CHAPTER II
LITERATURE REVIEW

2.1 Background on Surfactants and Biosurfactants

2.1.1 Surfactants

Surfactants (surface-active compounds) are amphipathic molecules with both hydrophilic and hydrophobic (generally hydrocarbon) moieties that partition preferentially at the interface between fluid phases with different degrees of polarity and hydrogen bonding such as oil/water or air/water interfaces. These properties render surfactants capable of reducing surface and interfacial tension and forming microemulsion where hydrocarbons can solubilize in water or where water can solubilize in hydrocarbons. Such characteristics confer excellent detergency, emulsifying, foaming, and dispersing traits, which makes surfactants some of the most versatile process chemicals (Desai and Banat, 1997).

They reduce the free energy of the system by replacing the bulk molecules of higher energy at an interface (Mulligan, 2005) that have different degree of polarity and hydrogen bonding, such as oil/water, or air/water and water/solid interfaces (Singh *et al.,* 2004). The surface and interfacial tensions at air/water and oil/water interfaces respectively are comparatively easily measured quantitatively, most commonly by instruments such as the Du Nouy tensiometer, and such measurements are the basis of most initial evaluations. Surface tension at water/solid interfaces is less conveniently quantifiable (Parkinson, 1985).

Current worldwide surfactant markets are around $9.4 billion per annum, and their demand is expected to increase at a rate of 35% toward the end of the century. Almost all surfactants currently in use are chemically derived from petroleum; however, interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environmentally friendly nature, the possibility of their production through fermentation, and their potential applications in the environmental protection, crude oil recovery, health care, and food-processing industries (Desai and Banat, 1997).

In Thailand, the market of surfactant is limited to the cosmetic and cleaning products industry. As a result, the market of surfactant depends on the growth of cosmetic industry and cleaning products manufacturing. Trend of cosmetic and cleaning product market is positive according to the population growth. Currently, there are more than 200 producers of surfactant, resulting in 4 times import value over export value. In 2001, the import value was 5.622 billion Baht where as the export value was 1.423 billion Baht. The import markets are composed of many developed markets such as Japan, U.S., Singapore, German, and Taiwan.

2.1.2 Biosurfactants

Biosurfactants or microbial surfactants are surface-active biomolecules containing both a lipophilic and hydrophilic moieties, like chemical surfactants, that are produced by a 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. 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. Biosurfactants have gained importance in the fields of enhanced oil recovery, environmental bioremediation, food processing and pharmaceuticals owing to their unique properties such as higher biodegradability and lower toxicity. Interest in the production of biosurfactants has steadily increased during the past decade (Morikawa *et al.*, 2000; Zouboulis *et al.*, 2003; and Muthusamy *et al.*, 2008).

2.1.2.1 Types of Biosurfactants

Biosurfactants are mainly classified into four categories based on their biochemical nature and the microbial species (Healy *et al.*, 1996). All of these are:

2.1.2.1.1 Glycolipids

Glycolipids (the most commonly studied biosurfactants) are carbohydrates in combination with long-chain aliphatic acids or hydroxyl aliphatic acids. The linkage is by means of either ether or an ester group. The main

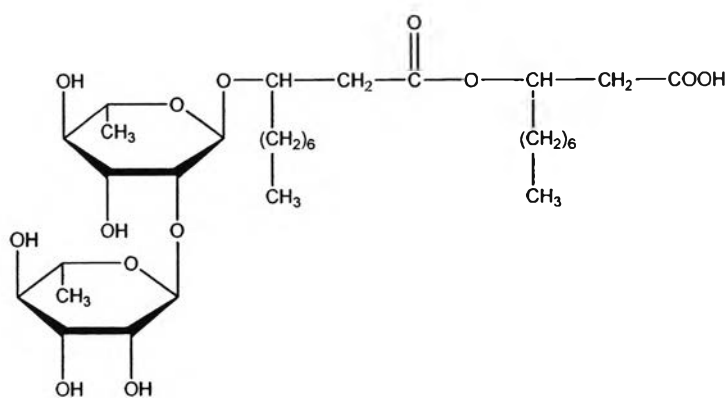
glycolipids which are studied from the point of view of surfactant characterization and properties are (A) rhamnolipid, (B) trehalose lipids, and (C) sophorolipids.

(A) Rhamnolipid

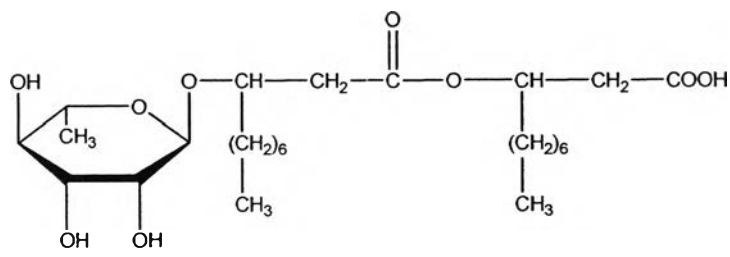
The rhamnolipids were first described in 1949, and their biosynthesis was studied in vivo by using radioactive precursors (Ochsner *et al.,* 1995). Rhamnolipid can be produced by several species of *Pseudomonas.* They produces mainly four types of rhamnolipid, consists of one or two molecules of rhamnose linked to one or two molecules of β-hydroxydecanoic acid, e.g. 3-[3-(rhamnosyl-rhamnosyl)-decanoyloxy]-decanoate, that called monorhamnolipid and dirhamnolipid respectively (Parkinson, 1985; Edwards *et al.,* 2002).

L-Rhamnosyl-L-rhamnosyl-b-hydroxydecanoyl-b-hydroxydecanoate and L-rhamnosyl-b-hydroxydecanoyl-b-hydroxydecanoate, referred to as rhamnolipid 1 and 2, respectively, are the principal glycolipids produced by *P. aeruginosa* The formation of rhamnolipid types 3 and 4 containing one b-hydroxydecanoic acid with one and two rhamnose units, respectively (Desai *et al.,* 1997). The schematic have demonstrated the structure of rhamnolipids as shown in Figure 2.1.

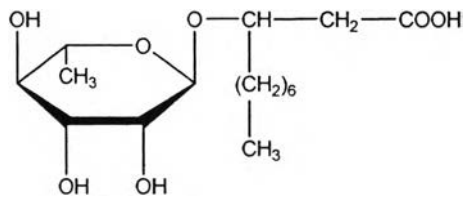
a) Rhamnolipid 1



b) Rhamnolipid 2



c) Rhamnolipid 3



d) Rhamnolipid 4

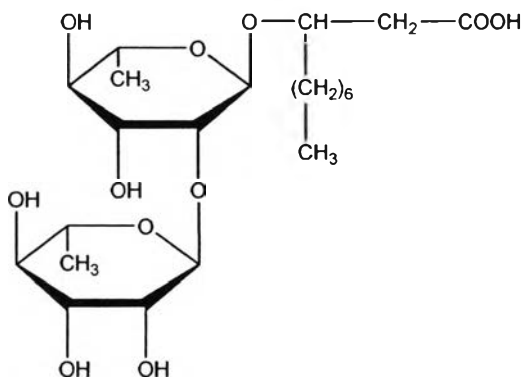


Figure 2.1 Schematic representation of four different rhamnolipids produced by *P.aerugonosa*.

(B) Trehalose Lipids

Several structural types of microbial trehalolipids biosurfactants have been reported (Figure 2.2). Disaccharide trehalose linked at C-6 and C-6' to mycolic acid is associated with most species of *Mycobacterium*, *Nocardia* and *Corynebacterium*. Mycolic acids are longchain, α-branched-β-hydroxy 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. Trehalose lipids from *Rhodococcus erythropolis* and *Arthrobacter* sp. lowered the surface and interfacial tension in culture broth from 25 to 40 and 1 to 5 mN/m respectively (Muthusamy *et al.*, 2008).

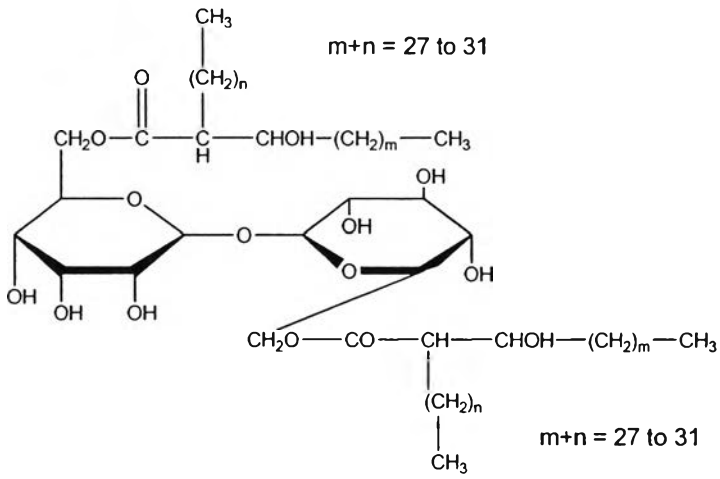
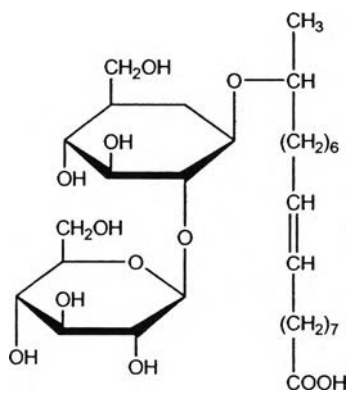


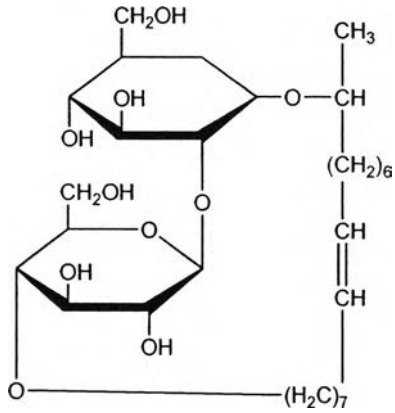
Figure 2.2 Trehalose dimycolate from *Rhodococcus erythropolis*, in which disaccharide trehalose is linked to two long-chain α-branched β-hydroxy fatty acids.

(C) Sophorolipids

These glycolipids, which are produced mainly by yeast such as *Torulopsis bombicola*, *T. petrophilum*, and *T. apicola* consist of a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid by glycosidic linkage. Generally, sophorolipids occur as a mixture of macrolactones and free acid form as see in Figure 2.3. It has been shown that the lactone form (Figure 2.3b) of the sophorolipid is necessary, or at least preferable, for many applications. These biosurfactants are a mixture of at least six to nine different hydrophobic sophorolipids (Muthusamy *et al.*, 2008).



(a) Structure of free-acid form of sophorolipids



(b) Structure of lactonized form of sophorolipids

Figure 2.3 (a) Structure of free-acid and (b) lactonized forms of Sophorolipids 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).

2.1.2.1.2 Lipoproteins or Lipopeptids

A large number of cyclic lipopetides, including decapeptide antibiotics (gramicidins) and lipopeptide antibiotics (polymyxins) are produced. These consist of a lipid attached to a polypeptide chain. One of the most effective cyclic lipopeptide biosurfactants is surfactin (SF), produced by *Bacillus subtilis* ATCC 21332. It is composed of a seven amino-acid ring structure coupled to a fatty-acid chain via lactone linkage. It lowers the surface tension from 72 to 27.9 mN/m at concentrations as low as 0.005% (Muthusamy *et al.*, 2008). Desai and Banat, 1997 showed that the structure of surfactin as in Figure2.4.

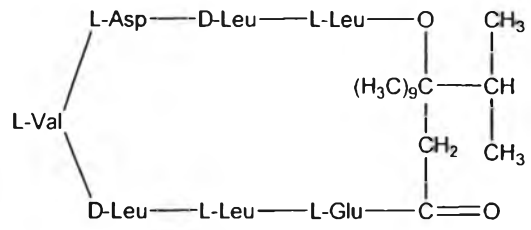


Figure 2.4 Structure of cyclic lipopeptide surfactin produced by *Bacillus subtilis.*

Lichenysin: Bacillus licheniformis produces several biosurfacants which act synergistically and exhibit excellent temperature, pH and salt stability. These are also similar in structural and physio-chemical properties to the surfactin26. The surfactants produced by *B. licheniformis* are capable of lowering a surface tension of water to 27 mN/m and an interfacial tension between water and *n*hexadecane to 0.36 mN/m.

2.1.2.1.3 Phospholipids and Fatty Acids

Large quantities of fatty acids and phospholipid surfactants are produced by several bacteria and yeast during growth on *n*-alkanes. The hydrophilic and lipophilic balance (HLB) is directly related to the length of the hydrocarbon chain in their structures. In *Acinetobacter* sp. strain HO1-N, phosphatidylethanolamine-rich vesicles are produced (Kappeli *et, al.,* 1979), which form optically clear microemulsions of alkanes in water. Phosphatidylethanolamine produced by *R. erythropolis* grown on *n*-alkane causes a lowering of interfacial tension between water and hexadecane to less than 1 mN/m and a critical micelle concentration (CMC) of 30 mg/l (Kretschmer *et, al.,* 1982).

Their structure are ester form between the alcohol group on a lipid and a phosphate. HLB value is directly related to the length of the hydrocarbon chain in their structures. *R.erythropolis* produced Phosphatidylethanolamine on *n*-alkane caused a reducing of interfacial tension (Desai and Banat, 1997). Figure 2.5 shows the structure of Phosphatidylethanolamine.

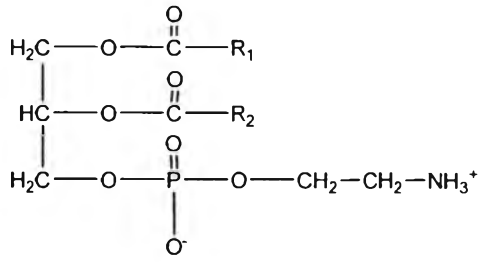


Figure 2.5 Structure of phosphatidylethanolamine, a potent biosurfactant produced by *Acinetobacter* sp. R_1 and R_2 are hydrocarbon chains of fatty acids.

2.1.2.1.4 Polymeric Biosurfactants

Polymeric 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.

(A) Emulsan

Emulsan is a very effective emulsifying agent for hydrocarbons in water even at a concentration as low as 0.001 to 0.01%. It is one of the most powerful emulsion stabilizers known today and resists inversion even at a water-to-oil ratio of 1: 4 (Gautam, *et al.*, 2006). Emulsan has been characterized as a polyanionic amphiphatic heteropolysaccharide as shown in Figure 2.6.

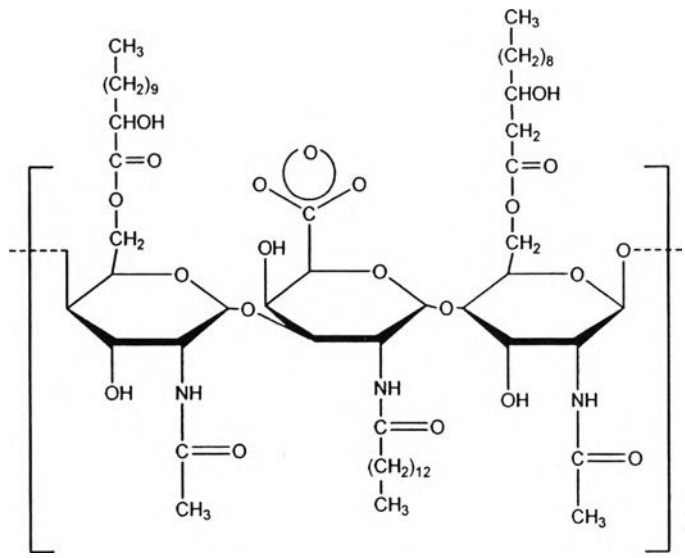


Figure 2.6 Structure of emulsan, produced by *Acinetobacter calcoaceticus*, in which fatty acids are linked to a heteropolysaccharide backbone.

(B) Biodispersan

Biodispersan is extracellular zwitterionic heteropolysaccharide. The active component of biodispersan is an anionic heteropolysaccharide, with an average molecular weight of 51,400. The biodispersan supports the breaking of water-suspended limestone particles, narrowing their size distribution to the 5-10/μm range. Its dispersing activity is pH dependent and negatively affected by Mg^{2+} and PO^{3-} ions. The dispersion process is currently tested in a large scale wet-milling process of limestone for utilizing the dispersed product in the paper industry (Shabtai, 1990; Desai *et al.*, 1997). Singh *et al.*, 2006 reported that biodispersan from *A. calcoaceticus* A2 has potential use in the paint industry.

(C) Liposan

Liposan is an extracellular water-soluble emulsifier synthesized by *Candida lipolytica* and is composed of 83% carbohydrate (heteropolysaccharide containing glucose, galactose, galactosamine, and galacturonic acid) and 17% protein (Gautam, *et al.*, 2006). Desai and Banat, (1997) showed the major types of biosurfactants, with their properties and microbial species of origin, are listed in Table 2.1.

Table 2.1 Microbial source and properties of important types of biosurfactants

Biosurfactant	Organisms	Surface tension (mN/m)	CMC	Interfacial tension (mN/m)
Glycolipids				
Rhamnolipids	P. aeruginosa	29		0.25
	Pseudomonas sp.	25–30	0.1–10	1
Trehalolipids	R. erythropolis	32–36	4	14-17
	N. erythropolis	30	20	3.5
	Mycobacterium sp.	38	0.3	15
Sophorolipids	T. bombicola	33		1.8
	T. apicola	30		0.9
	T. petrophilum			
Cellobiolipids	U. zeae, U. maydis			
Lipopeptides and lipoproteins				
Peptide-lipid	B. licheniformis	27	12–20	0.1-0.3
Serrawettin	S. marcescens	28-33		
Viscosin	P. fluorescens	26.5	150	
Surfactin	B. subtilis	27-32	23-160	1
Subtilisin	B. subtilis			
Gramicidins	B. brevis			
Polymyxins	B. polymyxa			
Fatty acids, neutral lipids, and phospholipids				
Fatty acids	C. lepus	30	150	2
Neutral lipids	N. erythropolis	32		3
Phospholipids	T. thiooxidans			
Polymeric surfactants				
Emulsan	A. calcoaceticus			
Biodispersan	A. calcoaceticus			
Mannan-lipid-protein	C. tropicalis			
Liposan	C. lipolytica			
Carbohydrate-protein-lipid	P. fluorescens	27	10	
	D. polymorphis			
Protein PA	P. aeruginosa			
Particulate biosurfactants				
Vesicles and fimbriae	A. calcoaceticus			
Whole cells	Variety of bacteria			

2.2 Factors Affecting Biosurfactants Production

2.2.1 Carbon Sources

Carbon sources are the raw materials that play an important role for using to produce rhamnolipid biosurfactants. It can be 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). Using the different carbon sources affected in the several proportions related to the types of rhamnolipids synthesized (monorhamnolipids and dirhamnolipids). In addition to the difference in the composition of the rhamnolipid species affects global surfactant properties (Santos *et al.,* 2002).

Guerra-Santos *et al.,* 1984 demonstrated that rhamnolipid mixtures are produced by *Pseudomonas aeruginosa* DSM2659 grow on glucose as a carbon in continuous culture.

Robert *et al.,* 1989 studied the affect of the carbon source on biosurfactant production by *Pseudomonas aeruginosa* 44T1. They use different carbon sources (glucose; fructose; sodium acetate; sodium succinate; sodium pyruvate; sodium citrate; glycerol; mannitol, were all supplied by Merck, olive oil by Cooperativa San Gregorio, Spain, and n-alkanes C_{10} to C_{16} by PETRESA (Petroquímica Española S.A.). And they found that *Pseudomonas* 44T1 was able to grow and produce rhamnolipids from glycerol, mannitol and glucose but fructose was not a good substrate for biosurfactant production. Type of rhamnolipids that produced from each carbon source was shown in Table 2.2. In addition to, Benincasa *et al.,* 2002 described the different oily substrates were produced different amounts of rhamnolipid biosurfactants.

Table 2.2 Surfactant production by *Pseudomonas aeruginosa* 44T1 with different carbon sources

Carbon Source	Rhamnolipid Type
C11	Rha-C10-C10, Rha-Rha-C10-C10.
C12	Rha-C10-C10, Rha-Rha-C10-C10.
Succinate	Rha-C10-C10, Rha-Rha-C10-C10.
Pyruvate	Rha-C10-C10, Rha-Rha-C10-C10.
Citrate	Rha-C10-C10, Rha-Rha-C10-C10.
Fructose	Rha-C10-C10, Rha-Rha-C10-C10.
Glucose	Rha-C10-C10, Rha-C10, Rha-Rha-C10-C10, Rha-Rha-C10.
Glycerol	Rha-C10-C10, Rha-Rha-C10-C10.
Mannitol	Rha-C10-C10, Rha-C10, Rha-Rha-C10-C10, Rha-Rha-C10.
Olive oil	Rha-C10-C10, Rha-Rha-C10-C10.

Zhang *et al.*, 2005 showed that rhamnolipid production by *pseudomonas aeruginosa* with glycerol is much higher than that of other substrates including glucose, vegetable oil and liquid paraffin, at an initial concentration of 30 g/L, but glucose is more effective to produce cell mass than the rest.

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 that 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 rape seed oil (both at a concentration of 6%) achieved a maximum concentration of 2.0–2.1 g/L.

Rashedi *et al.*, 2006b showed that 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.

Types of carbon sources were found to affect biosurfactant yield in biosurfactant production by *Pseudomonas aeruginosa* A41. 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 that higher oil displacement activity and rhamnose content than that of C18:1 (Thaniyavarn *et al.*, 2006).

Oliveira *et al.*, 2006 concluded the synthesis of surfactants by *P. aeruginosa FR* strain was verified. The bacterium could grow in three inorganic media using of palm oil as sole carbon source, producing surface-active compounds. The biosurfactants produced by bacterium growing in palm oil presents emulsification index of even 100%, when diesel was used as oily phase and temporal (72 h) stable emulsions were obtained.

Wu *et al.*, 2008 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 that 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.

2.2.2 Nitrogen Sources

The type of nitrogen source is also crucial to rhamnolipid production. 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 ($NaNO_3$, $(NH_4)_2SO_4$) on *Pseudomonas aeruginosa* growth and biosurfactant production. The medium 2 M 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 that 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 sodium nitrate appeared 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.

Chen *et al.* (2007) improved the production yield of rhamnolipid by used variety of nitrogen sources, including organic (urea) and inorganic (NH_4NO_3, $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl) ones, and examined for their effects on rhamnolipid production. The results show that nitrate-based nitrogen sources (NH_4NO_3 and $NaNO_3$) seemed to be better nitrogen sources for rhamnolipid production, giving a maximum rhamnolipid concentration of nearly 2300 mg/L. For NH_4NO_3, the maximum rhamnolipid yield reached the highest level after 72 h and essentially remained at that value until the end of the experiment, whereas, for $NaNO_3$, the rhamnolipid yield decreased progressively after a maximum level was reached at 100 h. Hence, NH_4NO_3 seems to be most favorable for rhamnolipid production with the *P. aeruginosa* S2 strain. In contrast, the performance of using $(NH_4)2SO_4$, NH_4Cl, and urea was poor when compared with nitrates.

Fonseca *et al.* (2007) showed that variation of surface tension and pH of *B. subtilis* YRE207 fermented media for different nitrogen sources. Among the nitrogen sources tested, ammonium nitrate, ammonium sulfate, and urea were the most favored ones for the biosurfactant production by *B. subtilis* YRE207. Nevertheless, ammonium nitrate was the best nitrogen source for surface-active compound synthesis, because the lowest surface tension value (66.7 to 31.5) and its higher percent reduction (52.8 ± 2.1) were achieved under this nutritional condition. The maximum lipopeptide production by a *B. subtilis* strain after 72 h of cultivation at 45°C with urea and nitrate ion on 3 g/L concentrations. Similarly, the cultivation of *Pseudomonas aeruginosa* strain in different nitrogen sources ($NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$, NH_4Cl), with concentrations varying from 2.0 to 5.0 g/L, presented satisfactory surface tension reduction of the medium in the presence of 2.0–3.0 g/L of $NaNO_3$

Wu *et al.* 2008 demonstrated the biosurfactant-producing potential of an indigenous *P. aeruginosa* EM1 isolate capable of producing rhamnolipid effectively from a wide range of nitrogen sources. Among the four nitrogen sources (NH_4Cl, $NaNO_3$, Urea, Yeast extract) examined, $NaNO_3$ was the best nitrogen source for rhamnolipid production because it gave the highest rhamnolipid yield (8.63 g/L),

this yield is nearly 20 fold of that obtained from using NH_4Cl as the nitrogen source. The carbon to nitrogen (C/N) ratio was found to play a crucial role in rhamnolipid production of the EM1 strain. The optimal C/N ratio varied slightly with the carbon source used, as the best C/N ratio was 26 and 52 for cultures containing a sole carbon substrate of glucose and glycerol, respectively. Moreover, this work also shows that poor rhamnolipid production performance was obtained when the C/N ratio was too high.

2.2.3 Environmental Factors

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

2.2.3.1 The pH

Guerra-Santos *et al.* (1984) showed that rhamnolipid production in *Pseudomonas* sp. 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, and decreased sharply above 7.0. Increased surfactant production was seen in cultures of *P. aeruginosa* UG2 maintained at neutral pH relative to cultures allowed to develop acidic conditions. In addition, surface tension and CMCs of a biosurfactant product remained stable over a wide range of pH values, whereas emulsification had a narrower pH range (Gautam and Tyagi. 2006).

2.2.3.2 Temperature

The studying rhamnolipid production by *P. aeruginosa* 44T1 on glucose, Robert *et al.* (1989) found that the best temperature for product formation was 37 °C. Nitschke *et al.* (2005) reported that it was possible to direct the rhamnolipid composition of the surfactant consisting of four compounds by temperature, when *n*-alkanes were used as substrate by *Pseudomonas* sp. DSM 2874.

Wei *et a.l.* (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.

2.2.3.3 Agitation and Aeration

Parkinson, (1985) reported the bacterium *Nocardia erythropolis* has been shown to be sensitive to shear force and the agitation rate apparently affects the kinetics of surfactant production.

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.*

An 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 (Wei *et al,*. 2005).

Gautam and Tyagi (2006) described an increase in agitation speed results in the reduction of biosurfactant yield due to the effect of shear in *Nocardia erythropolis*. On the other hand, in yeast, biosurfactant production increases when the agitation and aeration rates are increased.

2.3 Advantages and Disadvantages of Biosurfactants

The unique properties of biosurfactants allow their use and possible replacement of chemically synthesized surfactants in a great number of industrial operations. Surfactants are used by many industries and one could easily say that there is almost no modern industrial operation where properties of surfaces and surface active agents are not exploited. The potential application of biosurfactants in industries is also a reality.

2.3.1 Advantages (Kosaric, 1992).

There are many advantages of biosurfactants as compared to their chemically synthesized counterparts. Some of those are:

- Biodegradability.
- Generally low toxicity.
- Biocompatability and digestibility, which allows their application in cosmetics, pharmaceuticals and as functional food additives.
- Availability of raw materials. Biosurfactants can be produced from cheap raw materials which are available in large quantities. The carbon source may come from hydrocarbons, carbohydrates and/or lipids, which may be used separately or in combination with each other.
- Acceptable production economics. Depending,on the application, biosurfactants can also be produced from industrial wastes and by-products and this is of particular interest for bulk production (e.g. for use in petroleum related technologies).
- Use in environmental control. Biosurfactants can be efficiently used in handling industrial emulsions, control of oil spills, biodegradation and detoxification of industrial effluents and in bioremediation of contaminated soil.
- Specificity. Biosurfactants, being complex organic molecules with specific functional groups, are often specific in their action. This would be of particular interest in detoxification of specific pollutants, de-emulsification of industrial emulsions, specific cosmetic, pharmaceutical and food applications.

2.3.2 Disadvantages

- Low yield production of biosurfactants but large quantities are particularly needed in petroleum and environmental applications.
- Pure substances which is of particular importance in pharmaceutical, food and cosmetic applications.

2.4 Sequencing Batch Reactor on Biosurfactants Production (Irvine *et al.*, 2004)

The Sequencing Batch Reactor (SBR) is a biological wastewater treatment process. The SBR is a time-oriented system where each tank is filled for a discrete period of time and then operated as a batch reactor. Optimization of aeration and mixing strategies will lead to increased removal of carbon, nitrogen, phosphorus, and target organic compounds from industrial wastewaters. Since the SBR is a batch operation, it has significantly more flexibility than conventional systems allowing for more variances in the effluent levels. Given its structural simplicity, existing continuous-flow systems can be retrofitted to operate in batch. The SBR system is a set of tanks that operate on a fill-and-draw basis. Each tank in the SBR system is filled during a discrete period of time and then operated as a batch reactor. After desired treatment, the mixed liquor is allowed to settle and the clarified supernatant is then drawn from the tank.

2.4.1 The Cyclic Process of SBR

The cycle for each tank in a typical SBR is divided into five discrete time periods: Fill, React, Settle, Draw and Idle as shown in Figure 2.7. There are several types of Fill and React periods, which vary according to aeration and mixing procedures. Sludge wasting may take place near the end of React, or during Settle, Draw, or Idle. Central to SBR design is the use of a single tank for multiple aspects of wastewater treatment. A detailed discussion of each period of the SBR is provided in the following subsections, along with a description of typical process equipment and hardware associated with each.

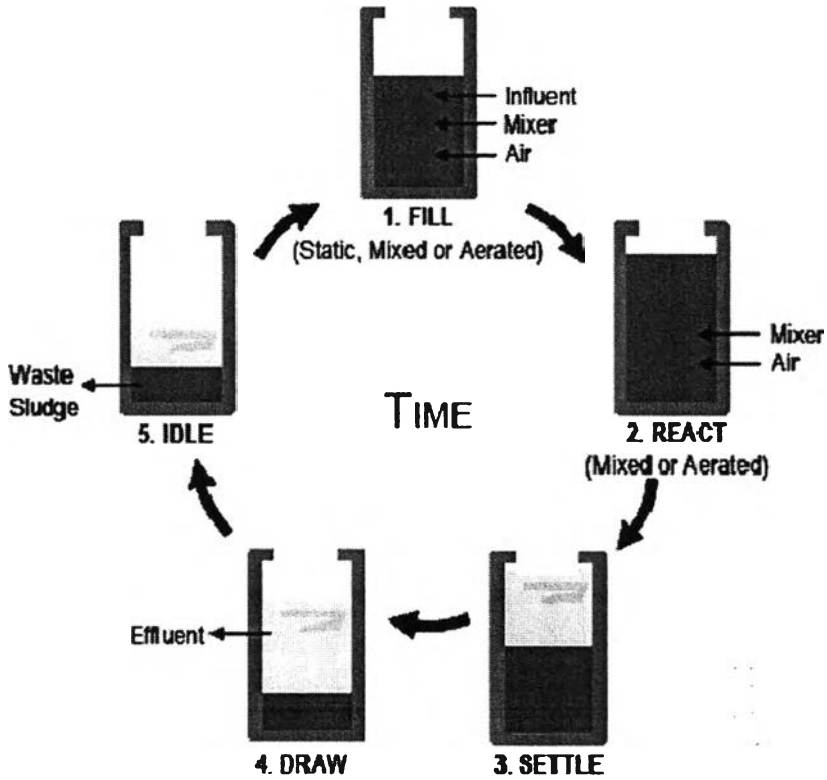


Figure 2.7 SBR operation for each tank for one cycle for the five discrete time periods of Fill, React, Settle, Draw, and Idle. (Irvine and Ketchum, 2004).

2.4.1.1 Fill

The influent to the tank may be either raw wastewater or primary effluent. It may be either pumped in or allowed to flow in by gravity. The feed volume is determined based on a number of factors including desired loading and detention time and expected settling characteristics of the organisms. The time of Fill depends upon the volume of each tank, the number of parallel tanks in operation, and the extent of diurnal variations in the wastewater flow rate.

2.4.1.2 React

Biological reactions, which were initiated during Fill, are completed during React. As in Fill, alternating conditions of low dissolved oxygen concentrations (e.g., Mixed React) and high dissolved oxygen concentrations (e.g. Aerated React) may be required. The liquid level remains at the maximum throughout react, sludge wasting can take place during this period as a simple means for con-

trolling the sludge age. By wasting during React, sludge is removed from the reactor as a means of maintaining or decreasing the volume of sludge in the reactor and decreases the solids volume. Time dedicated to react can be as high as 50% or more of total cycle time.

2.4.1.3 Settle

In the SBR, solids separation takes place under quiescent conditions (i.e., without inflow or outflow) in a tank, which may have a volume more than ten times that of the secondary clarifier used for conventional continuous-flow activated sludge plant. This major advantage in the clarification process results from the fact that the entire aeration tank serves as the clarifier during the period when no flow enters the tank. Because all of the biomass remains in the tank until some fraction must be wasted, there is no need for underflow hardware normally found in conventional clarifiers. By way of contrast, mixed liquor is continuously removed from a continuous flow activated-sludge aeration tank and passed through the clarifiers only to have a major portion of the sludge returned to the aeration tank.

2.4.1.4 Draw (Decant)

The withdrawal mechanism may take one of several forms, including a pipe fixed at some predetermined level with the flow regulated by an automatic valve or a pump, or an adjustable or floating weir at or just beneath the liquid surface. In any case, the withdrawal mechanism should be designed and operated in a manner that prevents floating matter from being discharged. The time dedicated to Draw can range from 5 to more than 30% of the total cycle time. The time in Draw, however, should not be overly extended because of possible problems with rising sludge.

2.4.1.5 Idle

The period between Draw and Fill is termed Idle. Despite its name, this "idle" time can be used effectively to waste settled sludge. While sludge wasting can be as infrequent as once every 2 to 3 months, more frequent sludge wasting programs are recommended to maintain process efficiency and sludge settling.

Cassidy *et al.* (2000) compared the performance of a continuous-flow stirred tank reactor (CSTR) and a soil slurry-sequencing batch reactor (SS-SBR) treating the same diesel fuel-contaminated soil. The result showed that the SS-SBR provided markedly enhanced contaminant degradation relative to the CSTR. Diesel fuel removal efficiency was 96% in the SS-SBR, compared with 75% in the CSTR and biosurfactant production was greater in the SS-SBR. Microbial growth was approximately 25% greater in the SS-SBR than the CSTR. However, signifcant biosurfactant production and foaming occurred in the SS-SBR, whereas none was observed in the CSTR. Converting the CSTR to an SS-SBR resulted in surfactant production and enhanced diesel fuel degradation. These results indicate that fill-and-draw operation (SS-SBR) selected for microbes with a greater ability to produce surfactants and degrade diesel fuel than the CSTR operation.

Cassidy and Hudak (2001) studied the microorganism selection and biosurfactant production in a CSTR and SS-SBR. They found that the SS-SBR operation favored the growth of biosurfactant-producing microorganisms relative to the CSTR. These results explain the enhanced biosurfactant production and diesel fuel (DF) biodegradation with SS-SBR operation relative to CSTR operation. Biosurfactant-producing species comprised 88% of the total microbial concentration in SS-SBR, and 23% in CSTR. However, the numbers of all five species (*C.tropicalis, B.casei, F.aquatile, P.aeruginosa*, and *P. fluorescens*) were significantly different with SS-SBR and CSTR operation except *P.aeruginosa*. *C tropicalis* concentrations achieved with SS-SBR operation were more than 3 orders of magnitude greater than with CSTR operation. Biosurfactants were produced in the SS-SBR to levels of nearly 70 times the critical micelle concentration (CMC) early in the cycle, but were completely degraded by the end of each cycle. The result also showed that biosurfactant production was not observed in the CSTR. DF biodegradation rates were over 40% greater and DF stripping was over five times lower in the SS-SBR than the CSTR. However, considerable foaming occurred in the SS-SBR.

Ong *et al.* (2003) evaluated the effect of Copper and the efficiency of the powered activated carbon (PAC) and activated rice husk (ARH) in reducing the toxic effect of copper on the activated sludge microorganisms. The SBR reactor were operated with FILL, REACT, SETTLE, DRAW and IDLE modes in ratio of

0.5: 3.5: 1: 0.75: 0.25 for a cycle time of 6 hours. The result showed that the efficiency of copper and COD removal is 90 and 85% respectively.

Andrea *et al.* (2004) studied the sequencing batch reactor (SBR) performance treating PAH contaminated sediments. The applicability of sediment slurry SBR to treat Venice lagoon sediments contaminated by polycyclic aromatic hydrocarbons (PAHs). The total PAH efficiency removal close to 55% was achieved for long (98 days), middle (70 days) and short (35 days) HRT of the SBR; moreover, although the addition of lactose (external carbon source) in the SBR has increased the biological activity. These results showed that the investigated biological treatment system can be pursued, with respect to the dredged sediment used.

Sarioglu (2005) investigated the effect of pure cultures on the enhancement of biological phosphorus removal capability of a Sequencing Batch Reactor (SBR) due to these reactor possibility of combined COD, nitrogen and phosphorus removal in the same tank therefore makes SBRs economically advantageous. Pure cultures of *Acinetobacter lwoffii*, *A. lwoffii–Pseudomonas aeruginosa* mixture and *P. aeruginosa* were added into the first, second and third reactors, respectively. This result has demonstrated that the addition of a pure culture of *A. lwoffii* resulted in 100% PO_4–P removal within one month. On the other hand, a mixture of *A. lwoffii* and *P. aeruginosa* and only *P. aeruginosa* pure culture addition reached steady-state PO_4–P removal efficiencies 25% and 20% within 19 days, respectively. The COD removal efficiency was 90% in all reactors. Decrease in PO_4–P removal in other two SBRs can be explained by different growth rates of these bacteria (*A. lwoffii and P. aeruginosa). P. aeruginosa* culture has a relatively slow growth rate in this system. Phosphorus removal data implied that population dynamics had a significant effect on phosphorus removal in all three SBRs.