CHAPTER III
EXPERIMENTAL

3.1 Materials

Acetobacter xylinum (TISTR 975) was purchased from Microbiological resources centre, Thailand Institute of Scientific and Technological Research (TISTR). Analytical grade D-Glucose anhydrous and yeast extract powder, which were used as culture medium, were purchased from Ajax Finechem (Austarlia) and HiMedia (India) respective. Analytical grade sodium hydroxide anhydrate pellet and glacial acetic acid, which were used for purification of BC, were purchased from Ajax Finechem (Austarlia) and Lab-scan (Ireland) respective. Aniline monomer (Merck, Germany) was purified by distillation. Ammonium persulfate (from Sigma-Aldrich, USA) was used as initiator for oxidative polymerization of PANI. Laboratory grade hydrochloric acid (J.T. Baker, Thailand) was used as doping agent. For magnetite nanoparticle synthesis, analytical grade ferric chlorides hexahydrate and ferrous sulfate heptahydrate (Sigma-Aldrich, USA) were used as precusors and laboratory grade ammonium hydroxide (Ajax Finechem, Austarlia) was used as precipifating agent.

3.2 Equipments

3.2.1 Fourier Transformation Infrared (FTIR) Spectroscopy

Fourier transformation infrared (FTIR) spectra of bacterial cellulose, polyaniline and bacterial cellulose containing polyaniline were recorded with a Thermo Nicolet Nexus 670 FTIR spectrometer equipped with default transition mode operate at 32 scan and a resolution of 4 cm^{-1} , covering a wave number range of 4000-400 cm^{-1}.

3.2.2 Scanning Electron Microscope (SEM)

Morphology of pure bacterial cellulose sheets, bacterial cellulose sheets containing polyaniline, bacterial cellulose sheets containing magnetite particles and bacterial cellulose sheets containing both of magnetite particles and

polyaniline were conducted on a JEOL JSM-5200 with an acceleration voltage of 15 kV, magnification in the range of 10,000-15,000 times.

3.2.3 Thermo Gravimetric-Dynamic Temperature Analyzer (TG-DTA)

Thermal stability and degradation temperature of pure bacterial cellulose sheets, pure polyaniline, bacterial cellulose sheets containing polyaniline, bacterial cellulose sheets containing magnetite particles and bacterial cellulose sheets containing both of magnetite particles and polyaniline were studied by a high resolution TG-DTA Pyris Diamond (Perkin Elmer). The samples were weighted in the range of 5-10 mg and loaded on a platinum pan. The mass change with increasing temperature was monitored and recorded from 30-800 °C with a heating rate of 10 °C/min under nitrogen flow.

3.2.4 Electrometer

DC electrical conductivity experiments were carried out with a two point probe arrangement. Two point probes, which were used to contact the samples, were coupled to Keithley electrometer model 6517A.

3.2.5 Custom-built Electromechanical Tester

Electromechanical actuation was investigated in terms of bending deformation. The transparent cell-box with a pair of parallel copper electrode plates were coupled with high DC voltage. The experiment was done in the voltage range of 0-750 V/mm. The video digital camera was used to record the bending deformation.

3.2.6 Vibrating sample magnetometer (VSM)

Magnetic properties of bacterial cellulose sheets containing magnetite particles with and without the incorporating of polyaniline were conducted on vibrating sample magnetometer (Lake Shore, model; 7404 VSM system with 4-inch Electromagnet). The experiment type was Hysteresis, in acquistion mode of continuous with 10 kOe maximum field at room temperature.

3.3 **Experimental Procedures**

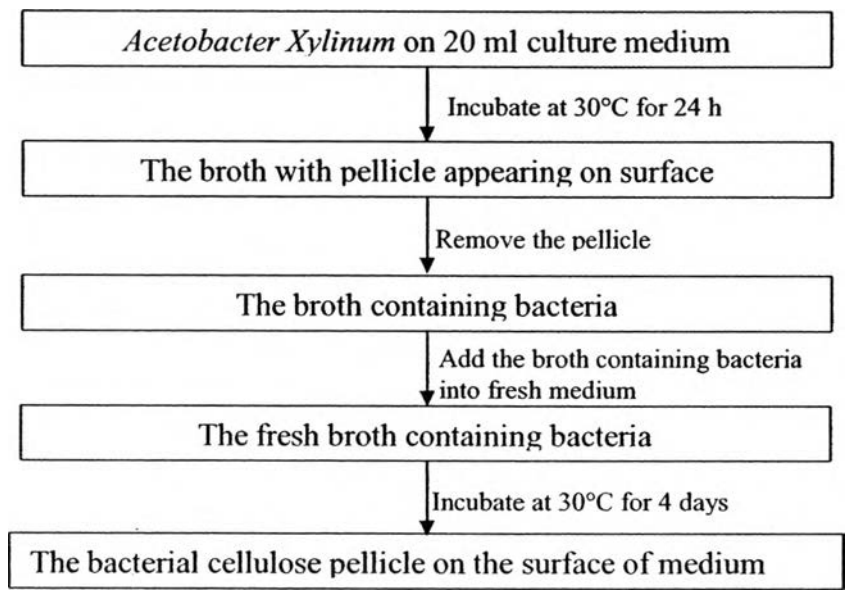
3.3.1 Production of Bacterial Cellulose

3.3.1.1 Culture Medium

Culture medium, which was used for the fermentation of *Acetobacter xylinum* to produce bacterial cellulose, consisted of D-Glucose anhydrous 4 g, extracted yeast powder 1 g, and distilled water 100 ml. After that prepared culture medium was sterilized by autoclaving at 115°C for 15 min.

3.3.1.2 Culture Condition

Pre-inoculum was prepared by transferring a single *Acetobacter xylinum* colony grown on agar culture medium into a 50 ml Erlenmeyer flask filled with 25 ml of liquid culture medium. After 24 h of cultivation at 30°C, the bacterial cellulose pellicle, which is produced on the surface of the culture medium, was either squeezed or vigorously shaken in order to remove active cells embedded in the bacterial cellulose membrane. Ten milliliter of the cell suspension was introduced into a 500-ml erlenmeyer flask containing 100 ml of a fresh liquid culture medium, and then erlenmeyer flask was covered by á porous paper and kept at 30°C for 4 days as shown in scheme 3.1 below.



Scheme 3.1 Procedure for producing of bacterial cellulose.

3.3.1.3 Purification

The bacterial cellulose pellicles was purified by boiling in sodium hydroxide (1.0 %w/v) for 2 h (2 times) to eliminate *Acetobacter xylinum* cells as well as components of the culture liquid, following with neutralizing with acetic acid (1.5 %w/v) for 30 min and then washed extensively with distilled water. Hydrogel sheet of bacterial cellulose was obtained.

3.3.2 Incorporating of Magnetite Particles (Fe_3O_4) into Bacterial Cellulose Sheets by Co-precipitation Method

3.3.2.1 Impregnation of Ferrous Ions (Fe^{2+}) and Ferric Ions (Fe^{3+}) into Bacterial Cellulose Sheets

The mixture solution of ferrous ions (Fe^{2+}) and ferric ions (Fe^{3+}) was prepared by ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in the ratio of 1 to 2. The bacterial cellulose sheets were immersed into the mixture solution at 60 °C for 1 hour to ensure that bacterial cellulose sheets saturated with these ions (the initial concentration of $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$ is shown in the table 3.1).

Table 3.1 Amount of precursors were used in this experiment

Initial concentraion of iron precursors (mol/l)	$FeSO_4 \cdot 7H_2O$ (g)	$FeCl_3 \cdot 6H_2O$(g)
0.01	0.459	0.895
0.05	2.294	4.477
0.10	4.587	8.953
0.20	9.174	17.906

3.3.2.2 Incorporation of Magnetite Nanoparticles (Fe_3O_4) into Bacterial Cellulose

The bacterial cellulose sheets, which saturated with ferrous ions (Fe^{2+}) and ferric ions (Fe^{3+}), were taken into the wide neck round bottom flask and then purge with N_2 gas for 60 min. Afterward, took off the N_2 gas valve and took

on the NH_3 gas valve for 30 min (The ammonia gas was produced from boiling ammonia solution). The color of the bacterial cellulose sheets were quickly changed from clear (no color) to black, indicating the formation of magnetite (Fe_3O_4) particles. And finally, the system was purged with N_2 again for 20 min.

3.3.2.3 Washing and Drying

The resultant bacterial cellulose sheets containing Fe_3O_4 were washed with distilled water three times. And then they were dried by using freeze dry technique.

3.3.3 Synthesis of Polyaniline by Oxidative Polymerization using Ammoniumpersulfate as Oxidizing Agent

3.3.3.1 Impregnation of Aniline Monomer into Bacterial Cellulose Sheets

Bacterial cellulose in the form of hydrogel sheets were weighed and added with desired amount of aniline monomer (15-40 %w/w) and then, sonicated them for 30 min to provide the good penetration of aniline monomer to bacterial cellulose sheets. After that, 20 ml. hydrochloric acid (0.48 M) was poured into the bacterial cellulose sheet with containing a small amount of aniline monomer and further sonicate for 30 min to increase the penetration efficiency of aniline monomer into bacterial cellulose sheets.

3.3.3.2 Synthesis of Polyaniline into Bacterial Cellulose Sheets

Polyaniline was synthesized in aqueous solution by the oxidative polymerization of aniline, using ammonium peroxide (APS) as an oxidant, in the presence of bacterial cellulose sheet. The synthesis procedure was described as follows: fourty milliliter of distilled water was poured into bacterial cellulose sheet with containing aniline monomer in the reaction system, which consists of bacterial cellulose sheets containing aniline monomer was cooled down to 0 °C with mechanical stirring for 1 h, twenty milliliter of 0.48 M HCl was added drop-wise into the reaction and the reaction will be maintained with mechanical stirring for 1 h. Next, a pre-cooled solution, at a temperature below 5 °C, containing APS in 40 ml of 0.48 M HCl was added drop-wise, and the reaction was stirred at 0°C for 4 h to complete the polymerization.

3.3.3.3 Washing and Drying

The bacterial cellulose sheets containing polyaniline were washed with an excess amount of distilled water, until becoming neutral. Finally, they were dried and kept in desiccators prior to use.