CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Surfactants

The term surfactant was coined by Antara Products in 1950, which is a blend of word "SURface ACTive AgeNT". Surfactant is amphiphathic molecules with both hydrophobic (tail) and hydrophilic (head) moieties as shown in the Figure 2.1 Therefore, they can help to reduce surface tension and capable interact with phase boundary between two phase in a heterogeneous system (Myers, 1992). A micelle - the lipophilic ends of the surfactant molecules dissolve in the oil, while the hydrophilic charged ends remain outside, shielding the rest of the hydrophobic micelle.

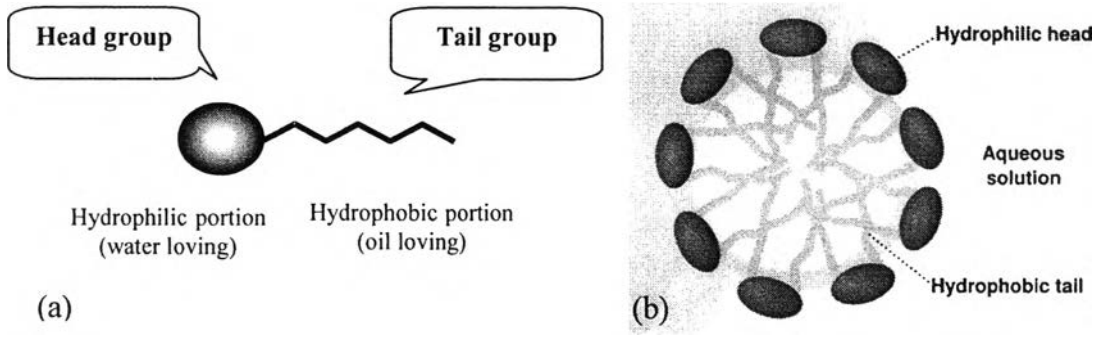


Figure 2.1 Schematic of a surfactant molecule (a) and a micelle (b)

2.1.1 Operation and Effects of Surfactants

Surfactants reduce the surface tension of water by adsorbing at the liquid-gas interface. They also reduce the interfacial tension between oil and water by adsorbing at the liquid-liquid interface. Many surfactants can also assemble in the bulk solution into aggregates. Some of these aggregates are known as micelles. The concentration at which surfactants begin to form micelles is known as the critical micelle concentration or CMC. When micelles form in water, their tails form a core that can encapsulate an oil droplet, and their (ionic/polar) heads form an outer shell

that maintains favorable contact with water. When surfactants assemble in oil, the aggregate is referred to as a reverse micelle. In a reverse micelle, the heads are in the core and the tails maintain favorable contact with oil.

The effectiveness of a surfactant is determined by its ability to lower the surface tension which is a measure of the surface free energy per unit area require to bring a molecule from the bulk phase to the surface (Rosen, 1978). The surface tension correlates concentration of surfactant until the critical micelle concentration (CMC) is reached as show in Figure 2.2 (Mulligan *et al.*, 2001).

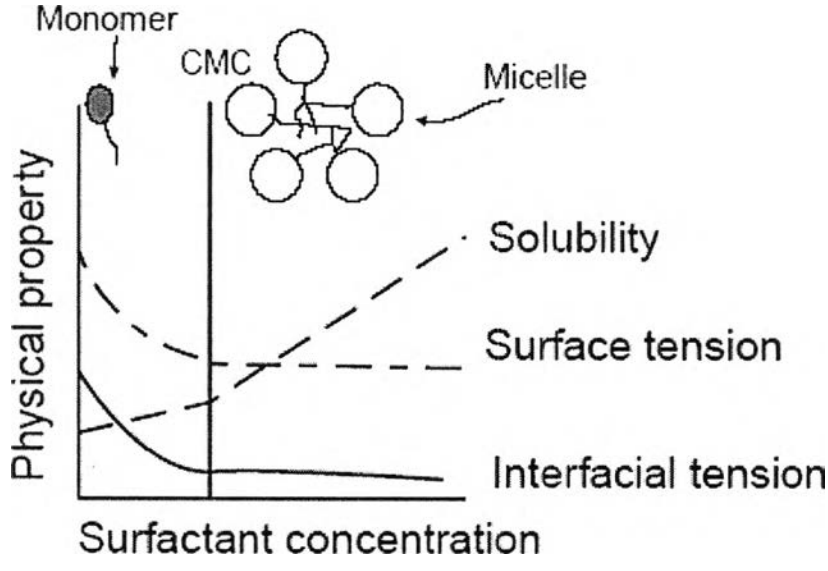


Figure 2.2 Schematic diagram of the variation of surface tension, interfacial and contaminant solubility with surfactant concentration.

2.1.2 Classification of Surfactants

Surfactants are often classified into four primary groups; anionic, cationic, non-ionic, and zwitterionic (dual charge). The classification is based on the nature of hydrophile, with subgroups being based on the nature of the hydroprobe. The four basic classes of surfactant are defined as below (Myers, 1992):

1. Anionic, with the hydrophilic group carrying a negative charge such as carboxyl ($RCOO^-M^+$), sulfonate ($RSO_3^-M^+$), or sulfate ($ROSO_3^-M^+$).

2. Cationic, with the hydrophilic bearing a positive charge, as for example, the quaternary ammonium halides ($R_4N^+Cl^-$).

3. Nonionic, where the hydrophilic has no charge, but derives its water solubility from highly polar group such as poloyoxyethylene ($-OCH_2CH_2O-$) or polyol groups.

4. Amphoteric (and zwitterionic), in which the molecule contains, or can potentially contain, both a negative and positive charge, such as the sulfobetaines $RN^+(CH_3)_2CH_2CH_2SO_3^-$

Moreover, surfactant can be classified into two main groups: synthetic surfactants and biosurfactants.

2.2 Biosurfactants

Due to the environmental problem from synthetic surfactant, many researcher pay attention on biosurfactants—producded from variety of microorganism such as bacteria, fungi and yeast—because of the numerous advantages of biosurfactants, such as mild production conditions, lower toxicity, higher biodegradability, biocampatibitity and environmental compatibility. All of these have prompted applications not only in the food, cosmetic, pharmaceutical industries and medical field (Rodrigues *et al.*, 2006) but also in environmental protection and energy-saving technology (Banat *et al.*, 2000; Cameotra and Makkar., 1998).

Biosurfactants (microbial surface active agents) are biomolecules containing both a lipophilic and hydrophilic moieties like chemical surfactants. The lipophilic portion is the hydrocarbon chain of a fatty acid or sterol ring whereas the hydrophilic or polar portion is the carboxyl group of fatty acids or amino acids, the phosphoryl group of phospholipids, hydroxyl group of saccharides, and peptides (Morikawa *et al.*, 2000). They produced by a wide variety of microorganisms (e.g. bacteria, yeasts, and filamentous fungi) which are able to grow on water-insoluble substrates like *n*-alkanes or vegetable oils, using it as a carbon source. These microorganisms have been adapted to feed by using the substrates, producing biosurfactant, which help them to adsorb, emulsify, wetting, solubilizing or dispersing the water-immiscible material (Zouboulis *et al.*, 2003).

Biosurfactants are commonly differentiated on the basis of their biochemical nature and the microbial species producing them, Major classes of biosurfactants include: Glycolipids type, Phospholipids type, Lipopeptides and lipoproteins type and Polymeric type (Healy *et al.*, 1996)

2.2.1 Types of Biosurfactants

Biosurfactants are mainly classified into four categories based on their biochemical nature and the microbial species. All of these are:

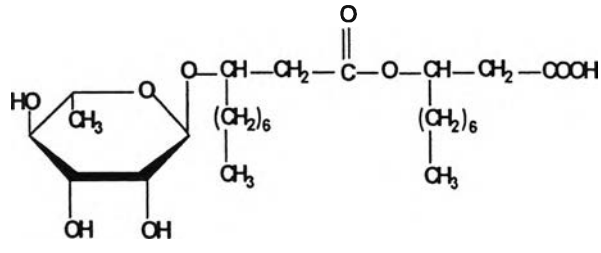
2.2.1.1 *Glycolipids*

Glycolipid (the most commonly studied biosurfactants) are carbohydrates in combination with long-chain aliphatic acids or hydroxyl aliphatic acids. The main glycolipids which are studied from the point of view of surfactant characterization and properties are (A) rhamnolipid, (B) trehalose lipids, and (C) sophorolipids.

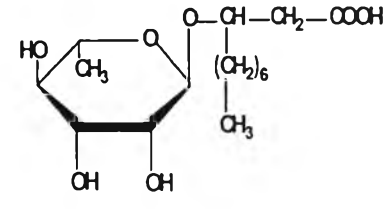
2.2.1.1.1 *Rhamnolipid*

Rhamnolipid is the one of best-studied glycolipids, produced by several species of *Pseudomonas.* They produces mainly two types of rhamnolipid, consists of one or two molecules of rhamnose linked to one or two molecules of β-hydroxydecanoic acid that called monorhamnolipid and dirhamnolipid respectively (Edwards *et al.*, 2002). The amounts of different types in the culture liquid was about 90% rhamnolipid 3 and 10% rhamnolipid 1 whereas rhamnolipid 2 and rhamnolipid 4 occurred only in trace amounts. The schematic reported by Kosaric, 1993 have demonstrated the structure of rhamnolipids as show in figure 2.3.

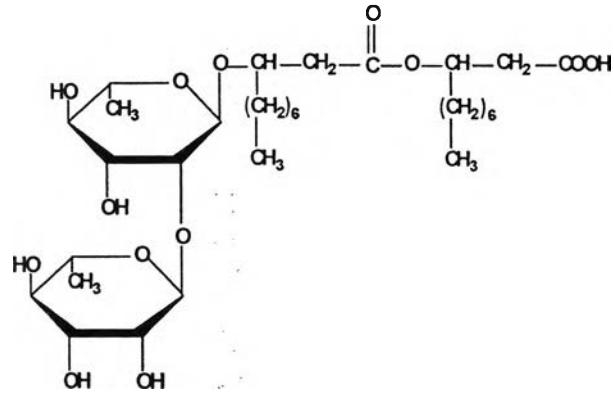
a) Rhamnolipid 1



b) Rhamnolipid 2



c) Rhamnolipid 3



d) Rhamnolipid 4

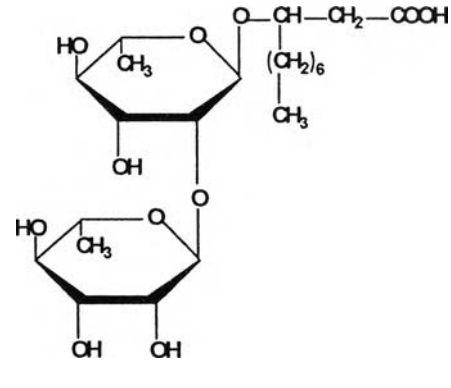


Figure 2.3 Schematic representation of four different rhamnolipids produced by *P.aerugonosa.*

2.2.1.1.2 *Trehalose Lipids*

The structure of trehalose lipids are found to be widely distributed. Their disaccharide trehalose linked at C6 and C6′ to mycolic acids is associated with most species of *Mycobacterium, Nocardia, Corynebacterium Arthrobacter* and *Brevibacterium.* Mycolic acids are long-chain, a-branched-bhydroxy fatty acids. Trehalolipids from different organisms differ in the size and structure of mycolic acid, the number of carbon atoms, and the degree of unsaturation. Desai and Banat, (1997) showed the structure of trehalose dimycolate produced by *Rhodococcus erythropolis* as in Figure 2.4.

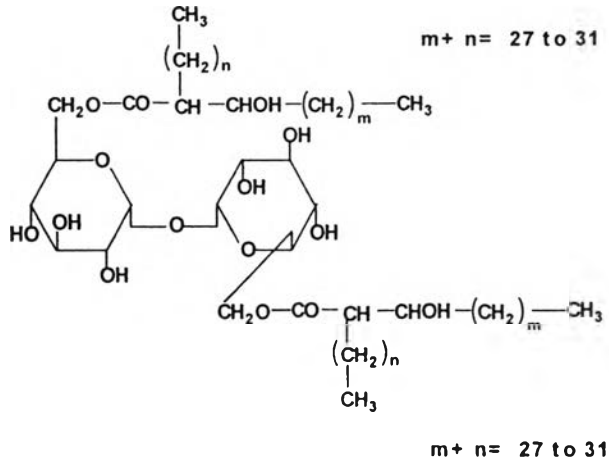


Figure 2.4 Trehalose dimycolate from *Rhodococcus erythropolis*, in which disaccharide trehalose is linked to two long-chain α-branched β-hydroxy fatty acids.

2.2.1.1.3 *Sophorolipids*

Sophorolipid biosurfactants, mostly produced by yeasts such as *Torulopsis bombicola*, *Torulopsis petrophilum,* and *Torulopsis apicola*, consist of a dimeric carbohydrate sophorose linked to a long-chain hydroxy fatty acid as see in Figure 2.5.

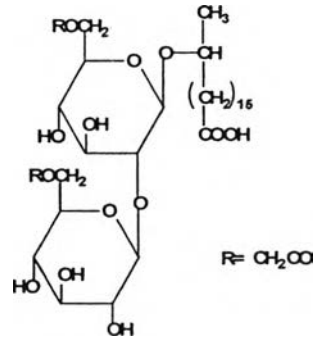


Figure 2.5 Sophorolipid from *Torulopsis bombicola* in which dimeric sophorose is linked to a long-chain (C18) hydroxy fatty acid.

Sophorolipids produced by *T. petrophilum* which grow on water-insoluble substrates such as alkanes and vegetable oils whereas some sophorolipids, which were chemically identical to those produced by *T. bombicola*, did not

emulsify alkanes or vegetable oils. Although sophorolipids can lower surface and interfacial tension, they are not effective emulsifying agents (Desai and Banat, 1997).

2.2.1.2 Lipoproteins or lipopeptids

These consist of a lipid attached to a polypeptide chain. One of the most effective cyclic lipopeptide biosurfactants is surfactin (SF) produced by *Bacilus subtilis* ATCC 21332. It is capable of lowering the surface tension from 72 to 27.9 mN/m at a concentration as low as 0.005%. Desai and Banat, (1997) showed the structure of surfactin as in Figure 2.6.

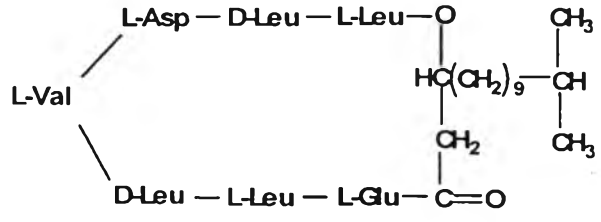


Figure 2.6 Structure of cyclic lipopeptide surfactin produced by *Bacillus subtilis.*

2.2.1.3 Phospholipids and Fatty acids

Phospholipids and Fatty acids are produced by bacteria and yeasts when grown on *n*-alkanes. Their structure are ester form between the alcohol group on a lipid and a phosphate. *Rhodococcus erythropoli* produced Phosphatidyle-thanolamine on *n*-alkane caused a reducing of interfacial tension (Desai and Banat, 1997). In Figure 2.7, showed the structure of Phosphatidylethanolamine.

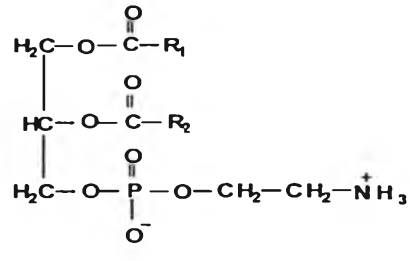


Figure 2.7 Structure of phosphatidylethanolamine, a potent biosurfactant produced by *Acinetobacter* sp. R_1 and R_2 are hydrocarbon chains of fatty acids.

2.2.1.4 Polymeric biosurfactants

These kind of biosurfactants have high molecular weight biopolymer generally demonstrate useful properties such as, high viscosity, tensile strength, and resistance to shear. Accordingly, polymeric biosurfactants have found a variety of industrial uses. Emulsan, liposan, mannoprotein, and other polysaccharide-protein complexes are the best studied of these biosurfactants.

2.2.1.4.1 *Emulsan*

Emulsan has been characterized as a polyanionic amphiphatic heteropolysaccharide as show in Figure 2.8.

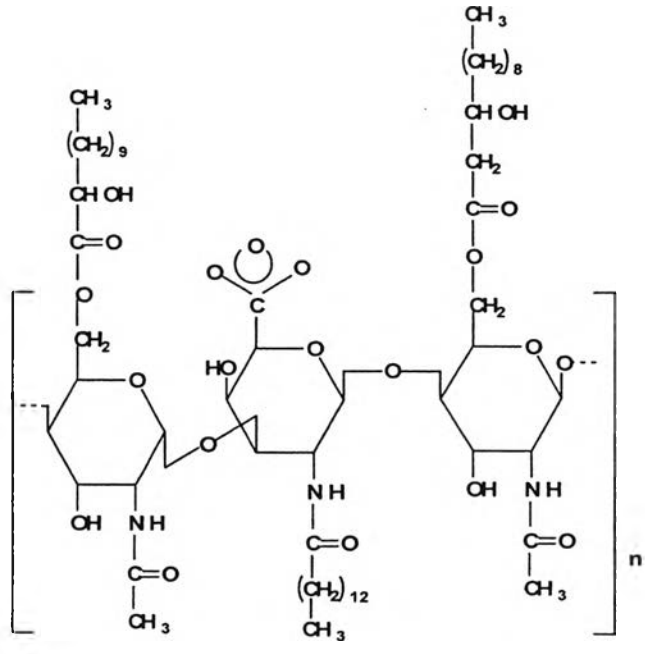


Figure 2.8 Structure of emulsan, produced by *Acinetobacter calcoaceticus*, in which fatty acids are linked to a heteropolysaccharide backbone.

2.2.1.4.2 *Biodispersan*

Biodispersan is an extracellular, nondialyzable dispersing agent produced by *A.calcoaceticus* A2. The active component of biodispersan is an anionic heteropolysaccharide, with an average molecular weight of 51,400 and four reducing sugars (glucosamine, 6-methylaminohexose, galactosamine uronic acid, and an unidentified amino sugar).

2.2.1.4.3 *Liposan*

Liposan is an extracellular water-soluble emulsifier synthesized by *Candida lipolytica* which composed of 83% carbohydrate (heteropolysaccharide containing glucose, galactose, galactosamine, and galacturonic acid) and 17% protein.

Among of these, the most studies focused on Glycolipids—rhamnolipids produced by Pseudomonas aeruginosa strains (Cameotra and Makkar, 2004). As mention above, this type of biosurfactant is composed of carbohydrates in a combination with long-chain aliphatic acids or hydroxyl aliphatic acids. As shown in Figure 2.9 result in extremely efficiency of reducing surface tension and increasing emulsion index with low critical micelle concentration(CMC), Furthermore, it exhibited good thermal and pH stability (Pornsunthorntawee *et al.*, 2008).

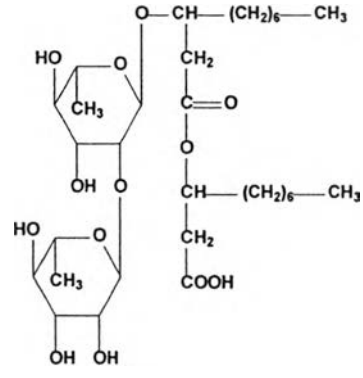


Figure 2.9 The biosurfactant structure(Di-Rhamnolipid produced by *Pseudomonas* spp.)

2.3 Factors Affecting Biosurfactants Production

2.3.1 Carbon Sources

Carbon sources, the raw materials used to produce rhamnolipid biosurfactants. It can divided into water-soluble carbon sources (e.g. glycerol, glucose, mannitol, and ethanol) and water-immiscible substrates (e.g. n-alkanes and vegetable oil) (Rashedi *et al.*, 2006). They influences biosurfactant synthesis by either induction or repression (Makkar and Cameotra, 2002). The different carbon sources in the

medium affected the composition of biosurfactant production in *Pseudomonas* spp. substrates with different chain lengths exhibited no effect on the chain lengths of fatty acid moieties in glycolipids.

Mata-Sandoval *et al.* (1999) demonstrated that rhamnolipid mixture ($RhC_{10}C_{10}$, $Rh_2C_{10}C_{10}$, $Rh_2C_{10}C_{12}$, $Rh_2C_{10}C_{12}H_2$) produced by *Pseudomonas aeruginosa* UG2 grow on corn oil as a sole carbon.

Benincasa *et al.* (2002) studied the effect of the carbon source on rhamnolipid production by *Pseudomonas aeruginosa* LBI. The different oily substrates were produced different amounts of rhamnolipid biosurfactants. The most suitable substrates were the residues containing soapstock (the waste from sunflower oil process), 12 g/l of rhamnolipids. When crude oleic acid, soy bean oil and sunflower oil, olive oil were supplied 4.5, 4.8, 4.9, 5.4 g/l of rhamnolipids were produced, respectively.

Wei *et al.* (2005) evaluated a variety of carbon substrates, including hydrophilic substrates (glucose, glycerol), vegetable oils (sunflower oil, grape seed oil, and olive oil), and mineral oils (diesel and kerosene) were examined for their effectiveness on rhamnolipid production from *Pseudomonas aeruginosa* J4. The results showed a general trend that rhamnolipid production initially increased with increasing carbon substrate concentration, until it reached a maximum value and then leveled off. However, only glycerol behaved differently, as the rhamnolipid level decreased sharply when glycerol concentration was over 2% resulting in negligible rhamnolipid production in the culture. Olive oil was an excellent carbon source for rhamnolipid production with a maximum rhamnolipid concentration of nearly 3.6 g/L occurred at an olive concentration of 10%. Sunflower oil and grape seed oil (both at a concentration of 6%) achieved a maximum concentration of 2.0–2.1 g/L.

Rashedi *et al.* (2006) showed rhamnolipid production by *P. aeruginosa* MM1011 using sugar beet molasses as a carbon and energy source. With a medium containing 24.2 g/l of glucose, a biosurfactant mass concentration (expressed as rhamnolipids) of up to 1.1 g/l was obtained in the cell-free culture liquid. The rhamnolipid mass concentration was 7.5 mg/ml.

Thaniyavarn *et al.* (2006) concluded the biosurfactant production by *Pseudomonas aeruginosa* A41 that types of carbon sources were found to affect bio-

surfactant yield. The yields of rhamnolipid biosurfactant were 6.58 g/L, 2.93 g/L and 2.91 g/L determined as rhamnose content when olive oil, coconut oil, and palm oil respectively, were used as a carbon source. These yield of biosurfactant steadily increased even after a stationary phase. Among them, biosurfactant obtained from palm oil was the best in lowering surface tension of the medium. Increase in biosurfactant activities in terms of oil displacement test and rhamnose content were observed to be higher with shorter chain fatty acids than that of the longer chains (C12>C14>C16). In addition, highly unsaturated fatty acid of C18:2, showed higher oil displacement activity and rhamnose content than that of C18:1.

Paisanjit *et al.* (2006) isolated *Pseudomonas aeruginosa* SP4 from petroleum-contaminated soil, and determined the optimum culture condition. They reported that nutrient broth containing 2% palm oil was shown to be the best carbon source for culturing *Pseudomonas aeruginosa* SP4 which gave the best percentage reduction of surface tension was 43.2% and an oil displacement test (ODT) was 132.8 cm^2.

Costa *et al.* (2006) used Oils from Buriti (Mauritia flexuosa), Cupuaçu (Theobroma grandiflora), Passion Fruit (Passiflora alata), Andiroba (Carapa guianensis), Brazilian Nut (Bertholletia excelsa) and Babassu (Orbignya spp.) as carbon sources for rhamnolipid production by *Pseudomonas aeruginosa* LBI. They found that the highest rhamnolipid concentrations were obtained from Brazilian Nut (9.9 g/l) and Passion Fruit (9.2 g/l) oils. Surface tension varied from 29.8 to 31.5 mN/m, critical micelle concentration from 55 to 163 mg/l and the emulsifying activity was higher against toluene (93–100%) than against kerosene (70–92%).

Wu *et al.*, (2007) examined the effect of carbon substrates, nitrogen sources and carbon to nitrogen (C/N) ratio on rhamnolipid production with a isolated strain *Pseudomonas aeruginosa* EM1 originating from an oil-contaminated site. The carbon sources tested included carbohydrates (glucose and sucrose), glycerol, vegetable oils (olive oil and soybean oil), fatty acid (oleic acid), and hydrocarbon (hexane) whereas, nitrogen sources included inorganic (NH_4Cl and $NaNO_3$) and organic (urea and yeast extract). The results show the effect of carbon sources that glucose and glycerol were effective for rhamnolipid production. After cultivation for 7 days, the culture with glucose, glycerol, olive oil, soybean oil, oleic acid, hexane and sucrose

produced 7.50, and 4.93 g/L, 3.70, 2.63 g/L, 0.55 g/L, 0.12 g/L and 0.07 g/L of rhamnolipid, respectively. These *P. aeruginosa* EM1 strain showed a different trend due to some reports show that vegetable oils were more efficient substrates in rhamnolipid production from *P. aeruginosa* strains when compared with glucose, glycerol, and hydrocarbons. This suggests that the carbon source preference for rhamnolipid production seems to be strain dependent.

2.3.2 Nitrogen Sources

The nitrogen source can be an important key to the regulation of biosurfactant synthesis. Nitrogen limitation not only causes overproduction of biosurfactant but also changes the composition of the biosurfactant produced. Among the inorganic salts tested, ammonium salts and urea were preferred nitrogen sources for biosurfactant production by *Arthrobacter paraffineus* whereas nitrate supported maximum surfactant production in *Pseudomonas aeruginosa* and *Rhodococcus* spp.

Guerra-Santos *et al.* (1984) studied the influence of nitrogen source ($NaNO_3$, $(NH_4)_2S0_4$) on *Pseudomonas aeruginosa* growth and biosurfactant production. The medium 2M with glucose concentration of 18.2 g/l served as the basic medium for the optimization experiments. Using nitrate as a nitrogen source can be lower surface and interfacial tension values of the culture broth than ammonium. The influence of C/N ratio on biosurfactant production showed maximum rhamnolipid production after nitrogen limitation at a C/N ratio of 18:1. A decrease or increase in the concentration of nitrate was expressed in a lower rhamnose concentration. At C/N ratio below 11:1 rhamnose was no longer detected or no biosurfactant production.

Robert *et al.* (1989) observed nitrate to be the best source of nitrogen for biosurfactant production by *Pseudomonas* strain 44T1. Olive oil as a carbon source (2%) supported the highest amount of growth (5 g/l) and surfactant production (CMC^{-1} = 20). Rhamnolipid production started soon after incubation (14 h), when nitrogen limiting conditions were reached, but increased dramatically, for 58 hours.

Abu-Ruwalda *et al.* (1991) studied the effect of different nitrogen sources on growth of *Rhodococcus* sp. ST-5 The result showed that nitrate to be the

best source of nitrogen for biosurfactant production by using 2% (v/v) n-paraffin as a carbon source.

Wu *et al.* (2007) reported that nitrate ($NaNO_3$) was the better inorganic nitrogen source than ammonium ion (NH_4Cl) for *P. aeruginosa* EM1 to rhamnolipid production. Giving a high rhamnolipid yield of 8.63 g/L and optimal C/N ratio of 26 and 52 was obtained for glucose- and glycerol-based culture, respectively. The effect of C/N ratio on rhamnolipid production may be slightly different when the carbon source was different. Moreover, this work also show that poor rhamnolipid production performance was obtained when the C/N ratio was too high.

2.3.3 Nutrition Sources

Nutrional requirements for high biosurfactant productivities with a *P. aeruginosa*. Paisanjit, (2006) has studied the effect of mediums to *Pseudomonas aeruginosa* SP4. The experiment used 3 mediums that-nutrient broth (NB), basal medium (BM), and defined medium (DM)-each of the mediums was varied of the percentage of palm oil from 2 to 10. The culture mediums were incubated at 37°C in a shaking incubator at 200 rpm for 72 hours. The result showed that nutrient broth containing 2% palm oil was shown to be the best culture medium for culturing *Pseudomonas aeruginosa* SP4.

Guerra-Santos *et al.* (1986) showed nutritional affecting biosurfactant production on *Pseudomonas aeruginosa* DSM 2659. The empirical adjustments of the mineral medium formulation showed that better yields of the active compounds, rhamnolipids, are obtained by minimizing the concentration of the respective salts of calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), sodium (Na^{+}) and the trace elements.

2.3.4 Environmental Factors

The pH, temperature, agitation, and oxygen as an environmental factors and growth conditions also affect biosurfactants production. Due to their effects on cellular growth or activity.

2.3.4.1 The pH

Gobbert *et al.* (1984) discussed the pH medium plays an important role in sophorolipid production by *T. bombicola.* High production of sophorolipid showed in pH values of 3.5 whereas Guerra-Santos *et al.*(1984) showed

rhamnolipid production in *Pseudomonas* spp. was at its maximum at a pH range from 6 to 6.5. An optimum pH for biosurfactant formation was obtained at a value of 6.25. Any change to both lower or higher pH values caused an appreciable drop in the productivity of biosurfactant. However, above pH 7, rhamnose concentration was decreased rapidly.

2.3.4.2 Temperature

Banat, (1995) showed that a thermophilic *Bacillus* sp. grew and produced biosurfactant at temperatures of 45°C. Heat treatment of some biosurfactants caused no appreciable change in biosurfactant properties such as the lowering of surface tension and interfacial tension and the emulsification efficiency, all of which remained stable after autoclaving at 120°C for 15 min.

Wei *et al.* (2005) reported that rhamnolipid production increased with temperature from 25 to 30°C, remained nearly constant for 30 and 37°C, and decreased slightly when temperature was further increased to 42°C. *P. aeruginosa* J4 was unable to grow at 47°C. These results suggest that the optimal temperature for rhamnolipid production with the J4 strain was in the range of 30–37°C.

2.3.4.3 Agitation and Aeration

Sheppard and Cooper (1990) studied the effects of a biosurfactant on oxygen transfer in a cyclone column reactor and concluded that oxygen transfer is one of the key parameters for the process optimization and scale-up of surfactin production in *B. subtilis.*

Wei *et al.* (2005) showed agitation rate affects the mass transfer efficiency of both oxygen and medium components and is considered crucial to the cell growth and biosurfactant formation of the strictly aerobic bacterium *P. aeruginosa* J4, especially when it was grown in a shake flask. Results from batch fermentation under different agitation rates (50–250 rpm) show that as the agitation rate increased, rhamnolipid production increased nearly 80% and the dissolved oxygen (DO) level in the batch culture also increased from approximately 0.12–0.55 mg/L.

Gautam and Tyagi (2006) described an increase in agitation speed results in the reduction of biosurfactant yield due to the effect of shear in *No-*

cardia erythropolis. On the other hand, in yeast, biosurfactant production increases when the agitation and aeration rates are increased.

2.4 Extraction of Crude Biosurfactant

In separation and extraction step, Pornsunthorntawee *et al.*, (2008) used the conventional method, the culture broth was centrifuged at 4°C and 8500 rpm for 20 min in order to remove the bacterial cells. The obtained supernatant was further treated by acidification to pH 2.0 using 6 M hydrochloric acid solution, and the acidified supernatant was left overnight at 4°C for the complete precipitation of the biosurfactants (Yakimov *et al.*, 1996). After centrifugation, the precipitate was then dissolved in a 0.1 M sodium bicarbonate solution, followed by the biosurfactant extraction step with a solvent having a 2:1 chloroform-ethanol ratio at room temperature (Zhang and Miller, 1992). The organic phase was transferred to a round-bottom flask connected to a rotary evaporator to remove the solvent, yielding a viscous honey-colored biosurfactant product. In additional, some studied used ethyl acetate as organic solvent for biosurfactant extraction (Santos *et al.*, 2002; Wei *et al.*, 2005).

Mata-Sandoval *et al.* (1999) extracted rhamnolipid biosurfactant using 10M NaOH adjusted the cultured broth and biomass was removed by centrifugation for 20 min at 10,000 g. The supernatant pH was adjusted to 2 (using 3M H_2SO_4) and an equal volume of chloroform–methanol (2:1) was added. The mixture was shaken for 10 min. Centrifugation was performed for 10 min at 10,000 g, and the organic phase removed. The extraction operation was repeated once more. The rhamnolipid product was concentrated from the pooled organic phases using a rotary evaporator. Then the thick yellowish product was dissolved in methanol, filtered and concentrated again using the rotary evaporator.

2.5 Characteristics of Crude Biosurfactant

Déziel *et al.*, (2000) developed two rapid and simple methods for the characterisation and quantification of rhamnolipids produced by a growing culture of the *Pseudomonas aeruginosa* strain 57RP. Two rhamnolipids were purified and their re-

sponse factors determined. The various rhamnolipids produced were then measured using liquid chromatography/mass spectrometry. The culture supernatants were injected directly, without prior purification, in a HPLC equipped with a C_{18} reverse-phase column. In order to shorten the analysis time, these method was developed which did not require chromatographic separation of the rhamnolipids prior to their detection. Quantification of rhamnolipids using the direct infusion method gave results very similar to those obtained with HPLC separation. These two methods were very well correlated with the standard colorimetric orcinol method.

Monteiro *et al.* (2007) produced rhamnolipids (3.9 g/L) by *Pseudomonas aeruginosa* DAUPE 614. The structure of the carbohydrate moiety of the glycolipid was determined by gas chromatography–mass spectroscopy (GC–MS) analysis allied to electrospray ionization mass spectrometry. The hydroxyl fatty acids were analyzed by GC–MS as hydroxy-acetylated fatty acid methyl ester derivatives. The positions of the fatty acids in the lipid moiety were variable, with 6 mono-rhamnolipid homologues (Rha-C_{10}-C_{10}; Rha-C_{10}-C_8; Rha-C_8-C_{10}; Rha-C_{10}-$C_{12:1}$; Rha-C_{12}-C_{10}; Rha-C_{10}-C_{12}) and 6 di-rhamnolipid homologues (Rha_2-C_{10}-C_{10}; Rha_2-C_{10}-C_8; Rha_2-C8-C10; Rha_2-C_{10}-$C_{12:1}$; Rha_2-C_{12}-C_{10}; Rha_2-C_{10}-C_{12}). The ratio of Rha_2-C_{10}-C_{10} to Rha-C_{10}-C_{10} was higher than has been reported in previous studies. This work allowed us to distinguish between the isomeric pairs Rha-C_{10}-C_8/Rha-C_8-C_{10}, Rha-C_{10}-C_{12}/Rha-C_{12}-C_{10}, Rha_2-C_{10}-C_8/Rha_2-C_8-C_{10} and Rha_2-C_{12}-C_{10}/Rha_2-C_{10}-C_{12}. For each isomeric pair, the congener with the shorter chain adjacent to the sugar was always more abundant than the congener with longer chain.

2.6 Foam

2.6.1 Foam Formations

Foam is produced when air or some other gas is introduced beneath the surfactant of a liquid that expands to enclose the gas is the film to liquid. Foam is a gas dispersed in a liquid (Rosen, 1988). The foam is unstable unless there are barriers to prevent coalescence when two gas bubble touch (Sebba, 1987). The barrier is produced by the present of a water-soluble of surfactant.

The formation of the foam from a bulk involves the expansion of the surfactant area due to the work acting upon the system. As surface tension is this work involved in creating a new system, then the amount of new area formed will be grater the low surface tension. Therefore, the surfactant is required for foam formation because it can reduce the surface tension of new surface area.

In accordance with Gibbs adsorption equation, surfactant will be adsorbed at this surface to produce an expanded monolayer. The gas will rise to the upper surface of water that also has a similar monolayer of surfactant on it. Because the head group of surfactant carries a charge that is the same sign as that of surfactant at the surfactant at the water surface, there will be repulsive force by the bubble. If in has enough momentum, it will penetrate the surface by lifting up a thin film of water and floating on the water as shown in Figure 2.10

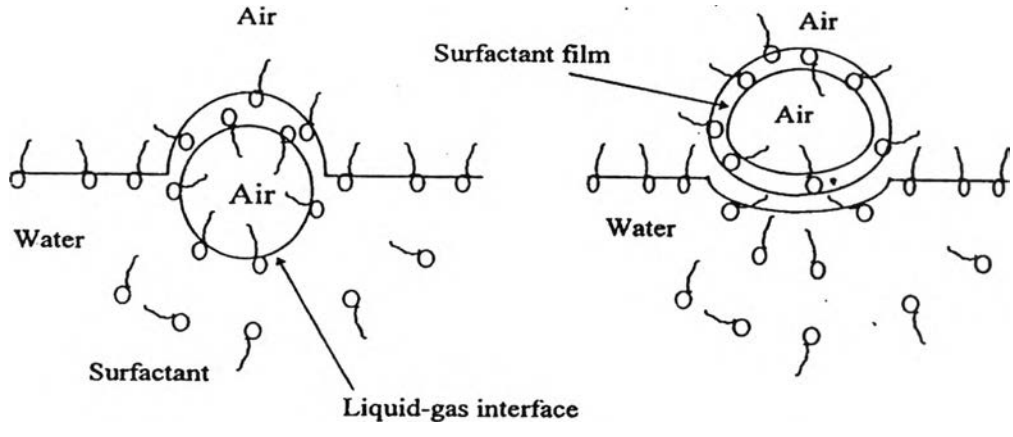


Figure 2.10 Formation of foam (Rosen, 1988)

2.6.2 Structure of Foam

The structure of foam is quite complicated. Foam consists of a ther-modynamically unstable two-phase system, which have high volume fraction of gas dispersed in a liquid. Liquid is a continuous phase. The structure of gas cell consist of thin liquid film and there are two-side of films that are called the lamellae of the foam where three or more bubbles meet, the lamellae are curved, concave to the gas cell, forming what is called the Plateau border or Gibbs triangle as seen in figure 2.11.

Most of the water in the continuous phase will be found in Plateau border will play an important role in the drainage of water in foams (Rosen, 1988; Adamson, 1990).

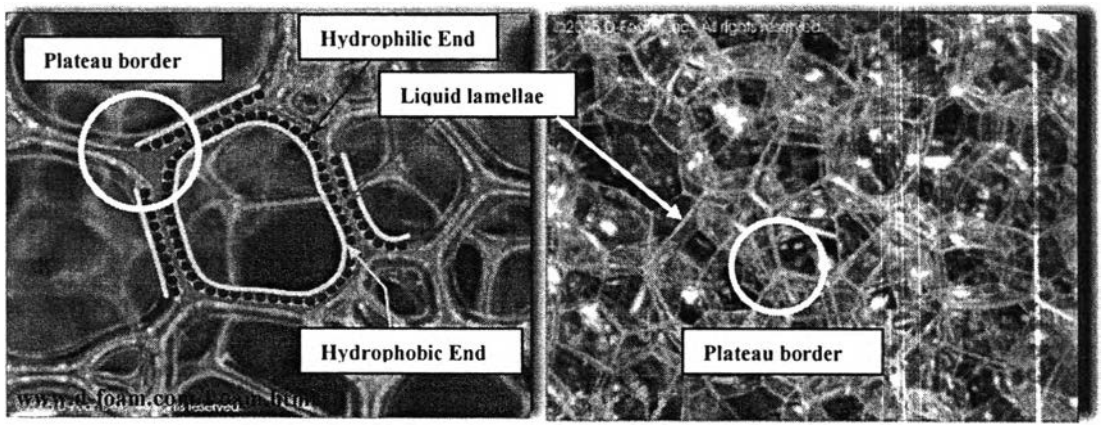


Figure 2.11 The structure of liquid foam.

Two main types of foams may be distinguished:

(1) Spherical foam (''Kugel Schaum''), consisting of gas bubbles separated by thick films of viscous liquid produced in freshly prepared systems. This may be considered as a temporary dilute dispersion of bubbles in the liquid.

(2) Polyhedral gas cells produced on aging; thin flat ''walls'' are produced with junction points of the interconnecting channels (plateau borders).

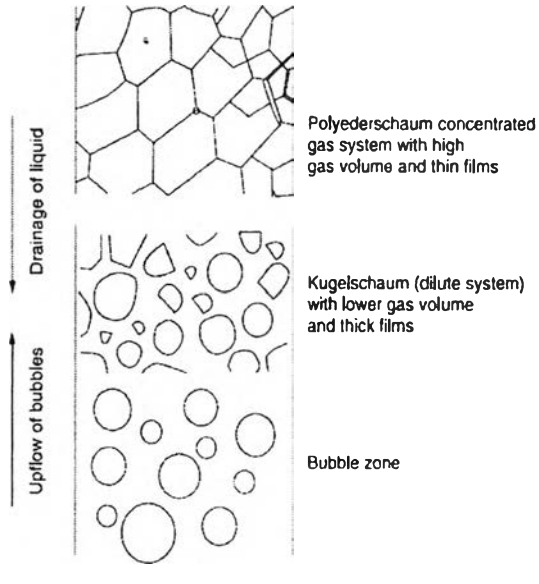


Figure 2.12 Schematic of foam structure in a column. (Tadros, 2005)

In a foam column, several transitional structures may be distinguished (Figure 2.12). Near the surface, high gas content (polyhedral foam) is formed, with a much lower gas content structure near the base of the column (bubble zone). A transition state may be distinguished between the upper and bottom layers.

2.6.3 Foam Stability

Foam is destroyed when the liquid drains out between the two parallel surfaces of the lamellae causing it to become thinner. At a certain critical thickness the film collapses and the bubble will burst. The stability of the film will depend on many factors. There are two major affecting factors the stability of the foam.

1. Film elasticity: Film elasticity indicates how easily the foam is formed that can be explained by two theories. One is the Gibbs effect that based on the change in surface tension with changing concentration of the surface-active solute. The other is the Marangoni effect based on the changing the surface tension with time. Both film elasticity theories postulate that elasticity due to the local increase in surface tension with extension of the film. As a local spot in the film thins and stretches and the area of the film in that region (Figure. 2.13) increases, its surface tension increases and a gradient of tension is set up that causes liquid to flow toward the thin spot from the thicker portion around it. The thinning spot thereby automatically draws liquid from its perimeter and prevents further thinning of the film. In addition, the movement of surface material carries with it underlying material that help heal and thicken the thinned spot by a surface transport mechanism (Rosen, 1988).Both theories can be explained that the surface tension increase in the thin lamellae. There is now restoring force from the Gibbs elasticity and the Marongoni effect bringing surfactant molecules back into the region of high surface tension (Porter, 1994), as show in Figure 2.14

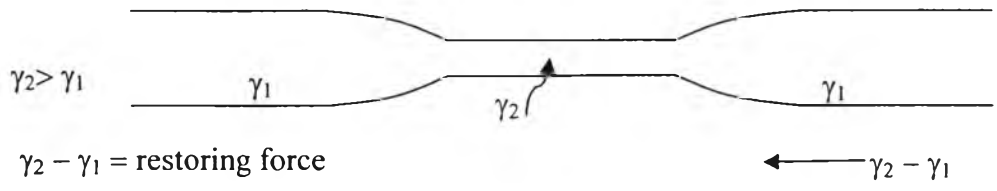
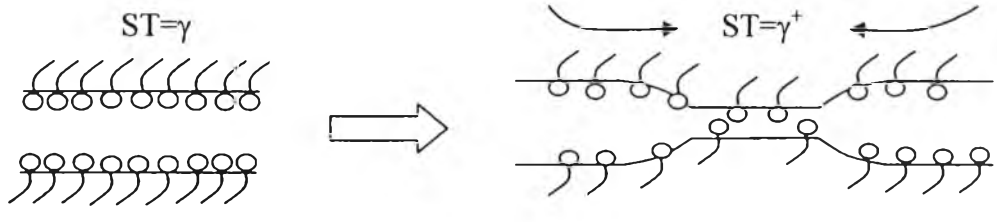
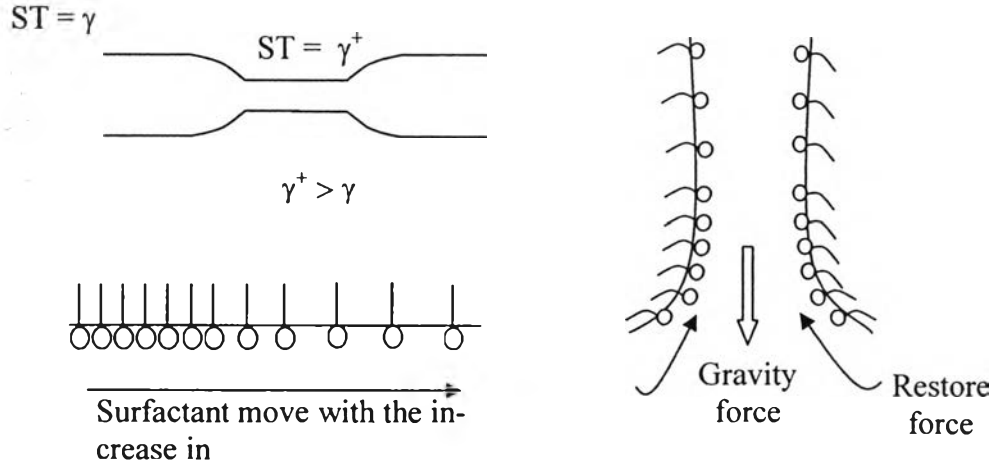


Figure 2.13 Stretch portions of foam lamellae, illustrating mechanism of film elasticity. (Rosen, 1988)



a) Gibbs Film Elasticity



b) Marangoni Effect

Figure 2.14 Marangoni Effect and Gibbs Film Elasticity.

2. Film drainage: Film drainage is the factor that indicates how rapidly the foam breaks once formed. Drainage of the film occur under two influences. The first drainage by gravity that is important mainly in very thick lamellae, such as is present when the foam is first formed. The bulk viscosity when very stable foams are desired. At a high concentration of the surfactant the viscosity of the bulk solution is

also high therefore the drainage rate in the lamellae is decreased with the amount of surfactant in the lamellar is increased.

The second is drainage by surface tension difference that is more important when the lamellae are thin. Since the curvature in the lamellae is greatest in the plateau borders, there is a greater pressure across the interface in these regions than elsewhere in the foam. Since the gas pressure inside on individual gas cell is everywhere the same, the liquid pressure inside the lamellae at the highly curved Plateau Borders (point A) must be lower than in adjacent, less curved regions (point B) of the plateau area. Thus, the continuous phase liquid drains from the thin film (point B) to the adjoining Plateau Borders (point A) as shown in Figure 2.15. The difference pressure (ΔP) can be calculated by the following equation;

$$\Delta P = \gamma\left(\frac{1}{R_A} + \frac{1}{R_B}\right) \qquad (1)$$

Where γ is surface tension.

R_A is the radii of the curvature of the liquid surface at point A.

R_B is the radii of the curvature of the liquid surface at point B.

The greater the difference between R_A and R_B, and the greater the surface tension of the solution in the lamellae, the greater the pressure difference causing drainage (Rosen, 1988).

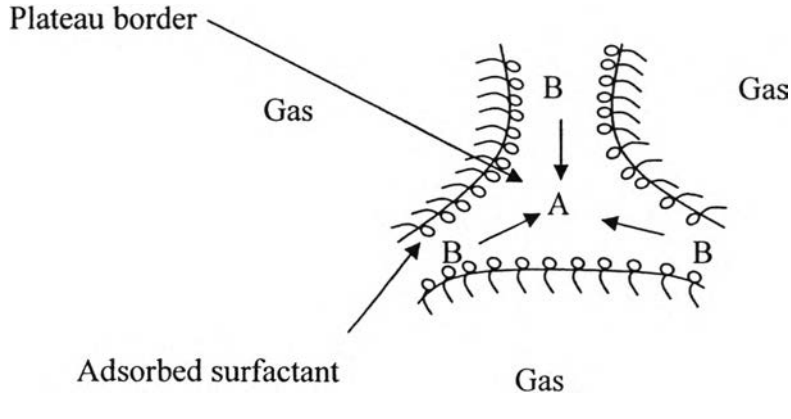


Figure 2.15 Liquid drainage in lamellae by curvature effect.

2.7 Principle of Foam Fractionation

The foam separation technique is adsorptive bubble separation that describe a process which solute species is adsorbed at an interface between a dispersed phase(bubble) and continuous phase(bulk liquid), then bubble with adsorbed substance is collected. The foam separation process can be divide into two types as shown in Figure 2.17, froth flotation and foam fractionation. Froth flotation separates insoluble materials by frothing, whereas foam fractionation separates soluble species by foams (Carleson, T.E.,1989).

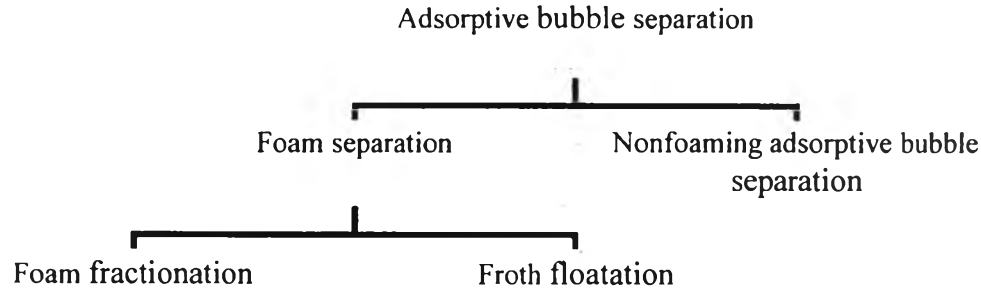


Figure 2.16 Classification of bubble separation techniques (Carleson, T.E.,1989).

In foam fractionation, air is sparged to produce bubbles which rise to the top of liquid column. Surfactant adsorbs at the air-liquid interface as the bubbles travel through the liquid phase shown in Figure 2.17b and Figure 2.17c. When the air bubble emerges from a cell in the foam honeycomb (Figure 2.17d), the thin film in the foam (lamellae) is stabilized by the adsorbed surfactant (Sebba, 1987). Drainage of liquid in the lamellae due to the gravity, Plateau border and surface tension difference cause the foam to eventually break or collapse (Rosen, 1988). The collapsed foamate solution that is collected from the top of column has higher concentration of the surfactant than the initial solution.

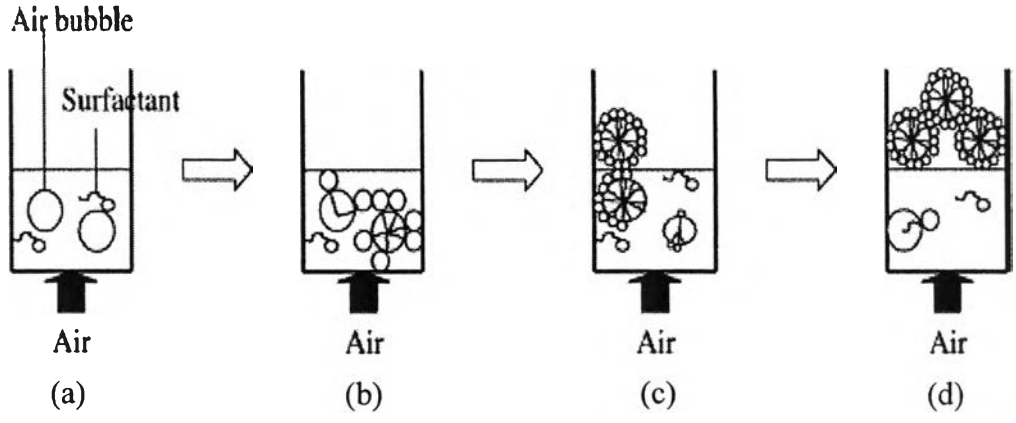
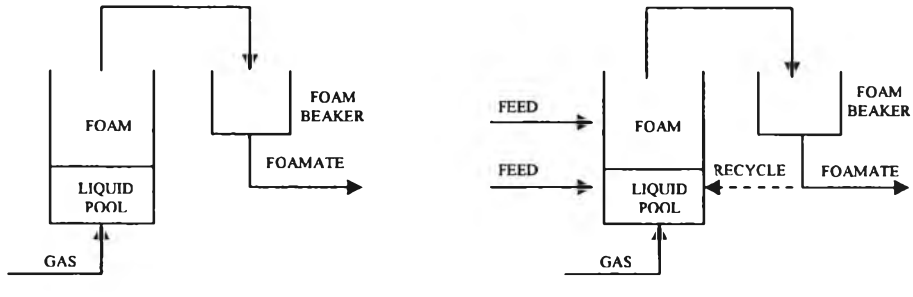


Figure 2.17 Principle of foam formation (Simmler,1972).

Foam fractionation can be devided into two modes: batch mode and continuous mode. In a batch operation, a solution of the surface-active species is stripped of the species by introduction of bubbles from a sparger. The rising foam is collected and coalesced to produce the overhead stream as shown in Figure 2.18a. Some of the coalesced foam may be returned to the foam column similar to that in a batch distillation column. In a continuous operation, the entering liquid may be introduced into the liquid pool or into the foam section of the column. Overhead product is drawn off as the top of the column and underflow from the liquid pool as illustrated in Figure 2.18b. Some of the overhead may be recycled analogous to a distillation column (Carleson, 1989). In this work a batch mode of operation is used to recover the surfactant from water.



a) batch mode b) continuous mode

Figure 2.18 Experimental configurations for foam separation (Carleson, 1989)

2.8 Applications of Foam Fractionation Process

The most applications of foam fractionation process are in the fields of wastewater treatment and recovery of components such as protein or surfactants from solution. Furthermore, advantage of this technique is suitable for surfactant removal at the concentration less than the critical micelle concentration (CMC); however, the performance of foam fractionation units depend on many operational parameters such as feed concentration, air flow rate, bubble size, and foam height, etc. as shown in many report.

For the batch mode, Yamagiwa *et al.*, (2001) investigated the effect of external fomate reflux and temperature on foam fractionation of PVA. With rising temperature, the enrichment and separation factor increase. External foamate reflux was essential foam fractionation when treating a highly foaming solution.

Tharapiwattananon *et al.*, (1996) investigated a simple continuous mode of foam fractionation to remove surfactant from water; two anionic surfactants (DADS and SDS) and one cationic surfactant (CPC) were studied. The effect of air flow rate, foam height, liquid height, surfactant concentration, and sparger porosity were investigated. The results shown that, the effectiveness of the foam fractionation process in recovering CPC can be reduced by 90% in single stage, and better than for DADS or SDS. Liquid height had little effect on the separation process. The enrichment ratio decreases with increasing air flow, surfactants concentration, and decreasing pore size of the sparger. Kumpabooth *et al.*, (1999) used the same surfactant as Tharapiwattananon and also investigated the effect of temperature and added salt. It was found that foam flow rate and enrichment ratio increase whereas the foam wetness and the rate of surfactant recovery decrease with increasing temperature. Increasing the concentration of added salt decreased the CMC of the surfactants. The foam flow rate, foam wetness and the rate of surfactant recovery increase while the enrichment ratio decreased with increasing concentration of salt.

For multistage mode, Darton *et al.*, (2004) have developed equipment and a process able to supply a number of stages of separation, working with an inert stripping gas. They found that the measured liquid compositions were in good agreement

with a model which describes the equilibrium using an adsorption isotherm, and make a mass balancer for each stage in the column. The effect of liquid reflux was shown to be important.

Boonyasuwat *et al.*, (2003) have studied the recovery of a cationic (CPC) and anionic (SDS) surfactant form water by using multistage foam fractionation in a buble-cap trayed column with one to four stages operated in steady-state mode for surfactant concentrations less than the critical micelle concentration (CMC). They found that enrichment ratio increased with decreasing air flow rate, increasing foam height of the top tray, increasing number of stages. The fractional surfactant removal increases with decreasing air flow rate, increasing foam height per tray , increasing feed liquid flowrate, increasing feed surfactant concentration, and increasing number of stages. The effectiveness of the foam fractionation process in recovering CPC was better than for SDS.

Triroj (2005) investigated the recovery surfactants foam aqueous solution in both single (CPC and $OPEO_{10}$) and mixed system (CPC/$OPEO_{10}$). From the experimental results, the enrichment ratio increased with decreasing air and liquid feed flow rate, and with increasing foam height. The effect of foam height on the surfactant recovery was not as significant as it was on the enrichment ratio. In contrast, surfactant recovery was strongly affected by the changes in air and liquid flow rates. In single surfactant system, both surfactant recovery and enrichment ratio obtained in the $OPEO_{10}$ system were higher than in the CPC system. Synergism was observed in the mixed surfactants system, which led to a total recovery of $OPEO_{10}$. Then, Sripituk (2006) used the same surfactant as Triroj and investigated the effect of feed position, reflux position and reflux ratio on surfactant recovery. For the two single surfactant systems, both the surfactant recovery and the enrichment ratio were strongly affected by feed position. The surfactant recovery decreased with increasing reflux position and reflux ratio. The results of mixed surfactant system showed that the recovery of CPC was lower than that of the pure CPC system. Interestingly, for the case of $OPEO_{10}$, it was higher than that of the pure $OPEO_{10}$ system due to the synergism effect.

For forth flotation technique, Watcharasing *et al.*, (2008) have investigated the relationship between the froth characteristics, the system interfacial tension (IFT),

and the efficiency of diesel oil removal by froth flotation under colloidal gas aphron (CGA) conditions. The air bubble size measurement was carried out in order to correlate the air bubble size to the froth flotation performance. Branched alcohol propoxylate sulfate sodium salt ($C_{14\text{-}15}(PO)_5SO_4Na$) as an extended surfactant, was used to form CGA and microemulsions with diesel oil. The results showed that the use of CGA enhanced the process performance of froth flotation in terms of both the removal and the enrichment ratio of diesel oil and surfactant separation since CGA increased both froth formation and stability, which play an important role in the removal of diesel oil and surfactant; more than the air bubble diameter effect.

However most of these studies have been used to concentrate and remove synthesis surfactant from aqueous solutions synthesis surfactant, whereas the use of foam fractionation to separate biosurfactants have seldom been reported.

Chen *et al.*, (2006) have investigated methods of producing the biosurfactant surfactin from cultures of *Bacillus subtilis* (BBK006). A reactor with integrated foam fractionation was used in batch mode, and the performance compared with that of the same culture in shaken flasks. In the batch reactor, significant foaming occurred between 12.5 h and 14.5 h of culture time. During this period, the foam was routed through the foam fractionation column to a mechanical foam breaker, and a biosurfactant-enriched foamate was collected. Concentration of surfactin in the foamate product was around 50 times greater than that in the culture medium. Using the integrated reactor, 136mg/L of surfactin was produced, significantly more than was achieved in shaken flasks (92mg/L).

Davis *et al.*, (2000) have studied a foam fractionation method for the recovery of the lipopeptide biosurfactant Surfactin from *B. subtilis* ATCC 21332 cell culture broths. They found that foam recovery was an efficient method for the recovery of surface-active products from culture broths. In non-integrated semi-batch mode, where foaming took place after the cell culture stage, Foam fractionation was considered for both cell free and cell containing broths, Foaming was less efficient for Surfactin concentration when cells were present as compared to cell-free systems. The maximum Surfactin enrichments from cell-free and cell-containing systems were 8.4 and 51.6 respectively. This is because cells increased the foamability of the solution, resulting in more liquid being carried up into the foam. The highest Surfactin

enrichments (up to 51.6) occurred during the latter stages of foaming, when the volume of liquid collected in foam per unit time was lowest. Low Surfactin enrichments (1.2–1.6) occurred during the initial foaming stages, where the majority of Surfactin was recovered (up to 70%).