CHAPTER IV

EXPERIMENTAL

4.1 Materials

4.1.1 Microbial Strains

The *A. xylinum* strain was isolated from *nata de coco*. The stock culture was kindly supplied by Pramote Tammarat, the Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand.

4.1.2 Other Chemicals

The details of chemicals used in this experiment are shown in Table

Table 4.1 The chemicals used in this experiment

Chemical	Supplier
Sucrose	Ajax Finechem
Sodium alginate	Carlo Erba
Ammonium sulfate	Carlo Erba
Sodium hydroxides	Carlo Erba
Sodium alginate	Carlo Erba
Acetic acid	BDH
Ethanol absolute	BDH

4.1.3 Equipments

-Scanning electron microscopy, SEM (JOEL JSM-5410LV, Japan).

-Fourier Transform Infrared (FTIR) spectrometer (Nicolet SX-170, USA).

-Universal testing machine (LLOYD 2000R, UK).

-Brunauer-Emmett-Teller (BET) surface area analyzer (Model ASAP 2020, USA).

-Autoclave (Model Tomy Autoclave SS-325, Ner ima-ku, Tokyo, Japan).

-Vacuum pump (Model RV5 Edwards, England).

4.2 Membrane preparation

The medium for the inoculum was coconut-water supplemented with 5.0% sucrose, 0.5% ammonium sulfate $(NH_4)_2SO_4$, 1.0% acetic acid, and different alginate concentration. The experiment was designed to test effects of supplementation of alginate (0%, 0.5%, 0.75%, 1% w/v). Precultures were prepared by a transfer of 50 ml stock culture to 1000 ml in 1500 ml bottle and incubated statically at 30 ^{o}C for 7 days. After the surface pellicle was removed, the 5% (v/v) preculture broth was added to the main culture a medium with different alginate content. The 75 ml of activated medium was inoculated in a Preti-dish and kept at 30 ^{0}C for 7 days.

All sample membranes were first purified by washing with DI water and then was treated with NaOH at room temperature to remove bacterial cells followed by a rinse with DI water and 1%acetic acid (w/v) until pH came to 7. Afterward, the BC

membrane was air-dried at room temperature (30 ^{0}C) and stored in plastic film at room temperature.

4.3 Characterization of BC-alginate membrane

4.3.1 Scanning electron microscope (SEM)

The examination of the surface properties was performed by scanning electron microscopy (SEM). Scanning electron micrographs were taken with JOEL JSM-5410LV microscope at Scientific and technological research equipment centre, Chulalongkorn University. The BC membranes were frozen in liquid nitrogen, immediately snapped, and vacuum-dried. Then, the membranes were sputtered with gold and photographed. The coated specimens were kept in dry place before experiment. SEM was obtained at 15 kV which was considered to be a suitable condition since too high energy can be burnt the samples.

4.3.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was used primarily to identify the chemical structure of the sample. FTIR spectra of the membranes were recorded with a Nicolet FT-IR Spectrometer (SX-170) in the region of 4000–500 cm^{-1}, at Scientific and technological research equipment centre, Chulalongkorn University.

4.3.3 Tensile properties testing

In this study, the tensile strength of the membrane was measured by Instron Testing Machine (5567, NY, USA) at Polymer Engineering Laboratory, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University. The test

conditions follow ASTM D882. The determination of tensile property was done under BC membrane was cut into strip-shaped specimens 10 mm in width and 10 cm in length. At least five specimens were used for each blend composition.

4.3.4 BET Surface analysis

The pore size and surface area of the membranes were measured by a Brunauer-Emmett-Teller (BET) surface area analyzer (Model ASAP 2020). The samples were placed in the sample cell, which was then heated up to 75 °C and held at this temperature for 2 hours. The samples were cooled down to room temperature and ready to measure the surface area. There were three steps to measure the surface area: adsorption step, desorption step and calibration step.

4.3.5 The degree of swelling measurement

The degree of swelling of membranes was determined by immersing the dried membrane in distilled water and 95% ethanol solution (v/v) at room temperature until equilibration. The membrane was then removed from the water and ethanol solution. After that, the surface of the membrane was blotted out with tissue paper, the weight of the swollen membrane was measured and the procedure was repeated until there was no further weight change. The degree of swelling was calculated using the following formula:

$$Swelling(\%) = \frac{W_h - W_d}{W_d} x100$$

Where W_h and W_d denoted the weight of hydrate and dry membrane, respectively.

4.3.6 The water vapor permeability measurement

Water vapor transmission rate (WVTR) of the BC membrane with area of 50.00 cm^2, were measured on water vapor permeation tester; Lyssy L80-4000 (at Thailand institute of scientific and technological research). The test conditions follow ISO 15106-1. The determination of WVTR was done under the following conditions: temperature, 38 °C; % Relative Humidity, 90%. The principle of this electronic tester is similar to that of conventional method. One side of the membrane was exposed to the water vapor. As water solubilizes into the membrane and permeates through the sample material, nitrogen sweeps the opposite side of the membrane and transports the transmitted water vapor molecules to the calibrated infrared sensor. The response was reported as a transmission rate.

4.3.6 The oxygen permeability measurement

Oxygen transmission rate (OTR) of the BC membrane were measured on oxygen permeation tester; ILLINOIS MODEL 8000 (at Thailand institute of scientific and technological research). The test conditions follow ASTM D3985. The determination of OTR was done under the following conditions: temperature, 23 °C; % Relative Humidity, 0%. The test BC membrane was held in such a manner that it separates two side of a test chamber. One side was exposed to a nitrogen atmosphere and the other side was exposed to an oxygen atmosphere. Testing was completed when the concentration of oxygen in the nitrogen side was constant.

4.4 Pervaporation

4.4.1 Pervaporation experiment

Figure 4.1 shows the diagram of pervaporation unit for dehydration of an aqueous ethanol solution in this study. The experimented procedure for determination of the optimal condition for the pervaporation dehydration of an aqueous ethanol solution is as follows.

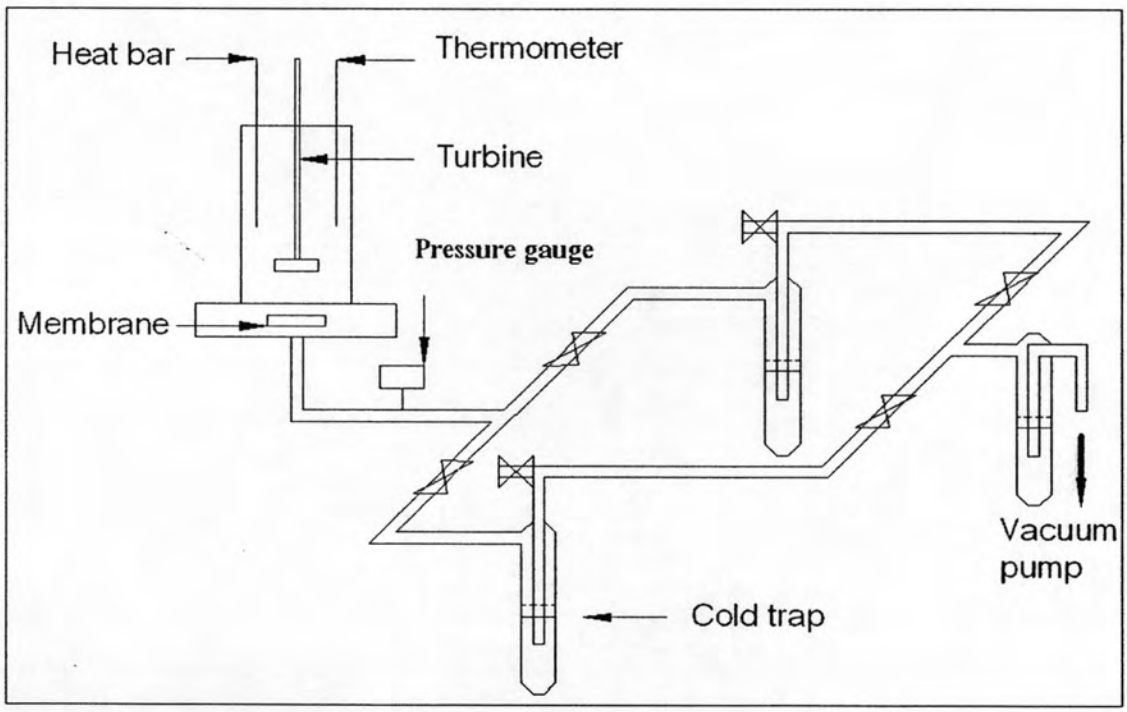

Figure 4.1 The diagram of pervaporation unit for dehydration of an aqueous ethanol solution

A 900 ml working volume stirred batch reactor with a pervaporation membrane on the bottom was used for the examination of pervaporative performance of the membranes. The effective area of the membrane was 19.625 cm^2. The feed solution was placed on one side of the membrane. The other side of membrane was evacuated with a vacuum pump. The permeate vapor was trapped in a cold trap using liquid nitrogen. The permeate pressure was kept below 10 mmHg. The concentration of ethanol in the feed solution was varied from 70, 80, 90 and 95% (w/w). The study for the effects of temperature was performance in the range of 30, 40, 50 and 60 °C .

4.4.2 Sample analysis

Ethanol assay was conducted by gas chromatography using a Shimadzu Model GC 7AG equipped with Flame Ionization Detector (FID). A column with length of 2 m, outer diameter of 3.3 mm, and packed with Porapak Q 80-100 mesh was used in collaboration with N2 as carrier gas. Flow rate of N_2 was 50 ml/min. The oven and detector temperature were 190 °C and 240 °C respectively. The samples are injected with volume of 1 µl and injection temperature of 240 °C.