CHAPTER III
EXPERIMENTAL

3.1 Materials

3.1.1 Bacterial Strain

Pseudomonas aeruginosa strain SP4 was isolated from petroleum contaminated soil in Thailand. The isolated strain was maintained on nutrient agar slants at 37 °C and was sub cultured every 2 weeks.

3.2.2 Other Chemicals

Ammonium peroxydisulfate (APS) and 1-Methyl-2-pyrrolidinone (NMR) of AR grade from Sigma-Aldrich Co., Ltd (Germany). The hydrochloric acid and ethanol of AR grade from J.T.Baker Co., Ltd (Malaysia). Chloroform of AR grade purchased from Labsan Co., Ltd(Thailand). Sodium bicarbonate and sodium hydroxide pellets were purchased from Ajax Finechem Pty Ltd (Australia). Nutrient Broth was purchased from Difco Co., Ltd (France). Agar power (Bacteriological) was purchased from Himedia Co., Ltd (India). And palm oil was purchased from Morakot Co.,Ltd (Thailand).

3.2 Equipment

3.2.1 FTIR Spectrophotometer

The FTIR spectra of biosurfactant and polyaniline were recorded with Thermo Nicolet Nexus 670 FTIR Spectroscopy with 16 scans at a resolution of 4 cm^{-1}. A frequency range of 4000-400 cm^{-1} was observed by using a deuterated triglycerinesulfate detector (DTGS) with specific detectivity of 1 x 10^9 $cm \cdot Hz^{1/2} \cdot w^{-1}$.

3.2.2 UV/Visible Spectrophotometer

UV-Visible spectrum of a synthesized polyaniline was obtained from a Shimadzu UV-VIS spectrometer model 2550 in the wavelength range 200-800 nm. The light source was a deuterium lamp. N-Methyl-2-pyrrolidone was used as the solvent to prepare the sample polyaniline solution in the emeraldine base form, insulating state of polyaniline.

3.2.3 Thermogravimetric Analyzer (TGA)

Thermogravimetric analysis (TGA) by Dupont Instrument TGA 5.1 model 2950 used to evaluate the thermal stability and determined the decomposition temperature of biosurfacatnt and polyaniline. The temperature range studied was 50-800°C at a heating rate of 10°C/min under a nitrogen gas atmosphere.

3.2.4 Scanning Electron Microscope (SEM)

A scanning electron microscope (JOEL model JSM-5410LV) is used to investigate the morphology of polyaniline. The small amount of polyaniline particle in water solution was drop on a brass-stub. The samples on stub are coated with thin layer of gold by using a JFC-1100E ion sputtering device.

3.2.5 Transmission Electron Microscopy (TEM)

The affect of aniline and hydrochloric acid on the aggregation behavior of the biosurfactant was observed with transmission electron microscopy (TEM) by using the negative-staining technique. A drop of the test biosurfactant solution sample was placed onto a copper grid, and was stained with 1% uranyl acetate aqueous solution. The excess of the biosurfactant solution was removed by adsorbing the drop with a piece of filter paper. The grid was dried in a vacuum desiccators for at least 6 h. The samples were imaged under a transmission electron microscope (JEOL, JEM-2100).

3.2.6 Wide-angle X-Ray Diffractometer (WXRD)

The X-ray diffraction (Rigaku, model D/MAX-2000) was used to characterize the crystalline structure of polyaniline after doped with 1.5M HCl. The measurements were carried out in the continuous mode with a scan speed of 5°/min covering the angles 2θ between 5 and 50°. Cu $K\alpha_1$ was used as the X-ray source

3.2.7 Electrometer

The electrical conductivity of the polyaniline was measured at room temperature and 50% relative humidity using a conventional two-probe technique with 6517A Electrometer/ High Resistance Meter (Keithley, model 7517A).

3.2.8 Dynamic Light Scattering (DLS)

The dynamic light scattering (DLS) technique was employed to measure the sizes of biosurfactant vesicles formed at various conditions. The Zetasizer Nanoseries model S4700 (Malvern Instrument, UK) was conducted in

range 0.6-6000 nm at 25±1°C. The sizes of biosurfactant vesicles were calculated from a computer analysis using the software provided with the instrument.

3.2.9 Tensiometer

A plate-type tensiometer (Krüss, K10T) was used to measure the surface tension of the aqueous solution at different surfactant concentrations at 25±1 °C.

3.3 Methodology

3.3.1 Production and Extraction of Crude Biosurfactant

- An inoculum prepared by transferring the bacterial colonies into a nutrient broth, and the culture was incubated at 37 °C in a shaking incubator at 200 rpm for 22 h.
- A nutrient broth containing 2% inoculum and 2% palm oil was incubated at 37°C under aerobic condition in a shaking incubator at 200 rpm for 48 h to obtain the highest microbial and surfactant concentration.
- The solution 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.
- 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-to-ethanol ratio at room temperature.
- 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.

3.3.2 Surface Tension Measurement

The surface tension of the aqueous solution at different surfactant concentrations was measured by using a plate-type tensiometer (Krüss, K10T). The surface tension measurement was carried out at 25±1 °C after dipping the platinum ring in the solution for a while in order to attain equilibrium conditions. The

measurement was repeated three times and an average value was obtained. The critical micelle concentration (CMC) was then determined from the break point of the surface tension versus log of initial biosurfactant concentration. For the calibration of the instrument, the surface tension of the pure distillated water was measured before each set of experiments.

3.3.3 Aggregation Behavior of Biosurfactant

Transmission electron microscopy (TEM) and Dynamic Light Scattering (DLS) was use to investigate the aggregation behavior and size of biosurfactant under condition of ANI content and 0.1M HCl content.

3.3.4 Synthesis of Polyaniline in the Presence of Biosurfactant.

- The emulsion was formed by adding aniline monomer into biosurfactant solution with vigorous stirring for 22 h.
- The mixture solution was cooled to $0^{o}C$ with mechanical stirring at 300 rpm for 1 h.
- HCl acid was added drop-wise into the mixture solution within 30 min and maintained mechanical stirring for 30 min.
- During waiting for step 3, the pre-cool solution (at a temperature below 5 ^{o}C) of $NH_4S_2O_8$ in distillation water was prepared.
- A pre-cool $NH_4S_2O_8$ was added into the mixture solution drop-wise within 30 min in order to initiate the polymerization reaction.
- The reaction was carried out for 6 h to complete polymerization reaction.
- The obtained suspension was neutralized by dialysis with mixture solution of distillation water and ethanol for 3 days.
- The suspension product was centrifuged and the precipitated product dried in vacuum oven for 3 days, finally, the resulting PANI was obtained.

3.3.5 Dedoping and Doping of Polyaniline

Firstly, the power of resulting PANI was dedoped with 50 $cm^3$of 0.4M NAOH for 3.5 h followed by dry process in vacuum oven for 3 days. After that, powder of dedope PANI was immersed for 24 h in an aqueous HCl solution (1.5M) and subsequently allowed to dry in vacuum oven for 3 days.

3.3.6 Polyaniline Nanostructures Characterization

3.3.6.1 Structural Characterization

The FTIR spectra of PANI in emeraldine salt (PANI ES) and emeraldine base (PANI EB) form were recorded with Thermo Nicolet Nexus 670 FTIR spectrometer.

3.3.6.2 Morphology

JOEL model JSM-5410LV Scanning electron microscope at 20 kV was use to investigate the morphology of synthesized PANI.

3.3.6.3 Thermogravimetric Analysis

Thermal stability and degradation temperature of obtained PANI were characterized by using temperature in ranging 50-800^{o}C at a heating rate of 10^{o}C/min under a nitrogen gas atmosphere.

3.3.6.4 Crystalline Structure

Wide angle X-Ray Diffractometer was used to studied the crystalline structures of polyaniline after doped with 1.5M HCl for 24 h, additionally, the resulting product synthesized by template route and conventional route were compared.

3.3.6.5 Oxidation State

The oxidation state of synthesized PANI was investigated by Shimadzu UV-VIS spectrometer model 2550 in the wavelength range 200-800 nm.