CHAPTER III
METHODOLOGY

3.1 Materials

Acetobacter xylinum (strain TISTR 975), an isolated strain in Thailand, was supplied from the microbiological Resources Center, Thailand Institute of Scientific and Technological Research (TISTR). Analytical grade of anhydrous D-glucose, sodium chloride, sodium hydroxide, methylene blue was purchased from Ajax Finechem. Bacteriological grade of yest extract powder, beef extract powder and agar powder was purchased from HiMedia. Bacteriological peptone was (Laboratorios Conda, Pronadisa, Micro & Molecular Biology). Titanium tetraisopropoxide (TTIP, 97%) was purchased from Sigma Aldrich. Analytical grade of absolute ethanol 99.8% assay and glacial acetic acid was purchased from Carlo Erba.

3.2 Measurements

3.2.1 Fourier-transform Infrared Spectroscopy (FTIR)

Functional group of chemical structure was characterized by a Thermo Nicolet NEXUS 670 Fourier-transform infrared spectrometer.

3.2.2 Thermal Gravimetric Analysis (TGA)

Thermal decomposition temperature was determined by a Perkin Elmer Pyris Diamond thermogravimetric/differential thermal analyzer using a heat program of 30 °C to 1000 °C at a heating rate of 20 °C min^{-1} under air atmosphere and using alumina pan.

3.2.3 X-ray Diffraction Analysis (XRD)

The crystallographic form of TiO_2 was verified by X-ray diffraction (XRD) Bruker AXS, German model D8 advance. The samples were scanned from $2\theta = 10°$ to $2\theta = 80°$ at scanning rate of 1 degree per minute.

3.2.4 Scanning Electron Microscope (SEM)

The morphology of bacterial cellulose and TiO_2 impregnated bacterial cellulose were observed by using a scanning electron microscope (FE-SEM) (S-4800, Hitachi High-Technologies Corporation, Ibarakiken, Japan).

3.2.5 The Photocatalytic Activity

The photocatalytic activity on methylene blue decolorization was determined by using UV-visible spectrophotometer (Shimadzu model UV-1800).

3.3 Methodology

3.3.1 Production of Bacterial Cellulose

A lyophilized *Acetobacter Xylinum* from Thailand Institute of Scientific and Technological Research was transferred to 20 mL of culture medium consisting of glucose (4 %w/v) and yeast extract (1 %w/v) dissolving in distilled water. Then, the culture broth was incubated at 30 °C for 1 day. After that a thin piece of pellicle appearing on surface of culture broth was removed before adding 10 mL of culture broth into 100 mL of a fresh culture medium, followed by incubating at 30 °C for 4 day. The bacterial cellulose pellicle was on the surface of culture broth. Afterward, the bacterial cellulose pellicle was steriled by autoclave at 120 °C for 15 min. Next, the remaining of bacteria cells in bacterial cellulose pellicle was removed by boiling in sodium hydroxide solution (1%v/v) at 80 °C for 2 hr with stirring. This process was repeated for 3 times for the compleate removal of bacteria cells. The bacterial cellulose pellicle was then neutralized by boiling in acetic acid solution (1.5%v/v) at 80 °C for 30 min. After that the bacterial cellulose pellicle was immerged in distilled water 1 day, followed by immerging in commercial ethanol for 1 day and then changed to absolute ethanol (99.8%v/v). After immersion in absolute ethanol for 2 day by changing absolute ethanol everyday, the bacterial cellulose pellicle was sonicated in fresh absolute ethanol (99.8%v/v) for 30 min. in order to get ethanol-saturated bacterial cellulose pellicle.

3.3.2 Synthesis of TiO_2 into Bacterial Cellulose Pellicle

Titanium tetraisopropoxide (TTIP, Aldrich) was used as a titanium source. A different amount of TTIP was added to 300 mL of absolute ethanol

consisting of 99.8% EtOH and 0.2% H_2O in an Erlenmeyer flask by conditions studied vary molar ratio TTIP to water as shown in table 4.1. Next, the bacterial cellulose pellicle was added into the TTIP solution. The mixture was heated in an autoclave at 127 °C for 7 hr to achieve the conversion of TTIP to TiO_2 by hydrolysis. Then, the bacterial cellulose pellicle containing TiO_2 was washed by sonicating in absolute ethanol and sonicating in distilled water for 30 min, respectively. Finally, the TiO_2 impregnated bacterial cellulose was dried in a vacuum chamber overnight.

3.3.3 Study on Photocatalytic Activity under UV Source

Decolorization of methylene blue (MB), a basic dye, was used to determine photocatalytic activity of TiO_2 impregnated bacterial cellulose under UV source. Photocatalytic degradation of MB by TiO_2 impregnated bacterial cellulose was performed according to the following procedure: TiO_2 impregnated bacterial cellulose sample (2 mg calculating from section 4.3) was put into a MB solution, 0.5 g in distilled water 1 L, in a plastic cuvette and kept in a dark condition for 1 day. Then, the UV radiation was provided from the top of the plastic cuvette by using a UV lamp of 300 watt 230 volt. During the photoreaction, samples were taken from the reaction mixture at 5, 10, 20, 30, 60 min and then every 30 min for determination of photocatalytic activity by UV-vis spectroscopy at a wavelength of 664 nm

The Phocatalytic activity of TiO_2 impregnated bacterial cellulose prepared at different molar ratio was determined base on Pelton *et al*., (2006)

$$\text{The degradation MB efficiency (\%)} = [(I_0 - I) / I_0] \times 100$$

Where I_0 = initial absorption intensity of MB at λ_{max} = 664 nm

I = intensity at λ_{max} after illumination at any time.

3.3.4 Antibacterial Activity Test

Antibacterial activity of TiO_2 impregnated bacterial cellulose was investigated by using the colony forming count method according to the following procedure. A colony of *Escherichia coli*, a gram negative bacterium, or *Staphylococcus aureus*, a gram positive bacterium, was put into 20 mL of culture medium containing 0.5%w/v peptone and 0.3%w/v beef extract dissolved in distilled

water. The culture broth was incubated in a shaking incubator at 37 °C and 110 rpm for 24 hr. Then the cell dilution was performed by transferring 0.1 mL of culture broth to 9.9 mL of a fresh medium. The dilution process was performed until an appropriate amount of cell concentration was obtained. Next, TiO_2 impregnated bacterial cellulose, 1.5 cm in diameter, was put into a culture broth containing an appropriate amount of bacteria, followed by incubating in a shaking incubator at 37 °C and 110 rpm for 24 hr with the exposure to an UV lamp (300 watt, 230 volt). After incubation, 0.1 mL of culture broth was spread on a nutrient agar plate containing 0.3%w/v beef extract, 0.5%w/v peptone and 1.5%w/v agar dissolved in distilled water, followed by incubating at 37 °C for 24 hr The colony-forming unit (CFU) was calculated and the average was taken of the three plates. The antibacterial activity against either *S. aureus* or *E. coli* of TiO_2 impregnated bacterial cellulose was determined from the percent reduction in the number of viable bacterial cells (Maneerung *et al.*, 2008).