CHAPTER II
BACKGROUND AND LITERATURE REVIEW

2.1 Ethanol fermentation

The production of ethanol from renewable resources (corn, wheat, cellulosic biomass etc.) is currently a hot topic. There are clear benefits of using fuel ethanol in developed countries, where the availability of feedstocks for ethanol fermentation could greatly reduce dependence on foreign oil. The cleaner burning and greenhouse gas neutrality of ethanol is also considered environmentally friendly. However for economic consideration its production process must be highly efficient (Allain, 2007).

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.

2.1.2 Microorganisms

There are many kind of microorganism that can produce ethanol such as lactic and acetic acid bacteria, fungi and different strains of yeast. Most of these organisms grow optimally at a temperature range of 25 - 37°C. However, the 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 produces 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*. The *Z. mibilis* 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 produces 2 mol of ethanol, 2 mol of carbon dioxide and 1 mol of ATP per mol of fermented d-glucose (Bai et al., 2008).

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 resulted in higher ethanol yield (Veliky et al., 1994). 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 from *S. cerevisiae*. Although these advantages, *Z. mobilis* is not suitable for 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. Ethanol fermentation industries 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 (Bai et al., 2008).

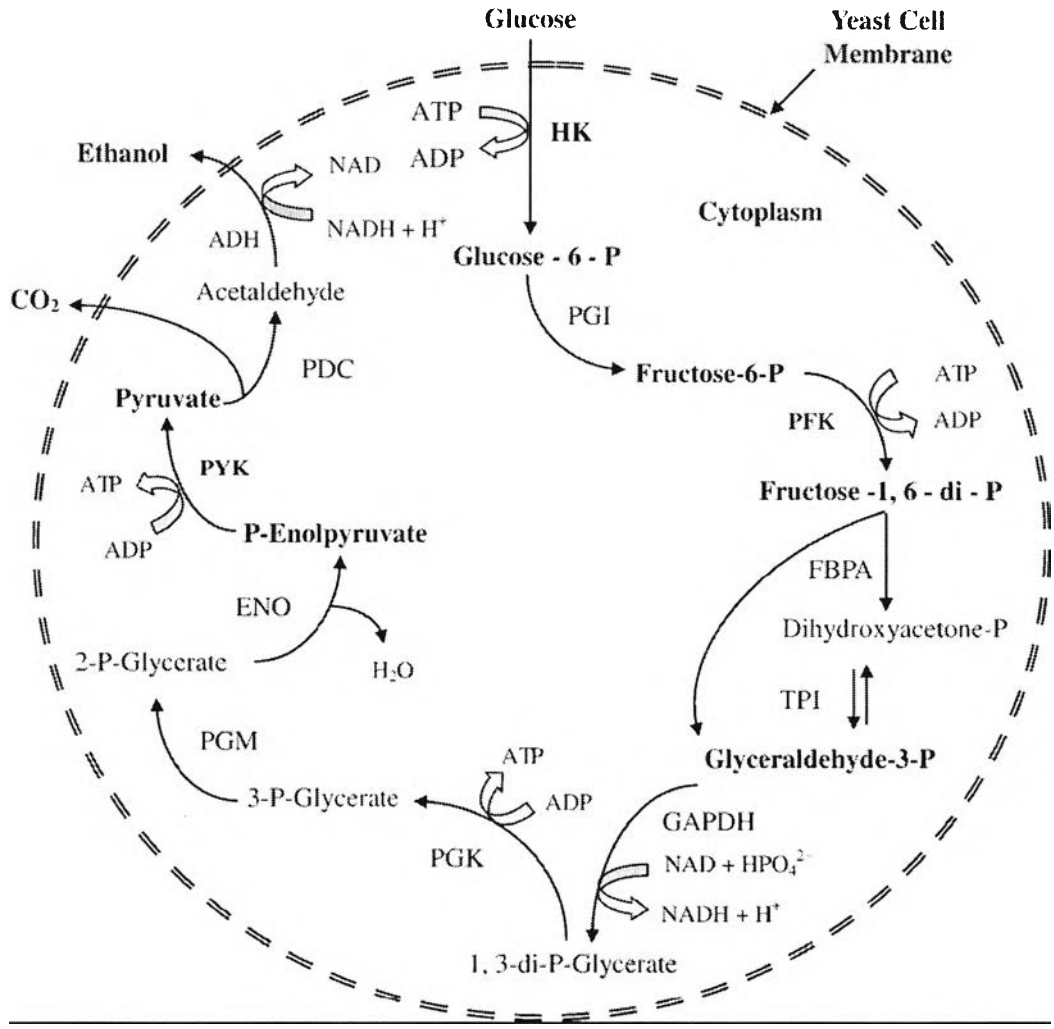

Figure 2.1 Embden-Meyerhof pathway (Bai et al., 2008)

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

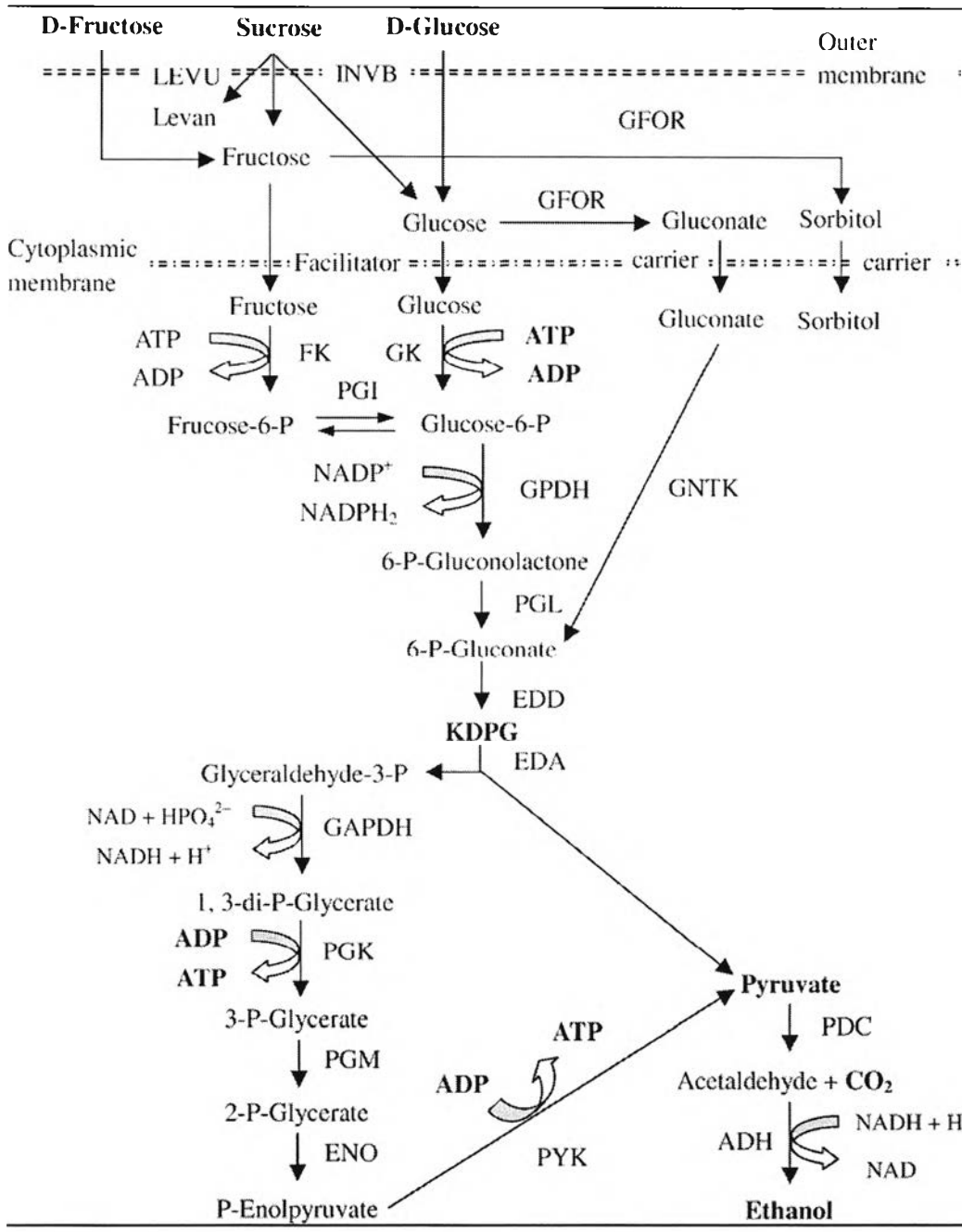

Figure 2.2 Entner–Doudoroff pathway (Bai et al., 2008)

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.

All of these processes need to be increased in productivity at the maximum possible temperatures at which *S. cerevisiae* can grow. Hence, if fermentation could be performed at higher temperatures using thermotolerant yeasts, several cost reductions would likely be achieved. Among the known yeast species used in fermentation processes, *Kluyveromyces marxianus* is thought to have the best performance in terms of growth and fermentation at high temperatures. It has also been experimentally studied that many thermotolerant stains of *K. marxianus* grow well at temperature as high as 45°C to 52°C and can efficiently ferment ethanol at temperatures of 37°C and 45°C (Anderson et al., 1986; Banat et al., 1992; Fleming et al., 1993; Banat and Marchant, 1995; Barron et al., 1995; Boyle et al., 1997; Singh et al., 1998; Lark et al., 1997; Abdel-fattah et al., 2000; Limtong et al., 2007; Nonklang et al., 2008; Babiker et al., 2010).

K. marxianus DMKU 3-1042 strain was isolated by Limtong et al. (2007) at Kasetsart University, Thailand, and the ethanol production performance of this strain at high temperatures was examined using molasses, which the results showed that *K. marxianus* DMKU 3-1042 has an optimal performance at 40°C, whereas *S. cerevisiae* M30 showed the best performance at 33°C (Phisalaphong et al., 2006).

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 by variety of microbial metabolites (Veliky and Mclean, 1994; Ramakrishna and Prakasham, 2006). Motivation for development of immobilized cell systems emerged from their potential advantages. Some potential advantages characteristics of immobilized cell over suspension fermentations include (Kourkoutasa et al., 2004).

1. The increase of cell activity and stability from a protective agent of immobilization support against physicochemical effects of pH, temperature, solvents or even heavy metals.
2. The increase of cell densities per unit bioreactor volume resulting in high volumetric productivity, shorter fermentation times and elimination of non-productive cell growth phases.
3. The enhanced substrate consumption and the improved yield.
4. The increase of tolerance of high substrate concentration and the reduction of end product inhibition.
5. The diminished risk of microbial contamination due to high cell densities and fermentation activity.
6. The elimination of washout problem which in turn enables the fermentation to be carried out at higher dilution rate.
7. The ability to regenerate and reuse the biocatalyst for extended periods in batch operations.
8. The simplified downstream processing.
9. The smaller bioreactor size 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 heterogeneity in the system. Moreover viable cells, growth and gas evolution can lead to significant mechanical disruption of the immobilizing matrix (Dursun and Tepe, 2005).

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 (Junter and Jouenne, 2004). 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 (Ramakrishna and Prakasham, 2006).

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 (Kourkoutasa et al., 2004);

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) (Kourkoutasa et al., 2004):

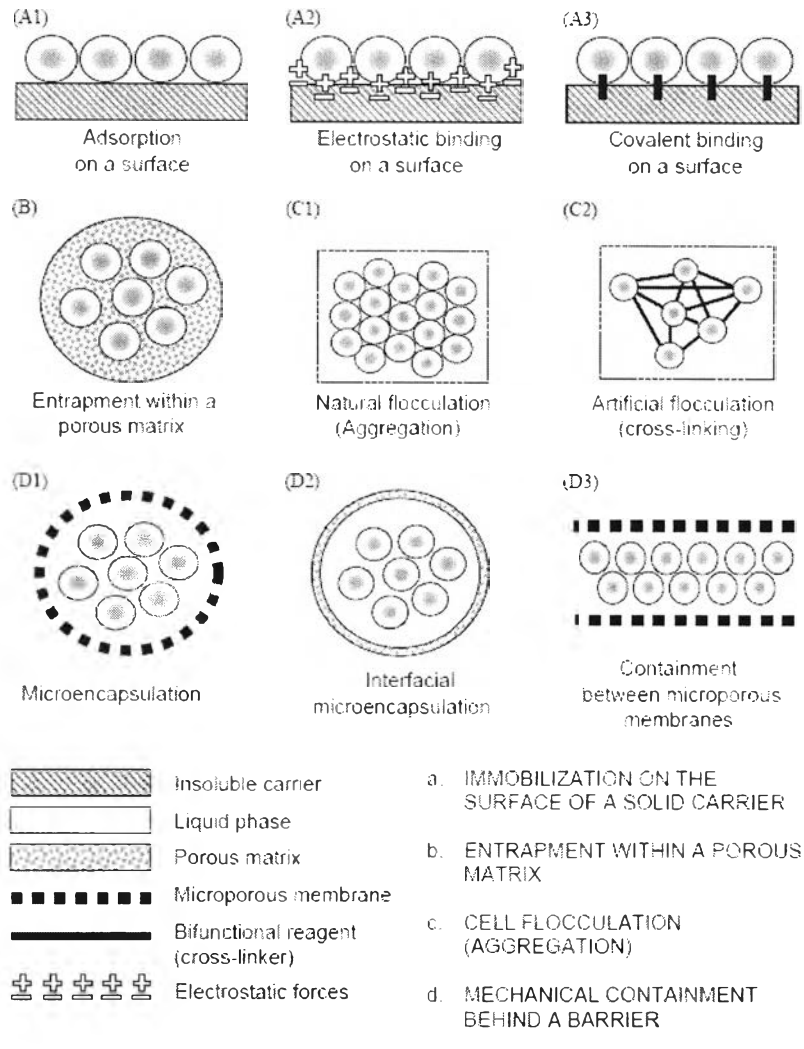

Fig. 1. Basic methods of cell immobilization.

Figure 2.3 Basic methods of cell immobilization (Kourkoutasa et al., 2004).

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 (Verbelen et al., 2006). 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 (Veliky and Mclean, 1994).

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 (Verbelen et al., 2006).

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 (Veliky and Mclean; Verbelen et al., 2006).

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, 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 (Veliky and Mclean, 1994; Verbelen et al., 2006).

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 (Verbelen et al., 2006).

2.3 Bioreactor

The overall productivity of an immobilized bioparticle process, and hence the feasibility of its industrial use, depend 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 higher production and investment costs (Nffiez and Lema, 1987).

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**.

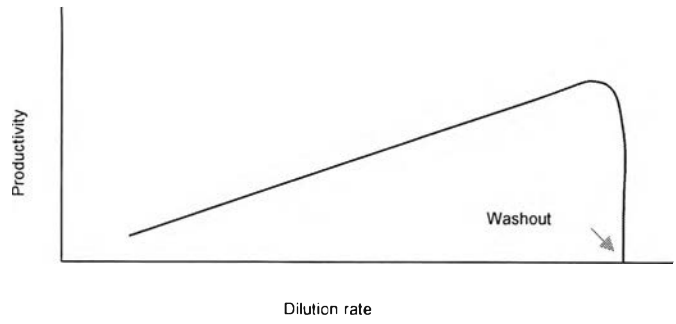

Figure 2.4 Productivity versus dilution rate curve

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 (Blanch and Clark, 1997).

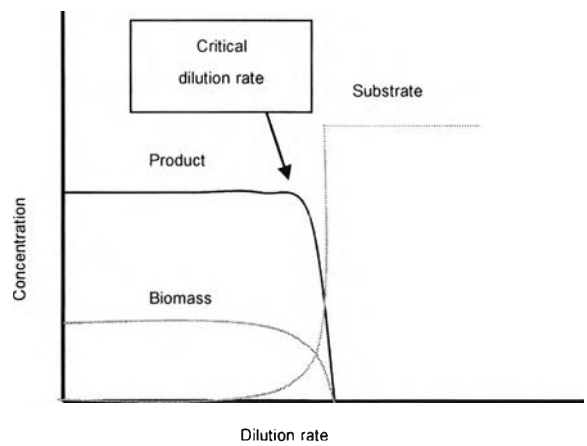

Figure 2.5 Concentration profile with variable dilution rate

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 (Nffiez and Lema, 1987)

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 (Nffiez and Lema, 1987; Wang and Zhong, 2007).

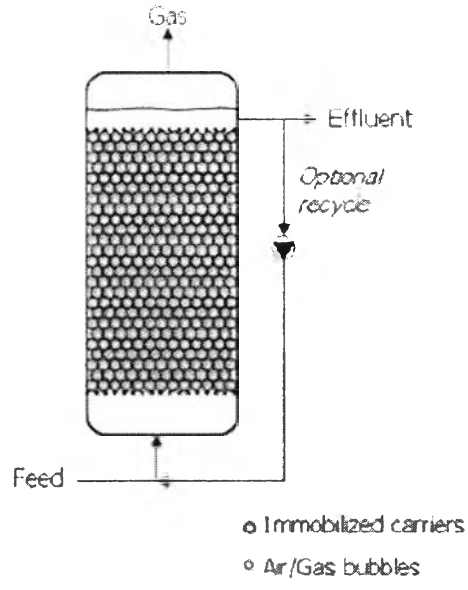

Figure 2.6 Packed bed reactor (Verbelen et al., 2006).

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 (Wang and Zhong, 2007).

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.

2.4 Review of ethanol fermentation by immobilization system

Abate et al. (1996) studied pure and mixed cultures of *Zymomonas mobilis* and *Saccharomyces* sp. For ethanol production using sucrose as the carbon source. Both strains, isolated from spontaneously fermenting sugar cane juice, are flocculent and alcohol-tolerant. The best results were obtained using a mixed culture, with a yield of 0.5 g ethanol/g sugar consumed and a volumetric productivity of 1.5 g ethanol l^{-1} h^{-1}.

The thermotolerant, ethanol-producing yeast strain *Kluyveromyces marxianus* IMB3 was immobilized in calcium alginate and used in a continuous flow bioreactor to produce ethanol from molasses at 45°C (Gough et al., 1998). Although maximum ethanol concentrations were obtained using sugar concentrations of 140 g/l, within 10 h of introducing the feed to the column bioreactors, those ethanol concentrations subsequently decreased to lower levels over a 48 h period. Examination of viable yeast cell number within the immobilization matrix indicated a dramatic reduction over this time period. At lower molasses concentrations, ethanol production by the continuous flow system remained relatively constant over this time period. At a fixed molasses sugar concentration (120 g/l), a residence time of 0.66 h was found to be

optimal on the basis of volumetric productivity. Efficiencies of the continuous flow bioreactor configuration used in these studies ranged from 31-76%.

Limtong et al. (2007) had reported production of fuel ethanol at high temperature from sugar cane juice by a newly isolated *Kluyveromyces marxianus*. Studied of the effect of temperature on ethanol fermentation was carried out at 30, 37, 40 and 45°C in the basal sugar cane juice medium. Effects of nutrient composition, including sugar concentrations and sources of nitrogen, phosphate and magnesium were also studied. Sugar cane juice media with adjusted pHs at 4, 4.5, 5 and 5.5 were used for the study of the effect of pH on ethanol fermentation. The results of this study demonstrated that the newly isolated *K. marxianus* strain DMKU 3-1042 was an effective strain that could be employed for ethanol production at elevated temperature when sugar cane juice was used as a raw material. It produced 8.7% (w/v) ethanol at 37°C and 6.8% (w/v) at 40°C from sugar cane juice medium composed of 22% total sugars by shaking flask cultivation.

Yu et al. (2007) studied sorghum bagasse without any treatment for the immobilization of *Saccharomyces cerevisiae* at 0.6 ± 0.2 g dry cell weight (DCW)/g dry sorghum bagasse weight (DSW) through solid-state or semi-solid state incubation. The scanning electron microscopy (SEM) of the carriers revealed that the immobilization is adsorption and embedding. The ethanol productivity of the immobilized cells was 2.24 times higher than the free cells. In repeated batch fermentation with an initial sugar concentration of 200 g/L, nearly 100% total sugar was consumed after 16 h. The ethanol yield and productivity were 4.9 g/g consumed sugar and 5.72 g/(L h), respectively. The immobilized cell reactor was operated over a period of 20 days without breakage of the carriers, while the free cell concentration in the effluent remained less than 5 g/L throughout the fermentation. The maximum ethanol productivity of 16.68 g/(L h) appeared at the dilution rate of 0.3 h^{-1}.

Cáceres-Farfán et al. (2008) studied ethanol production from henequen (*Agave fourcroydes Lem.*) juice and molasses by a mixture of two yeasts: *Kluyveromyces marxianus* (isolated from the henequen plant) and *Saccharomyces cerevisiae* (commercial strain). An ethanol production of 5.22 ± 1.087% v/v was obtained with residual reducing sugars of 2-4 g/l. A decrease on ethanol production was observed with the use of the *K. marxianus* strain. The best results were obtained when a

mixture of 25% *K. marxianus* and 75% *S. cerevisiae* or *S. cerevisiae* alone were used with an initial inoculum concentration of 3×10^7 cell mL^{-1}.

Paul Peiris and Nan Fu. (2008) studied co-fermentation of a mixture of glucose and xylose to ethanol by *Zymomonas mobilis* and *Pachysolen tannophilus*. It was found that the addition of co-fermentation process showed potential for the conversion of the sugar mixture to ethanol. Ethanol yield from xylose was improved from 29 to 33% from the single strain fermentation to the co-fermentation. However, the decreased sugar utilization rate and cell growth indicated ethanol inhibition of the growth of *P.tannophilus*. *Z.mobilis* showed excellent capacity for ethanol production from glucose and had potential to be used in co-fermentation processes especially in glucose-rich hydrolysates. It was suggested that the reduction of ethanol inhibition and the manipulation of oxygen levels in the xylose fermentation stage could be significant factors for the improvement of ethanol yields.

Guo et al. (2008) studied ethanol production by mixed immobilized cells of *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*. From the experimental study at 30°C, the use of the mixed culture of immobilized cells of *K.marxianus* and *S.cerevisiae* improved ethanol fermentation using cheese whey powder (CWP) as medium. This system achieved higher ethanol productivity than that from fermentations by free cells or coimmobilized cells.

Nonklang et al. (2008) demonstrated herein the ability of *Kluyveromyces marxianus* to be an efficient ethanol producer and host for expressing heterologous proteins as an alternative to *Saccharomyces cerevisiae*. Growth and ethanol production by strains of *K. marxianus* and *S. cerevisiae* were compared under the same conditions. *K. marxianus* DMKU 3-1042 was found to be the most suitable strain for high-temperature growth and ethanol production at 45°C. This strain, but not *S. cerevisiae*, utilized cellobiose, xylose, xylitol, arabinose, glycerol, and lactose. To develop a *K. marxianus* DMKU3-1042 derivative strain suitable for genetic engineering, a uracil auxotroph was isolated and transformed with a linear DNA of the *S. cerevisiae* Sc*URA3* gene. The results demonstrated that *K. marxianus* DMKU3-1042 could be an alternative cost-effective bioethanol producer and a host for transformation with linear DNA by use of *S.cerevisiae*-based molecular genetic tools.

Wilkins et al. (2008) studied on the fermentation of xylose by the thermotolerant yeast strains *K. marxianus* IMB2, IMB4, and IMB5 under anaerobic conditions. The effects of temperature and initial pH on anaerobic xylose utilization by the thermotolerant yeast strains *K.marxianus* IMB2, IMB4, and IMB5 were evaluated. The experimental result showed that *K.marxianus* IMB4 produced more ethanol at a greater yield than *K.marxianus* IMB2 and IMB5. Ethanol yields were greater at 40°C than at 45°C and they were greater at pH 5.5 than at pH 4.5 and 5.0. *K.marxianus* IMB2 and IMB5 produced more xylitol than IMB4. Xylitol yield was greater at 45°C than at 40°C for IMB2.