CHAPTER IV

RESULT AND DISCUSSION

1. Growth of *Clostridium thermoaceticum* in formulated thioglycollate broth media

Clostridium thermoaceticum ATCC 39073 was grown in 58°C in an anaerobic condition using formulated thioglycollate broth media which compose of 0.55% glucose (carbohydrate source), 0.5% yeast extract, 0.05% sodium thioglycollate, 0.25% sodium chloride, 0.5% L-cysteine and 1.5% casitone. The result indicated that growth was rapidly increasing within 3 days of fermentation and acetic acid was found at 1.44 mg/ml after 3 days (figure 7).

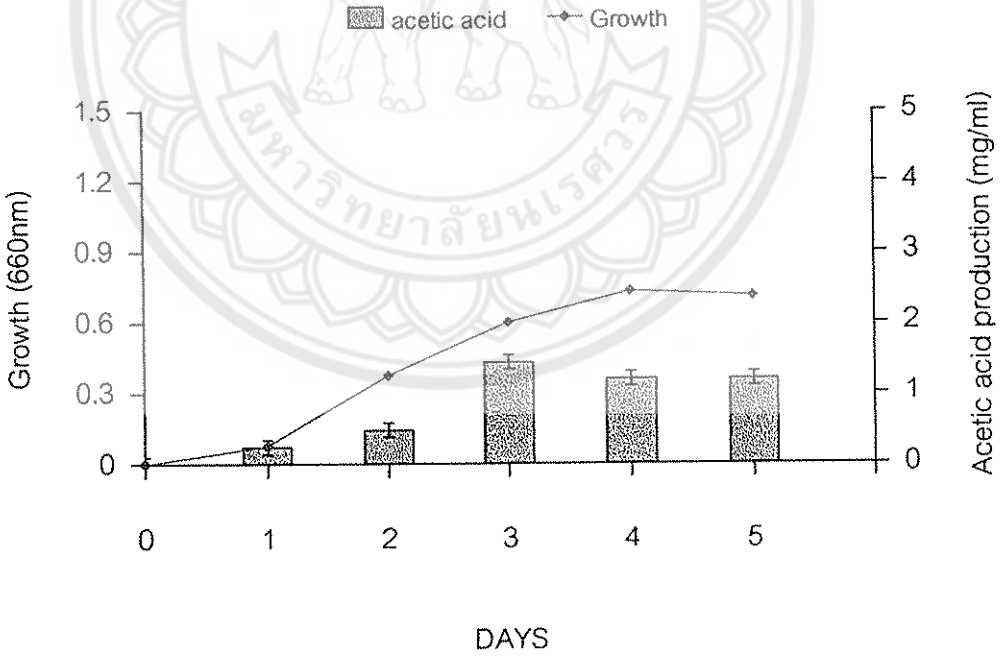


Figure 7 Growth and acetic acid production by *Clostridium thermoaceticum* in formulated thioglycollate broth media in an anaerobe jar at 58°C for 5 days.

2. Fermentation of *Clostridium thermoaceticum* in molasses

The use of molasses as substrate for *Clostridium thermoaceticum* was studied both as diluted molasses and non-diluted molasses. The result showed that *Clostridium thermoaceticum* can grow and produce acetic acid only in diluted molasses at dilution of 1:50 and 1:100. Acetic acid concentration was found at 0.12 mg/ml and 0.24 mg/ml in 1:50 and 1:100 diluted molasses respectively. The bacteria can not grow and produce acetic acid in non-diluted molasses (1:0) and 1:25 diluted molasses (figure 8).

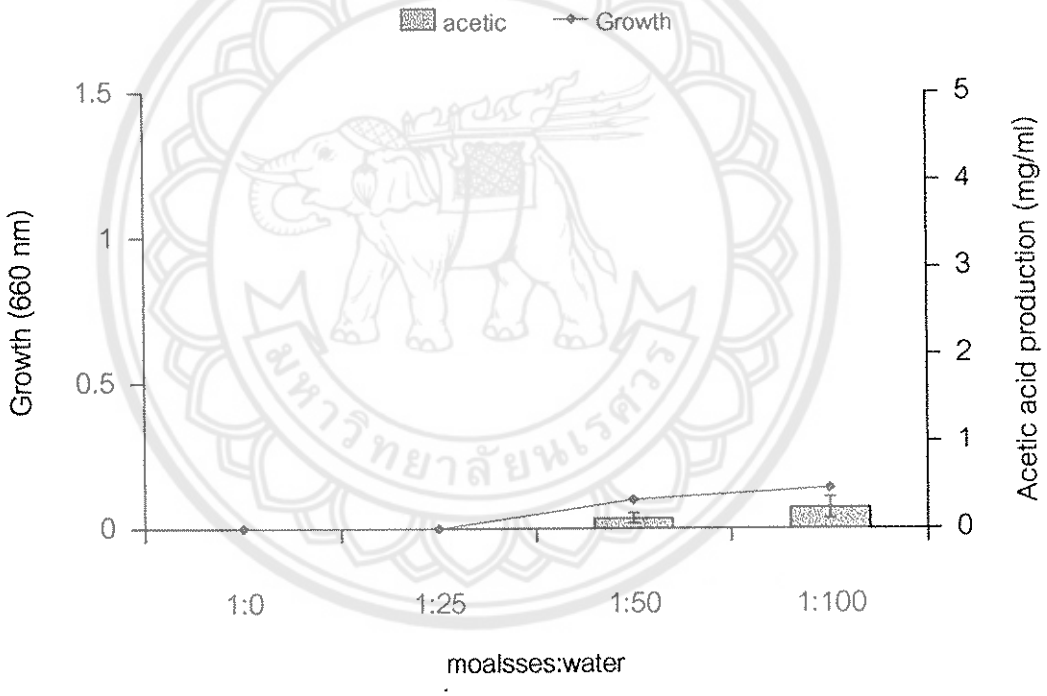


Figure 8 Growth and acetic acid production by *Clostridium thermoaceticum* in molasses at dilution of 1:0, 1:25, 1:50 and 1:100 in an anaerobe jar at 58°C for 5 days.

3. Charcoal pretreated molasses

Pretreatment of diluted molasses with activated charcoal was studied. The results showed that *Clostridium thermoaceticum* can grow and produce acetic acid in molasses at all dilution of 1:25, 1:50 and 1:100. Acetic acid concentration was found at 0.24, 0.30 and 0.24 mg/ml in 1:25, 1:50 and 1:100 diluted molasses respectively (figure 9).

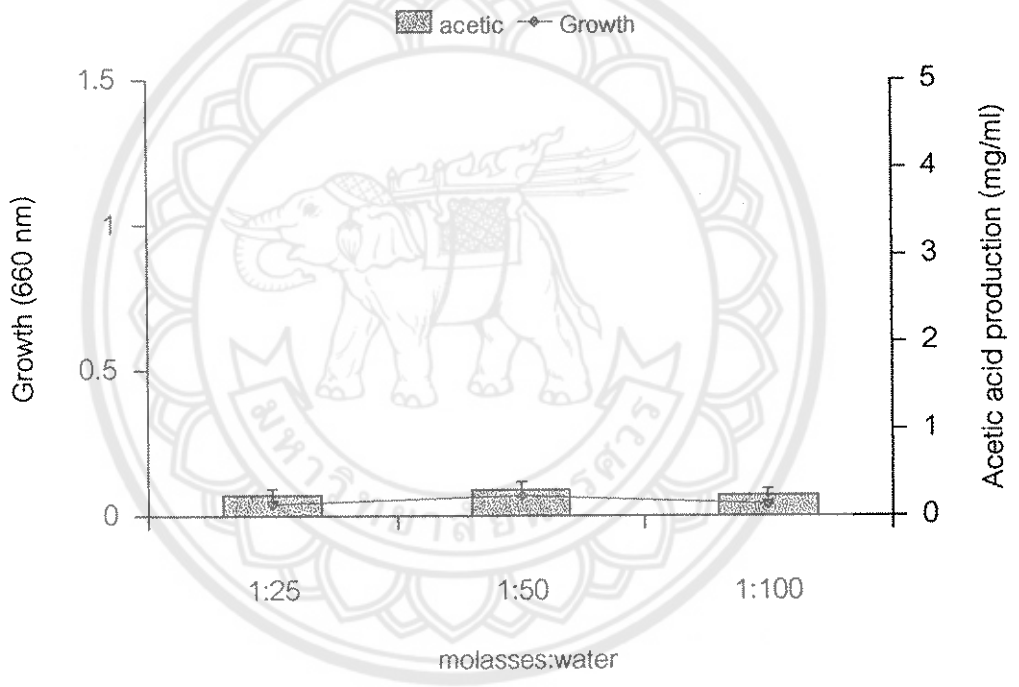


Figure 9 Growth and acetic acid production by *Clostridium thermoaceticum* in activated charcoal pretreatment molasses at dilution of 1:25, 1:50 and 1:100 in an anaerobe jar at 58°C for 5 days.

4. Molasses with Nutrient supplement

4.1. Diluted molasses at 1:25, 1:50 and 1:100 with nutrient supplement were studied. The results showed that growth and acetic acid production of *Clostridium thermoaceticum* were markedly increased in all dilution comparing to diluted molasses without nutrient supplement (figure 9). Acetic acid concentration was found at 0.30, 2.1 and 1.5 mg/ml in 1:25, 1:50 and 1:100 diluted molasses respectively (figure 10).

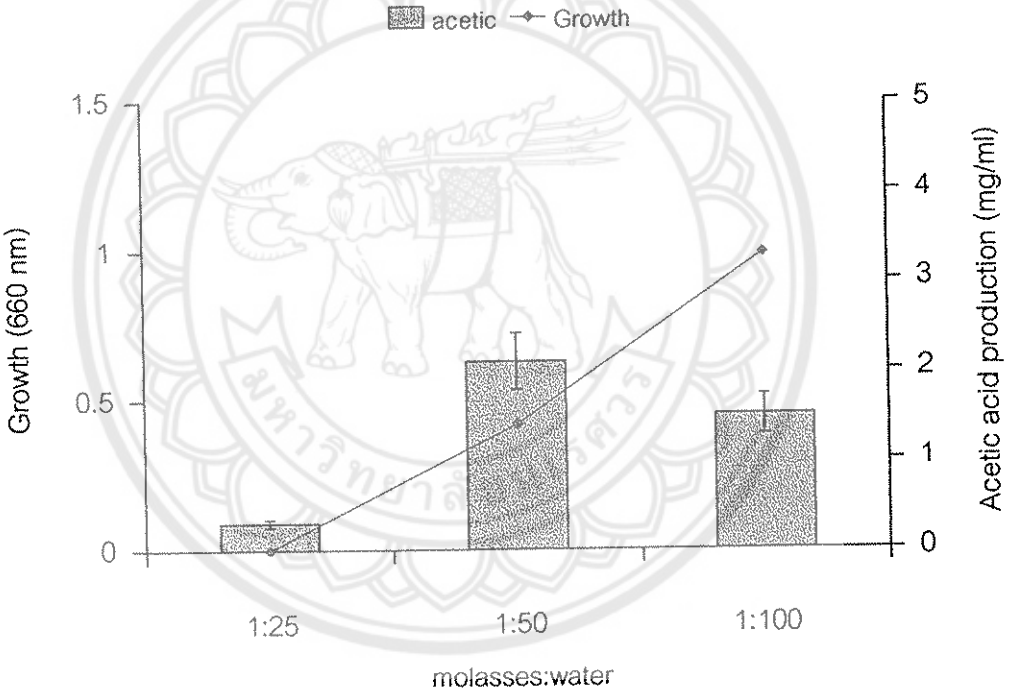


Figure 10 Growth and acetic acid production by *Clostridium thermoaceticum* in diluted molasses with ingredient of formulated thioglycollate broth media in an anaerobe jar at 58°C for 5 days.

4.2. Activated charcoal pretreatment of 1:50 diluted molasses with additional of ingredient was studied. The result showed in figure 11. Little amount of acetic acid concentration (0.4 mg/ml) was found in activated charcoal pretreatment alone. Combination of activated charcoal pretreatment with ingredient supplement slightly increased acetic acid production of *Clostridium thermoaceticum* in molasses. Acetic acid concentration was 1.92 and 2.04 mg/ml in molasses with ingredient supplement and combination of ingredient supplement with activated charcoal.

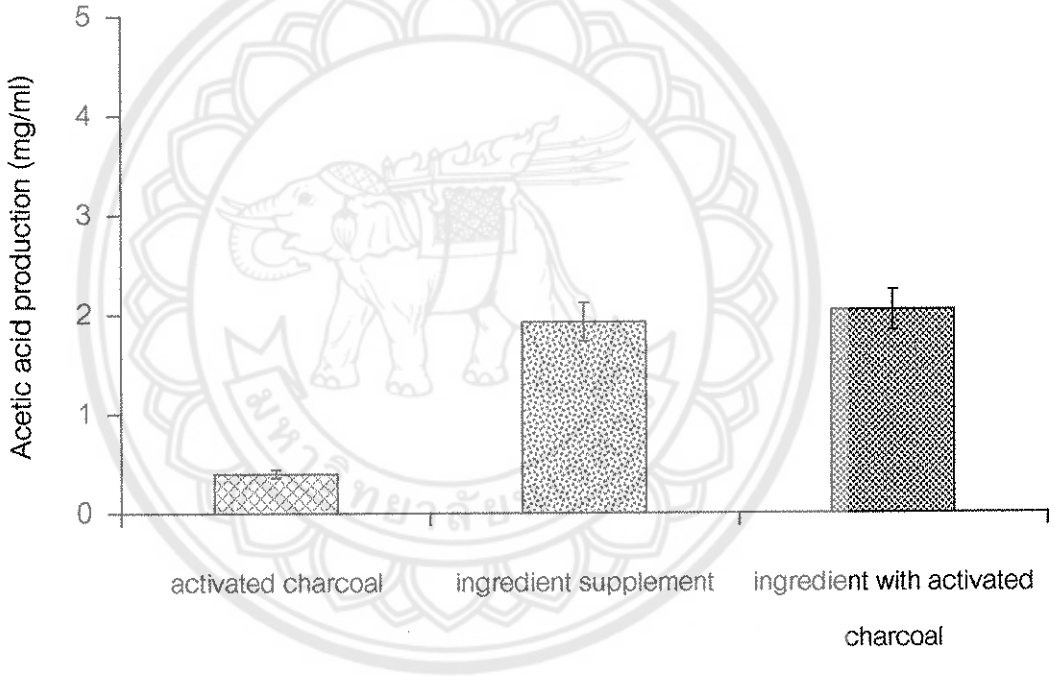
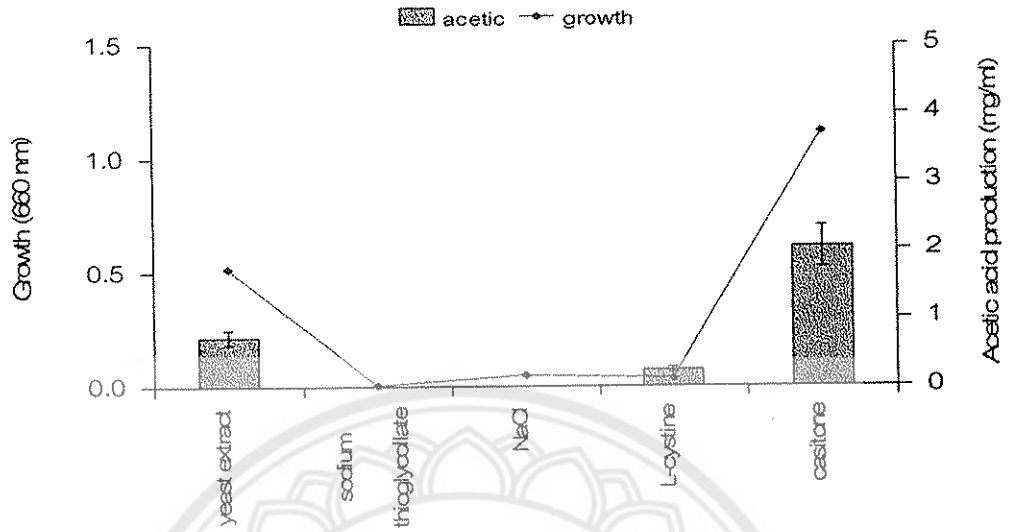


Figure 11 Acetic acid productions by *Clostridium thermoaceticum* from 1:50 diluted molasses ▨ pretreated with activated charcoal, ▨ addition of ingredient of STG, and ▨ pretreated with activated charcoal and ingredient supplement in an anaerobe jar at 58°C for 5 days.

4.3. Activated charcoal pretreatment of 1:50 diluted molasses with additional of separate ingredient was studied. Yeast extract, sodium chloride, sodium thioglycollate, L-cysteine or casitone was added into pretreated charcoal separately (figure 12a). The result showed that *Clostridium thermoaceticum* represented good growth and acetic acid production in the addition of yeast extract or casitone. Acetic acid concentration was found at 0.72 and 2.04 mg/ml in addition of yeast extract and casitone respectively. Two combinations of casitone with yeast extract, casitone with sodium thioglycollate, casitone with sodium chloride, casitone with L-cysteine and two hundred percent of casitone were added to study the most effective ingredient in acetic acid production from molasses (figure 12b). The result showed that *Clostridium thermoaceticum* represented good growth and acetic acid production in combination of casitone and yeast extract. Acetic acid concentration were found at 3.36, 1.68, 2.46, 2.46 and 3.24 in combination of casitone and yeast extract, casitone and sodium thioglycollate, casitone and sodium chloride, casitone and cysteine and two hundred percent of casitone respectively. In addition, high amount of casitone (2X casitone) adding into pretreated molasses did not have much effective in acetic acid production comparing to two combination of casitone and yeast extract (figure 12b).

a)



b)

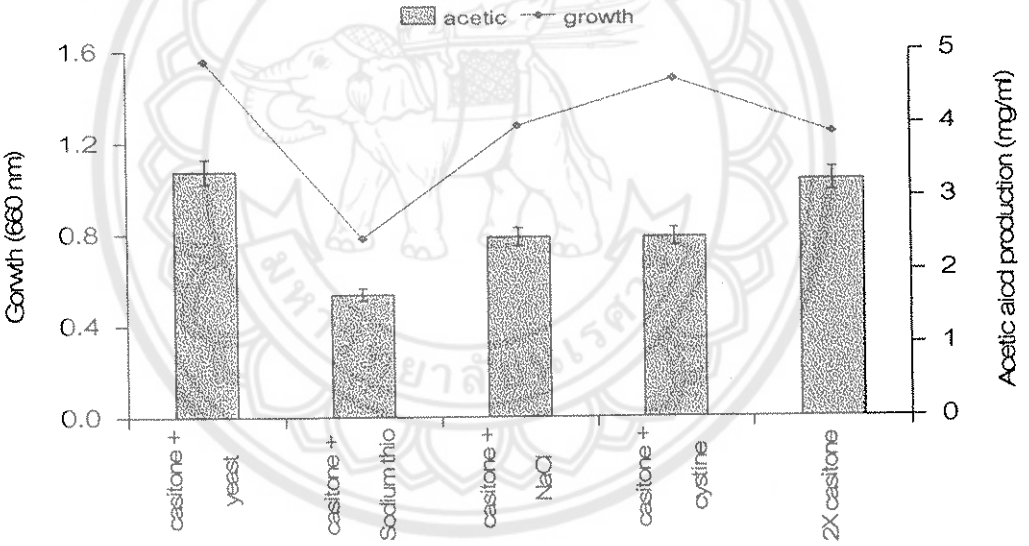


Figure 12 a) Growth and acetic acid production by *Clostridium thermoaceticum* in activated charcoal pretreatment of 1:50 diluted molasses with supplementation of yeast extract, sodium thioglycollate, sodium chloride, L-cysteine or casitone in an anaerobe jar at 58°C for 5 days.

b) Growth and acetic acid production by *Clostridium thermoaceticum* in activated charcoal pretreatment of 1:50 diluted molasses with two combinations of casitone and yeast extract, casitone and sodium thioglycollate, casitone and sodium chloride, casitone and cysteine and 2x casitone in an anaerobe jar at 58°C for 5 days.

5. Effect of phosphate

The effect of phosphate on growth and acetic acid production in pretreatment of 1:50 diluted molasses with nutrient supplement of STG was studied. The result showed that *Clostridium thermoaceticum* represented better growth and acetic acid production in the additional of 120 mM phosphate. Acetic acid concentration was found at 2.28, 3.54 and 4.56 mg/ml in the addition of 40 mM, 80mM and 120 mM phosphate respectively (figure 13).

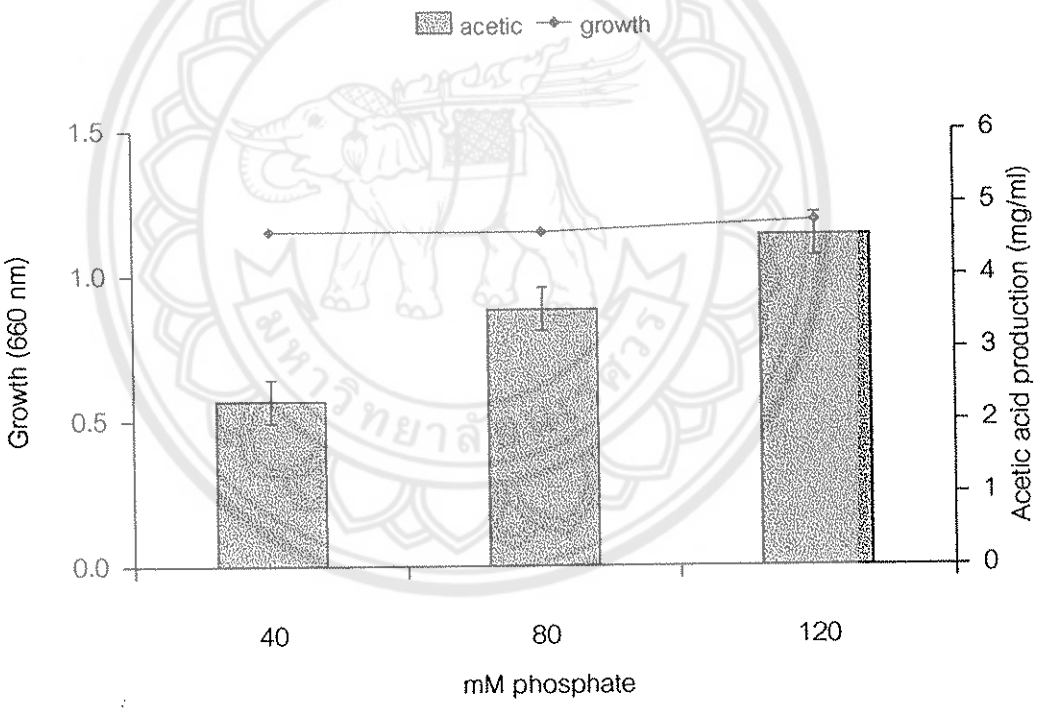


Figure 13 Acetic acid productions by *Clostridium thermoaceticum* in 1:50 diluted molasses with nutrient supplement of STG in an anaerobe jar at 58°C for 5 days.

6. Fermentation in batch fermenter

Two liter of activated charcoal pretreatment of 1:50 diluted molasses with the combination of casitone and yeast extract supplementation and the addition of 120 mM of phosphate were subjected to 2.5 liter batch fermenter. The result showed that growth of *Clostridium thermoaceticum* rapidly increased within 3 days and slightly increased after 3 days (figure 14). Acetic acid production was also increased rapidly within 2 days and slightly increased after 3 days. The maximum acetic acid production was found at 5.52 mg/ml after 5 days.

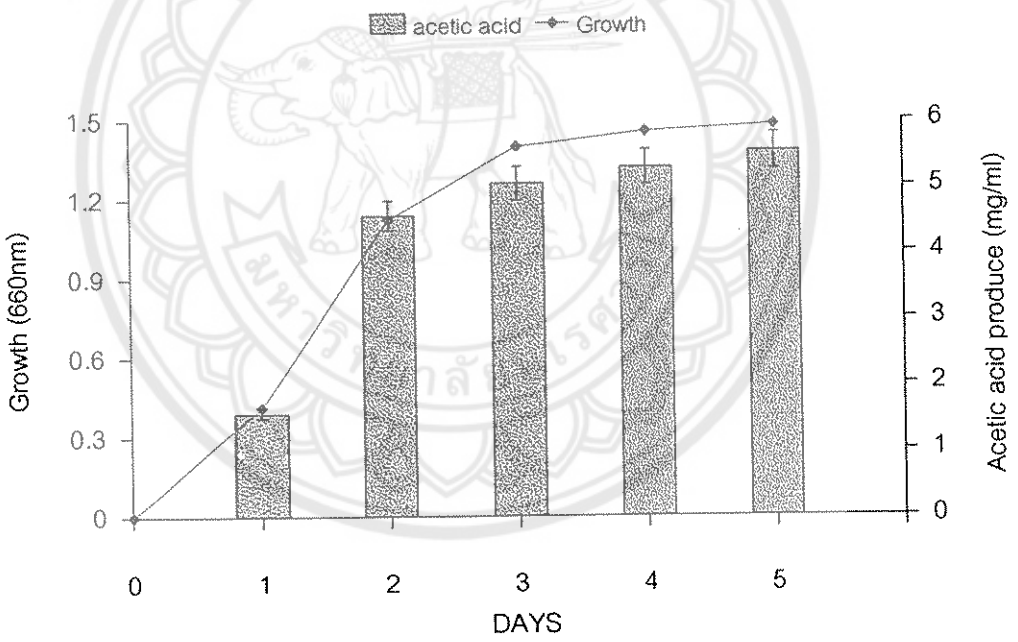


Figure 14 Growth and acetic acid production by *Clostridium thermoaceticum* in Batch fermenter under 100 rpm agitation with CO_2 at 58°C after 5 days.

7. Cost Analysis

The method for estimating the cost of the acetic acid production by batch fermentation system is shown as follow;

7.1. Method

In order to perform the calculation, the following data are required and the necessary assumptions are made.

Assumption data:

1. The economic life of the system	15	years
2. The interest rate for economic life of the system	5	%
3. The capital cost of the system (overall) (Vassel, Agitator, Water heater)	200,000	Baht
4. The cost of electricity	2.5	Baht
- Agitator 0.067 kwh	578.8	kWh/year
- Water heater 0.096 kwh	826.8	kWh/year
5. Operating and maintenance cost	-	
6. Savage value	-	
7. Labor cost	-	

Primary condition:

1. The capital cost of the Fermentation system (overall)	200,000	Baht
2. The cost of electricity for heater and agitator (120 batch/year)	3,515	Baht/year
3. The cost of CO_2	18,000	Baht/year
4. The cost of nutrient	4,946	Baht/year

7.2. The equation for calculating the cost of acetic acid and payback period

Cost of acetic acid;

$$\text{Cost of acetic acid production} = \frac{(\text{Capital cost x CRF}) + \text{Operating cost}}{\text{Quantity of acetic acid produced}} \quad \text{..... (j)}$$

$$CRF = [i\,(1+i)^n]/[(1+i)^n - 1] = 1/SPWF \quad \text{..... (k)}$$

Future amount;

$$F_n = \frac{(1+i)^n - 1}{i} \quad \text{..... (l)}$$

Payback period;

$$\text{Payback period (year)} = \frac{\text{Fixed cost}}{\text{Net income}} \quad \text{..... (m)}$$

Internal rate of return;

$$IRR\ (\%) = \frac{\text{Net income}}{\text{Fixed cost}} \text{ x } 100 \quad \text{..... (n)}$$

Where

F_n = Future amount

CRF = Capital recovery factor

SPWF = Series present worth factor

IRR = Internal rate of return

i = Interest rate

n = Number of year

Therefore, the cost of acetic acid production from molasses by *Clostridium thermoaceticum* in batch fermenter was calculated. Cost of acetic acid was 34.6 baht/g on shown in table 3.

Table 3 Cost of acetic acid production from molasses by *Clostridium thermoaceticum* in batch fermenter.

Production rate	11.00	g / batch
Batch number	120.00	batch/year
Operating time	8,640.00	hour/year
CRF (5%,15 year)	0.096	
Product (acetic acid)	1,320.00	g / year
Operating cost		
- operating and maintenance	0.00	baht/year
- electricity (agitator, water heater)	3,515.00	baht/year
- nutrient	4,946.00	baht/year
- CO_2	18,000.00	baht/year
Total	26,461.00	baht/year
Capital (machine)	200,000.00	baht
Product (acetic acid)	1,320.00	g / year
Operating Cost	26,461.00	baht / year
Cost of acetic acid	34.60	baht / g

The calculation of cost of acetic acid production by batch fermentation was based on an estimated operational life of fifteen years and 5% interest rate. The price of acetic acid by batch fermentation is 34.6 baht/g (table 3). The cost of acetic acid at 34.60 baht/g was the margin cost of acetic acid that was to bring about the payback period of 15 years (table4). However, comparing to commercial cost of acetic acid in Asia which is 540-550 U.S.$/metric ton (0.022 baht/g) [18], this production was very high cost consumed.

Considerable interest using solar hot water to replace use of electrical water heater will decrease price of acetic acid production. Electrical energy of electric water heater at 826.8 kWh/year will be deprived, as well as that of the operation was had income from the carbon sequestered about 250 baht/year, which direct CO_2 emission intensities from power stations which was 0.75 kg/kWh of electricity consumed in average [20]. The current price of carbon sequestered is approximately 5 to 10 U.S.$/metric ton [5].

Therefore, the cost of acetic acid production from molasses by *Clostridium thermoaceticum* in batch fermenter using solar hot water to replace use of electrical water heater was calculated. The result showed that the cost of acetic acid production by batch fermentation using solar hot water decrease cost of acetic acid production by 2 baht/g. The cost of acetic acid is 32.9 baht/g (table 5).

Table 4 Cash flow of acetic acid production by batch fermentation at 57.57 baht/g of acetic acid.

year	Capital cost	operating cost			Income
		Ingredients	Electric	CO2	
0	200,000				
1		4,946	3,515	18,000	45,672
2		4,946	3,515	18,000	45,672
3		4,946	3,515	18,000	45,672
4		4,946	3,515	18,000	45,672
5		4,946	3,515	18,000	45,672
6		4,946	3,515	18,000	45,672
7		4,946	3,515	18,000	45,672
8		4,946	3,515	18,000	45,672
9		4,946	3,515	18,000	45,672
10		4,946	3,515	18,000	45,672
11		4,946	3,515	18,000	45,672
12		4,946	3,515	18,000	45,672
13		4,946	3,515	18,000	45,672
14		4,946	3,515	18,000	45,672
15		4,946	3,515	18,000	45,672
Total payment in 15 years					396,915
Total income in 15 years					685,080
Annual cost, F(SFF,5%,15)					18,394
Annual income, F(SFF,5%,15)					31,748
Net income cost					13,354
Pay back period (years)					15
IRR (%)					7

Table 5 Cost of acetic acid production from molasses by *Clostridium thermoaceticum* in batch fermenter using solar hot water to replace use of electrical water heater.

Production rate	11.00	g / batch
Batch number	120.00	batch/year
Operating time	8,640.00	hour/year
CRF (5%,15 year)	0.096	
Product (acetic acid)	1,320.00	g / year
Operating cost		
- operating and maintenance	0.00	baht/year
- electricity (agitator)	1447.20	baht/year
- nutrient	4,946.00	baht/year
- CO_2	18,000.00	baht/year
Total	24,393.20	baht/year
Income		
- carbon sequestered	250.00	baht/yare

Capital (machine)	200,000.0	baht
Product (acetic acid)	1,320.0	g / year
Operating Cost	24,393.2	baht / year
Income	270.0	baht/year
Cost of acetic acid	32.9	baht / g