



CHAPTER II

LITERATURE REVIEW

2.1 Biosurfactant

Biosurfactant Molecules will show free carboxylic groups and behave as anions when pH is above 4.0. These compounds are soluble in methanol, chloroform, and ethyl ether and show also good solubility in an alkaline aqueous solution. Rhamnolipids provide good physicochemical properties in terms of surface activities, stabilities, and emulsification activities.

2.1.1 Surface Activities

In general, biosurfactants produced by *P.aeruginosa* strains were found to reduce the surface tension of pure water from 72 to 30 mN/m with CMCs in the range of 5-200 mg/l, depending on their components.

Parra *et al.* (1989) reported that rhamnolipid surfactants can reduce the surface tension of water from 72 mN/m to values below 30 mN/m and the interfacial tension of water/oil systems from 43 mN/m to value < 1 mN/m.

Pornsunthorntawee *et al.* (2008) also reported that the biosurfactants produced by *Pseudomonas aeruginosa* SP4 were found to reduce the surface tension of pure water to 28.3 mN/m with the CMC value of 120 mg/l. On the other hand, from the previous study, the crude biosurfactant reduced the surface tension of pure water to 29.0 mN/m with a CMC of 200 mg/l (Pornsunthorntawee *et al.*, 2008).

Wang *et al.* (2007) and Wei *et al.* (2005) found that rhamnolipid produced by *Pseudomonas aeruginosa* J4 able to reduce surface tension of water to less than 30 dynes/cm from 72 dynes/cm with critical micelle concentration (CMC) value of 50 mg/l.

Moreover, the oil displacement test is an indirect measurement of the surface activity of a surfactant sample tested against oil

Pornsunthorntawee *et al.* (2008) investigated that the surface activity of the crude biosurfactant was much higher than of the Pluronic F-68, but was slightly lower than that of SDS — both Pluronic F-68 and SDS as synthetic surfactants.

2.1.2 Emulsification Activities

The rhamnolipids produced by different *Pseudomonas* strains can effectively emulsify and stabilize emulsions with various types of hydrocarbons and oils.

Wei *et al.* (2005) revealed that the biosurfactant also achieved a maximum emulsion index of 70% and 78%, for diesel and kerosene, respectively, at a low concentration of about 300 mg/l.

Benincasa *et al.* (2004) also reported that the biosurfactant produced by *Pseudomonas aeruginosa* LBI could form stable emulsions with *i*-propyl palmitate, castor oil, almond oil, crude oil, kerosene, and benzene for 21 days, suggesting potential applications of the excreted rhamnolipids in the pharmaceutical and cosmetic industries, and environmental pollution treatment.

Haba *et al.* (2003) reported that culture fluid supernatants obtained from different *Pseudomonas* strains were able to form emulsions with kerosene that remained stable for 3 months.

Patel *et al.* (1997) Emulsions of *n*-alkanes, aromatic compounds, crude oil, kerosene, and coconut and olive oils were stabilized by rhamnolipids, showing a loss of 5-25% of stability after 24 h depending on the carbon source.

2.2 Vesicle Formation

From the previous data indicate that the biosurfactant can exhibit interesting aggregation characteristic upon increasing biosurfactant concentration. Increasing the concentration of biosurfactant above the critical micelle concentration (CMC) leading to vesicle formation

Sanchez *et al.* (2006) found that increasing the concentration of dirhamnolipid (diRL) above the CMC led to a sharp increase of the turbidity of the suspensions. This increase in turbidity might be due to an increase of the amount of micelles, or to the formation of larger aggregates which could be separated by centrifugation.

The structure formed by aggregation of surfactant monomers is dependent upon the ratio of the effective diameter of the polar head groups to the effective diameter of the nonpolar tails. If this ratio is close to 1, surfactant monomers line up side by side to form planer bilayers. As this ratio increases, the bilayer structure will curve to form vesicles. (Champion *et al.*, 1995)

2.3 Biosurfactant Applications

2.3.1 Bioremediation

Rahman *et al.* (2002) reported that since microorganisms require nitrogen, phosphorus and other mineral nutrients for incorporation into biomass, the availability of these nutrients limiting the hydrocarbon degradation. The addition of rhamnolipid biosurfactant enhanced bioremediation of gasoline-spilled soil. This was likely due to better solubilisation of hydrocarbons prior to microbial degradation.

2.3.2 Cosmetic Industry

Kao Chemical Corporation at present uses sophorolipids commercially as humectants for cosmetic makeup brands such as Sofina — Sophorolipids are produced both by *C. bombicola* KSM-36 and by *C. apicola*.

2.3.3 Food Industry

In the food industry, biosurfactants are used as emulsifiers for the processing of raw materials. Emulsification plays an important role in forming the right consistency and texture as well as in phase dispersion. Biosurfactants have various pharmacological applications such as inhibiting fibrin clot formation and hemolysis (Bernheimer and Avigard1970) and formation of ion channels in lipid membranes (Sheppard *et al.*, 1991).

2.3.4 Pharmaceutical Industry

Itokawa *et al.* (1994) have reported the potential of surfactin against human immunodeficiency virus 1 (HIV-1).

2.4 Polyaniline

PANI and its derivatives are generally synthesized by chemical or electrochemical oxidative polymerization of the monomers, although some other approaches such as plasma polymerization, electroless polymerization and solid-state polymerization were also reported.

Han *et al.* (2008) reported that nano-scaled PANI spherical particles were achieved from DBSA micelles with ammonium persulfate (APS) as an oxidant in hexane by one-step polymerization. The DBSA and anilinium-DBSA acted as both surfactants and doping agents to achieve nano-scaled DBSA-doped PANI with high conductivity.

Anilkumar *et al.* (2007) found that the amphiphilic azobenzenesulfonic acid molecule (act as surfactant and dopant) exist in the form of ~ 4.3 nm spherical micelles in water for templating polyaniline nanomaterials. The dopant micelles form spherical aggregates with APS in the aqueous layer and the diffusion of aniline (in the interfacial layer) into these spherical aggregates get oxidized to produce polyaniline spheres of 200-400 nm.

Zhang *et al.* (2002) reported that in the present of a SDBS surfactant, micelles formed by anilinium cations and surfactant anions were regarded as templates in the formation of the nanostructures. In the absence of a surfactant, on the other hand, micelles formed by anilinium cations were considered as templates. However, the size of PANI nanostructures was slightly affected by the addition of the surfactant during the polymerization.

Wei *et al.* (2001) found that the formation mechanism of PANI-NSA tubes was different when the $[\text{NSA}]/[\text{An}]$ ratio was changed. At high $[\text{NSA}]/[\text{An}]$ ratio (e.g., 2), for instance, the NSA-An salt precipitate acted in a “template-like” manner in forming the PANI-NSA microtubes, while at low $[\text{NSA}]/[\text{An}]$ ratio (e.g., 1/2) resulted in NSA-An micelles acted in a “template-like” manner in forming the PANI-NSA nanotubes.

Jeevananda *et al.* (2008) indicated that when the SDS (anionic surfactant) was mixed with water, micelles were formed, which might act as templates for the formation of the self-assembled PANI nanostructures. This complex might form a

spherical shape by aggregation. With the addition of APS solution in one step, the initiator molecules induce the formation of PANI by rapidly reacting with the aniline.

Hassan *et al.* (2004) reported that the positively charged nature of anilinium ion in AHC (aniline hydrochloride) further facilitates the adsorption of this molecule on the surface of anionic micelles through electrostatic interactions.

Thanpitcha *et al.* (2008) reported that aniline monomer is first suspended in the CM-chitin solution with agitation. When 1.5-M HCl is added into the solution (the pH of the mixture solution decreases below one), most aniline monomers are transformed into amphiphilic anilinium ions. The anilinium ion can migrate into the core-shell structure, oriented radially with the benzene ring towards the hydrophobic core and the ammonium group towards the polar shell, and subsequently form a nucleating site at the interface of the core-shell structure.

2.5 Factors Affecting the Morphology of PANI Nanoparticles

2.5.1 Effect of Concentration of Aniline

Hassan *et al.* (2004) observed that the size of the particles increased from 20 to 70 nm by doubling the concentration of monomer.

2.5.2 Effect of the Ratio of Dopant to Aniline

The ratio of dopant to aniline also plays an important role in the morphology of the PANI.

Zhang *et al.* (2002) reported that PANI-H₃PO₄, for example, showed granular morphology when [An]/[H₃PO₄] was 1:2 or 1:3. However, some [An]/[H₃PO₄] mole ratios (e.g., 1:0.5, 1:0.3, and 1:0.16) were more favorable for the formation of fibrous PANI-H₃PO₄ nanostructures.

2.5.3 Effect of the Reaction Temperature

Furthermore, the reaction temperature had an important influence on the morphology of the resulting PANI. Taking PANI-HCl as an example, besides the fibrous nanostructures, there were some granular solids when aniline was polymerized at room temperature ([An]/[acid] = 1:0.5). When the polymerization occurred at a lower temperature (0-4 °C), nearly all the products showed fibrous

nanostructures, and the formation probability of the nanotubes or rods was greatly enhanced.

2.5.4 Effect of Polymerization Time

Moreover, the polymerization time has a significant effect on the morphology of the nano-structured PANI.

Jeevananda *et al.* (2008) reported that PANI nanostructures were formed at an early stage in the polymerization process (1 hour). These nanostructures have an average diameter of 30–35 nm with lengths of several micrometers. As the polymerization proceeds further (2 hours), these nanostructures become scaffolds and finally turn into irregularly shaped agglomerates containing nanostructures and particulate particles of PANI.

2.5.5 Effect of the Molecular Structure of the Polymeric Acid

Zhang *et al.* (2008) reported that the molecular structure of the polymeric acid or dopant has a major impact on the morphology of the nanotubes that were formed. The PANI–PAA nanotubes (PAA, poly (acrylic acid)) obtained under the same conditions showed a much denser packing than those obtained using PSSA (poly (4-styrenesulfonic acid)) and PMVEA (poly (methyl vinyl ether-*alt*-maleic acid)). The length of these nanotubes was also much shorter and less regular than for the other two cases.

Zhang *et al.* (2002) reported that for instance, the diameters of PANI nanostructures varied from 150 to 340 nm, depending on the dopant used (e.g., HCl, H₂SO₄, HBF₄, and H₃PO₄). The result indicates that the size of PANI nanostructures is controllable by changing the dopant structure.

Park *et al.* (2004) found that the particle size of polyaniline nanoparticles (PAPSSA) was decreased with increasing the molecular weight of the dopant.

2.5.6 Effect of the Kind of Oxidizing Agent

The kind of oxidizing agent also plays an important role in the morphology of the PANI.

Saravanan *et al.* (2008) investigated the morphology of PANI synthesized by using various types of the oxidizing agent including sodium dodecyl sulfate (SDS), potassium persulfate (PPS) and ammonium persulfate (APS). The

result showed that nanoporous PANI bundles made of agglomerated spheres with typical sizes around 100 nm are observed in the case of SPS and PPS. In the case of APS, cauliflower like irregular porous polyaniline structures is obtained.

2.5.7 Effect of the Addition of the Template

The addition of the template does affect the size of PANI nanostructure. Thanpitcha *et al.* (2008) reported that, when using CM-chitin as a template to synthesize PANI nanoparticles whose surfaces are covered with radially aligned PANI dendrites were observed. In contrast, irregularly-shaped PANI aggregates were observed in the PANI synthesized by the conventional method.

2.5.8 Effect of the Template Structure and its Concentration

The influence of the template structure (or the surfactant structure) and its concentration on the morphology of PANI nanostructures was investigated.

Palanisamy *et al.* (2009) found that when used rhamnolipid as a template to synthesis nickel oxide (NiO) nanoparticles. The result showed that rhamnolipid can form micelles and these micelles are spherical in shape and favored the formation of spherical nanoparticles during synthesis.

Han *et al.* (2009) reported that in the polymerization of PANI-DBSA, DBSA molecules act as a dopant as well as a surfactant. DBSA molecules could form spherical micelle structures in hexane even at low concentrations. Inside the micelles, ANI could be polymerized into spherical nanoparticles. The shape of particles synthesized at the other concentrations in the present study was expected to deviate slightly from the spherical micellar structures.

2.5.9 Effect of PH of the Solution

Besides PANI nanoparticles were synthesized by using surfactants as a template, the NiO nanoparticles were successfully synthesized using rhamnolipids as a template, too. However, the pH of solution also effect on the size of nanoparticles.

Palanisamy *et al.* (2009) found that increasing the pH of the solution decreased the size of nanoparticles. NiO particles with the diameter of 86 ± 8 nm, 63 ± 6 nm and 47 ± 5 nm were obtained for pH values of 11.6, 12.0 and 12.5, respectively. From previous work on pH dependent variation in morphology of NiO particles, it was shown that, when the pH value of the synthesis was around 8, the

nanoparticles were flaky in nature. With increase in pH to 10, the nanoparticles morphology changed to spherical. In the case of calcined NiO particles, a different morphology was observed.

2.6 Factors Affecting the Electrical Properties of PANI

2.6.1 Effect of Doping Level

Among many factor, doping level could be considered as the primary controlling factor of electrical conductivity.

Kim *et al.* (2000) revealed that the doping level of PANI particles was determined by X-ray photoelectron spectra (XPS). From the results indicated that the peak area $[N^+]/[N]$ ratios could be correlated with the doping level. The doping level of PANI particles prepared in SDS micelles was highest compared with those of particles formed in aqueous and NP-9 micellar solution. The electrical conductivities of pellets formed with particles prepared both in bulk and in NP-9 micellar solution was about 4.0 S/cm. The pellet formed with particles prepared in SDS micellar solution had about 20.0 S/cm.

2.6.2 Effect of PH of Medium

By a suitable change in pH (protonation reaction), one can alter the electrical conductivity of these polymers to tune them up as an insulator, semiconductor, or conductor. The conductivity of PANI powder as a function of the pH value of the dispersions was considered.

Yu *et al.* (2002) found that the conductivity was 20 S/cm at pH 1. It decreased to 1.1×10^{-3} S/cm at pH 2. Conductivity decreased gradually with increasing pH value from 2 to 9. Its value arrived at 4.0×10^{-7} S/cm at pH 9, indicating that the PANI sample was not completely dedoped at this pH value. It was reported that the conductor–insulator transition of conventional PANI is at about pH 4. The higher transition pH value observed here may have been caused by the strong interaction between doped PANI and SDS anions.

2.6.3 Effect of the Polymerization Time

Moreover, the polymerization time also affect the electrical conductivity of PANI nanoparicles.

Jeevananda *et al.* (2008) indicated that the conductivity of the PANI-SDS nanostructured complex decreased from 1.15×10^{-1} to 6.61×10^{-2} S/cm as the reaction time increased from 2 to 4 h. — In the absence of any added acid.

Han *et al.* (2008) investigated the conductivity of PANI-DBSA samples which were synthesized with different reaction times in hexane. As a result, the optimum reaction time was 2 h for the highest conductivity (3.7 S/cm). This value was higher than that reported in earlier studies reporting a long reaction time.

2.6.4 Effect of the Temperature of Reaction

Zhang *et al.* (2002) reported that the temperature dependence of conductivity of PANI doped with different inorganic acids ($[An]/[acid] = 1:0.5$, $[SDBS] = 2.4 \times 10^{-3}$ M, at 0-4 °C) was measured between 50 and 300 K. It was found that the conductivity of all measured samples decreased with decreasing temperature, exhibiting a typical semiconductor behavior.

2.6.5 Effect of the Reaction Conditions

Moreover, the reaction conditions (constant stirring or static) also were observed. Whether the reaction was carried out under constant stirring or without stirring (static condition), the conductivity decreased from 0.06 to 0.04 S/cm. (Saravanan *et al.*, 2008).

2.6.6 Effect of the Kind of Acid Used and the Oxidizing Agents

Saravanan *et al.* (2008) reported that the polymerization was carried out under stirring condition at 0–5 °C for 4.h using acids such as p-TSA, R-CSA, H₂SO₄ and HCl. The value of conductivity was found to be dependent on the kind of acid used. The highest conductivity (0.08 S/cm) of PANI was obtained with p-TSA as protonic acid. This result indicates that the oxidation and protonation effect of aniline are more efficient with the use of p-TSA as the acid. Among the oxidizing agents, ammonium persulfate (APS) yields the highest conductivity (0.2 S/cm).

2.6.7 Effect of the Ratio of Aniline to Doping Agent

The effect of $[An]/[H_3PO_4]$ on the conductivity of PANI-H₃PO₄ nanostructures was measured. However, there was no significant difference in

conductivity, around 10^{-2} S/cm, when $[\text{An}]/[\text{H}_3\text{PO}_4]$ varied from 1:0.5 to 1:2.5 $\times 10^{-2}$. As the aniline/ H_3PO_4 mole ratio reached 1:1.0 $\times 10^{-3}$, the conductivity decreased to 5.7×10^{-2} S/cm. This result is consistent with the doping degree. (Zhang *et al.*, 2002)

2.6.8 Effect of Degree of Crystallinity

Han *et al.* (2009) showed the result of X-ray diffraction analysis of DBSA-doped PANI which indicates a high degree of crystallinity, presumably due to the orientation behavior of the organized micelle systems. This high crystallinity of PANI is accompanied by high conductivity.