
CHAPTER III

MATERIAL AND METHODS

1. Culture and culture media

The lyophilized culture used in this study was *Clostridium thermoaceticum* (ATCC 39073). The *Clostridium thermoaceticum* was grown in formulated thioglycollate broth (FTGB) media. The FTGB was media preparing from the ingredient of thioglycollate broth which contained 0.55% glucose, 0.5% Yeast extract, 0.05% sodium thioglycollate, 0.25% sodium chloride, 0.50% L-cysteine and 1.5% casitone. The bacterium was cultivated in 40 ml of media in 50 ml Erlenmeyer flask at 58° C in anaerobic chamber for 5 day. It was serially transferred two or three times in FTGB media for 3 days prior to use in the experiments.

2. Fermentation

All fermentations were carried out in 40 ml of media in 50 ml Erlenmeyer flask. The inoculum used was 5% v/v of 3 day-old cultures. The cultures were incubated at 58°c in an anaerobic chamber. Growth of *Clostridium thermoaceticum* was determined by measuring the increase in absorbance at 660 nm (Spectronic Genesys 5. Milton Roy, U.S.A.). Acetic acid concentration was determined by titration technical.

2.1. Growth in formulated thioglycollate broth media (FTGB)

The media was formulated from thioglycollate broth which contained 0.55% glucose, 0.5% Yeast extract, 0.05% sodium thioglycollate, 0.25% sodium chloride, 0.50% L-cysteine and 1.5% casitone and so called formulated thioglycollate broth (FTGB). The inoculated cultures were incubated at 58°c in an anaerobic chamber. Growth and acetic acid production were measured after 5 day.

2.2. Fermentation in molasses

2.2.1. Without treatment

Molasses used in the experiments were Blackstrap molasses which were obtained from Phisanulok sugar factory, Bangkok, Phisanulok, Thailand. Molasses were diluted to 1:0, 1:25, 1:50 and 1:100 and filtered through Whatman paper no. 2. Pretreatment of molasses was then filtered sterilized prior to use as substrate for fermentation. These Molasses were inoculated with 5% v/v inoculum and were incubated at 58°C in an anaerobic chamber. Growth and acetic acid production were measured after 5 day.

2.2.2. Charcoal pretreated molasses

Pretreatment of 1:25, 1:50 and 1:100 diluted molasses with activated charcoal were conducted to remove colorant. Five gram activated charcoal was added into 100 ml molasses (1:20) basis. The mixture of molasses and activated charcoal was stirred for two hours and filtered through Whatman paper no. 2 and through Whatman paper no. 5 to remove charcoal. Pretreatment of molasses was then filtered sterilized prior to use as substrate for fermentation. Growth and acetic acid production were measured after 5 day.

2.2.3. Molasses with Nutrient supplement

Pretreatment of 1:25, 1:50 and 1:100 diluted molasses with nutrient supplement was studied. The ingredients of FTGB except sugar (STG) were autoclaved separately and added to molasses prior to use as the substrate fermentation. In addition, each separately ingredient of yeast extract, sodium chloride, sodium thioglycollate, L-cysteine and casitone as well as there combination of yeast extract, sodium chloride, sodium thioglycollate, L-cysteine and casitone were added to study the most effective ingredient in acetic acid production from molasses.

2.3. Additional of phosphate

The effect of phosphate on growth and acetic acid production in pretreatment of 1:50 diluted molasses with nutrient supplement was studied. Phosphate concentration of 40, 80 and 120 mM were added in 1:50 diluted molasses with nutrient supplement. The inoculated cultures were incubated at 58°C in an anaerobic chamber. Growth and acetic acid production were measured after 5 day.

2.4. Fermentation in Batch fermenter

Two liter of pretreatment of 1:50 diluted molasses with three combination of casitone, yeast extract and 120 mM of phosphate were subjected to 2.5 liter batch fermenter. The fermentation was carried under 100 rpm agitation and CO₂ bubble at 58°C. Ten milliliter of samples was taken out every 24 hrs and growth and acetic acid production were determined.

3. Cost analysis

Cost of acetic acid production from molasses was determined from the cost of chemical used including system management.

$$\text{Cost of acetic acid production} = \frac{\text{Total cost through project life time}}{\text{Quantity of acetic acid produced through project life time}} \dots\dots\dots (i)$$