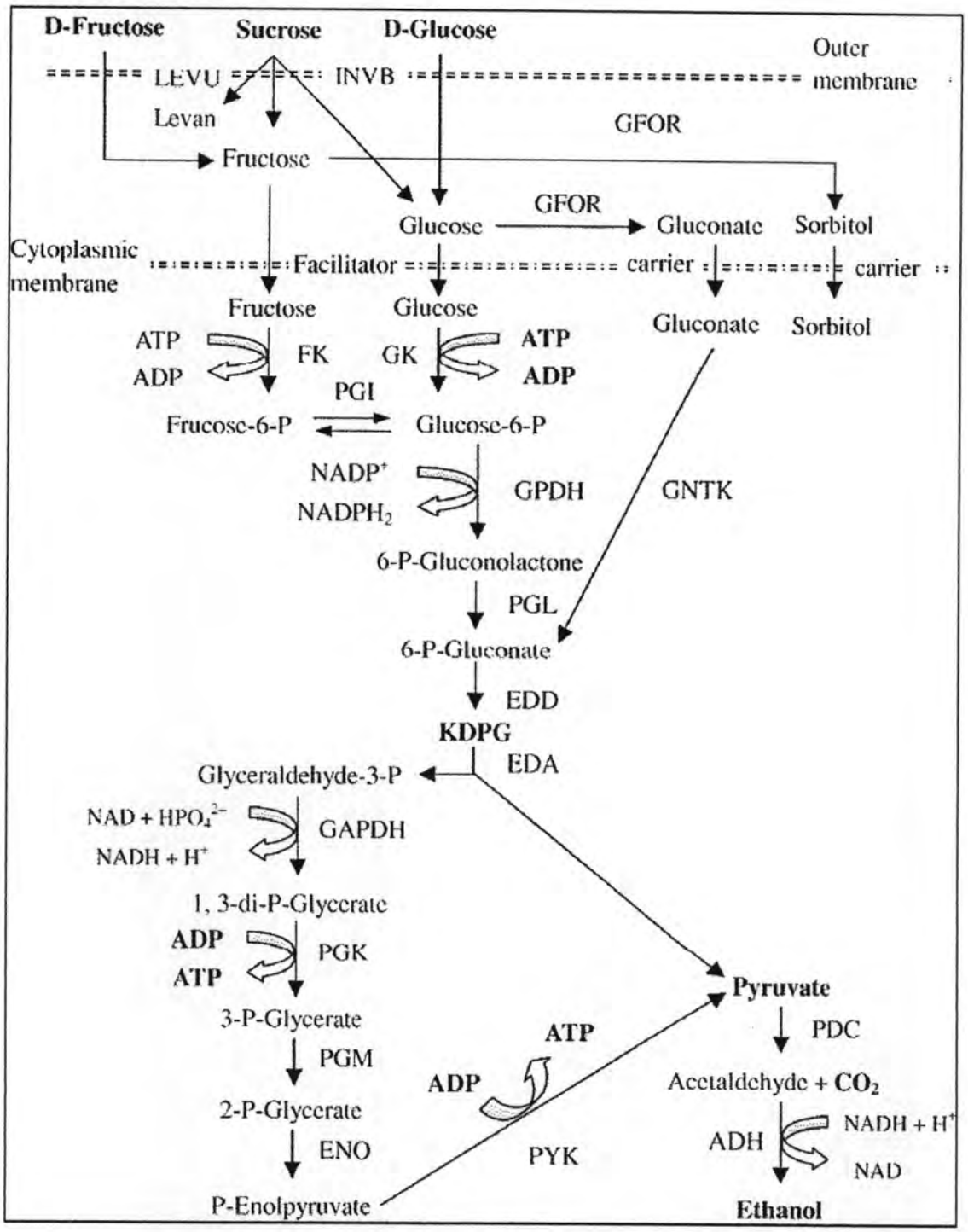

Figure 2.2 Entner–Doudoroff pathway [10]

Abbreviations:
LEVU: levansucrase, INVB: invertase, GFOR: glucose–fructose oxidoreductase, FK: fructokinase, GK: glucokinase, GPDH: glucose-6-phosphate dehydrogenase, PGL: phosphogluconolactonase, EDA: 2-keto-3-deoxy-gluconate aldolase,

KDPG: 2-keto-3-deoxy-6-phosphogluconate, EDD:6-phosphogluconate dehydratase, GNTK: gluconate kinase.
See Figure 2.1 for PGI, GAPDH, PGK, PGM, ENO, PYK, PDC and ADH.

2.2 Cell immobilization

Immobilization of cell is a trend in biochemical processing, and its full potential is just being recognized. It is the attachment of cells to solid support, fixed in the form of an active layer. When substrate passes over the surface, enzymatic reactions change the substrate to the desired product. This technique allows obtaining much more profit from the process, which may improve microbial performance, and provide good operational stability. Immobilized cells are used in manufacturing food flavors, additives, medicines, and other goods are produced by variety of microbial metabolites [2, 3, 11]. Motivation for development of immobilized cell systems emerged from their potential advantages. Some potential advantages characteristics of immobilized cell over suspension fermentations including [12];

1. The cell activity and stability increase because the immobilization support may act as a protective agent against physicochemical effects of pH, temperature, solvents or even heavy metals.
2. The cell densities per unit bioreactor volume increase, which leads to high volumetric productivity, shorter fermentation times and elimination of non-productive cell growth phases.
3. The substrate consumption is enhanced and the yield is improved.
4. The tolerance of high substrate concentration increases and the end product inhibition reduces.
5. The risk of microbial contamination is diminished due to high cell densities and fermentation activity.
6. It is possible to apply in continuous processing.
7. The washout problem is eliminated which in turn enables the fermentation to be carried out at higher dilution rate.

8. It is able to regenerate and reuse the biocatalyst for extended periods in batch operations, without removing it from the bioreactor.
9. The downstream processing is simplified.
10. The bioreactor size is smaller with simplified process designs and therefore lower capital costs.

Besides these advantages, the use of immobilized microorganism has some disadvantages. One of the major problems is susceptibility to diffusion limitation on reaction rate and possible loss in the yield of the desired product. In such case, the control of micro-environmental conditions is difficult because of the resulting heterogeneity in the system. With viable cells, growth and gas evolution can lead to significant mechanical disruption of the immobilizing matrix [13].

Immobilized systems can be classified into natural and artificial occurring ones. In nature, some microorganisms can form biofilm by attaching to one another or even to surfaces. This attachment is facilitated by secretion of adhesive substance called glycocalyx by the cells [14]. In artificial immobilized cell system, cells are immobilized by using carriers/supports. Proper selection of carrier is extremely important for immobilized cell application because it will affect greatly on the performance of the system. As every organism exhibits different interaction with different carriers, evaluation of carrier performance for an individual organism should be done in case by case basis [2].

2.2.1. Cell immobilization carriers and techniques

Various materials have been tested as cells carriers. Selection of supporting materials will depend upon many factors including the resistance to microbial degradation, mechanical strength, type of fluid, surface characteristics, and the cost of materials. The properties of carrier should be as following [12];

1. The carrier should have high surface area for cell attachment.
2. The carrier area should have good binding affinities to the cell.
3. The carrier must be easy to handle, not expensive and easy to scale up.

4. Cell viability and stability of the immobilized cell should be high and retained in a longer term.
5. The biological activity of the immobilized cells should not be negatively affected by the immobilization process.
6. The porosity of carrier should be uniform and controllable and the pore size should be suitable for the mass transport of substrates, products or gases in the system.
7. The carrier should have good mechanical, chemical, thermal and biological stability and not be easily degraded by enzymes, solvents, pressure changes or shearing forces.

Cell immobilization techniques can be divided into four major categories based on the physical mechanism employed (Figure 2.3) [12]:

2.2.1.1 Surface attachment

In this type of immobilization, yeast cells are allowed to attach to a solid support. Many different carrier materials are being used. Cellular attachment to the carrier can be induced using linking agents (such as metal oxides, glutaraldehyde or aminosilanes). However, for the production of beverages and ethanol, natural adhesion is often preferred over the use of (potentially harmful or unstable) inducers. Natural immobilization is very simple and the conditions are mild, but cell loadings are usually not as high as those obtained in systems in which the cells are entrapped [3]. Moreover, as there are no barriers between the cells and the solution, cell detachment and relocation is possible with potential establishment of equilibrium between adsorbed and freely suspended cells. Examples of solid carriers used in this type of immobilization are cellulosic materials (DEAE-cellulose, wood, sawdust, delignified sawdust), inorganic materials (polygorskite, montmorilonite, hydromica, porous porcelain, porous glass), etc. Solid materials like glass or cellulose can also be treated with polycations, chitosan or other chemicals (pre-formed carriers) to enhance their adsorption ability [11].

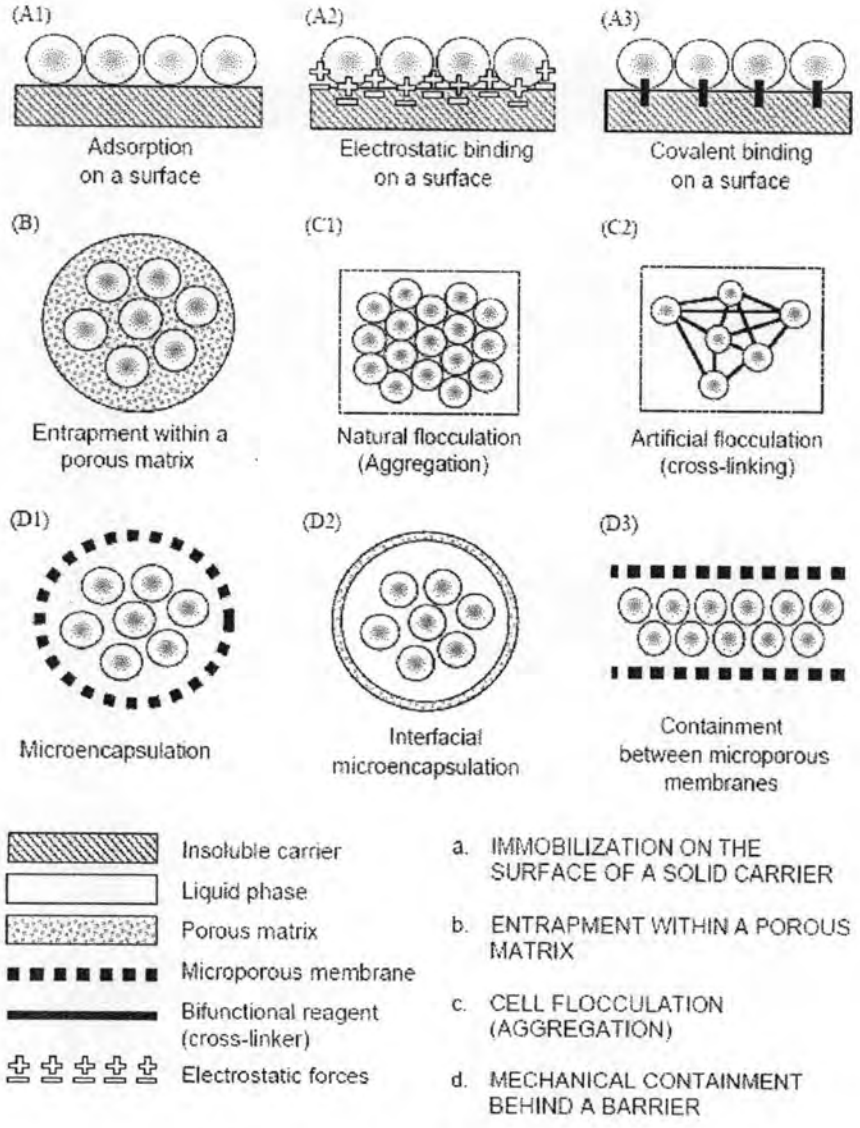

Figure 2.3 Basic methods of cell immobilization [12]

While the natural adhesion of yeast cells to substrates remains somewhat mysterious, several mechanisms have been proposed. The adhesion phenomenon could, for example, be conferred by electrostatic, ionic and hydrophobic interactions, but retention within carrier cavities and yeast flocculation can also play an important role in the immobilization process on preformed, roughly shaped carriers. Hence, the physicochemical properties of the yeast cell wall and the carrier, such as hydrophobicity,

charge, electron-donor and electron acceptor properties should be considered when designing new immobilization carriers [3].

2.2.1.2 Entrapment within porous matrix

The second major category of yeast immobilization is entrapment within porous matrices. Two methods of entrapment based on the inclusion of cells within a rigid network to prevent the cells from diffusing into the surrounding medium, while still allowing mass transfer of nutrients and metabolites. In the first, cells are allowed to diffuse into a preformed porous matrix. After the cells begin to grow, their mobility is hindered by the presence of other cells and the matrix and they are thus effectively entrapped. Attachment on the surface of this material is also possible. Sponge, sintered glass, ceramics, silicon carbide, polyurethane foam, chitosan and stainless steel fibers are commonly used materials.

In the second method, the porous matrix is synthesized in situ around the cells. Most often, natural and synthetic polymeric hydrogels such as Ca-alginate, carrageenan, agar, polyurethane, polystyrene and polyvinylalcohol are being used. These polymeric beads are usually spherical with diameters ranging from 0.3 to 3 mm. Cell growth in the porous matrix depends on diffusion limitations imposed by the porosity of the material and later by the impact of accumulating biomass. Although high biomass loadings can be obtained, gel entrapment has received less attention in the fermentation industry because of several drawbacks, such as diffusion limitations of nutrients, metabolites and oxygen due to the gel matrix and the high cell densities in the gel beads, the chemical and physical instability of the gel and the non-regenerability of the beads, making this immobilization type rather expensive. Recently, attempts are made to solve most of these drawbacks by the introduction of new techniques that are able to adjust the size (microbeads) and shape (lenticular shape) of the hydrogels [3, 11].

2.2.1.3 Yeast flocculation

Cell flocculation has been defined by many authors as an aggregation of cells to form a larger unit or the property of cells in suspensions to adhere in clumps and sediment rapidly. Yeast flocculation is a reversible, asexual and calcium dependent process in which cells adhere to form flocs consisting of thousands of cells. It involves lectin-like proteins, which stick out of the yeast cell wall and selectively bind mannose residues present on the cell walls of adjacent yeast cells. Yeast flocculation is a complex process that depends on the expression of several specific genes such as FLO1, FLO5, FLO8 and Lg-FLO1. Because of their macroscopic size and their mass, the yeast flocs rapidly sediment from the fermenting medium, thus providing a natural immobilization of the cells.

The use of flocculating yeast is very attractive, due to its simplicity and low cost. However, things are more complex than they may seem. Flocculation is affected by numerous parameters, such as nutrient conditions, agitation, Ca^{2+}-concentration, pH, fermentation temperature, yeast handling and storage conditions. Hence, the fermentation medium itself, and more specifically the content of glucose, sucrose and nitrogen compounds may influence the success of immobilization. However these parameters have not yet been systematically studied and it is hard to predict the impact of the medium on cell adhesion. Above all, flocculation is a strain-specific phenomenon. The ability of yeast cells to flocculate is of considerable importance for the brewing industry, as it affects fermentation productivity and beer quality in addition to yeast removal and recovery. The growing interest in flocculation bioreactors, because of the prospect of high cell densities in continuous processes, further intensifies the need for controlling yeast flocculation. In this case, constitutive flocculent yeast strains (by genetic engineering) are desired, because normal strains only flocculate in the stationary phase and thereby the exponentially growing cells would be washed out [3, 11].

2.2.1.4 Mechanical containment behind a barrier

Containment of yeast cells behind a barrier can be attained either by the use of microporous membrane filters or by entrapment of cells in microcapsules. This type of immobilization is most suited when a cell free product is required, or when high molecular weight products need to be separated from the effluent. Inherent problems of this technique are mass transfer limitations and possible membrane fouling caused by cell growth. This type of immobilization is attractive in terms of productivity, but it seems that the cost/benefit ratio for low-added-value fermentations like beer will remain unfavorable as long as high-performance membranes remain expensive. Several research groups have nevertheless investigated their use for the production of ethanol [3].

2.3 Bioreactor

The overall productivity of an immobilized bioparticle process, and hence the feasibility of its industrial use, depends to a large extent on the choice of the reactor system. In the case of the production of ethanol by fermentation, which is only attractive if the costs involved are no more than those of the usual petrochemical process, a recent study has concluded that replacement of batch processes by continuous immobilized cell systems may result in production and investment costs [15].

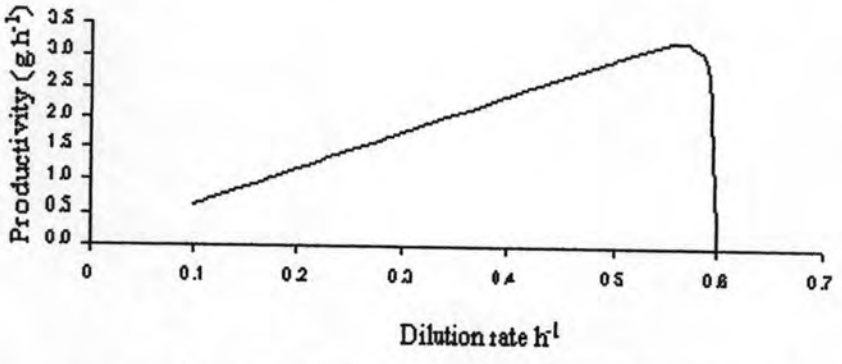

Figure 2.4 Productivity versus dilution rate curve [16]

In a continuous fermentation, productivity in general can be improved by increasing the flow rate of the system which is usually represented as dilution rate. Dilution rate is the ratio between flow rate and volume of reactor. The relationship between productivity and dilution rate is shown in Figure 2.4.

After reaching an optimum value, the productivity will decrease drastically until it reaches nearly zero. In some cases, the optimum bioreactor volume is set by the critical dilution rate which corresponds to the dilution rate at which washout occurs. Figure 2.5 shows a relationship between substrate, product, and biomass concentration with critical dilution rate [17].

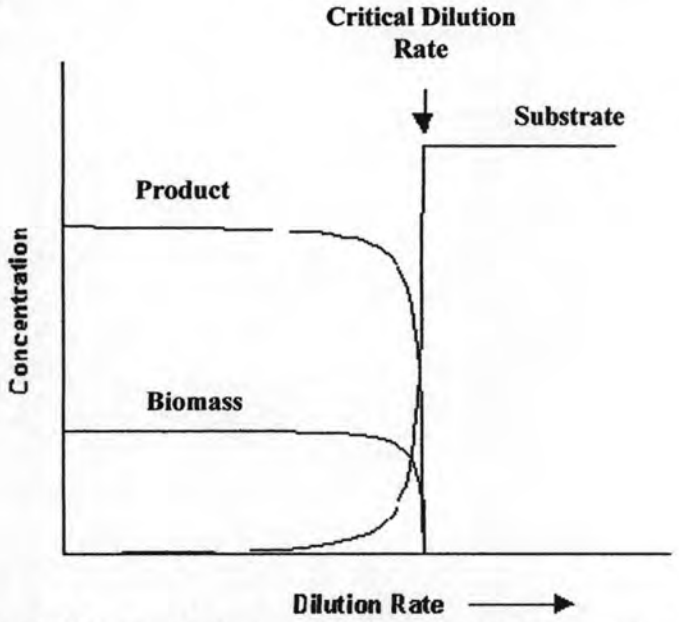

Figure 2.5 Concentration profile with variable dilution rate [16]

The design of fermentors must therefore take into account the need to maintain the activity of the fermenting microorganisms. Most of the continuous bioreactor systems currently being evaluated in laboratory experiments or pilot plants work with entrapped cells. The chief problem they face is that of ensuring adequate diffusion of substrate and oxygen through the matrix toward the immobilized cells, and adequate diffusion of carbon dioxide, ethanol and other metabolites out of the matrix. Poor diffusion may severely limit the productivity of a bioreactor [15].

As is well known, the production of alcohol by fermentation is inhibited by the products. Together with economic considerations, this circumstance is crucial in deciding on the type of reactor system in which to perform fermentation. In view of the autocatalytic nature of the fermentation process, continuous fermentation by free cells is generally carried out in continuous stirred tank reactors (CSTRs), but this system has the drawback of concentrating the product to a maximum, which means maximum inhibition too. By immobilizing the cells inside the bioreactor, high cells concentration can be achieved even though the dilution rate has exceeded its critical value. With the combination between high dilution rate and high cell densities inside the reactor, immobilized cells can maintain higher productivity as compared to conventional suspended cells culture [15, 18].

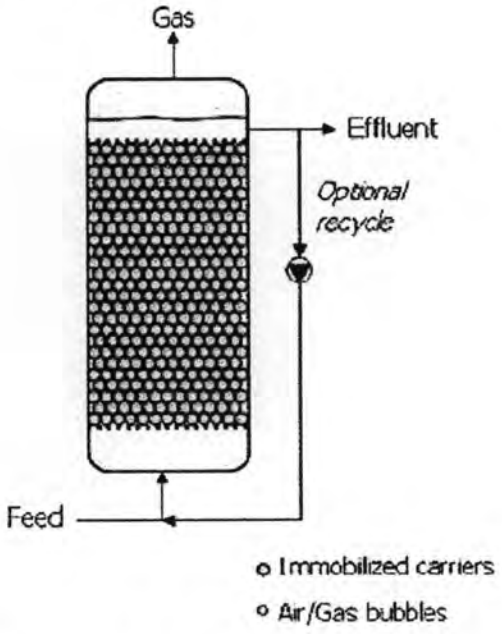

Figure 2.6 Packed bed reactor [3]

Packed bed reactors (PBR) are one of the most frequently employed types of bioreactor for immobilization systems. The reactor consists of a tube, usually vertical, packed with catalyst particles. Medium can be fed either at the top or bottom of the column and forms a continuous liquid phase between the particles. This type of bioreactor has the advantages of simplicity of operation and high reaction rates. Cells are

immobilized in appropriate carriers, which are packed in the fixed reactors, resulting in high solid-liquid specific interfacial contact areas, and the velocity of liquid creeping over the static solid particles substantially alleviates the film resistance to mass transfer. The major disadvantages of the PBR are mass transfer limitations, difficulties in CO_2 evacuation, compression of some carrier materials and fouling [18].

The most important characteristic of a PBR is that material flows through the reactor as a plug; they are also called plug flow reactors (PFR). Ideally, all of the substrate stream flows at the same velocity, parallel to the reactor axis with no back - mixing. All material present at any given reactor cross-section has had an identical residence time. The longitudinal position within the PBR is, therefore, proportional to the time spent within the reactor; all product emerging with the same residence time and all substrate molecule having an equal opportunity for reaction. The conversion efficiency of a PBR, with respect to its length, behaves in a manner similar to that of a well-stirred batch reactor with respect to its reaction time [19].

2.4 Review of ethanol fermentation by immobilization system

There have been many studies regarding ethanol fermentation by immobilized cells. Review on some of those studies is summarized in Table 2.1. Entrapment of microbial cells within the polymeric matrices is preferred by many researchers. Among the various methods, alginate gels have received maximum attention. There are several studies on the composition of alginate and their suitability for cell immobilization [2]. Alginate is widely used in food, pharmaceutical, textile, and paper products. The uses of alginate utilized in these products are for thickening, stabilizing, gel and film forming. Sodium alginate is a linear polysaccharide, normally isolated from many strains of marine brown seaweed and algae. Thus the name alginate, the copolymer consists of two uronic acids or polyuronic acid. It composed of primarily of D-mannuronic acid (M) and L-glucuronic acid (G). Alginic acid can be either water-soluble or non-water soluble depending on the type of the associated salt. Interchanging of sodium ions with calcium

ions in the solution may follow solidification of sodium alginate in calcium chloride solution. The sodium salt, other alkaline metals and ammonia are soluble in water, whereas the polyvalent cations salts, e.g., calcium, are not water-soluble, except the magnesium ions [20].

On the other hand, practical application of polymeric entrapment method has been limited by the problems of physical and chemical stability of gel as well as mass transfer limitation within the gel beads. Furthermore, large scale production of these beads requires complex and sophisticated equipment which in the end will increase overall manufacturing cost of the product [21].

From mass transfer point of view and ease of immobilization procedure, passive adsorption to solid surfaces (attachment method) is preferred to entrapment method [2]. However, cell adsorption alone is rarely satisfactory since the governing forces are usually not strong enough to prevent cell leakage and detachment from the carriers. Thus, very low cell loading is often observed with consequent low productivity. In view of this, incorporation of additional component into the gel matrix to improve the mechanical strength has been tried. Several components such as silica, sand, alumina and various gums are generally used [21].

Many of the reports on immobilization cell used γ-Al_2O_3 as an inorganic supports. High porosity and good mechanical strength of alumina seem to be responsible for both good productivity and storage stabilities. While immobilized systems using organic supports generally exhibit shorter biological half-lives (8-90 days), reactor lifetime with cells fixed on inorganic supports seems to be unlimited [22]. Furthermore, the positive effect of γ- Al_2O_3 on growth and fermentation efficiency of yeast was reported by M. Kanellaki et al. (1989) [4]. This result was supported by the finding of Kana et al. (1989), the reaction at lower pH is favorable for the electrostatic attraction between the γ- Al_2O_3 particles and the yeast cell because of γ- Al_2O_3 and *S. cerevisiae* have opposite electric charges within a lower pH range 3-6.5, as show in Figure 2.7 [5]. *S. cerevisiae* was immobilized on γ- Al_2O_3 particles with binder polymers using a spray-dryer. The optimum pH for the immobilized yeast was found to be 4. The pre-soaking of γ-Al_2O_3

particles in resin solution before immobilized by a spray-dryer improved immobilized and resulted in a high sucrose conversion to ethanol [23]. Recently, J.Pullsirisombat et al. (2007) developed *S. cerevisiae* M30 immobilized by γ-Al_2O_3 doped alginate gel as a new carrier which combines both of adsorption and entrapment technique. In repeated batch fermentation, this carrier demonstrated a good potential of reusability and ethanol production was found more stable than that of suspension cell culture [6]. The result shows that γ- Al_2O_3 doped alginate gel will be able to carry out in continuous process.

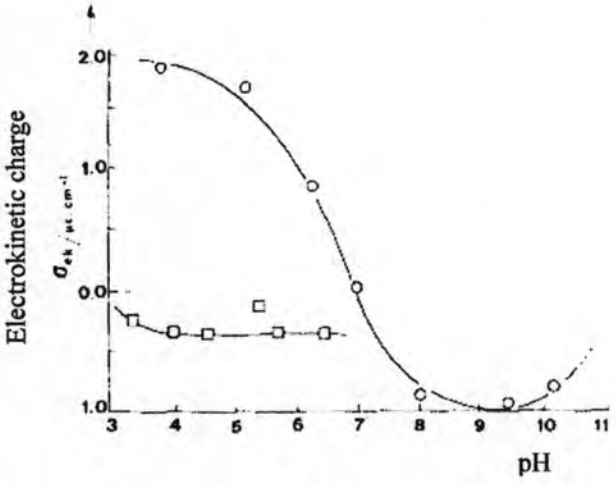

Figure 2.7 Electrokinetic charge versus pH for γ- Al_2O_3 pellets and cells of *S. cerevisiae*. Symbol: □, *S. cerevisiae* cells and ○, γ- Al_2O_3 [4]

The continuous process has significant advantages over the traditional batch ethanol fermenter, due mostly to the reduced cost of the bioreactor construction, reduced maintenance and operation requirements, better process control, and increased productivity. The high cell densities are achieved by cell immobilization techniques [3]. Ethanol fermentation with immobilized cells was generally performed in packed bed reactor due to simple design and operational control. However, the drawbacks of these systems are the mass transfer limitations, gas entrapment, compacting of the bed [24]. Attempts to solve this problem, Hamdy et al. (1989) developed the near-horizontal (15°angle) reactor column with immobilized yeast cells on to channeled alumina beads. This column demonstrated that efficiently removed CO_2, and increased ethanol

productivity when compared to the vertical column. Alumina beads could be used as a cells carrier for continuous ethanol fermentation in a maximum of one year without loss of activity [22].

Arasaratnam et al. (1994) studied on the optimization of parameters for continuous ethanol production by alginate entrapped *S. cerevisiae*. Problems of maintaining viable entrapped cells in a packed bed column have been overcome to some extent by using nutrients for cell viability and calcium for bead stabilization in addition to glucose. Immobilized *S. cerevisiae* performed better with medium containing nutrients and 0.05 M $CaCl_2$ (28 days) than with nutrients alone (10 days). Ethanol production decreased with an increase in alginate concentration however, the beads became more stable with respect to the retention of *S. cerevisiae* [25]. In addition, Göksungur et al. (2001) demonstrated that the suitable condition for ethanol production in packed bed reactor system consist of pH 3.9, diameter 2-2.4 mm, 2% Na-alginate solution and initial sugar concentration 10.90 %. When bead diameter and Na-alginate concentration were increase ethanol production will be decrease due to the diffusion limitation of the beads. Ethanol concentration, productivity and theoretical yield values decreased as sugar concentration of the medium was increased. The reason for this decrease in the overall fermentation performance was possibly from product and substrate inhibition. The result of the fermentation for 25 days demonstrated that the yield and productivity of the packed bed reactor were higher than these of the continuous stirred reactor [26].

Table 2.1 Study on ethanol production by immobilized cells

Author	Carrier		Dilution rate (h^{-1})	Ethanol Concentration (g/l)	Productivity (g/l • h)	Remark
	Method / Material	Shape / Size (mm)				
Kanellaki et al. (1989) [4]	Adsorption / γ-alumina pellets	Cylindrical L = 5 Ø = 2.5	-	-	3.33	1. γ- Al_2O_3 has a positive effect on growth and fermentation efficiency of yeast.
Kana et al. (1989) [5]	Adsorption / γ-alumina pellets	Cylindrical L = 5 Ø = 2.5	-	-	3.33	1. The electrostatic attachment of the *S. cerevisiae* cells on γ-alumina pellets is possible within a wide pH range 3-6.5. 2. γ-alumina pellets have in the promotion of repeated batch fermentation.

Author	Carrier		Dilution rate (h^{-1})	Ethanol Concentration (g/l)	Productivity (g/l • h)	Remark
	Method / Material	Shape / Size (mm)				
Hamdy et al. (1989) [22]	Adsorption / Alumina beads	Sphere Ø = 2	3.1	25	40	1. Continuous ethanol fermentation was successfully achieved using a bioreactor with cells immobilized on to alumina beads. 2. The near-horizontal (15°angle) column was superior for continuous ethanol production as compared to the vertical column. 3. The immobilized cell system was stored for a maximum of one year without loss of bioreactor activity.
Melzoch et al. (1993) [27]	Entrapment / Ca-alginate	Sphere Ø = 2	0.4	62	24.8	1. Immobilized cells retained high metabolic activity when long-term continuous anaerobic process performed in packed bed reactor (1100 h). 2. The immobilized cells in Ca-alginate bead were stored in 4°C water longer than 5 years that could grow and produce ethanol in batch process.

Author	Carrier		Dilution rate (h^{-1})	Ethanol Concentration (g/l)	Productivity (g/l • h)	Remark
	Method / Material	Shape / Size (mm)				
Arasaratnam et al. (1994) [25]	Entrapment / Ca-alginate	Sphere	0.6	41.3	10.5	1. Ethanol production decreased with increasing alginate concentration. 2. $CaCl_2$ was added in the glucose feed that could increase the stability of the bead.
Isono et al. (1995) [23]	Adsorption/ γ-alumina	Sphere Ø = 2	-	-	-	1. Presoaking of alumina particles in resin (3% w/v SMC) solution increased immobilization yield and sucrose conversion to ethanol. 2. The optimum pH for fermentation shifted to acid (pH 4). This restricted contamination and facilitated electrostatic attraction between alumina and cells.

Author	Carrier		Dilution rate (h^{-1})	Ethanol Concentration (g/l)	Productivity (g/l • h)	Remark
	Method / Material	Shape / Size (mm)				
Xu et al. (1996) [28]	Entrapment / Ca-alginate	Sphere	-	Batch = 46.6 Add nutrient = 75.8 alginate = 101.1 step feeding = 118.5	Batch = 0.55 Add nutrient = 0.90 alginate = 1.20 step feeding = 1.41	1. The method of ethanol fermentation by using glucose step-feeding with immobilized yeasts was the best performance when compared to the method of addition nutrient to standard medium and immobilization cells in alginate.
Ogbonna et al. (2000) [29]	Adhesion / Loofa sponge	Cylindrical L = 12 cm Ø = 9 cm			11	1. The fermentation was scale up to 50 liters bubble column and no significant different in ethanol productivity and yield when compare with 2 liters bubble column.

Author	Carrier		Dilution rate (h^{-1})	Ethanol Concentration (g/l)	Productivity (g/l • h)	Remark
	Method / Material	Shape / Size (mm)				
Göksungur et al. (2001) [26]	Entrapment / Ca-alginate	Sphere Ø = 2-2.4	0.22	46.2	10.16	1. The suitable condition for ethanol production in packed bed reactor system consist of pH 3.9, diameter 2-2.4 mm, 2% Na-alginate solution and initial sugar concentration 10.90 %. 2. Packed bed reactor with ethanol production, theoretical yield and productivity higher than continuous stirred reactor was performed when the system was run for 25 days.
Najafpour et al. (2004) [20]	Entrapment / Ca-alginate	Sphere Ø = 5	0.14	47	6.58	1. The immobilized cells reactor system exhibited a higher yield compared to the batch system. 2. The suitable alginate concentration based on activity of the beads for ethanol production was 2%.

Author	Carrier		Dilution rate (h^{-1})	Ethanol Concentration (g/l)	Productivity (g/l • h)	Remark
	Method / Material	Shape / Size (mm)				
Valach et al. (2005) [24]	Entrapment / 1. Ca-alginate 2. Ca-Pactate	Sphere Ø = 2-3	0.16	PBR = 44.4 Gas life = 47.3	PBR = 7.11 Gas life = 7.57	1. The gas-life reactor was more efficient than packed bed reactor due to a better mass transport between the phases. 2. Ca-pactate gel was more suitable than Ca-alginate gel because of mechanical resistance and favorable diffusion parameter. 3. Abrasion effect in Ca-alginate gel was stronger than Ca-pactate gel.
Baptista et al. (2005) [30]	Adhesion / Polyurethane foam	Cube	0.4	Adhesion = 40 Non-adhesion = 28	Adhesion = 16 Non-adhesion = 11	1. Non-adhesion yeast strain could produce continue longer than adhesion yeast strain but productivity lower than adhesion yeast strain.

Author	Carrier		Dilution rate (h^{-1})	Ethanol Concentration (g/l)	Productivity (g/l • h)	Remark
	Method / Material	Shape / Size (mm)				
Yu et al. (2007) [31]	Adsorption / Sorghum bagasse	Cube 10x10x10	0.3	Batch = 68.6 PBR = 55.6	Batch = 5.72 PBR = 16.68	1. Immobilization of *S. cerevisiae* to sorghum bagasse have increase the immobilized cells concentration and enhance the stability of the fermentation system effectively. 2. Complete conversion of total sugar to ethanol was obtained at a dilution rate 0.1 h^{-1}.
Budiraharjo et al. (2007) [21]	Adsorption / Loofa and Entrapment / Ca-alginate	8x8x1	-	Suspension = 91.7 EALC = 77.8	Suspension = 1.91 EALC = 1.62	1. Chitosan based carrier were unfavorable for yeast immobilization because of their inhibitory effect on cell activity. 2. Entrapment-Alginate-Loofa Cube (EALC) culture was more stable than suspension cell culture.
Pullsirisom-bat et al. (2007) [6]	Adsorption / γ-alumina and Entrapment / Ca-alginate	Sphere Ø = 3	-	74.6	1.55	1. New carrier, *S. cerevisiae* immobilized by γ-alumina doped alginate gel demonstrated that a good potential of reusability and ethanol production was more stable than suspension cell culture.