CHAPTER IV

EXPERIMENTS

The purpose of this experiment was to study the control of biomass concentration in a continuous membrane reactor. The detail of the experimental investigation is as follows:

4.1 Strain

The strain used in this study was Clostridium Acetobutylicum ATCC 824 stored as liquid samples from non-pH regulated cultures at 0 ^{o}C in glass tubes.

4.2 Medium

The synthetic medium contained per litre of distill water :

K_2HPO_4	0.5	g
KH_2PO_4	0.5	g
$MgSO_4.7\ H_2O$	0.2	g
$MnSO_4.7H_2O$	0.01	g
$FeSO_4.7H_2O$	0.01	g
NaCl	0.01	g

Yeast extract	6.0	g
D(+) Glucose anhydrous	50	g
Silicone antifoam emulsion ,DFF-372	0.2	ml

Silicone antiform emulsion was added to the fermentation broth for foam reduction.

4.3 Experimental Equipments

4.3.1 Fermentor

A Eylla-M100 1 litre glass fermentor was used. The pH of the experimental fermentation broth was controlled by a pH controller which controlled an alkali feed pump. The temperature of the system was controlled at 35°C by a recirculated water-bath. Cell concentration was controlled by the turbidity meter FSC402.

4.3.2 Ceramic Filter (Figure 4.1)

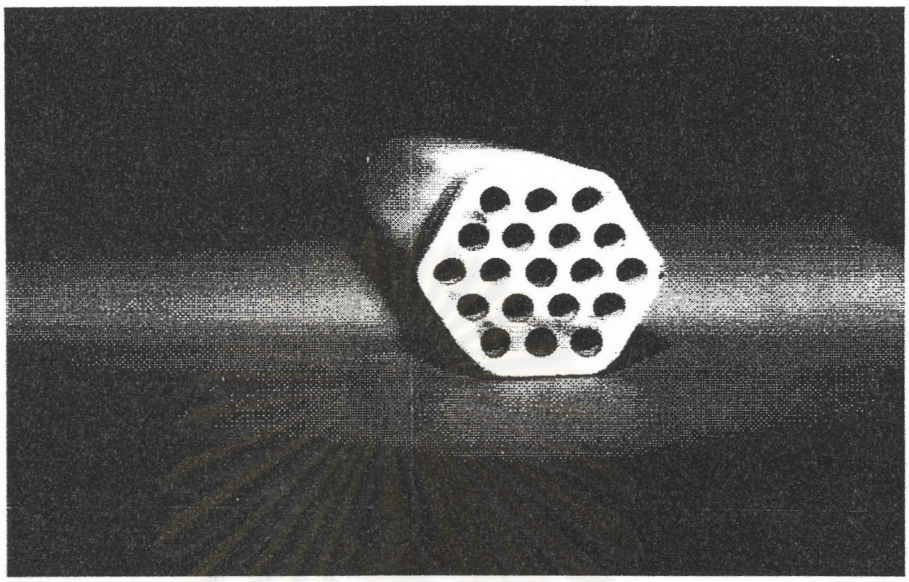
The microfilter was the ceramic filter(type 1M-1,Japan) consisting of 19 carbon tubes with microfiltering ceramic coat inside. The tubes were 4 mm. inside diameter ,85 cm. long and 0.2030 m^2 total filtration area. The pore diameter was 0.2 μm. (molecular weight cut off 100,000 approximately).

4.3.2.1 Cleaning and Regenerating the filter.

With the microfiltration membranes, that are composed of α-alumina, it can be cleaned by chemical reagent using acid and alkali. In this experiment, the used filter was washed with demineralized water, and then dip it into chemical solution (0.5 to 2% NaOH) for 12 to 24 hours, and then washed with demineralized water again.

4.3.3 The Cell Recycling System

A peristaltic pump was used for pumping the nutrient which was controlled by a level controller. The fermentation broth was circulated through the microfiltration module with a sanitary rotary pump(0.75 kW). The circulation velocity was 0.465 m/s. The permeate was continuously discharged at a fixed flow rate F and collected in a storage tank. The retentate was directly recycled to the fermentor.



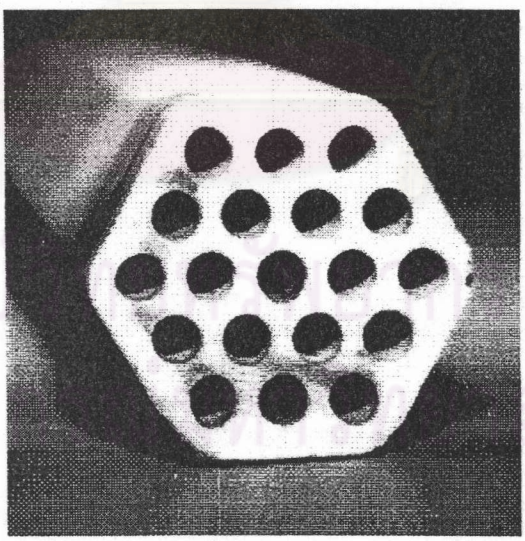


Figure 4.1 A ceramic filter (type 1M-1)

4.4 Experimental Procedure

4.4.1 Sterilization and Cleaning.

The cell recycling system was cleaned by a chemical solution and rinsed by demineralized water until the permeate flow rate was not less than the set point (7.27×10^{-2} m^3/hr at recirculation flow rate 0.4 m^3/hr, pressure 0 kg_f/cm^2 at 33°C). The system was steam sterilised by flushing with saturated steam at 100°C for more than 30 minute for each parts. The medium and other equipments were sterilised by keeping in the autoclave at 1.5 bar, 121°C for 15 minute.

4.4.2 Preparation of the Clostridium acetobutylicum ATCC 824 Cultures

A 10 ml of Clostridium acetobutylicum ATCC 824 stored as liquid at 0°C in a glass tube was aseptically transfered to 100 ml of the sterilised medium in a 500 ml flask. The medium was kept anaerobic by bubbling pure nitrogen for 15 minute, and was then kept at 35°C for a 18 hours growth. Culture tube and inoculum flask were shown in figures 4.2 and 4.3.

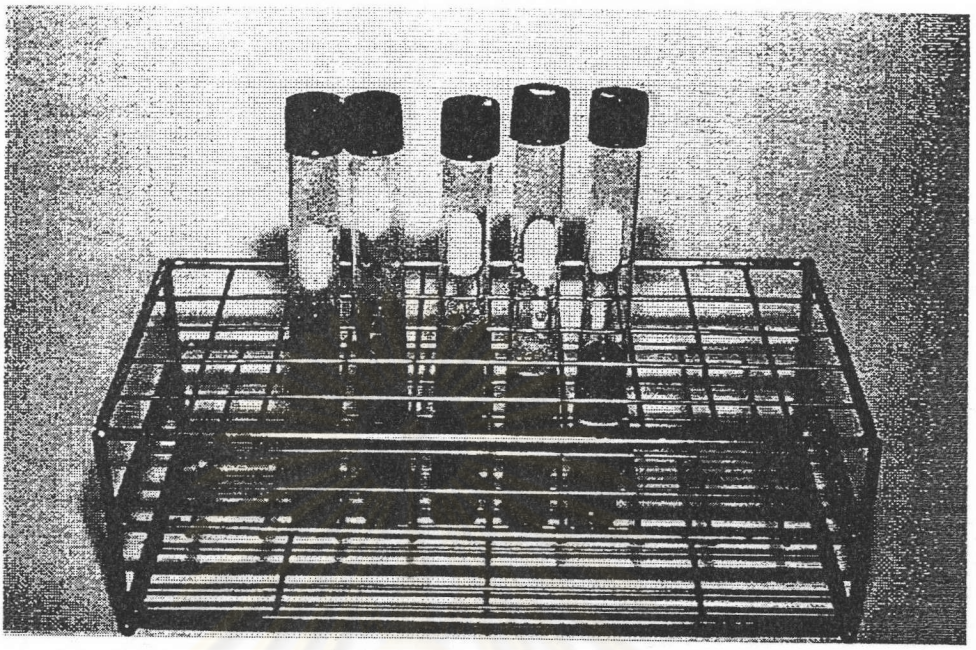


Figure 4.2 Culture tubes.

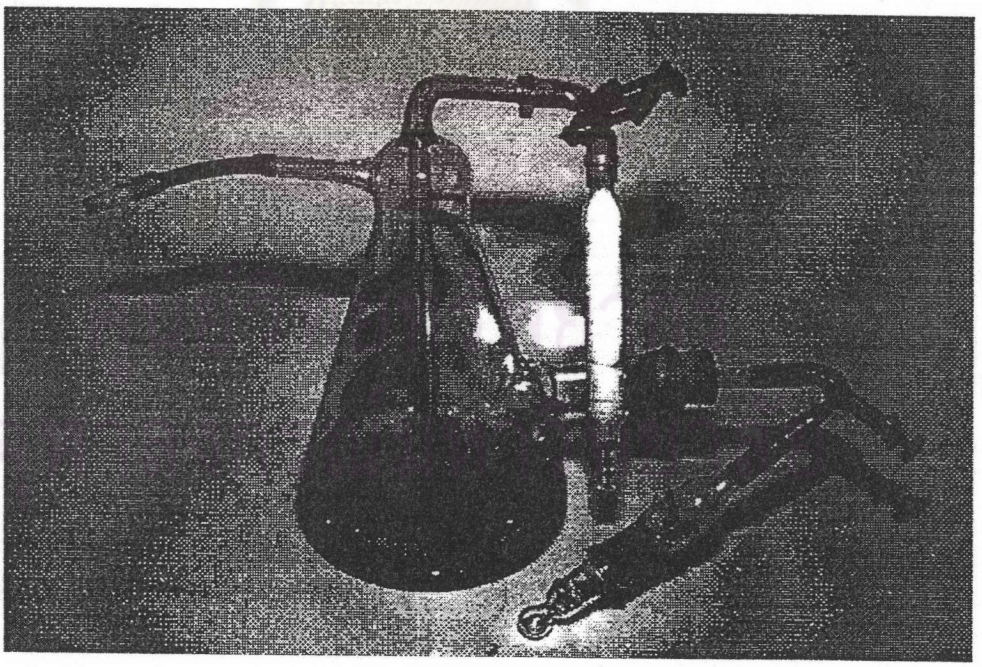


Figure 4.3 An inoculum flask.

4.4.3 Batch Operation in the Cell Recycling System

The 100 ml of the prepared culture (from 4.4.2) was aseptically transferred to 1 liter of the sterilised medium in the fermentor. The agitator speed was maintained at about 200 rpm and the temperature was controlled at 35°C. The pH of medium was controlled not to be less than 4.8 by automatic addition of 1N NH_4OH. The medium was kept anaerobic by bubbling of pure nitrogen.

After the residual substrate concentration reached about 18 g/l or until the biomass concentration was constant, 50 g/l glucose of the sterilised medium was added by medium feed pump. Every 6 hours of the operation, 5 ml of fermentation broth was aseptically taken from the fermentor for determination of products, biomass concentrations and glucose concentrations.

4.4.4 Continuous Operation in the Cell Recycling System.

The continuous operation was started after the residual substrate concentration reached about 18 g/l or until the biomass concentration was constant. After that, a sterilised medium was fed into the fermentor by a peristaltic pump controlled with a level controller, while the permeate was continuously discharged at a fixed flow rate to the product storage tank. The pH of the medium was controlled not to be less than 4.8 by automatic addition of 1N NH_4OH. The temperature was controlled at 35°C.

4.4.5 Study of Controlled Biomass Concentration in A Continuous Fermentation.

The aim of this study was to find the steady state optimum solvent productivity. At a uncontrolled biomass concentration system, the optimum productivity was not constant, since solvent productivity depends on cell concentration. At high biomass concentration, the solvent produced inhibits growth rate. Then the biomass concentration was decreased. Consequently,the solvent productivity was decreased too. The important parameters under our study were the dilution rate and biomass concentration. Other parameters such as temperature, pH, agitator speed, glucose concentration were fixed at the optimum point in a batch acetone-butanol fermentation process. Schematic diagram and photograph of this operation were shown in Figure 4.4 and 4.5, respectively. From continuous fermentation with cell recycling unit, biomass concentration was started to control at 20 g/l. The biomass concentration was kept constant by turbidity controller. Firstly, the dilution rate was fixed at 0.1 hr^{-1} until the residual glucose, biomass concentration, and glucose concentration were constant at steady state. The system was continuously maintained for 18 hours. For 20 g/l biomass concentration, the dilution rate was changed to 0.13 hr^{-1} and 0.19 hr^{-1}, respectively. After that, biomass concentration was changed from 20 g/l to 40 g/l. The dilution rate at 40 g/l biomass concentration were 0.3 hr^{-1} and 0.5 hr^{-1}, and then biomass concentration increased up to 60 g/l at the dilution rate of 0.5 hr^{-1}

The final biomass concentration in this experiment was 80 g/l at the dilution rate of 0.5 hr^{-1}. Every 6 hours of operation, 5 ml of fermentation broth was aseptically taken from the fermentor for determination of biomass concentration and 10 ml of permeate was taken for determination of products and glucose concentrations.

4.5 Analysis

4.5.1 Determination of Biomass concentration.

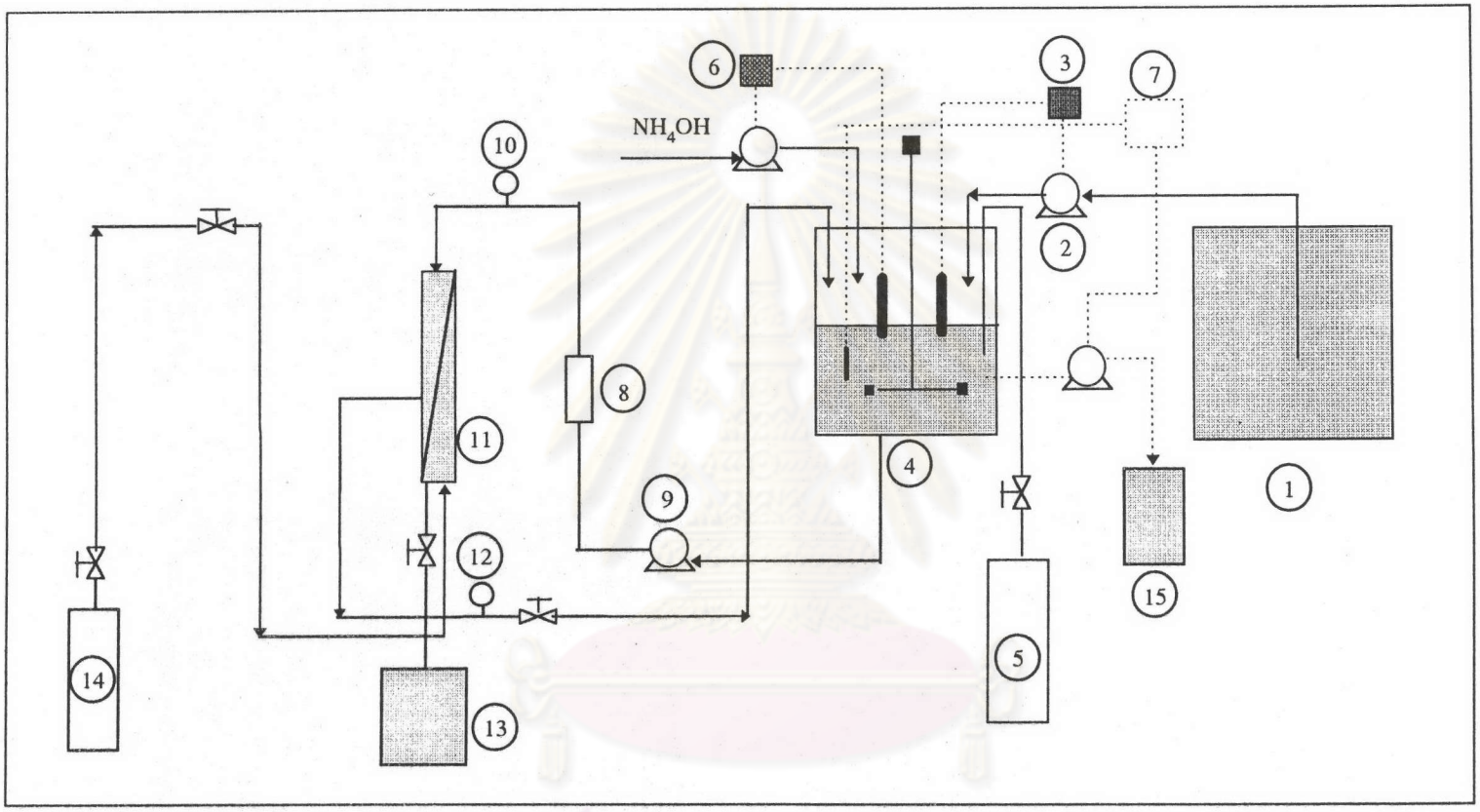
Biomass concentration was determined by two independent method:

1. Optical density measurement at 625 nm with a spectrophotometer: The samples were diluted in order to work in the linear range.

2. Dry weight: A 5 ml sample of fermentation broth was centrifuged at 3,400 rpm for 30 minute. The cell pellet was resuspended and was washed twice with distilled water and then was dried for 48 hours, at 90 °C and was weighed.

4.5.2 Determination of Product Concentrations and Glucose Concentration.

Acetone, n-butanol, ethanol, acitic acid, and butyric acid were determined by gas chromatography using a Shimazu Model G_C7A_G equipped with a flame ionization detector. Separation took place in a 2 m x 0.125 inch stainless steel column packed with Porapak Q 80-100 mesh at 185°C and nitrogen was used as carrier gas. The injector temperature was 210°C and the detector temperature was 210°C. The analysis of the chromatogram was with a Chromatopac C-R6A recorder. Flow rate of carrier gas was 50 ml/min, retention time of butanol, acetone, ethanol, acetic acid , and butyric acid were 5.38, 1.88, 1.28, 2.79, and 11.99 minutes, respectively. Glucose was determined by a YSI Model 27 glucose analyzer.



1.feed tank
2.feed pump
3.level controller
4.fermentor
5.N_2storage tank
6.pH meter
7.turbidity controller
8.flow meter
9.recirculate pump
10.inlet pressure gauge
11.ceramic filter
12.outlet pressure gauge
13.storage tank.
14.N_2 storage tank
15.waste

Figure 4.2 Schematic diagram of control of biomass concentration in a continuous fermentation.

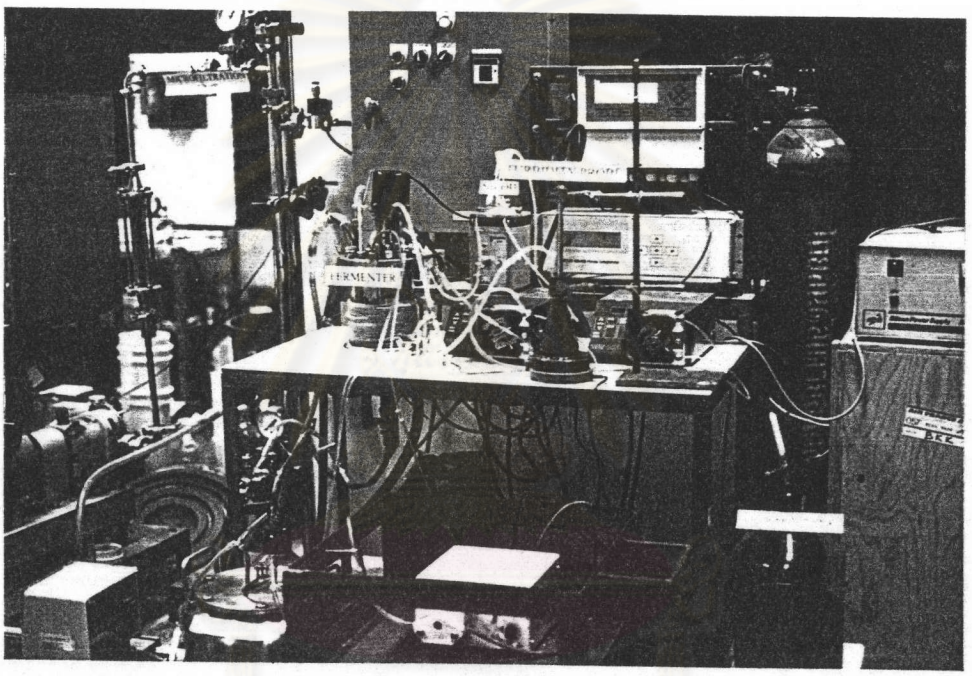


Figure 4.5 Photograph of Controlled biomass microfiltration system.