CHAPTER III

THEORY

This chapter will be focused on laying theoretical ground work to be employed as a basis for interpretation of the experimental results. The general information of protease and starch hydrolysation are stated in section 3.1 and section 3.2, respectively. In section 3.3, general description of microfiltration system based on gel polarization model is given and important parameters of the system are identified. In section 3.4, general description of mathematical analysis of continuous fermentation process coupling with microfiltration is provided.

3.1 Protease

3.1.1 General description

The process where protein is broken down is called proteolysis where proteo stands for protein and lysis for breakdown. The major enzymes concerned are proteases, e.g. rennin, pepsin and trypsin. Enzymes are biological catalysts that are protein molecules in nature. They are absolutely essential as catalysts in chemical reactions because almost every reaction in a living cell requires the presence of specific enzyme [19]. Protease will degrade proteins into peptides, which are then degraded by various peptidases to smaller peptides and free amino acids.

Apart from catalytic functions in microorganism cell, they can also function out of cell. A major function of enzymes is to catalyze the making or breaking of chemical bonds. Enzymes can increase rate of reaction by reducing the

activation energy from ΔE_{NE} to ΔE_E as illustrated in Figure 3-1 without themselves undergoing permanent chemical changes.

Generally, the rate of reaction which enzymes catalyze will depend on the range of working temperature and pH including enzyme and substrate concentration as illustrated in Figure 3-2.

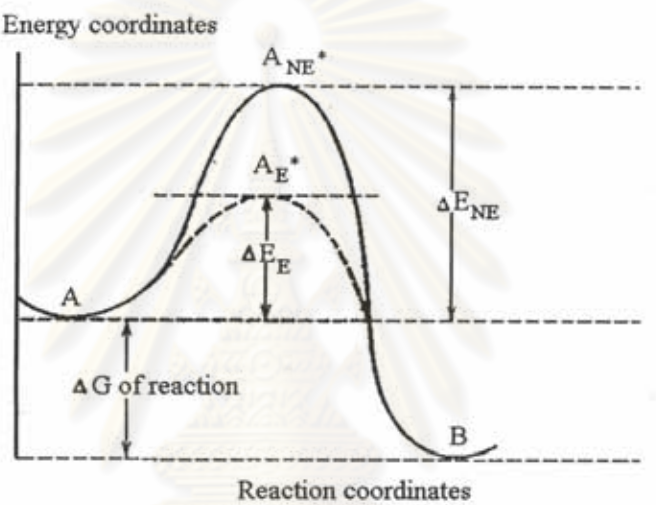


Figure 3-1 Reduction of activation energy by enzymes

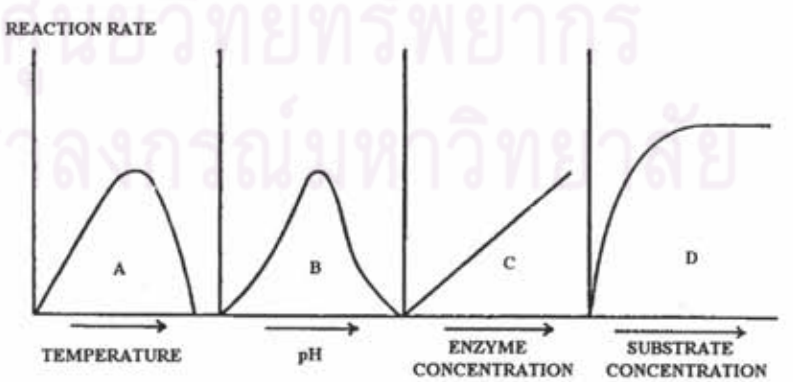


Figure 3-2 Effects of A) temperature, B) pH, C) enzyme and D) substrate concentration on reaction rate

In spite of the fact that there are many classification systems of enzymes, the most generally accepted one, classified by the International Enzyme Commission, comprise six major classes of enzymes which depend on types of chemical reaction they catalyze. These classes are oxidoreductases, transferases, hydrolyses, lyases, isomerases and ligases. Each enzyme can be designated by a four-numerical code system, the first number stands for major class and the last three numbers represent for subclass, doublesubclass and triplesubclass, respectively.

In order to form an absolutely perfect enzyme-substrate complex, some enzymes, called apoenzyme, do need coenzymes to be incorporated with as illustrated in Figure 3-3.

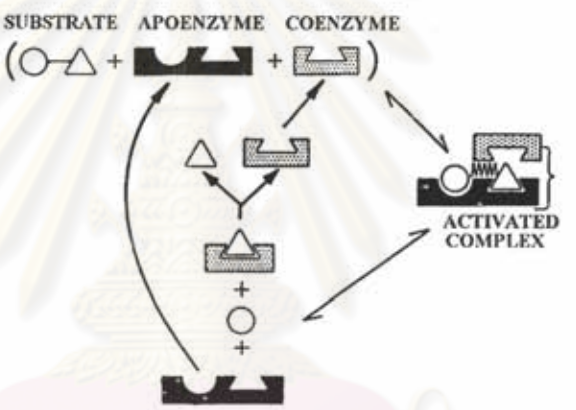


Figure 3.3 Enzyme hydrolyzation

The catalytic ability of enzymes depends on their particular protein structure. A specific chemical reaction is catalyzed at a small portion on the surface of enzyme termed "Active Site". With optimum condition ,enzymes will have the best ability to trigger chemical reactions and to affect the course and speed of such reactions. Their activity drops if the temperature is increased beyond optimum and only if enzymes approach a high critical condition (high temperature or extreme pH value), they are more completely denatured (inactivated). The temperature of inactivation varies from one type of enzyme to another.

Enzyme reactions are different from chemical reactions [19] as follows :

1. An enzyme catalyst is highly specific and catalyzes only one or a small number of chemical reactions. A great variety of enzymes exist, which can catalyze a very wide range of reactions.
2. The rate of an enzyme-catalyzed reaction is usually much faster than that of the same reaction when directed by nonbiological catalysts. Only a small amount of enzyme is required to produce a desired effect.
3. The conditions (temperature, pressure, pH and etc.) for the enzyme reactions are very mild.
4. Enzymes are comparatively sensitive or unstable molecules and require care in their use.

Hydrolase, one of the six major classes, is an important group of enzymes in biochemical engineering field, they facilitate the breakdown of macromolecule into micromolecule such as amylases break starch into sugars, lipases break lipid into fatty acids and proteases also break protein into amino acids.

Proteases are protein hydrolyzing enzymes which constitute a very large and complex group of enzymes which differ in properties such as substrate specificity, active site and catalytic mechanism, pH and temperature activity and stability profiles. The major function of proteases is to hydrolyze peptide bond in protein molecule to be smaller units called amino acids.

The specificity of proteases is primarily related to the nature of the amino acid sidechain and other groups close to the bond being hydrolyzed. Proteases can be separated into two major groups based on their ability to hydrolyze peptide bond which are at the outside (exopeptidase) or at the middle (endopeptidase) of amino acid chain [20]

3.1.2 History

Proteases have been used to transform milk into products such as cheese since 5000 BC. Besides proteases were used in leather industry for all stages of leather preparation --initial soaking, dehairing and bating of hides-- and as presoak detergents since 1910, they were also used in baking industry to provide flour with a sufficiently low protein content and thus render the dough more workable including enhancement of flavor and taste [1].

In brewing industry, proteases were needed in malt preparation step to achieve sufficient breakdown of protein and in chillproof process to prevent undesirable haze.

Other existing or potential applications for proteases include desizing textiles, meat tenderization, animal feed preparation and pharmaceutical and clinical usage as digestive aids. There are many industrial usage of proteases that makes them account for nearly 60% of the industrial enzyme market. Moreover, proteases are in the first range of Thailand imported enzyme group in 1987 [21].

3.1.3 Sources

Proteases of commercial importance are produced from animal, plant and microbial sources as follow [20]

3.1.3.1 Animal proteases

Rennet enzyme usually comes from the slaughterhouses by grinding calf stomachs and extracting with 5-10% salt solution. Pancreatin, a mixture of enzyme including proteases, is extracted from the pancreas by defatting, mincing, drying and removal of insoluble material. These enzymes are generally produced industrially as by-products of insulin production. Pepsin is prepared from the fundus portion of hog stomachs by acid extraction and filtration.

3.1.3.2 Plant proteases

Papain and ficin are prepared by water extraction of crude material from Carica papaya and Ficus carica , respectively. Their purification steps involve filtration, solvent precipitation and drying. Bromelain is obtained from the stem of pineapple plant by extraction and fractional solvent precipitation.

3.1.3.3 Microbial proteases

For various technological and economic reasons microorganisms are superseding the other sources of supply. Microbial proteases are produced extracellularly from high yielding strains by fermentation under controlled conditions in surface or submerged culture and their recovery involves separation of cell free liquor by microfiltration or centrifugation. Depending on the product and the degree of purity required, further purification might involve steps such as concentration, precipitation and stabilization.

A wide range of microorganisms has been shown to produce proteases but commercialization is quite restricted because of the many practical and legal considerations. For successful commercialization the following criteria have to be considered :

- The microorganism must grow on cheap and readily available raw materials and produce proteases in high yield in a short fermentation time which should be extracellular enzymes.
- The crude enzyme must be easily isolated with a good recovery.
- The microorganism must be non-pathogenic or susceptible to bacteriophage and should not produce any toxins.
- The enzymes produced should have characteristics which make them most suitable for the intended application.

Three microorganisms --Bacillus subtilis, Aspergillus niger and Aspergillus oryzae-- were allowed to be used under these considerations together with the US Food and Drug Administration restriction on GRAS (generally regarded as safe).

Apart from the same usages as those of animal and plant, bacterial proteases were further used in many vital chemical industries such as enzyme-containing toothpaste and mouth washes, spot removal in dry cleaning of clothing and recovery of silver from spent photographic film by digestion of the gelatin emulsion coating whereas the idea of using proteases in detergents was first developed in 1913 [22].

Extracellular protease production by microorganism has long been used as a method of classification and differentiation between them. All of the industrially significant proteases are endopeptidases rather than exopeptidases. The microbial proteases fall into four main groups of endopeptidases classified by their diverse catalytic nature and industrial importance under the headings of acid, thiol, metallo and alkaline proteases respectively as follows :

3.1.3.3.1. Acid Proteases

Acid proteases are chiefly of fungal origin which have properties as same as those of animal. Many of them contains aspartate residue at their active site. Although most of them are similar to pepsin in most respects, a few of them resemble renin, having a marked specificity in casein hydrolysis, which leads to the clotting milk and potential application in cheese manufacture. These proteases are most active in the pH range of 2.0-5.0 but are stable in the wider pH range (pH. 2.0-6.0) ; however their actual maximum range depends on the particular enzyme and substrate used. They are insensitive to inhibitors such as sulfydryl reagants, metal-chelating agents, heavy metals and diisopropyl fluorophosphate (DFP). With a low isoelectric point characteristic, the proteases have been isolated from mold fermented beer by the protein fractionation.

Aspergillus sp., Rhizopus sp. and Mucor sp. are such predominant microorganism that can produce acid protease well.

3.1.3.3.2. Thiol Proteases

These enzymes have cysteine at their active site. Their main utilization is in brewing, textile and general protein hydrolysation. Neutral pH is optimal condition for thiol protease. They are normally activated by reducing agents such as hydrogen cyanide and inhibited by oxidizing agents such as sulfhydryl reagents. The best known microbial thiol proteases are clostripain and Streptococcal proteases obtaining by Clostridium sp. and Streptococcus sp., respectively but these are not of direct commercial interest.

3.1.3.3.3. Metallo Proteases

These proteases are zinc-containing metalloprotein which are produced by both fungal and bacteria. Their applications are chiefly of baking, food processing, leather and pharmaceutical industry. They all have pH optimum around 7-8 and are sensitive to metal chelating agents such as EDTA and ortho- phenanthroline but are not inhibited by DFP or thiol reagents. Their stability decreases rapidly below pH 6.0 and above 9.0 but they are stabilized by the presence of calcium. The molecular weights of these group were found to be in the range of 35,000-45,000 . Several of these enzymes have been extensively purified by fractionation and chromatography. Many bacteria secrete neutral proteases also produce alkaline one. The most extensively used enzymes of this group are from Bacillus subtilis, B. thermoproteolyticus, B. megaterium, B. cereus, Streptomyces naraensis and Aspergillus oryzae.

3.1.3.3.4. Alkaline Proteases

Alkaline proteases are widespread in bacteria and fungi, and resemble the animal enzymes typsin and chymotrypsin. The most extensively studied enzymes in the group are the subtilisins Novo and subtilisins Carlsberg produced by a variety of Bacillus sp. The alkaline proteases or serine proteases have both serine and

histidine at the active site and have molecular weights from 26,000 to 34,000, slightly below the range of the neutral proteases. The alkaline protease have found extensive uses in the detergent and food industries and in particular as detergent additives.

The alkaline proteases have broad pH activity profiles from pH range of 7 to 11, although they generally have maximum activity at pH range of 9.5 to 10.5. Most of them are stable from pH range of 5 to 10 at low temperatures, but lose activity rapidly at 65 °C. However, in the presence of the calcium, the stability was increased so that more than 50 % of the original was maintained on heating at 65 °C for 15 minutes. The alkaline proteases are not generally inhibited by soya bean trypsin inhibitor, but the subtilisins are inhibited by a potato inhibitor. Thiol poisons have little effect on them and neither do metal-chelating agents. Diisopropyl fluorophosphate (DFP), Phenlymethylsulfonyl fluoride and tosyl-L-lysine chloromethyl ketone (TLCK) are also their inhibitors but not chelating agents (EDTA). The alkaline proteases of a series of Bacilli have been isolated by salt or solvent fractionation followed by ion-exchange chromatography on Duolite C-10 resin or carboxymethyl cellulose.

The best known enzymes in this group are the subtilisins produced by various Bacilli such as Bacillus subtilis, Bacillus licheniformis and alkalophilic bacillus. The others are produced by Streptomyces fradiae, S. griseus and Aspergillus oryzae.

3.1.4 Protease synthesis in Bacillus sp.

Strains of B. subtilis produce either alkaline protease alone or a mixture of neutral and alkaline ones. The alkaline proteases are called subtilisins Carlberg when alkaline proteases alone is produced, and subtilisins Novo when the mixture is produced [1]. Members of the genus Bacillus are Gram-positive, endospore forming, rod-shaped bacteria (1.5 - 1.8 μm.), which can form a spore (1 μm.) inside a normal cell (vegetative cell) in the post logarithmic phase or where an unsuitable condition for growth occurs as shown in Figure 3-4. Usually, they are found in a short rod-shaped chain which moves by using lateral flagella.

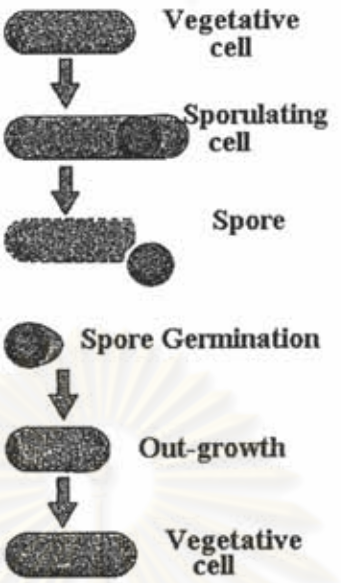


Figure 3-4 Sporulation of Bacillus sp.

The surface of spores are stainable, glossy, and pigment lacking. Anaerobically, in complex media containing glucose, growth and fermentation are weak, on the other hand, admission of oxygen permits abundant growth at pH 5.5 - 8.5 with the fermentation of 2,3-butanediol, acetoin and CO_2 as major products [23]. Involving the fermentation process, Bacillus sp. need, carbon and nitrogen sources for growth together with enough oxygen concentration.

The synthesis and secretion of protease in complex cultures of Bacillus sp. are related with cell growth and always occur in postlogarithmic phase [24]. Protease synthesis is circumscribed by nucleic acids, which are genetic materials such as DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid). In log phase, most of nucleic acid is employed to duplicate another genetic materials for a new cell and after a certain amount of cell reaches its maximum concentration at stationary phase, there are some nucleic acid left. The surplus nucleic acid is employed to synthesize protease [25]. The protease is synthesized by the transcription of mRNA (messenger Ribonucleic acid) from specific protease gene sequence in DNA then, follow by the translation of the mRNA, which mRNA will initially bind with rRNA (ribosomal RNA) and tRNA (transfer RNA) escorting with amino acid will attach at its site,

sequentially. The amino acid brought with tRNA will be sequentially ordered as same as the amino acid sequence of protease gene in DNA as shown in Figure 3-5. The mentioned amino acid are formed by Glycolysis pathway and Kreb's cycle as illustrated in Figure 3-6. The synthesized proteases always have to be cut the leading amino acid sequence in order to secrete out of the cell. For more stabilization, the proteases have to fold themselves to be in the most stable form.

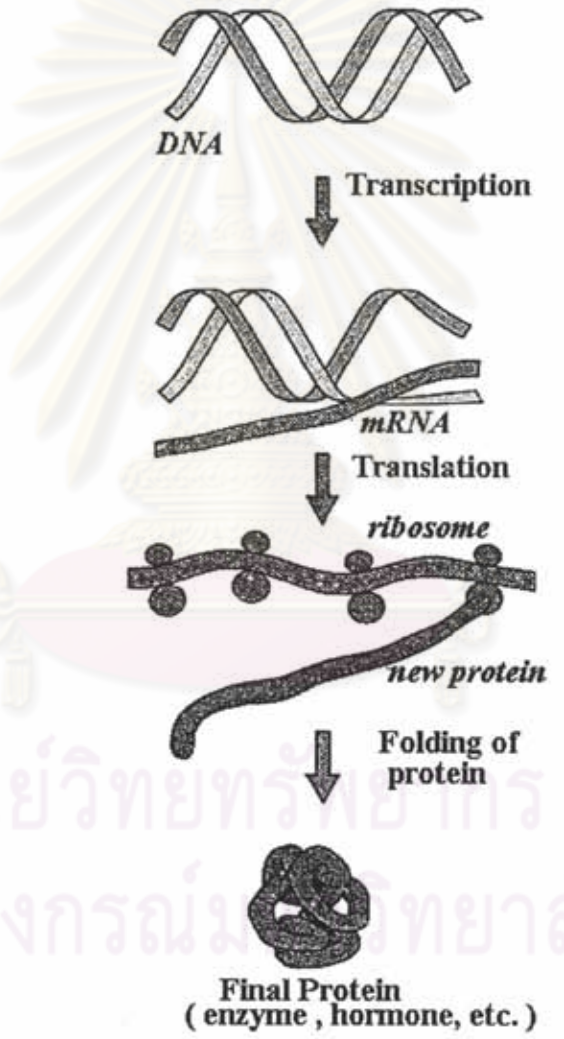


Figure 3-5 The synthesis of protease

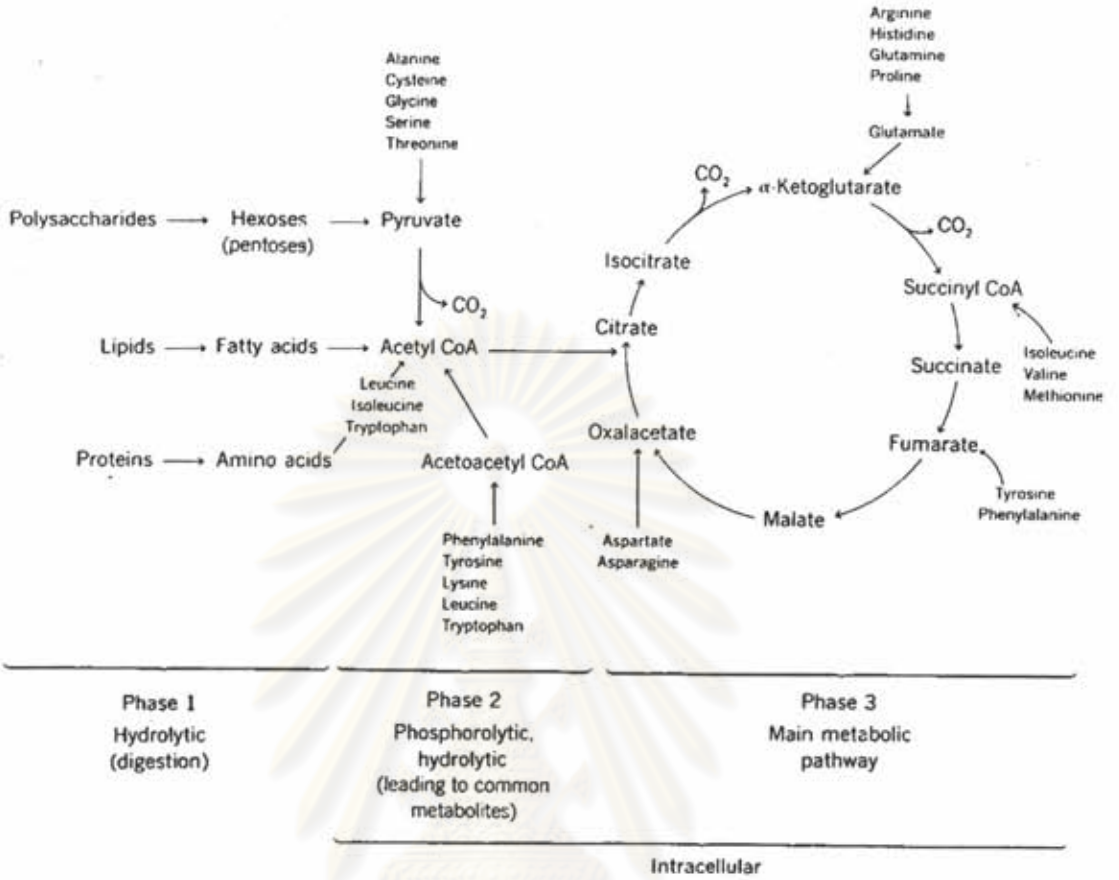


Figure 3-6 Glycolysis and Kreb's cycle

3.1.5 Extractions

A general production or extraction of enzymes from the supply source have many steps [20]. Some are identical but some are not, as shown in Figure 3-7. Due to the less proteases in animal and plant tissue comparing to that of production from microorganism, the cost of extraction from animal and plant tissue was so high that nowadays, the major commercially used proteases are obtained from microorganism source. The total value of the protease market increases from $400 to $600 million between 1981 and 1984. And the estimated world sales of industrial enzymes in 1986

indicate that microbial proteases account for nearly 60% of the total industrial protease market [2] as illustrated in Table 3-1.

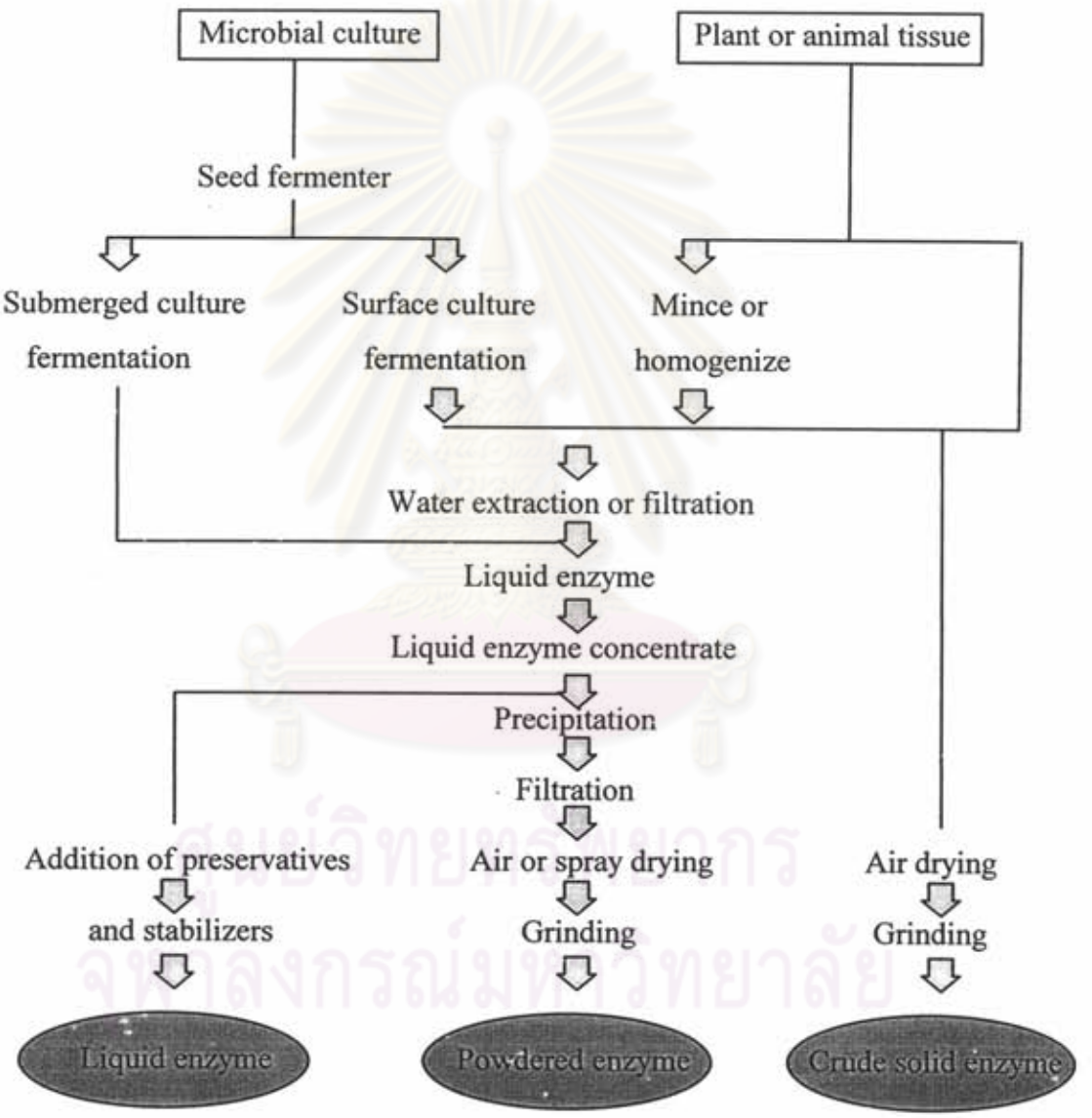


Figure 3-7 A general flow diagram for production of enzymes

Table 3-1 The value and market share of protease in 1986.

Proteases	Value (million$)	Market Share (%)
Bacteria proteases	145	60
Animal rennet	50	21
Papain	15	6
Microbial rennet	12	5
Pancreatin	12	5
Bromelain	5	2
Fungal proteases	3	1
Total	242	100

3.2 Starch

Starch is almost always a mixture of polysaccharides composed of chains of D-glucopyranose units known as glucose joined together by 1-4,α -D linkage as shown in Figure 3-8, Generally, it is accepted that there are at least two major components termed amylose and amylopectin as illustrated in Figure 3-9 and 3-10, respectively. The cassava starch contains about 75-80 % amylose and 20-25 % amylopectin. These two components can be separated by precipitation of amylose with the use of a selective precipitant such as butanol.

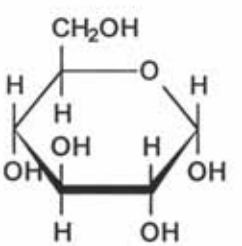


Figure 3-8 A glucose unit

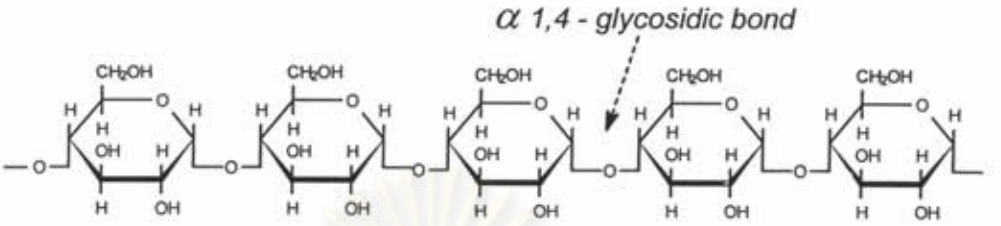


Figure 3-9 Amylose

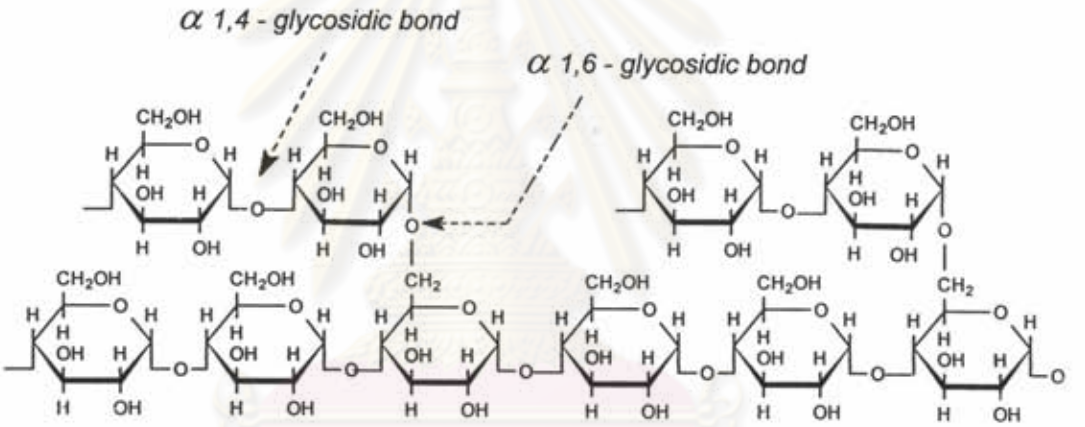


Figure 3-10 Amylopectin

Like proteases, α - amylases are also hydrolyzing enzymes that crack starch molecules, not proteins, into small polysaccharides and then, glucoamylases will crack the small polysaccharides into glucose units with higher conversion than that of acid hydrolyzation. Cassava is a major raw material for starch production in Thailand which gives a high polysaccharide content. Normally, glucose form cassava is always employed by enzymatically hydrolysation method.

3.3 Principles of Microfiltration

Microfiltration (MF) is a pressure-driven membrane separation technique for suspended macromolecules or macrosolute based on molecular size. Feed solution which is the substances to be concentrated or fractionated is forced through membrane under applied pressure. The feed solution flows over a membrane and solid, larger substances comparing to filter pore size, are retained while permeate, smaller substances, is removed. This separation technique involves no phase change and no chemical reaction process with little energy consumption. Membrane are categorized by their molecular weight cutoff, supposedly the molecular weight of the smallest molecule that cannot pass through the membrane. However, owing to various interactions, a membrane cannot be purely selected on the basis of molecular weight cutoff [26]. With pressure exerted on solution between 1 and 5 bar as a driving force, microfiltration is employed to separate particles with molecular weight from 100,000 to 1,000,000 or with linear dimensions in the approximate range of 0.02 to 10 μm. This size range encompasses a wide variety of natural and industrial particles [27]. The spectrum of application of membrane separation processes is shown in Figure 3-11.

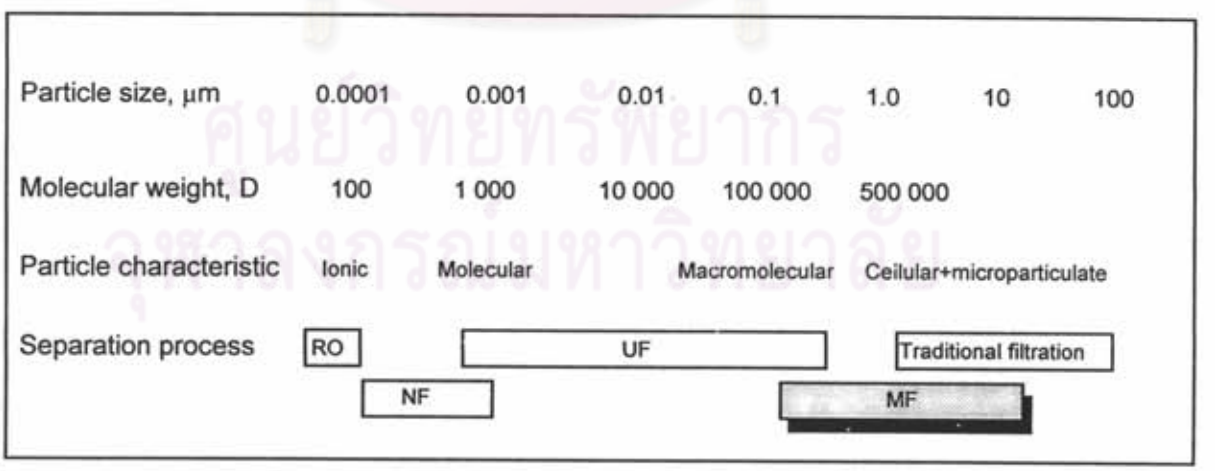


Figure 3-11 Spectrum of application of membrane separation processes in the industry

Note : RO, UF and NF stands for Reverse Osmosis, Ultrafiltration and Nanofiltration, respectively.

It should be mentioned here that traditional or conventional filtration, also called dead-end filtration, is usually used for separation of suspended particles larger than 1 μm. Several differences can be noted between conventional filtration and membrane microfiltration; conventional filters are thick with open structures and typically use papers as filter media whereas membrane microfiltration filters are thin and fairly controlled pore size and normally use polymers or ceramics as filter media [26].

In conventional filtration, gravity is the main force affecting particle separation. Pressure may be applied only to accelerate the process. The feed flow is perpendicular to the filter medium, and filtration can be conducted in open systems. The retained particles building up with time form a cake layer, and this results in an increased resistance to filtration and causes declination in permeate flux rate if pressure drop is held constant, or causes the increasing of pressure drop to increase if the flux rate is held constant. As a result, the dead-end filtration process must be terminated periodically in order to remove the retained particle or to replace the filter medium.

In membrane filtration, the use of pressure is essential as the driving force for separation and a cross-flow or tangential flow design is followed. Filtration must be carried out in a closed system. In cross-flow design, the feed solution runs parallel to membrane surface and the permeate flows perpendicular to the filtration membrane. The permeate flow carries particles to the membrane surface, where they are rejected and form a thin cake layer. Unlike dead-end filtration, this cake layer does not build up indefinitely. Instead, the high shear exerted by the suspension flowing tangential to the membrane surface sweeps the deposited particles toward the filter exit so that the cake layer remains relatively thin. A steady or quasi-steady flux is achieved once the cake layer has reached its steady-state thickness. In practice, long-term flux decline is sometimes observed even after cake buildup has stopped. This may be the result of cake or membrane compaction or of membrane fouling. Examples of different membrane configurations are shown in Figure 3-12. And the schematics of dead-end filtration and cross-flow filtration are illustrated in Figure 3-13.

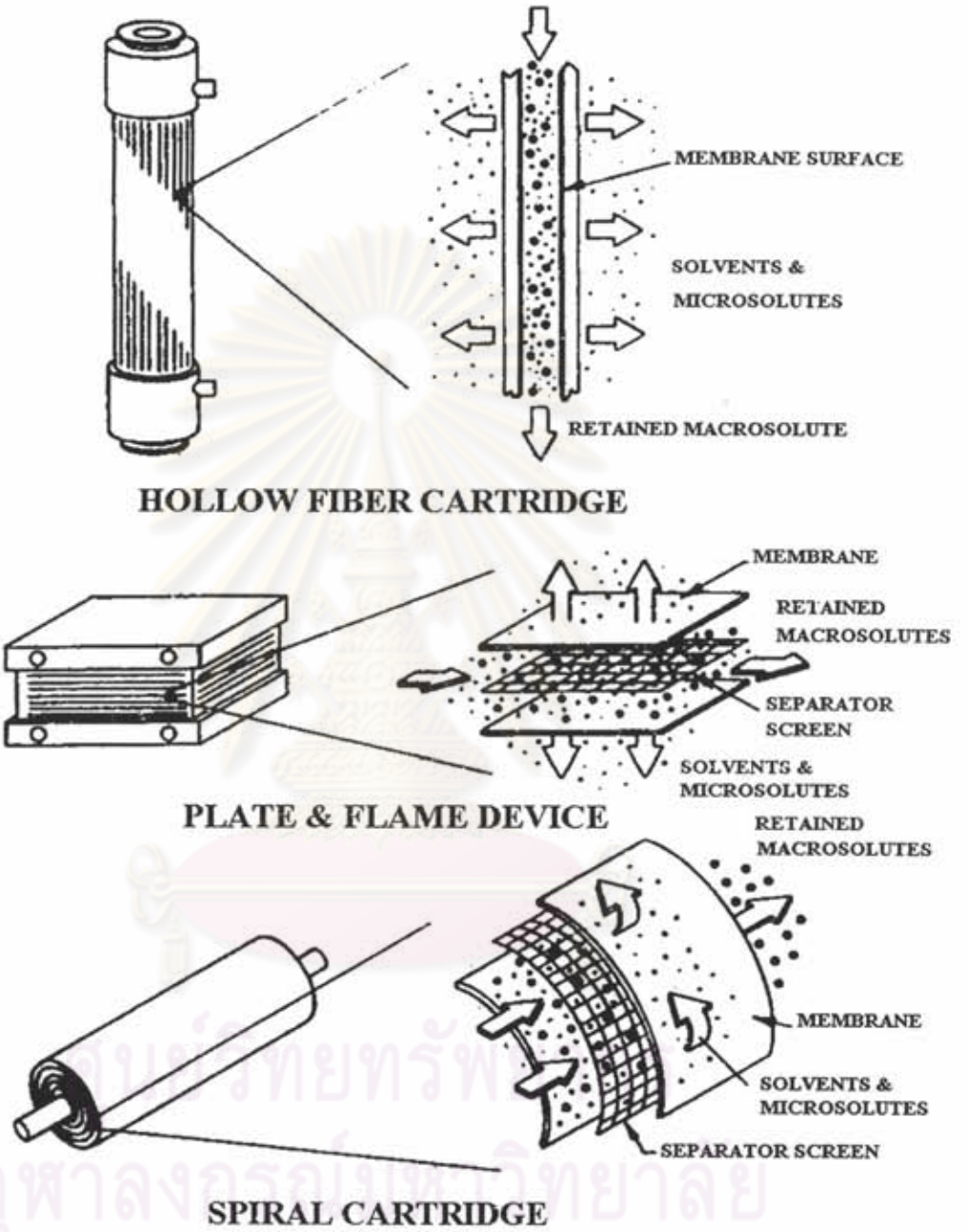


Figure 3-12 The examples of different membrane configurations

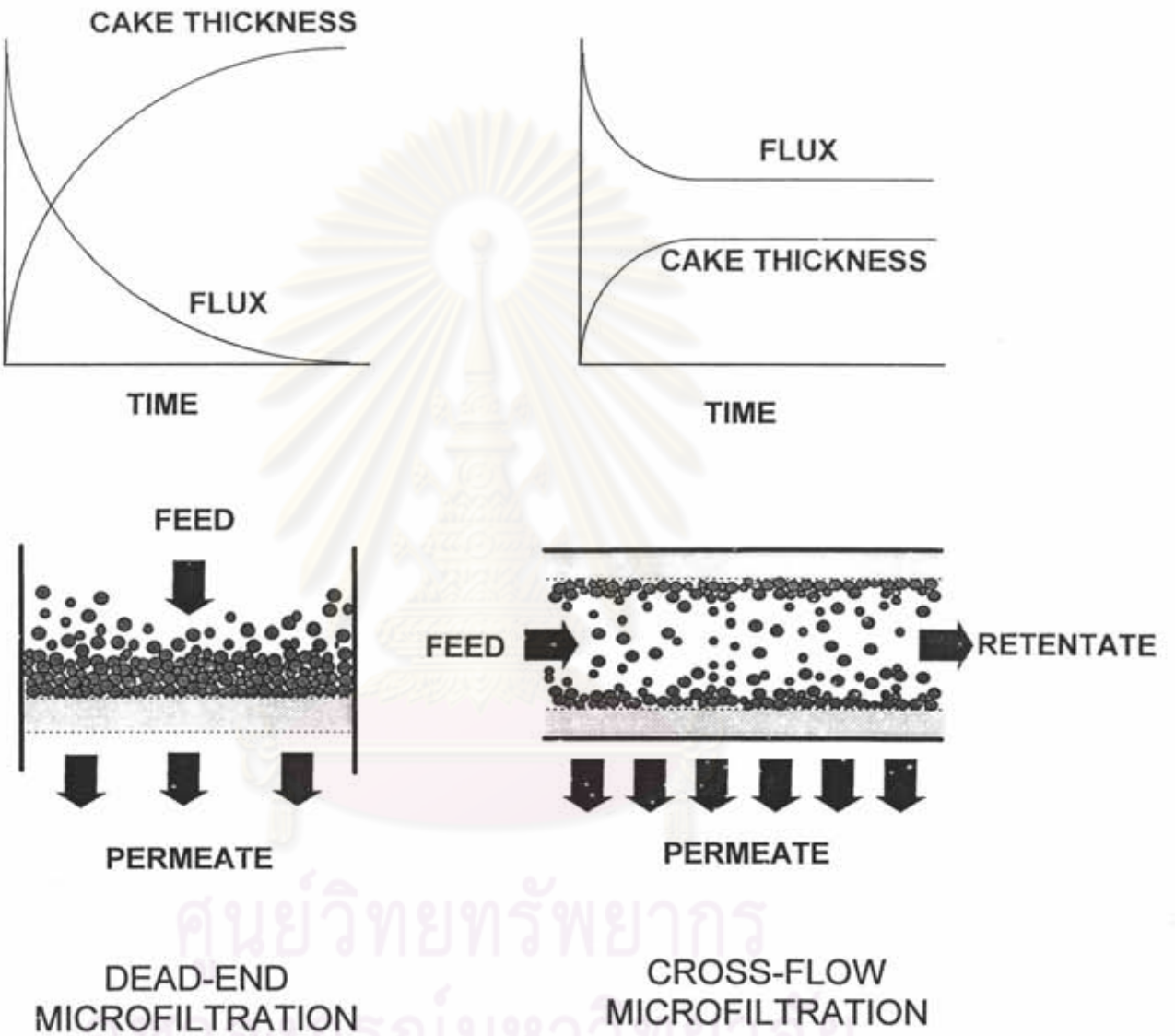


Figure 3-13 The schematics of dead-end filtration and cross-flow filtration

3.3.1 Mass transfer and gel polarization

Deposition of macromolecule on the surface causes concentration gradient between membrane surface and bulk fluid. This phenomenon is known as concentration polarization which is back diffusion of macromolecules or macrosolutes from the membrane surface, gel layer, to the bulk solution (Figure 3-14) due to the concentration gradient described above.

At steady state condition, the mass balance equation of solute concentration is affected by the convective mass transfer toward the membrane (JC) and the back diffusive movement (DdC/dx) as follows :

$$JC - \frac{DdC}{dx} = JC_p \qquad (3\text{-}1)$$

where

J	=	water flux or permeate flux, in case of broth used ($m^3/m^2.hr$)
C	=	macromolecule concentration at x position (g/l)
D	=	macromolecule diffusivity (m^2/hr)
x	=	fluid boundary layer thickness (m)
C_p	=	macromolecule concentration at permeate side (g/l)

Integration of Equation 3-1 beyond the boundary conditions of $C = C_b$ at $x = 0$ and $C = C_w$ at $x = \delta$ with assumption of no macromolecule concentration at permeate side yields :

$$J = D/\delta \ln (C_w/C_b) \qquad (3\text{-}2)$$

or

$$J = k \ln (C_w/C_b) \qquad (3\text{-}3)$$

where

δ = film thickness (m)

k = mass transfer coefficient (m/hr)

C_w, C_b = macromolecule concentration in water and bulk fluid (g/l)

In practice, as the flux increases, the value of C_w is increased as well and the maximum value of C_w is C_g where gel layer is formed so a mass balance equation for a gel formation (Figure 3-14 B) can be obtained from substitution of C_w with C_g as shown in Equation 3-4. Apart from macrosolute concentration, gel formation depends on the nature of solute, pH and pressure. When a gel layer is formed, the permeate flux decreases logarithmically with increasing macrosolute concentration in bulk fluid because the gel layer causes hydraulic resistance against flow and acts somewhat like a second membrane.

$$J_v = k \ln \{C_g/C_b\} \quad (3\text{-}4)$$

where

C_g = maximum value of C_w

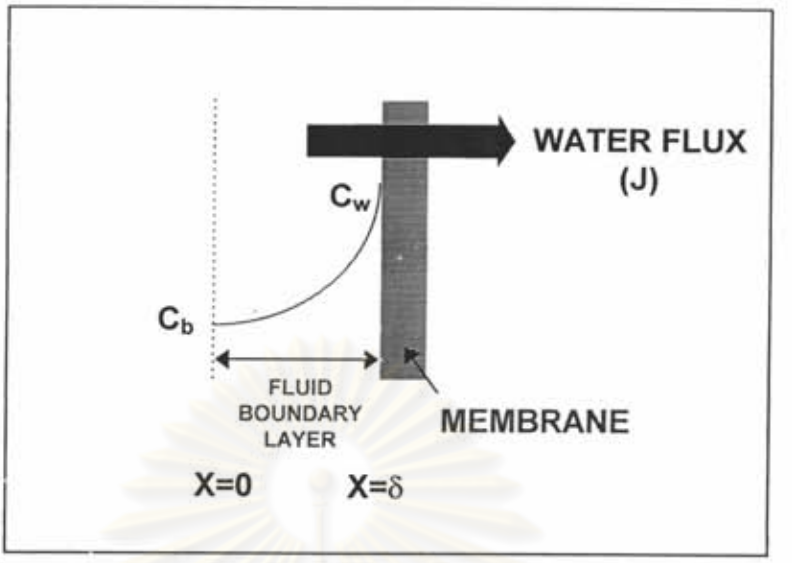
C_g is dependent upon operating pressure, temperature, solubility, filter channel shape, fluid flow and pH. Actually, C_g is the concentration where osmotic back-pressure is high enough to prevent the permeate flux. The mass transfer coefficient (k) is normally not a function of macrosolute concentration but depends on the driving pressure and any fluid flow across the membrane as illustrated in various terms of dimensionless number in Equation 3-5

$$Sh = dk/D_e = A\,Re^B\,Sc^{1/3} \quad (3\text{-}5)$$

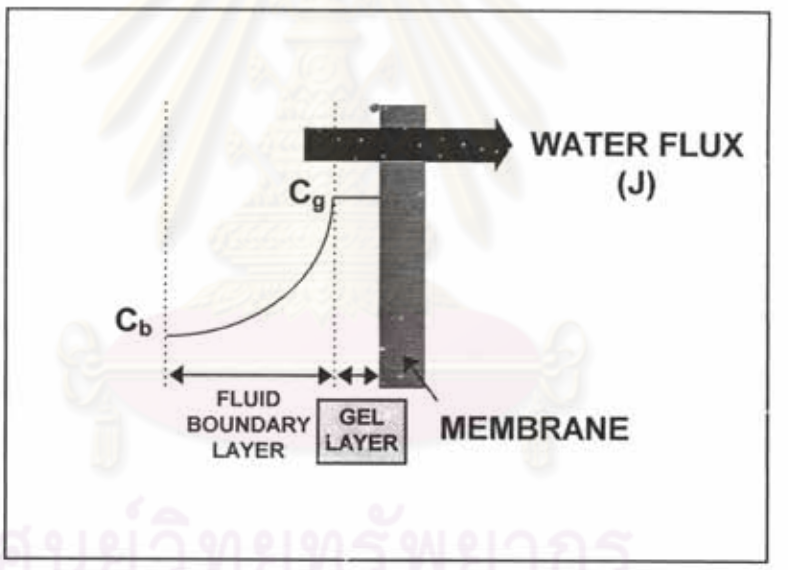
and

$$Re = (dU\rho)/\beta \quad (3\text{-}6)$$

$$Sc = \beta/(\rho D_e) \quad (3\text{-}7)$$



(A)



(B)

Figure 3-14 Concentration gradient in microfiltration process

(A) without gel formation (B) with gel formation

where

Sh	=	Sherwood number (-)
d	=	fluid channel height over the membrane (m)
A	=	constant number (-)
B	=	0.5 (-) for laminar flow and 1.0 (-) for turbulent flow
Re	=	Reynold number
Sc	=	Schmidt number
U	=	velocity of feed fluid (m/hr)
ρ	=	density of feed fluid (g/m^3)
β	=	viscosity of feed fluid (g/m-s)

In conclusion, k is proportional to square root of fluid flow for the laminar system, and is proportional to the fluid flow for the turbulent one.

Gel formation can be partially eliminated by cross-flow filtration, where pressure is not applied directly perpendicular to the membrane, but parallel to it. That is why this operation governs the movement of a macrosolute away from the membrane, and therefore reduces the thickness of the gel layer as demonstrated in Figure 3-15 . Mechanical agitation or vibration of the membrane surface can also be employed to alleviate the gel formation.

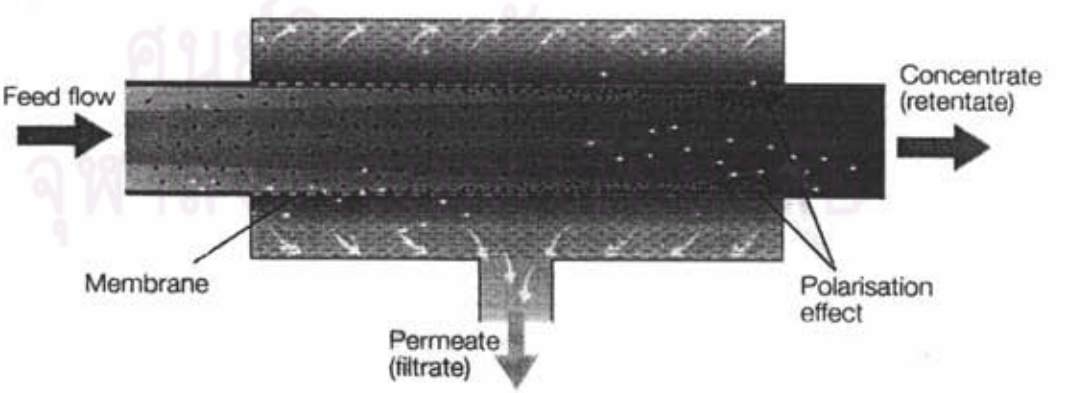


Figure 3-15 Cross-flow microfiltraion model

The main principle of cross-flow microfiltration is the fact that the feed flows tangentially with pressure difference as shown in Figure 3-16

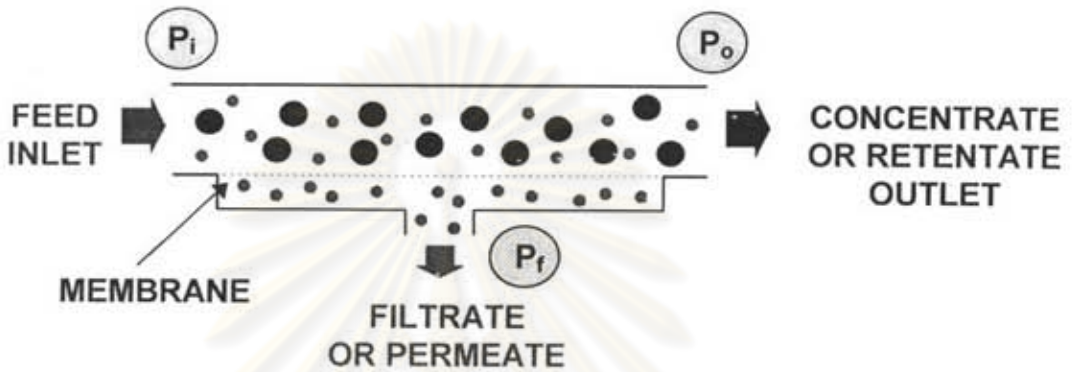


Figure 3-16 Cross-flow filtration principle

Being a driving force, the transmembrane pressure drop (ΔPTM) can be evaluated from pressure drop (ΔP) as follows :

$$\Delta PTM = \{(P_i + P_o)/2\} - P_f \quad (3\text{-}8)$$

$$\Delta P = P_i - P_o \quad (3\text{-}9)$$

in case of permeating out at atmospheric pressure

$$\Delta PTM = P_i - (\Delta P/2) \quad (3\text{-}10)$$

For laminar flow, with the use of Hagen - Poiseuille equation

$$\Delta P = (C_1 \beta L V)/d^2 = (C_2 \beta L Q)/d^4 \quad (3\text{-}11)$$

where

β = viscosity of feed fluid (g/m.hr)

V = velocity of feed fluid (m/hr)

Q = feed flow rate (m^3/hr)

L = length of filter (m)

C_1, C_2 = channel shape factor (-)

For turbulent flow,

$$\Delta P = (C_3 f L U^2)/d = (C_4 f L Q^2)/d^5 \quad (3\text{-}12)$$

where

f = Fanning factor (-) ,be a function of Re

C_3, C_4 = channel shape factor (-)

The flux evaluated from ΔPTM is shown in the Equation 3-13

$$J = \Delta PTM/(R_M + R_G) \quad (3\text{-}13)$$

where

R_M = Hydraulic resistance of membrane

R_G = Hydraulic resistance of gel layer

Hydraulic resistance of membrane is constant in any membrane; however, hydraulic resistance of gel layer varies with the macrosolute concentration and tangential velocity across the membrane which can retard or eliminate gel formation. The filtration flux is a function of fluid velocity, as described by Equation 3-10 to 3-13. Usually, there is an optimal fluid velocity range maximizing the filtration rate. At low fluid velocities, the mass transfer coefficient is low resulting in high gel resistance, and low filtrate flux. At high fluid velocities, ΔP is high resulting in low ΔPTM, and therefore low filtrate flux. In the same way, there are optimal values of ΔPTM maximizing the filtration rate. The inlet pressure (P_i) is circumscribed by the physical properties of membrane. With modern membranes, especially new ceramic membrane, it is possible to apply P_i to a very high value. Besides that, permeate flux

increases with ΔPTM when the range of low ΔPTM is applied; however, at high ΔPTM, gel formation occurs resulting in rising gel resistance along with ΔPTM, and this results in relatively constant filtration flux.

In conclusion, permeate flux of suspended solution depends on four parameters as follows :

1. Pressure (P_i) : Equation 3-10 and 3-13

The permeate flux is proportional to pressure up to the point when a gel layer is formed. The further increase in pressure will increase the gel thickness until the constant permeate flux is achieved.

2. Recirculation flow rate (v) : Equation 3-4 , 3-5 and 3-10

Mass transfer coefficient is proportional to recirculation velocity. Moreover, increasing recirculation velocity will cause the sweeping of some macromolecules over membrane surface by increasing shear force, hence gel thickness and gel resistant are decreased. However the average ΔPTM will decrease with increasing recirculation velocity.

3. Temperature (T)

Being the function of temperature, mass transfer coefficient increases with temperature resulting in an increase of the permeate flux rate.

4. Concentration (C) : Equation 3-4

Generally, the more solute concentration increases in feed solution, the less permeate flux is detected which means the poorer filtration has occurred.

3.3.2 Membrane rejection

$$R = (C_b - C_p)/C_b = 1 - (C_p/C_b) \qquad (3\text{-}14)$$

The rejection coefficient of microfiltration is defined by Equation 3-14 which indicates that its value must be in the range between 0 to 1. If the filtration is complete which means every solute is rejected across the membrane (C_p=0), the rejection coefficient will be 1. On the other hand, when the rejection coefficient is zero, macrosolute is detected in permeate side. Therefore R is dependent on membrane selectivity and macrosolute concentration.

3.4 Fermentation process and mathematical analysis

Fermentation is a bioprocess involving microbial cells. Raw materials, usually biomass, are treated and mixed with other ingredients that are required for cells to grow well. The liquid mixture, the medium, is sterilized to eliminate all other living microorganisms and introduced to a large cylindrical vessel, bioreactor or fermenter, typically equipped with agitators, baffles, air spargers, and various sensing devices for the control of fermentation conditions. A pure strain of microorganisms is introduced into the vessel. The number of cells will start to multiply exponentially after a certain period of lag time and reach a maximum cell concentration as the medium is depleted. The fermentation will be stopped and the contents will be pumped out for the product recovery and purification. This process can be operated either by batch or continuously [19].

3.4.1 Basic mathematical equation for fermentation process

The general material balance equations for growth, substrate utilization and product formation are defined as the following equations.

Cell (X) growth ;

Cell mass accumulation	=	Cell in	-	Cell out	+	Cell growth	-	Cell death	
dX/dt	=	FX_o/V	-	FX/V	+	μX	-	γX	(3-15)

Substrate (S) utilization ;

Substrate mass accumulation = Substrate in - Substrate out - Substrate for growth - Substrate for *M* - Substrate for *P*

where *M* and *P* stand for Maintenance and Product formation, respectively.

$$dS/dt = FS_o/V - FS/V - \mu X/Y_{x/s} - mX - \upsilon X/Y_{p/s} \quad (3\text{-}16)$$

Product (P) formation ;

Product mass accumulation	=	Product in	-	Product out	+	Product synthesis	-	Product destruction	
dP/dt	=	FP_o/V	-	FP/V	+	υX	-	KP	(3.17)

where

X	=	Cell concentration (g-cell/l)
F	=	Volumetric flow rate (l/hr)
V	=	Working volume (l)
K	=	Product destruction rate (hr^{-1})
S	=	Substrate concentration (g-substrate/l)
μ	=	Specific growth rate (hr^{-1})
γ	=	Specific dead rate (hr^{-1})
υ	=	Specific product rate (g-product/g-cell-hr)
m	=	Maintenance factor (hr^{-1})

$Y_{x/s}$ = Cell Yield (g-cell/g-substrate) = $\frac{\Delta X}{\Delta S}$

$Y_{p/s}$ = Product Yield (g-product/g-substrate) = $\frac{\Delta P}{\Delta S}$

Subscript (o) stands for initial condition

The equations mentioned above are applicable for both steady and unsteady fermentation process.

3.4.1.1 Batch fermentation

For simplicity, some assumptions are made as the following items.

1. At the exponential growth phase :

$$\mu >> \gamma \qquad (3\text{-}18)$$

2. At the product formation phase :

$$\upsilon >> K \qquad (3\text{-}19)$$

3. At the substrate consumption phase :

$$\mu X/Y_{x/s} >> mX \qquad (3\text{-}20)$$

There are no inlet and outlet feed rate in batch operation hence F is zero. And together with Equation 3-18 to 3-20, the Equation 3-15 can be simplify as follow :

$$\frac{1}{X}\frac{dX}{dt} = \mu \qquad (3\text{-}21)$$

integration of equation 3.21 yields

$$\ln\frac{X}{X_0} = \mu t \qquad (3\text{-}22)$$

This equation can be applied to obtain the doubling time (t_d) for the binary fission of microorganisms in log phase as follows :

$$t_d = 0.693/\mu \quad (3\text{-}23)$$

With the same fashion, the Equation 3-16 and 3-17 can be simplify as follows

$$\upsilon = \frac{1}{X}\frac{dP}{dt} \quad (3\text{-}24)$$

$$\upsilon_S = \frac{1}{X}\frac{dS}{dt} \quad (3\text{-}25)$$

$$Y_{X/S} = \frac{\Delta X}{\Delta S} \quad (3\text{-}26)$$

$$Y_{P/S} = \frac{\Delta P}{\Delta S} \quad (3\text{-}27)$$

where

υ_S = Specific consumption rate (g-substrate/g-cell-hr)

Monod Equation

$$\mu = \frac{\mu max.S}{Ks + S} \quad (3\text{-}28)$$

3.4.1.2 Continuous fermentation coupling with microfiltration

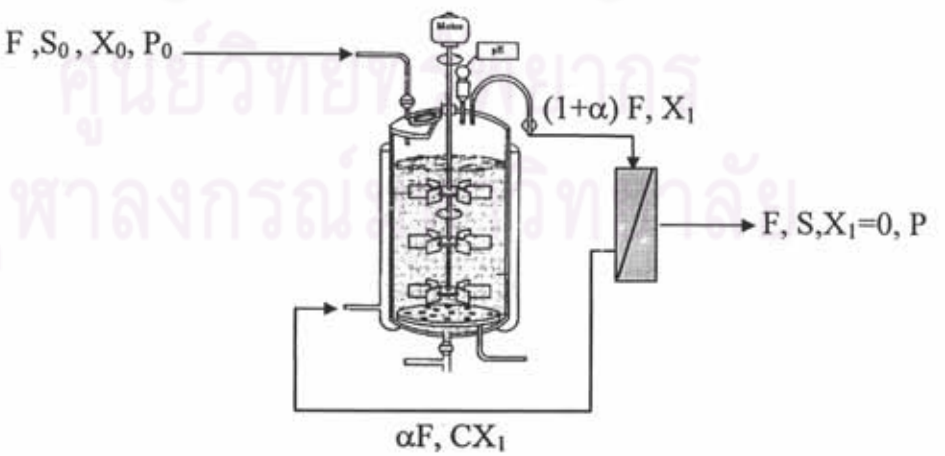


Figure 3- 17 Continuous fermentation coupling with microfiltration

From Figure 3-17, the general material balance equations for growth, substrate utilization and product formation are defined as the following equations.

Cell (X) growth ;

Cell mass accumulation = Cell in + Cell recycle - Cell out + Cell growth - Cell death

$$\frac{dX_1}{dt} = \frac{FX_0}{V} + \frac{\alpha F.CX_1}{V} - \frac{(1+\alpha)F.X_1}{V} + \mu X_1 - \gamma X_1 \quad (3\text{-}29)$$

Substrate (S) utilization ;

Substrate mass accumulation = Substrate in + Substrate recycle - Substrate out - Substrate for growth - Substrate for *M* - Substrate for *P*

where *M* and *P* stand for Maintenance and Product formation, respectively.

$$dS/dt = \frac{FS_o}{V} + \frac{\alpha FS}{V} - \frac{(1+\alpha)FS}{V} - \mu X_1/Y_{x/s} - mX_1 - \upsilon X_1/Y_{p/s} \quad (3\text{-}30)$$

Product (P) formation ;

Product mass accumulation = Product in + Product recycle - Product out + Product synthesis - Product destruction

$$dP/dt = \frac{FP_o}{V} + \frac{\alpha FS}{V} - \frac{(1+\alpha)FP}{V} + \upsilon X_1 - KP \quad (3\text{-}31)$$

For simplicity, some assumptions are made as the following items.

1. At the exponential growth phase :

$$\mu >> \gamma \qquad (3\text{-}18)$$

2. At the product formation phase :

$$\upsilon >> K \qquad (3\text{-}19)$$

3. At the substrate consumption phase :

$$\mu X/Y_{x/s} >> mX \qquad (3\text{-}20)$$

Under these assumptions, the Equation 3-29 to 3-31 are modified as follows:

$$dX/dt = (\mu + \alpha CD - (1+\alpha)D)\,X \qquad (3\text{-}32)$$

$$dS/dt = D(S_o - S) - \mu X/Y_{x/s} - \upsilon X/Y_{p/s} \qquad (3\text{-}33)$$

$$dP/dt = \upsilon X - DP \qquad (3\text{-}34)$$

At steady state

$$\mu = (1+\alpha-\alpha C)D \qquad (3\text{-}35)$$

$$\upsilon = (1+\alpha-\alpha C)DP/X \qquad (3\text{-}36)$$

$$Y_{x/s} = \mu X/((S_o-S)D-\upsilon X/Y_{p/s}) \qquad (3\text{-}37)$$