EFFECT OF TIME AND TEMPERATURE ON BUTANOL PRODUCTION FROM PALM OIL MILL EFFLUENT (POME) BY ANAEROBIC FERMENTATION USING *CLOSTRIDIUM BEIJERINCKII*

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JUDUL : EFFECT OF TIME AND TEMPERATURE ON BUTANOL PRODUCTION FROM PALM OIL MILL EFFLUENT (POME) BY ANAEROBIC FERMENTATION USING CLOSTRIDIUM BEIJERINCKII

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A thesis submitted in fulfillment
of the requirements for the award of the Degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
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NOVEMBER 2010

I declare that this thesis entitled "Effect of Time and Temperature on Butanol Production from Palm Oil Mill Effluent (POME) by Anaerobic Fermentation Using *Clostridium Beijerinckii*" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

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Special Dedication to my beloved family,

my friends, my fellow colleague

and all faculty members

For all your care, support and believe in me.

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In the name of Allah S.W.T. the most gracious and most merciful, Lord of the universe, with His permission Alhamdulillah the study has been completed. Praise to Prophet Muhammad S.A.W., His companions and to those on the path as what He preached upon, might Allah Almighty keep us His blessing and tenders.

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ABSTRACT

Owing to the increasing volume of palm oil mill effluent (POME) wastewater generated, disposal remains as perennial problem and its bioconversion has been considered as an option for pollution control (C.N. Hipolito *et al.,* 2008). Thus, the availability of an inexpensive raw material; POME which will used in this research is essential if solvent fermentation is to become economically viable. In Malaysia, POME represents an alternative cheap carbon source for fermentation processes that is attractive in both economic and geographical considerations. The mainly research purpose is to study the production of butanol from the anareobic fermentation of solventogenic bacteria (*clostridium beijerinkii,* ATCC 51743) by using POME as the fermentation media. The scope of the research is to study the effect of fermentation time and temperature to the butanol production. The fermentation is based on batch fermentation in schott bottle. It is an anaerobic fermentation and the strain, *Clostridium Beijerinckii* (ATCC 51743) was treated strictly in an anaerobic condition. Optimum conditions that were maintained in this research are the substrate concentration and agitation rate. POME and Reinforced Clostridia Media (RCM) were used as the growth medium in this batch culture. The fermentation temperature that were used in this study are 35°C, 40°C and 45°C while the fermentation time that were used are 48 to 72 hours respectively. The results indicated that the concentration of butanol will decreases as the temperature increases. The highest concentration of butanol that produced by POME was 0.224 g L^{-1} at 35°C by 72 hours of fermentation. From this study, it is showed that POME can produce butanol at optimum fermentation temperature at 35°C in 72 hours of fermentation time. Hence, the result also showed that POME is viable to use as growth medium to produce butanol.

ABSTARK

Penambahan sisa buangan kelapa sawit (POME) yang terhasil, masalah pembuangan berterusan dan penukaran biologi sisa ini dianggap sebagai salah satu bentuk pencemaran (C.N. Hipolito *et al.,* 2008). Namun begitu, bahan asas yang murah sedia ada; POME yang diguna di dalam kajian ini sesuai untuk penapaian pelarut menjadi salah satu kajian yang ekonomi. Di Malaysia, POME merupakan alternatif yang murah sebagai sumber karbon untuk proses penapaian yang juga sesuai dalam segi ekonomi dan geografi. Tujuan utama kajian ini ialah untuk menkaji penghasilan butanol daripada proses penapaian tanpa oksigen menggunakan bacteria yang boleh menghasilkan pelarut (*clostridium beijerinkii,* ATCC 51743) dengan mengaplikasikan penggunaan POME sebagai media untuk penapaian. Skop kajian ini adalah kesan masa dan suhu penapaian terhadap penghasilan butanol. Penapaian ini adalah penapaian berbentuk terkumpul di dalam botol schott. Penapaian ini juga adalah penapaian tanpa oksigen dan bakteria yang digunakan adalah *Clostridium Beijerinckii* (ATCC 51743) yang juga bakteria yang hanya boleh hidup di dalam keadaan tanpa oksigen. Keadaan yang optimum dikekalkan di dalam kajian ini adalah kepekatan cecair dan tahap gerakan. POME dan Reinforce Clostridia Media (RCM) digunakan sebagai media tumbesaran di dalam pembentukan terkumpul ini. Suhu penapaian yang digunakan di dalam kajian ini adalah 35°C, 40°C dan 45°C sementara masa penapaian yang digunakan adalah 48 hingga 72 jam. Data menunjukan, kepekatan butanol akan menurun jika suhu meningkat. Kepekatan butanol yang paling tinggi yang dihasilkan daripada POME adalah 0.224 g L^{-1} pada suhu 35°C selama 72 jam penapaian. Kajian ini menunjukan, POME boleh menghasilkan butanol pada suhu penapaian optimum iaitu 35°C dalam masa 72 jam penapaian. Namun begitu, data kajian juga membuktikan POME boleh digunakan sebagai media tumbesaran untuk menghasilkan butanol.

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LIST OF ABBREVIATIONS

ABE - Acetone-Butanol-Ethanol

CPO - Crude Palm Oil

DNS - Dinitrosalicylic Acid

FFB - Fresh Fruit Bunch

FID - Flame Ionization Detector

GC - Gas Chromatography

HPLC - High Performance Liquid Chromatography

min - Minute

OD - Optical Density

POME - Palm Oil Mill Effluent

RCM - Reinforce Clostridia Medium

UV-VIS - Ultraviolet–visible Spectroscopy

LIST OF SYMBOLS

$ - dolar

% - percentage

cm - centimeter

ft - feet

g - gram

h - hour

kg - kilogram

L - liter

mL - mililiter

mm - millimeter

nm - nanometer

^{o}C - degree Celcius

rpm - revolutions per minute

w/v - weight per volume

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CHAPTER 1

INTRODUCTION

1.1 Research Background

In the new era of the world, scientist and engineer were pushed by the world government to develop a technology to substitute the usage of fuel in transportable because of the crude for fuel processing is almost depleted. Also with the urgency of environmentalist, a green technology as an alternative of the usage of fuel is also being researching for almost decade.

Palm oil mill effluent (POME) treatment was being experimented in many ways to become treated or to become a product. The high compositions and concentrations of carbohydrate, protein, nitrogenous compounds, lipids and minerals in POME (Hwang *et al*., 1978; Phang, 1990; Habib *et al*., 1997) render it possible to reuse the effluent for biotechnological means. Production of butanol is one of the treatments that can be done to the POME; the alternatives fuel that being develop for the mean of green technology.

Various attempts were being made to achieve high yield of butanol production from POME using solvent fermentation seems impossible. Due to this fact, this paper is

being study to come up with the best parameter with the use of clostridium beijerinckii to yield higher percentage of butanol from POME using solvent fermentation technique.

1.2 Problem Statement

There has been an increased interest in research on the bioconversion of agricultural biomass into fuels and chemical feedstock's for two primary reasons, one being the limited supply of fossil fuels and petroleum, and the other, the increasing and fluctuating prices of oil. In order to overcome these problems, research is focused on developing bioconversion processes for fuels and chemicals (N. Qureshi *et al.,* 2008).

Owing to the increasing volume of palm oil mill effluent (POME) wastewater generated, disposal remains a perennial problem and its bioconversion has been considered as an option for pollution control (C.N. Hipolito *et al.,* 2008). Thus, the availability of an inexpensive raw material; POME which will used in this research is essential if solvent fermentation is to become economically viable. In Malaysia, POME represents an alternative cheap carbon source for fermentation processes that is attractive in both economic and geographical considerations.

Unfortunately based on researches in the market, the problem with the technology is to produce high yield of butanol from POME. Therefore, this study is focusing on finding the suitable parameters for solvent fermentation that can produce higher yield of butanol from POME using *clostridium beijerinkii*.

1.3 Objective

The objective of this research is to study effect of time and temperature on butanol production from palm oil mill effluent (POME) by anaerobic fermentation using *Clostridium Beijerinckii*.

1.4 Scopes

To achieve the objective, four scopes have been identified in this research:

i. To determine the growth pattern of the *Clostridium Beijerinckii* strain in POME substrate
ii. To study the effect of temperature (35 - 45°C) to the butanol production
iii. To study the effect of fermentation time (48 hours – 72 hours) to the butanol production
iv. To study the effect of the parameters on glucose consumption of the substrate

1.5 Rationale & Significance

The significant of this research is to study the possibility of POME as a good substrate for solvents fermentation and hence give an alternative substrate for the solvents fermentation purpose. Besides that, this study will help to establish a suitable parameter to overcome the lower yield of butanol production in solvent fermentation from POME.

CHAPTER 2

LITERATURE REVIEW

2.1 Palm Oil Mill Effluent (POME)

The production of palm oil results in the generation of huge quantities of highly polluting wastewater termed as Palm Oil Mill Effluent (POME).

The extraction process for crude palm oil (CPO) starts from the local palm oil mills throughout Malaysia. The mills processes FFB received from the oil palm plantations into CPO and other by-products. A schematic process flow of palm oil milling for the extraction of crude palm oil and sources of waste generation is shown in **Fig. 2.1**. Palm oil mills typically generate large quantities of extremely oily organic contented liquid (Industrial Processes and The Environment, 1999).

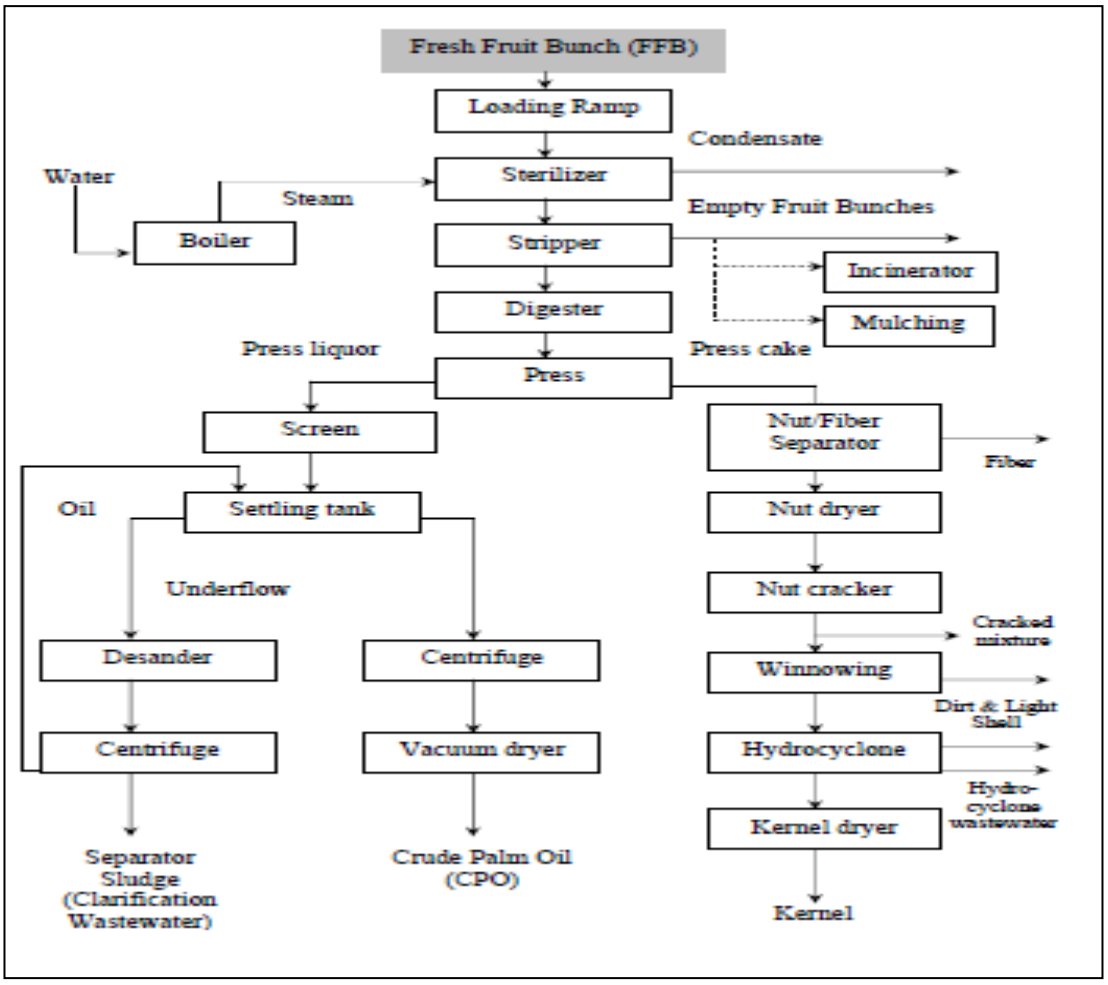

Figure 2.1 Process flow of typical palm oil milling (Industrial Processes and The Environment, 1999)

Large quantities of water are used during the crude oil extraction process. Up to about 1.5 cubic meters of water are characteristically used to process one tonne of FFB. From this quantity, about 50% of the water results in the POME, the other 50% being lost as steam, mainly through sterilizer exhaust, piping leakages, as well as wash waters (Oil Palm & The Environment A Malaysian Perspective, 1999). POME comprises a combination of the wastewaters which are principally generated and discharged from the following major processing operations as seen early in Fig. 2.1, such as; sterilization of FFB - sterilizer condensate is about 36% of total POME, clarification of the extracted CPO - clarification wastewater is about 60% of total POME and hydrocyclone

separation of cracked mixture of kernel and shell-hydrocyclone wastewater is about 4% of total POME.

Based on previous research by bachelor student, Einayah (2009), sugar compositions in POME are being analyzed by implemented high performance liquid chromatography (HPLC) function using capillary column Supelcosil LC-NH_2. **Table 2.1** and **Figure 2.2** showed the concentration of each sugar groups in POME.

Table 2.1 Sugar concentration in POME

Sample	Sugar Group	Concentration(g/L)
POME	Fructose	1.967
	Glucose	3.668
	Galactose	8.859
	Sucrose	2.24
	Lactose	1.313

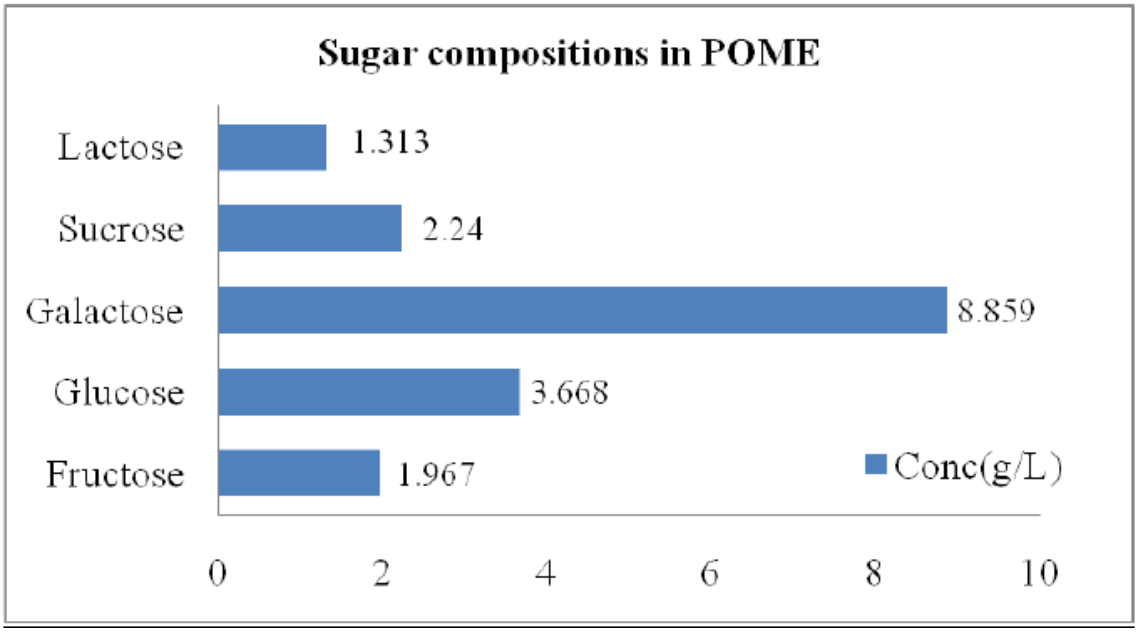

Figure 2.2 Sugar concentrations in POME

The possibility of reusing POME as fermentation media is largely due to the fact that POME contains high concentrations of carbohydrate, protein, nitrogenous compounds, lipids and minerals (Hwang *et al*., 1978; Phang *et al*., 1990; Habib *et al*., 1997; Suwandi *et al*., 1991; Wu *et al*., 2006b) pointed out the possibility of recovering and concentrating the available bioresources in POME by an ultrafiltration process in order for the concentrated bioresources to be reused more effectively as fermentation media. According to Wu *et al*. (2006b) POME and its derivatives have been exploited as fermentation media to produce various products/metabolites such as antibiotics, bioinsecticides, solvents, polyhydroxyalkanoates, organic acids as well as enzymes to varying degrees of success. The hydrogen production from POME during anaerobic treatment has also been intensively studied (Atif *et al*., 2005; Vijayaraghavan *et al*., 2006) since the generated hydrogen and its combustion products do not count as green house gases (Koroneos *et al*., 2004). However, it has been reported that POME also contains certain powerful water-soluble antioxidants, phenolic acids and flavonoids (Wattanapenpaiboon *et al*., 2003) that may inhibit the growth development in microorganisms (Lin *et al*., 2005; Uzel *et al*., 2005).

2.2 Butanol

Butanol is a 4-carbon alcohol originally central to a number of industrial chemical processes. It is now recognised as an important transport fuel - with superior characteristics to ethanol.

With four carbons, butanol has more energy than ethanol - 25% more energy per unit volume. Butanol has a lower vapour pressure and higher flashpoint than ethanol, making it easier to store and safer to handle. Butanol is not hygroscopic while ethanol attracts water. Ethanol has to be blended with petrol shortly before use. Butanol can be blended at a refinery without requiring modifications in blending facilities, storage tanks or retail station pumps. Butanol can run in unmodified engines at any blend with petrol.

Ethanol can only be blended up to 85% and requires engine modification. Unlike ethanol, butanol may also be blended with diesel and biodiesel. Butanol is less corrosive than ethanol and can be transported using existing infrastructures.

While current utilization strategies for biomass have focused on ethanol production, producing butanol instead of ethanol offers several advantages for biofuel-gasoline blending. With lower vapour pressure but higher energy content makes butanol safer for blending with gasoline as well as offering better fuel economy than ethanol-gasoline blends. In addition, with the higher tolerance to water contamination in gasoline blends and therefore butanol-gasoline blends are less susceptible to separation and that facilitates its use in existing gasoline supply and distribution channels. Therefore, optimizing ABE fermentation to enhance butanol production over ethanol appears to be the more commercially and technologically attractive option (C.N. Hipolito *et al.,* 2008).

2.3 Fermentation

2.3.1 Anaerobic Fermentation

Anaerobic fermentation is the process of fermentation without using any oxygen. One of advantages of the anaerobic process is the recovery of the useful matters such as solvents (Hwang *et al.,* 2004).

The most important economic factor in solvent fermentation is the cost of substrate, which made up about 60% of the overall cost of production. (Liew *et al.*, 2006). Biobutanol production is an anaerobic two-stage fermentation process where acetic and butyric acids, carbon dioxide and hydrogen are first produced in the acidogenic phase. Then the culture undergoes metabolic shift to solventogenic phase and acids are converted into acetone, ethanol and butanol. At the end of the fermentation, products are recovered from the cell mass, other suspended solids, and by-products (Pakkila *et al*., 2009).

2.3.2 Solvent Fermentation

Acetone-butanol-ethanol (ABE) fermentation by microbial is one of the oldest known industrial fermentations. It was ranked second only to ethanol fermentation by yeast in its scale of production, and is one of the largest biotechnological processes ever known. The actual fermentation, however, has been quite complicated and difficult to control. ABE fermentation has declined continuously since the 1950s, and almost all butanol is now produced via petrochemical routes. Butanol is an important industrial solvent and potentially a better fuel extender than ethanol. Current butanol prices as a chemical are at $3.75 per gallon, with a worldwide market of 370 million gallons per year. The market demand is expected to increase dramatically if green butanol can be produced economically from low cost biomass.

2.4 Solventogenic Clostridia

C. beijerinckii is a saccharolytic, strictly anaerobic, mesophylic, motile, rod-shaped bacteria with oval, sub-terminal spores. It exhibits peritrichous flagella. During fermentation, *C. beijerinckii* produces a number of products including acetate, butyrate, lactate, hydrogen gas, carbon dioxide, acetone, butanol, ethanol, acetoin and acetyl methyl carbonil. The morphology of the cell changes over the growth cycle of the organism; at early exponential phase, the cells are long, filamentous and very motile. As the culture approaches the solventogenic stage, which corresponds with the stationary phase, cells shorten, become plumper and exhibit a lower level of motility *C. beijerinckii* species are ubiquitous in nature and routinely isolated from soil samples (US Department of Energy Joint Genome).

C. beijerinckii has great biotechnological potential for the production of butanol, acetone, and/or isopropanol because of its broad substrate range (pentoses, hexoses, starch, and others), its sustained production of solvents well into log-phase, its stability with respects to strain degeneration and the adaptability it shows to continuous

processes. Pilot plant studies confirmed that *C. beijerinckii* grows well and is easy to handle in simple, inexpensive media that is realistic for industrial use. *C. beijerinckii* has also shown responsiveness for genetic improvement. The exceptional solvent productivity of the strain *C. beijerinckii*, produced after only one episode of mutagenesis, has demonstrated the enormous potential of derivatives of C. beijerinckii in solvent production (US Department of Energy Joint Genome).

The sequence of *C. beijerinckii* will make possible the application of DNA microarrays for gene expression profiling and comparative genomics in order to understand the phenotypic differences apparent between C. beijerinckii and other important saccharolytic strains, such as C. acetobutylicum. That may lead to the unraveling of the general principles of saccharide utilization and solvent production and therefore, to rational approaches to strain construction and optimization of the acetone-butanol fermentation.

CHAPTER 3

METHODOLOGY

3.1 Material

3.1.1 Bacterial strains and culture maintenance

Clostridium beijerinckii was used for these studies. Laboratory stocks of *C. beijerinckii* were maintained as spore suspensions in glycerol stock vial at -80°C. For prepare for pre-culturing the strain, spores were allowed to liquefy for 10 minutes at room temperature in anaerobic chamber to maintain the anaerobic condition. 1 mL of the stock culture was transferred into 150 mL Reinforced Clostridia Media (RCM) and incubated for 24 hours until it active to be used.

Streaking technique used to isolate pure *C. beijerinckii* strain from the active culture to be grown in Reinforced Clostridia Agar plate that has been sterilized. After streak the active strain on the sterilized agar plate, the plates were incubated for another 18 – 24 hours at 35°C in anaerobic condition. From this technique, the growth of *C. beijerinckii* can be explored, identified and studied.

3.1.2 Preparation of medium

RCM was used as pre-culture medium, as main culture and as control medium for batch fermentation. 38 g RCM powder was suspended in 1 liter of distilled water and brings to the boil to dissolve completely. The solution was sterilising by autoclaving at 121°C for 15 minutes.

While RCM used as control medium, Palm Oil Mill Effluent (POME) was used as experimental medium for the batch fermentation. POME was collected from Kilang Kelapa Sawit Felda Lepar Hilir, Gambang, Pahang. Fresh POME was sediment passively in heat resistant bottle and stored at 4°C for 24 hours. After 24 hours, the upper layer (supernatant) was decanted and lower layer (POME sludge) use as the experimental medium. The pH of POME is adjusted to 5.8 using 5M of NaOH and then was sterilized in autoclave at 121°C for 15 minutes.

3.1.3 Inoculum development

For producing pure culture of C. beijerinckii strain, after distinguish visible colonies of C. beijerinckii on the incubated agar plate, few loops of the colonies transferred to 150 mL of RCM in a flask and place in anaerobic condition for 18 – 24 hours at 36 ± 1 °C for inoculums development. After the incubation time, the culture broth was prepared for fermentation process. The culture broth was centrifuge for 10 minutes at 10000 rpm in microcentrifuge. The supernatant was decanted and re-suspend the cell with 100 ml of sterile saline solution, 0.85% (w/v) NaCl for cell washing. The cell washing was performed twice to wash out all the remaining broth from the cell. For the final cell suspension, the optical density (OD) value of 1.3 at 600nm was set and read the OD value using UV-VIS. If the OD value exceeds 1.3, add more saline solution. If the OD value less than 1.3, add more cell. Use the final cell suspension with OD value of 1.3 ± 0.1 as the inoculum for subsequent works (10% of the working solution).

3.2 Experimental Procedure

3.2.1 Batch fermentation

Batch fermentations were carried out in 500 mL screw-capped (300 mL of medium) and was been operate in hybrid incubator shaker. The medium contained RCM and POME at 243 mL adding up with 27 mL of sterilized distilled water for 90% concentration of substrate, and was autoclaved for sterilization condition. This was followed by the addition of final cell suspension at inoculation rate of 10%. After inoculation, the broth was spurge with filtered oxygen-free nitrogen gas for 30 minutes to maintain strict anaerobic conditions. The fermentation were experiment on two parameters on two different fermentation medium; fermentation time and fermentation temperature (**Table 3.1**) on optimum agitation rate (200 rpm) and concentration of substrate (90%). Samples were periodically withdrawn every 6 hours.

Table 3.1 Batch fermentation

		FERMENTATION	
RUNS	MEDIUM	TEMPERATURE, °C	TIME, hours
1	RCM	35	48
	POME		
2	RCM		72
	POME		
3	RCM	40	48
	POME		
4	RCM		72
	POME		
5	RCM	45	48
	POME		
6	RCM		72
	POME		

3.2.2 Analyses

i. Growth profile of strain

Growth of *C. beijerinckii* in RCM and POME on optimum fermentation condition was experimented to check ability of the strain in these medium. The working volume with final cell suspension was identical as the experimental procedure. The optimum condition of the *C. beijerinckii* is at temperature of 35 ± 2°C, agitation rate of 200 rpm and 90% concentration of the substrate. Samples were periodically withdrawn every 12 hours and analyze the OD (wavelength 600 nm) by using UV-VIS.

ii. Cell concentration

Cell concentration of fermentation broth was estimated by an OD (wavelength 600 nm) methods using UV-VIS every 6 hours.

iii. Glucose utilization

Total reducing sugar concentration was measured according to the 3,5-dinitrosalicyclic acid method (DNS) reagent. 20 g of DNS reagent powder is suspended in 400 mL distilled water and continuously stirring the solution. 300 mL of sodium hydroxide solution is added and the volume is subsequently adjusted to 1500 mL by the addition of distilled water. Stirring process was continued until a clear solution is obtained. Next, 600 Rochelle salt (sodium potassium tartrate) is added and stirring with heating continued until dissolution of the salt. The volume of the solution was adjusted to 2000 mL and then was filtered for clear solution. The solution must keep at room temperature in dark place to protect against carbon dioxide absorption.

Total reducing sugar concentration of the fermentation broth was determined every collecting samples for 6 hours interval. 3 mL of DNS solution was added to 3 mL of sample from the fermentation broth. Then the test tubes was heated in 90°C of water for 10 minutes, proceed by cooled to room temperature. Then, the OD reading was determined by using UV-VIS at 540 nm wavelength.

iv. Solvent production

ABE production was determine using a 6890 Hewlett-Packard gas chromatography (Hewlett-Packard, Avondale, PA, USA) equipped with a flame ionization detector (FID) and 6 ft x 2 mm Inowax glass column. The carrier gas was nitrogen (1.2 kg cm^{-2}). The temperatures of injection and the detector were 250°C respectively. The column temperature was programmed to change from 50 to 170°C at a rate of 10°C/min. ABE productivity was calculated as ABE produced g L^{-1} divided by the fermentation time and is expressed as g L^{-1} h^{-1}.

CHAPTER 4

RESULT & DISCUSSION

In order to evaluate the effect of time and temperature on solventogenic clostridia fermentation, a batch fermentation using *Clostridium Beijerinckii* was run with Palm Oil Mill Effluent as experimental medium and Reinforce Clostridia Media as control medium. The effectiveness of the methodology to produce acetone-butanol-ethanol (ABE) are analyzed the fermentation broth by using Gas chromatography flame ionization detector (GC-FID). The reducing sugar is also one of important factor that manipulates the production of ABE. The reducing sugar is determined by using 3,5-dinitrosalicyclic acid method (DNS) reagent and UV-VIS used to read the optical density of sample with DNS reagent solution.

Table 4.1 Batch fermentation runs

RUN	FERMENTATION TEMPERATURE °C
1	35
2	40
3	45

4.1 Growth Profile

Growth of *C. beijerinckii* in RCM and POME on optimum fermentation condition was experimented to check the growth pattern of the strain in these medium. Samples were periodically withdrawn every 12 hours and analyze the OD (wavelength 600 nm) by using UV-VIS.

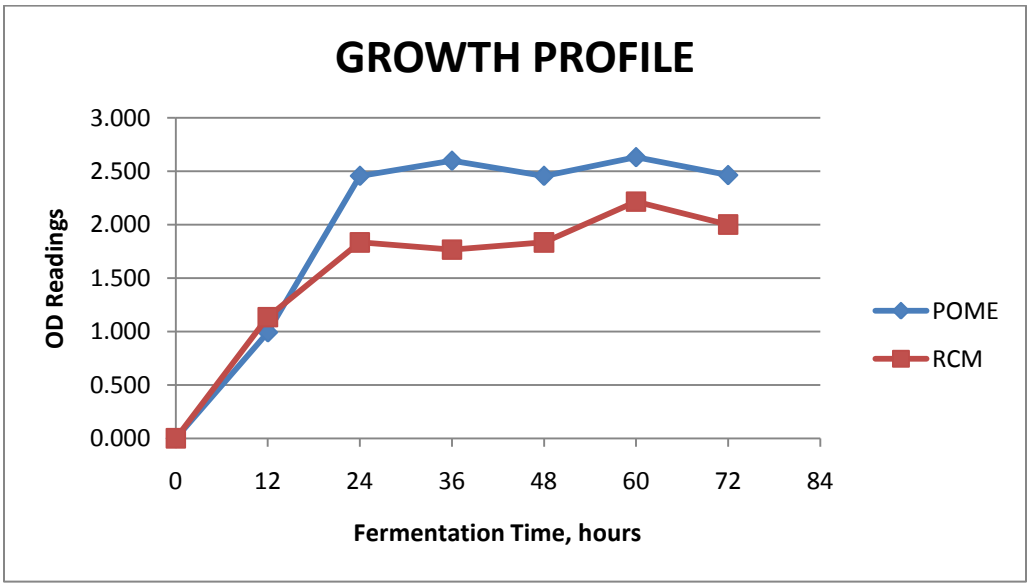

Figure 4.1 Growth

Figure 4.1 is referring to the growth of *C. Beijerinckii* and the utilization of POME and RCM as food supply for its growth. This figure can be related to biomass curve whereas there are three phases in the curve; exponential phase, stationary phase and death phase.

The growth pattern of *C. Beijerinckii* in both medium is approximately the same with the exponential phase, stationary phase and death phase, start and stop at the same hour. The growth pattern of *C. Beijerincki* in POME and RCM is used as reference in fermentation process in this study.

C. Beijerinckii in POME medium proceeds to cultivate quite rapidly from 0 hour to 24 hours where the maximum growth happened. *C. Beijerinckii* fully utilized the availability of nutrients in POME in this phase. Start from hour-24, the graph fluctuating before the stationary phase start. As the stationary phase start, the cultivation of *C. Beijerinckii* also starts to slow down and stable until hour-60. This phase referring to the production of new cellular material roughly offset but the total number of viable cell remains constant. The death phase happened when the availability of nutrients in POME is severely depleted which cause to the growth of *C. Beijerinckii* population become slower. The growth of *C. Beijerinckii* in RCM almost the same performance as the growth in POME.

The growth of *C. Beijerinckii* between both medium, showed the growth of cell in POME is more higher compared to the growth of cell in RCM. The OD reading of cell cultivation in POME can be reach until 2.600 the maximum while cell cultivation in RCM only can reach until 2.200 the maximum. The strain of this study, *C. beijerinckii* can be growth actively with optimum condition in POME rather than RCM.

4.2 Solvent Production

Solventogenic clostridia will produce solvents such as acetone, butanol and ethanol by fermentation. Production of solvent linked to the glucose utilization in medium and cell growth in fermentation broth. These three factors will be related to each other for discussion of this part.

4.2.1 Effect of fermentation time to butanol production

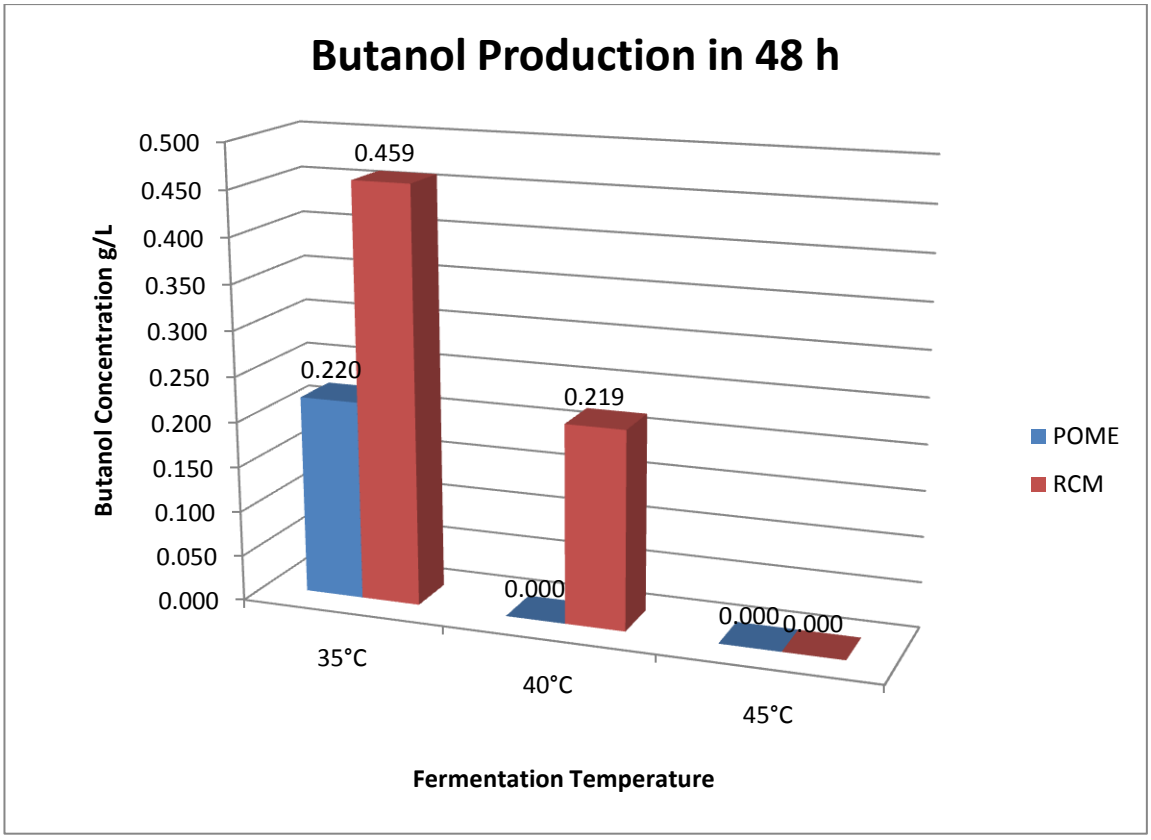

Figure 4.2 Butanol production based on temperature at 48 hour of fermentation

Based on **Figure 4.2**, butanol produced in POME substrate is limited either in 35°C, 40°C or 45°C rather than the butanol produced in RCM substrate. Among these three temperature points, butanol produced the most at 35°C of fermentation temperature. While in 45°C, neither in POME nor RCM, butanol did not produce. Yet,

only at fermentation temperature of 35°C, butanol produced in POME substrate which is half less of butanol produced in RCM substrate.

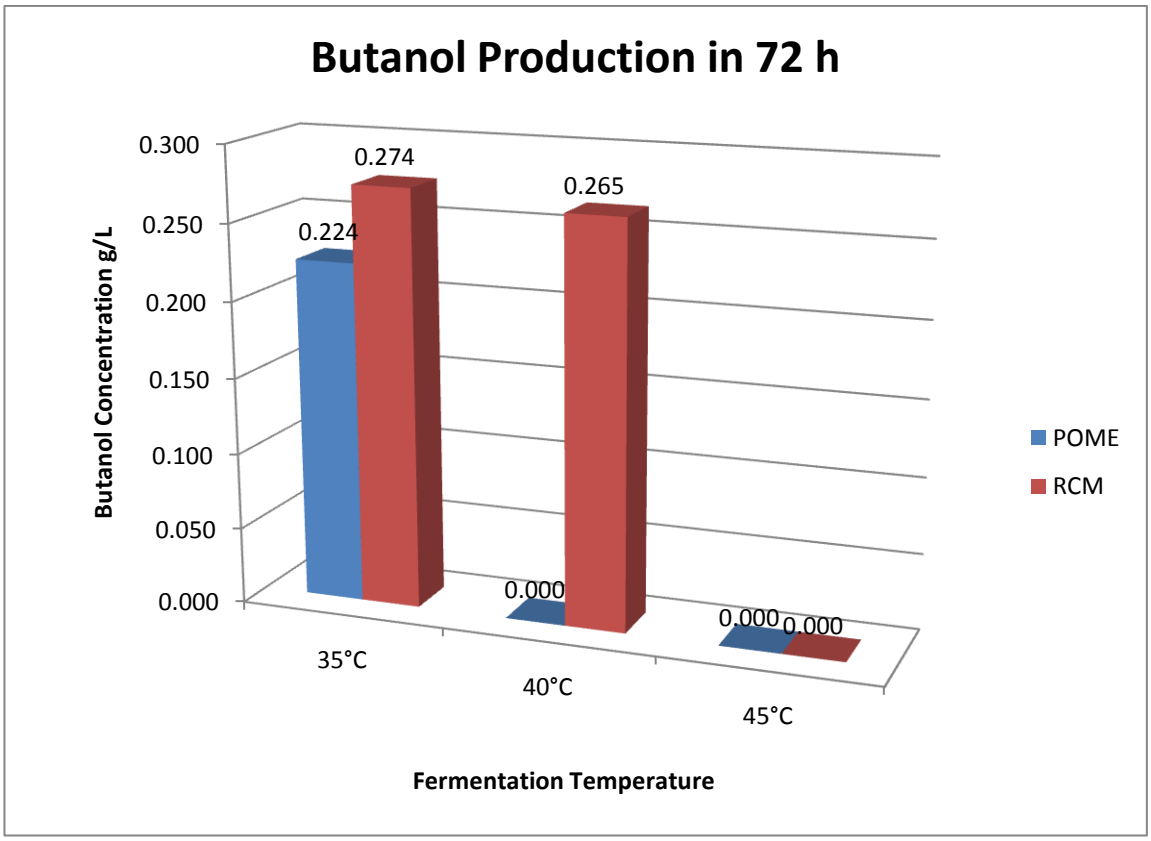

Figure 4.3 Butanol production based on temperature at 72 hour of fermentation

The patent of butanol produced in POME substrate in 72 hours of fermentation almost the same as in 48 hours of fermentation; butanol produced only at 35°C, nor at 40°C or at 45°C fermentation temperature. The patent of butanol production also the same as in RCM substrate. Unfortunately butanol produced in RCM substrate at 72 hours of fermentation not as much as produced at 48 hours of fermentation. The different butanol produced between in POME and RCM substrate at 35°C, is only slightly different, 0.050 g L^{-1}. While the different of butanol production in RCM substrate at 35°C and 40°C, also slightly diverse; 0.009 g L^{-1}.

From **Figure 4.2** and **Figure 4.3**, the patent of butanol production either in POME or RCM substrate showed that, by increasing fermentation temperature, the production will decreasing.

4.2.2 Effect of fermentation temperature to butanol production

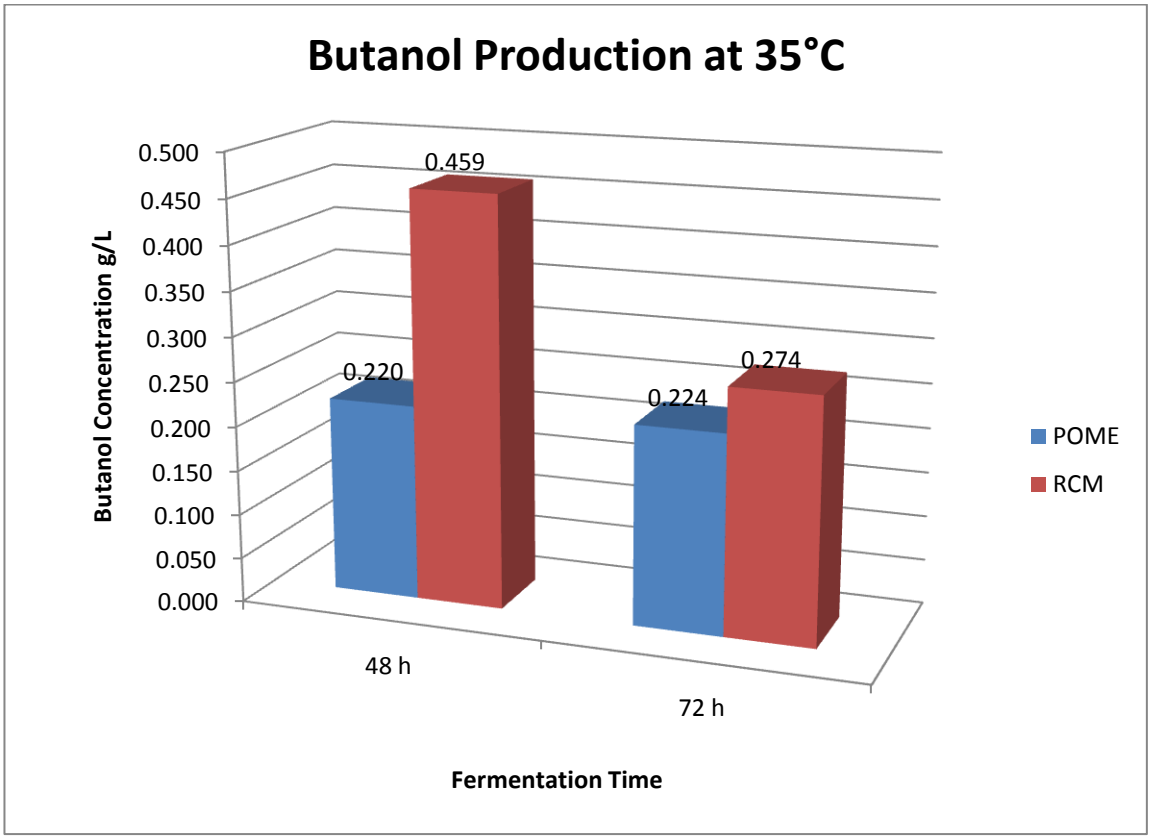

Figure 4.4 Butanol production based on fermentation time at 35°C

The different between butanol productions in POME substrate between these two time sets showed in **Figure 4.4,** can be neglected as the incremental from 48 hours of fermentation to 72 hours of fermentation is only 0.004 g L^{-1}. As time increasing, the cost of production also will be increasing, so if the production differences of these two time sets can be neglected, it is better choice to pick production in less hours of fermentation.

Unlike, incremental showed in production within POME substrate, the theory cannot be applied on RCM substrate as the production decreased as the time increased. Theoretically, production should be increase by increasing of fermentation time. Based on previous study, too many subcultures will decrease the inoculums productivity. The strain used up less sugar and produced progressively less butanol and isopropyl alcohol and more butyric acid. In clostridia fermentation, the sporulation occurs concomitantly with the solventogenesis (Gapes *et al.*, 1983). This theory may affect on this run of the 48 hours of fermentation.

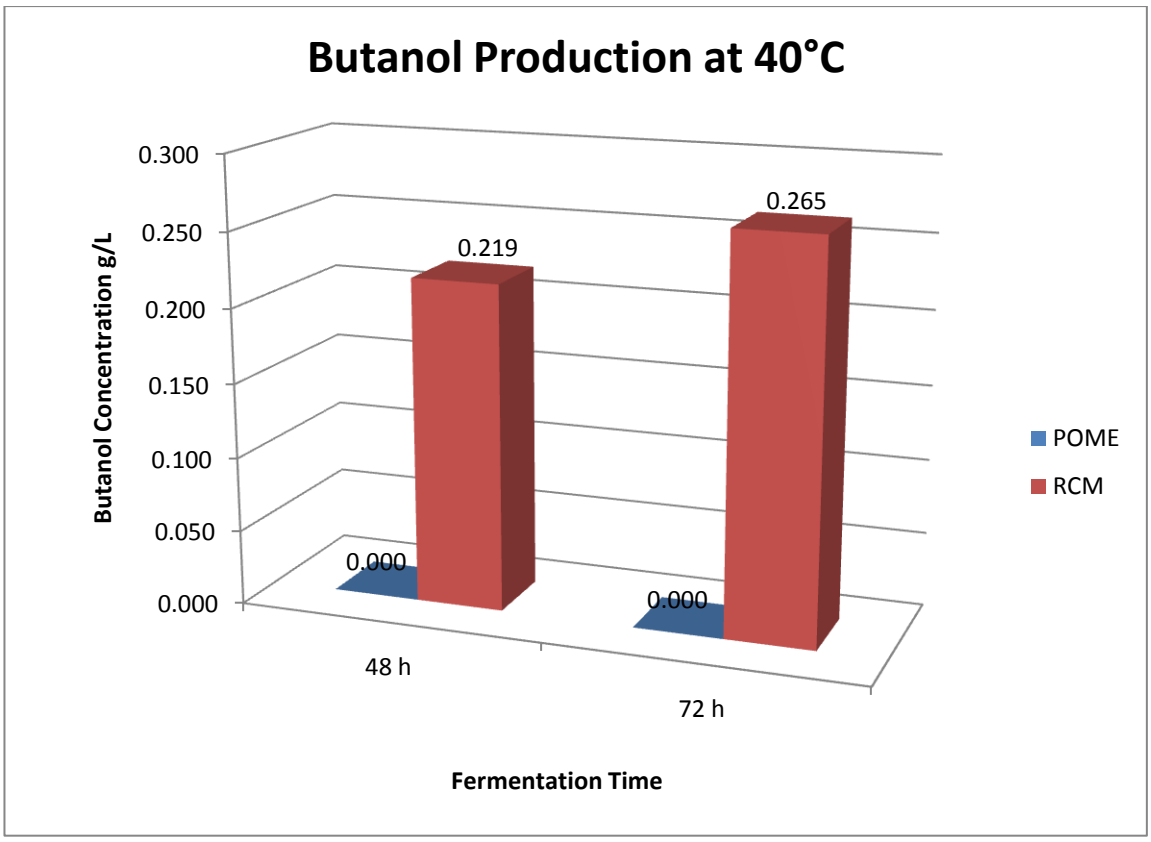

Figure 4.5 Butanol production based on fermentation time at 40°C

Figure 4.5 showed that the production in POME substrate at 40°C did not as productive as production in RCM substrate. There are no productions of butanol in POME substrate at both time sets. While in RCM substrate, the production increasing as

the time of fermentation also increased; with incremental of 0.046 g L^{-1} butanol in 24 hours fermentation time different.

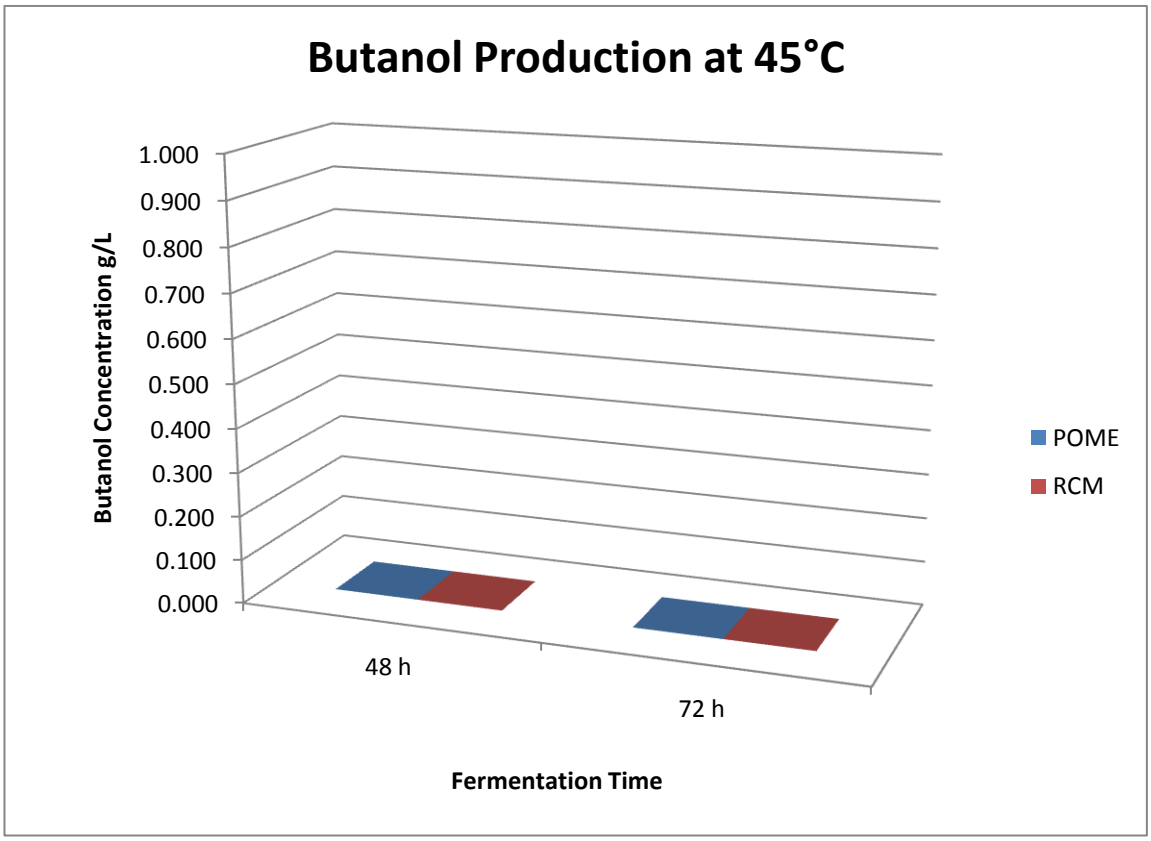

Figure 4.6 Butanol production based on fermentation time at 45°C

There are no butanol productions either in POME or RCM substrate as shown in **Figure 4.6**. This might due to the high temperature that not suitable for the strain to culture in these substrates.

4.2.3 Effect of fermentation time and temperature to butanol production

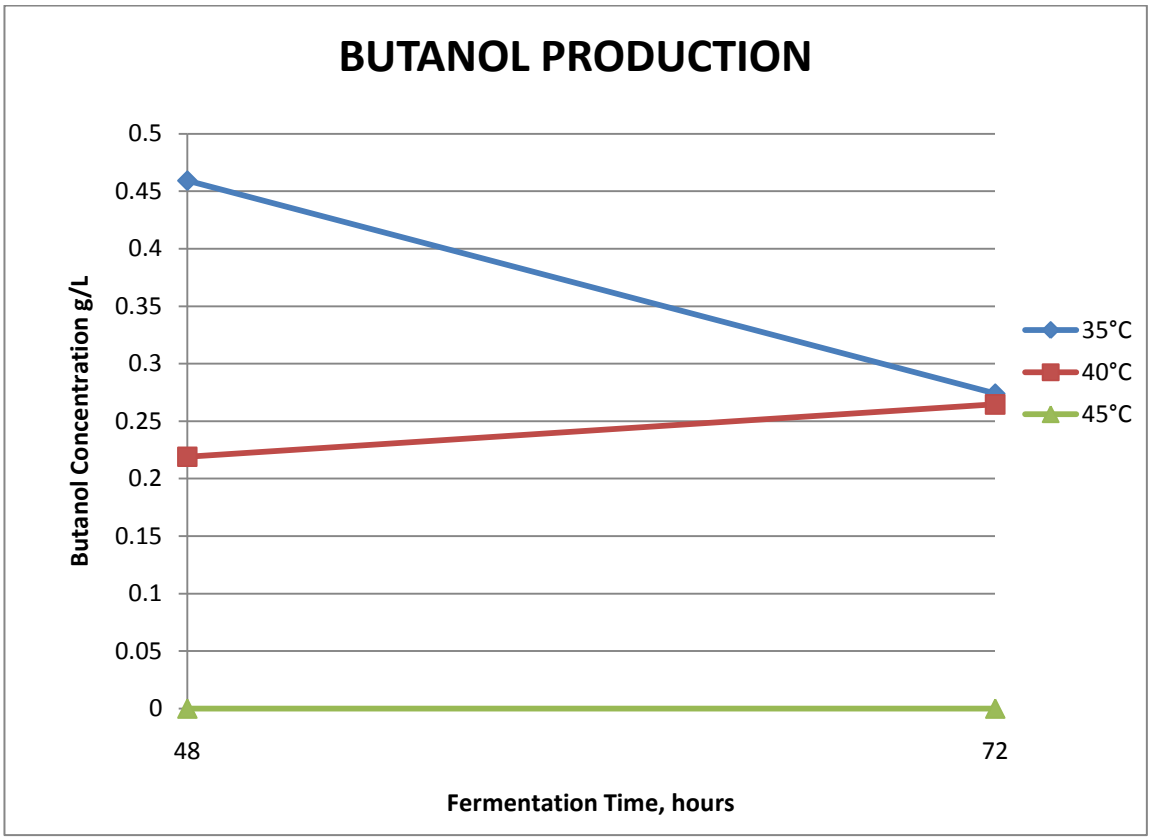

Figure 4.7 Butanol production based on time and temperature in RCM

As in **Figure 4.7** showed that, the production at 35°C produced the highest concentration of butanol while at 45°C, the are no production either in 48 hours or 72 hours of fermentation.

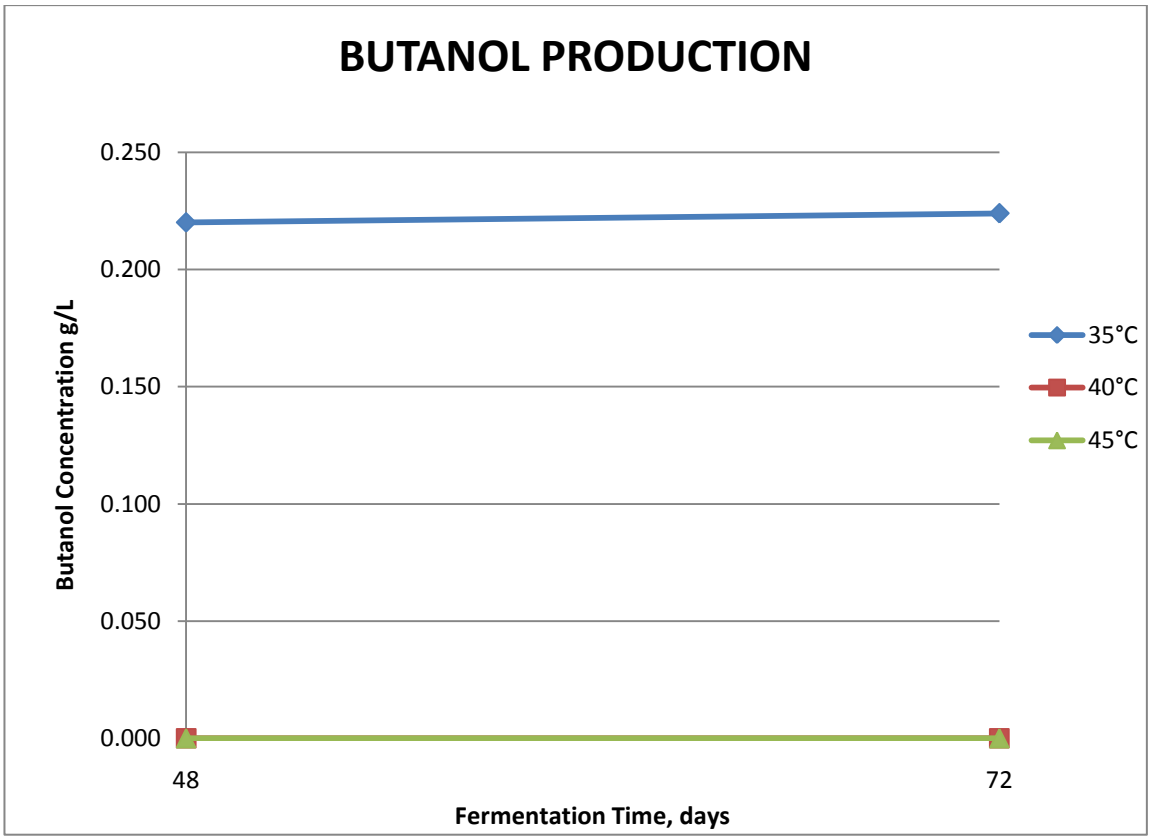

Figure 4.8 Butanol production based on time and temperature in POME

While in **Figure 4.8**, butanol only produced at fermentation temperature of 35°C in POME substrate.

4.2.4 Comparison butanol production in POME and RCM

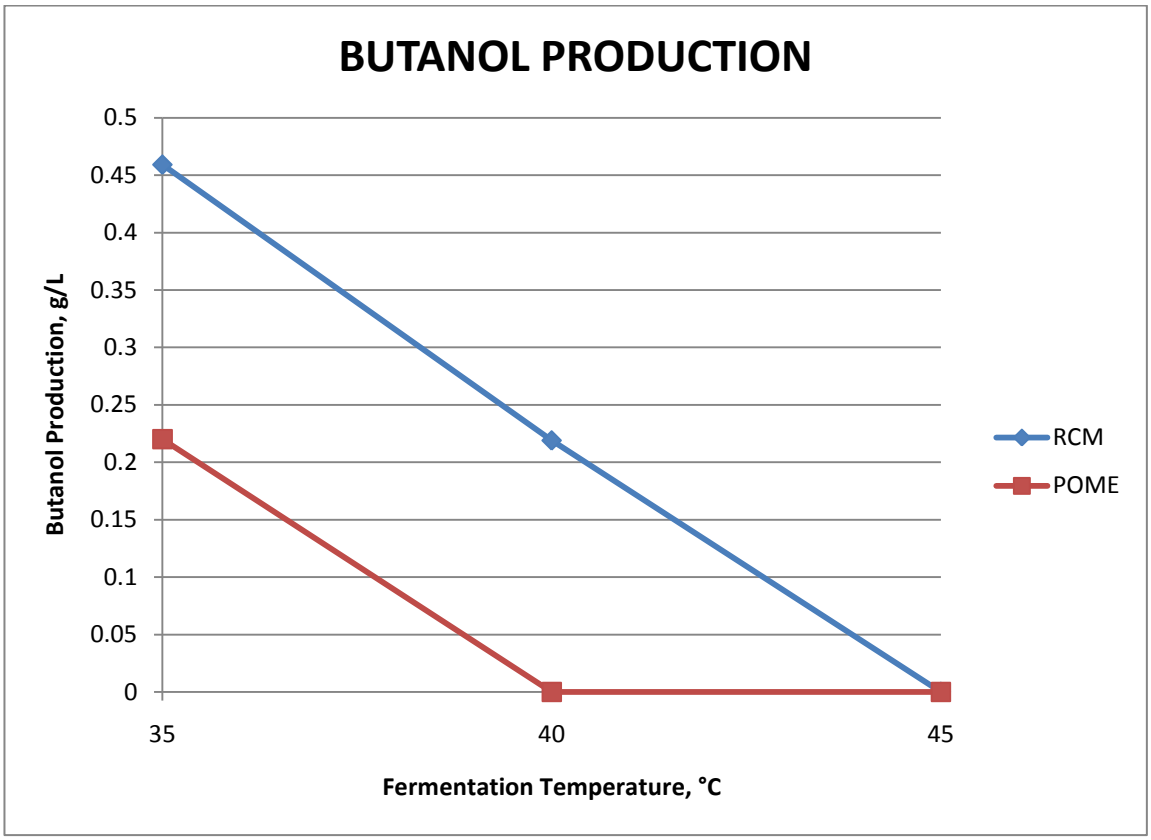

Figure 4.9 Butanol productions between POME and RCM in 48 hours

Figure 4.9 is the comparison butanol production in POME and RCM within 48 hours of fermentation. The graph showed that, RCM produce more butanol than POME. The production patent showed that, the production of butanol decreases as the fermentation temperature increases. Production of butanol in RCM produces a decreasing straight line from temperature 35°C to 40°C to 45°C. While production of butanol in POME only at temperature 35°C, no production at temperature 40°C and 45 °C. The difference between butanol produce in RCM is twice more than butanol produce in POME at temperature 35°C. While at 40°C, RCM produce same quantity of butanol as POME produce at 35°C. At 45°C, both RCM and POME did not produce any butanol might due to the high fermentation temperature.

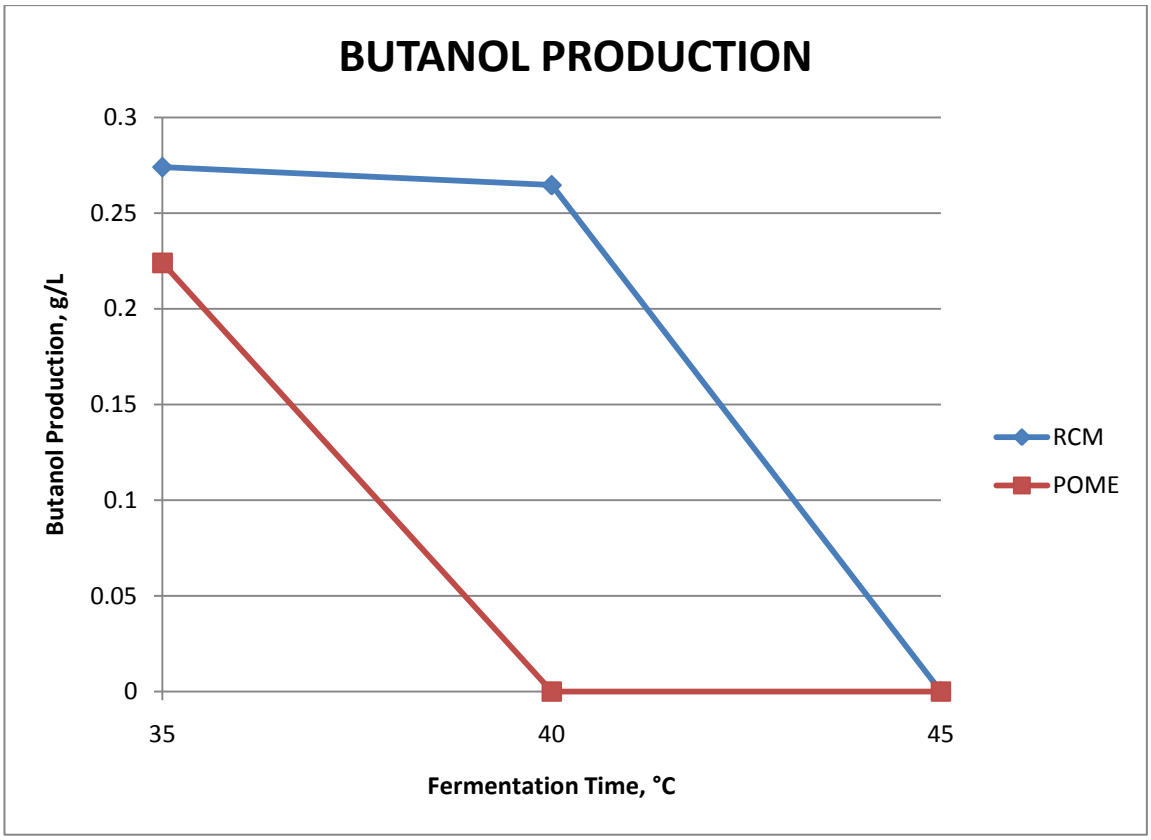

Figure 4.10 Butanol productions between POME and RCM in 72 hours

Butanol production in RCM of 72 hours fermentation lower than production in 48 hours fermentation, while butanol production in POME of 72 hours of fermentation still produce almost the amount as in 48 hours of fermentation. Still, production of butanol by POME and RCM showed same patent of production as in 48 hours of fermentation. Butanol only produce at 35°C of fermentation by POME, while butanol produce at both 35°C and 40°C of fermentation by RCM and there are no trace of butanol in production at 45°C of fermentation temperature. There is only slightly different between production of butanol in RCM at 35°C and 40°C.

4.3 Glucose Utilization

4.3.1 Effect of fermentation temperature on glucose utilization

i. In 48 hours of fermentation

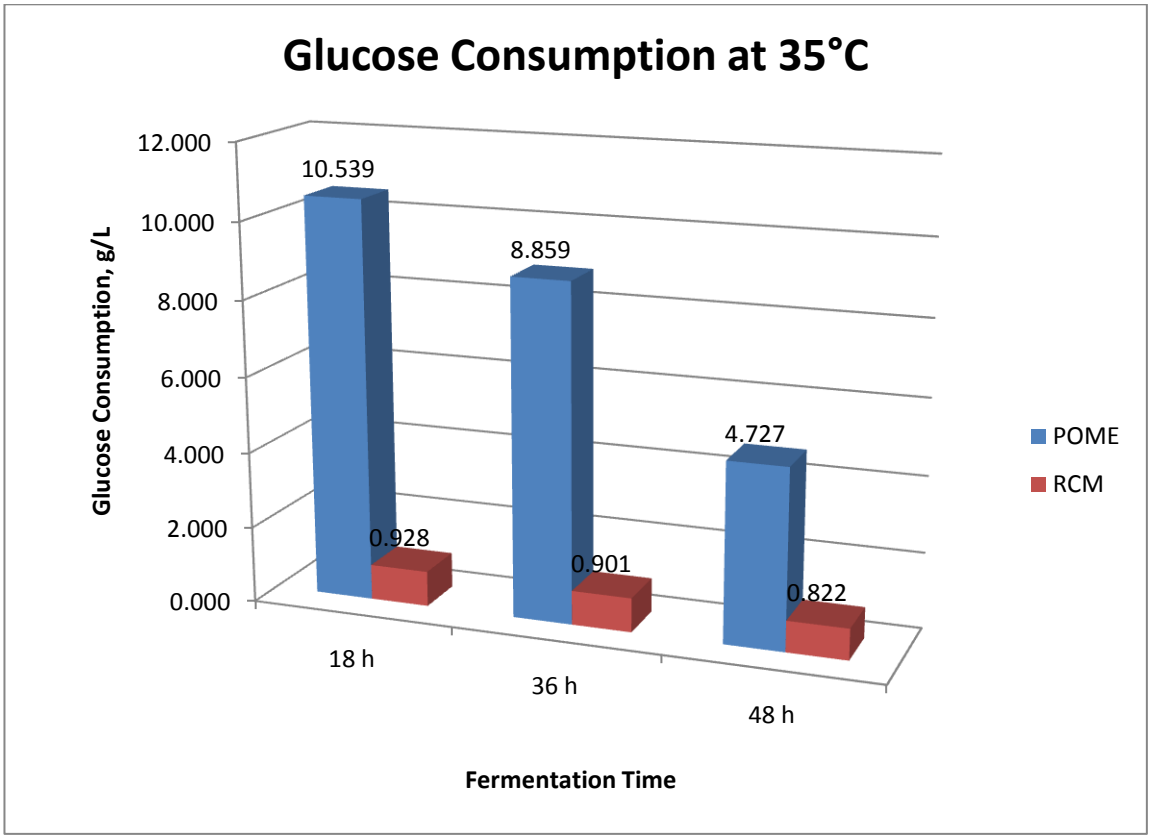

Figure 4.11 Glucose consumption based on fermentation time at 35°C

Figure 4.11 showed the reducing of glucose through time between POME and RCM. Both of substrate glucose level decreasing through time. Mostly, glucose in POME decreasing more than glucose in RCM through the time of fermentation.

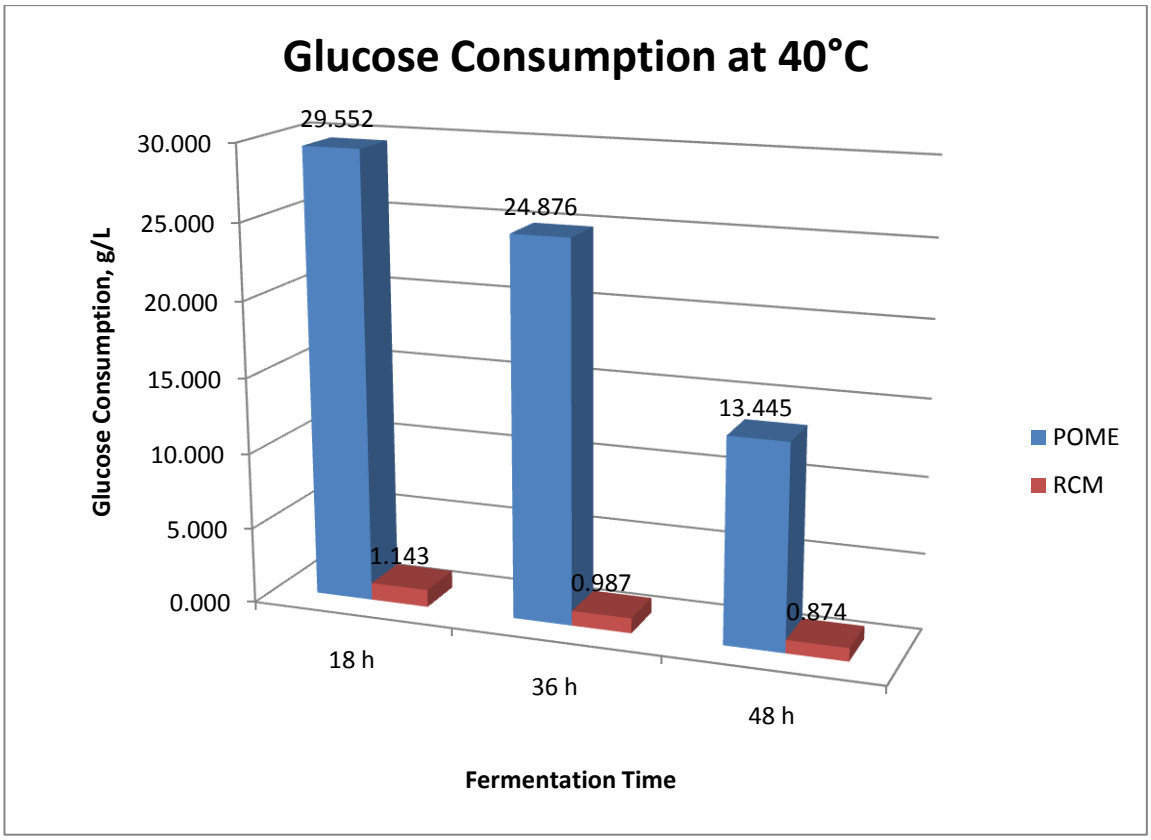

Figure 4.12 Glucose consumption based on fermentation time at 40°C

Glucose reducing through fermentation time at 40°C also showed in **Figure 4.12**. The concentration of glucose at 40°C higher than at 35°C either in POME or RCM substrate. This may be because; the strain is not actively culture in 40°C rather than in 35°C. This reason can be proof with the OD readings of cell in sample that collected in interval of time (refer Appendix C).

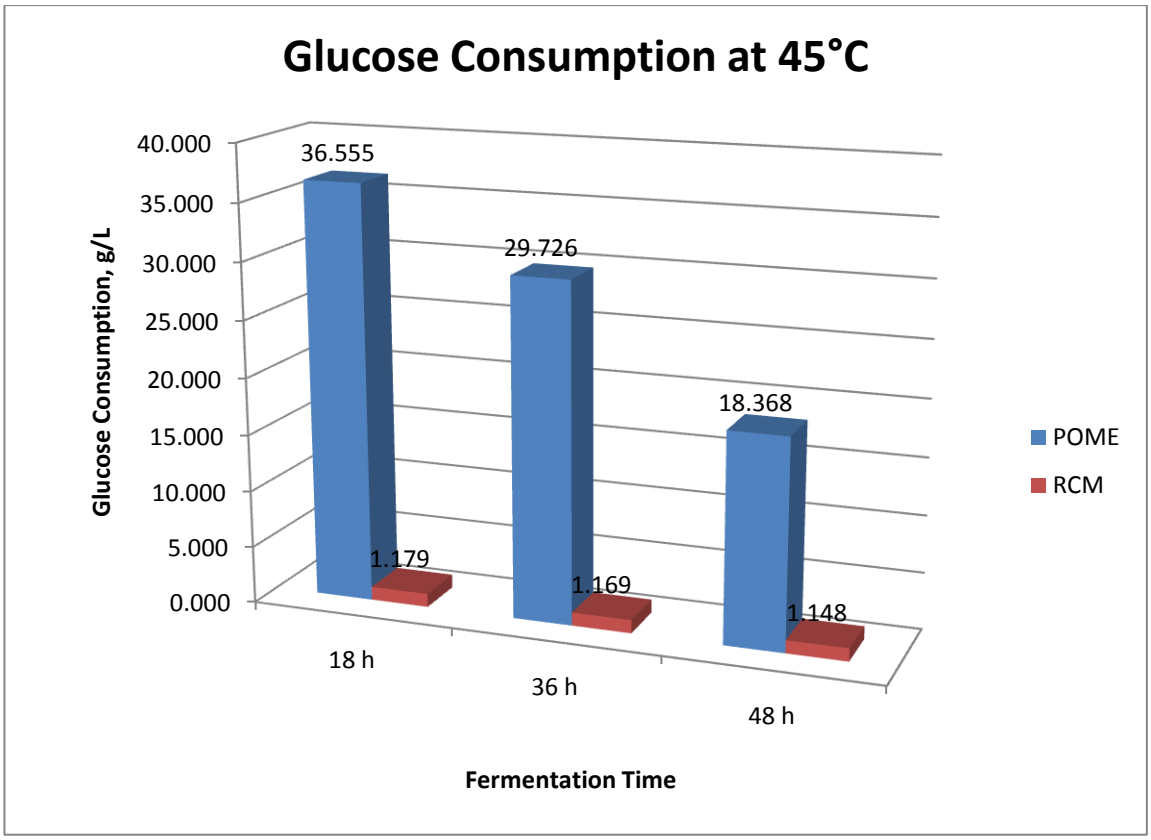

Figure 4.13 Glucose consumption based on fermentation time at 45°C

As discussed in **Figure 4.11** and **Figure 4.12**, the glucose consumption at 45°C fermentation temperature also decreasing through fermentation time. Based on the **Figure 4.13**, the concentration of glucose higher if differentiate between with consumption at 35°C and 40°C of fermentation temperature. As discussed before, the glucose consumption might be low because of the culture in the substrate is not active as the temperature increasing.

ii. In 72 hours of fermentation

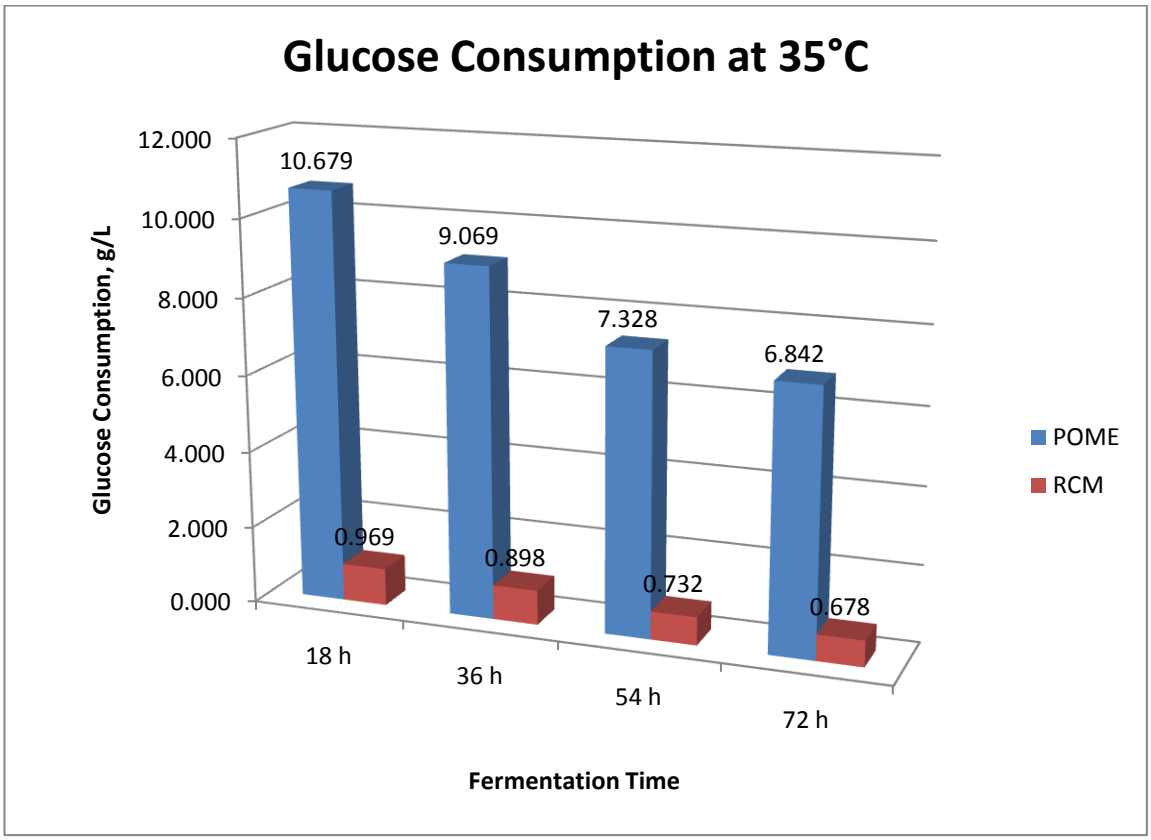

Figure 4.14 Glucose consumption based on fermentation time at 35°C

Figure 4.14 showed the glucose consumption at 35°C in 72 hours of fermentation. The reducing of glucose did not obvious as in 48 hours of fermentation. As discussed previously, too many subcultures will decrease the inoculums productivity. This might be the reason, the glucose consumption did not decreasing rapidly as the fermentation time increasing; the incremental of subcultures in the substrate interrupted the strain to actively produced and since that, the glucose cannot be absolutely utilized.

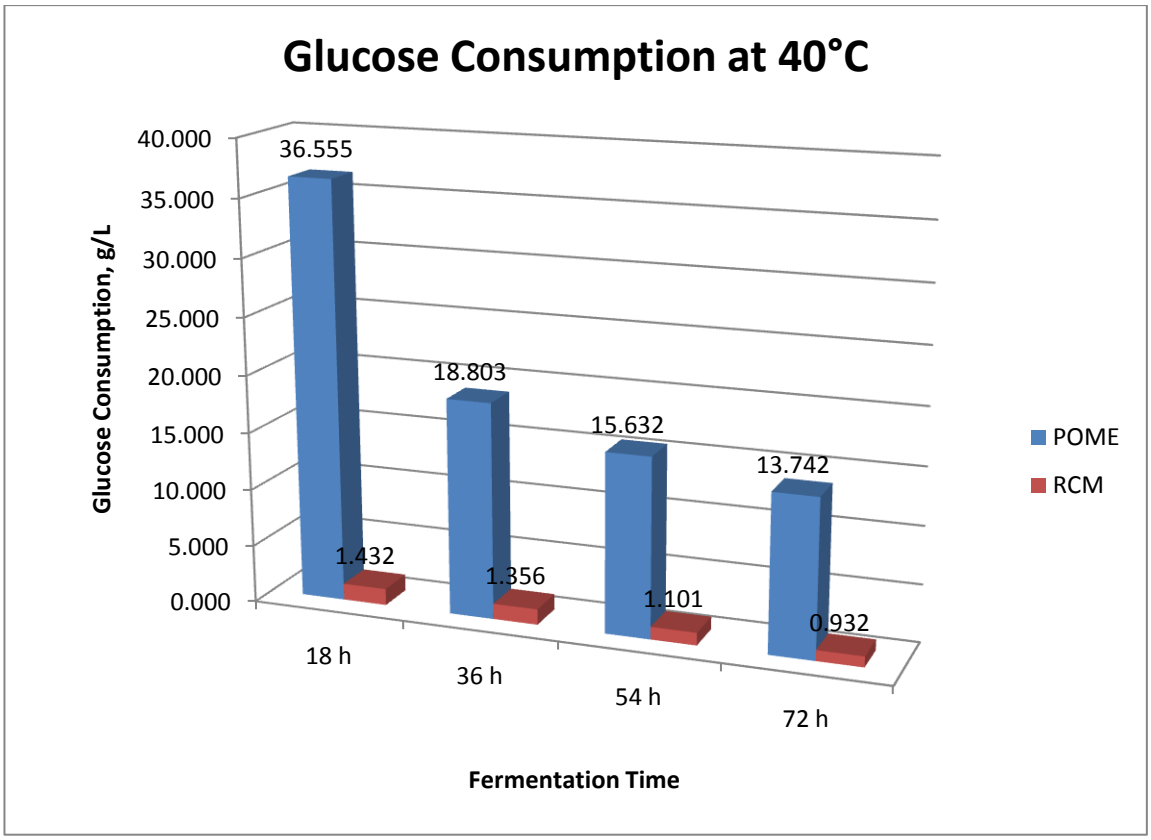

Figure 4.15 Glucose consumption based on fermentation time at 40°C

Glucose consumption at 40°C in POME substrate is decreasing rapidly at hour-18 to hour-36 as shown in **Figure 4.15**. Then the reducing became stable until the end of fermentation. While in RCM substrate, the glucose consumption stable decreases through time.

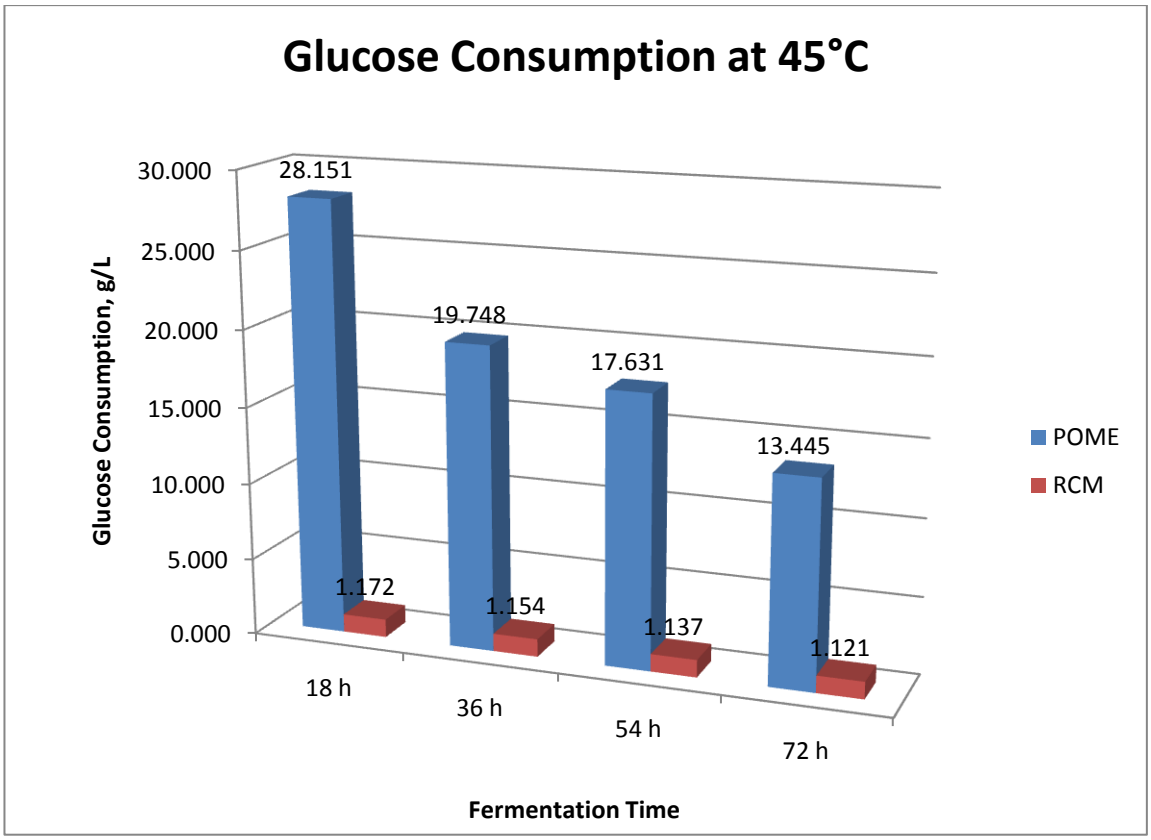

Figure 4.16 Glucose consumption based on fermentation time at 45°C

If differentiate between result in **Figure 4.15** with result in **Figure 4.16**, the glucose concentration in hour-18 at 45°C is more lower than hour-18 at 40°C in POME substrate. Then, the glucose consumption between these two temperature sets, the glucose consumption at 40°C is more rapidly decreasing than at 45°C. This may proof that, the temperature did play some role in strain culture and also the concentration of subculture as discussed previously also can be applied on such situation as above.

CHAPTER 5

CONCLUSION & RECOMMENDATIONS

5.1 Conclusion

From the result, showed that the solventogenic fermentation process utilized glucose in the substrates and different amounts of butanol. The highest concentration of butanol that produced by POME was 0.224 g L-1 at 35°C by 72 hours of fermentation, while highest concentration of butanol produced by RCM was 0.459 g L-1 at 35°C by 48 hours of fermentation. Biobutanol production still has some limitations including butanol toxicity to culture leading to low butanol yields. From this study, it is showed that POME can produce butanol at optimum fermentation temperature at 35°C in 72 hours of fermentation time. The results also conclude that POME is viable to use as growth medium to produce butanol.

5.2 Recommendations

Based on previous study by Gapes *et al.* (1983), too many subcultures will decrease the inoculums productivity. The strain used up less sugar and produced progressively less butanol and isopropyl alcohol and more butyric acid. In clostridia fermentation, the sporulation occurs concurrently with the solventogenesis. This theory may affect the fermentation with longer hours. As recommendation, new strain can be added to the fermentation broth, so that the strain inside can live longer and produced more product.

Strain is most important part of the fermentation. So the strain needed to be study the acceptability of the strain to the substrate characteristic and the fermentation parameter. Genetic alteration of *C. Beijerinckii* to construct tolerant of the strain and the product might increase the production of butanol.

Toxic and inhibitory products are disadvantages to the solventogenic fermentation. The longer the fermentation, the higher concentration of butanol should produce. The problem to produce more butanol is these two factors. The changes from acidogenic to solventogenic have to study as this factor indicates the actual timing for the acids to change to the solvents. As the time of transforming indicated, new strategies can be plan to make sure the toxic and inhibitory products do not interfere with the process of producing butanol.

Extractive fermentation is one of the strategy can be applied to the fermentation broth so that the butanol can continuously produced as the toxin being removed. Another techniques is by using in-situ extraction during the fermentation is being run. This technique enhances solvent production in ABE fermentation by reducing end product inhibition by butanol (Ishizaki *et al*., 1999).

One recommendation can be applied to increase the production is by maintaining the pH of substrate as the pH also can interfere with the cell to active in the substrate. As

mentioned previous study in Lepage *et al.* (1998), increased productivity could be achieved in a single-stage continuous fermentation when operated at low pH and dilution rate. So the suitability of the strain, *C. Beijerinckii*, to certain pH also need to be study to make sure the strain can actively culture in the substrate and produced more product.

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APPENDIX A

Table A.1 DNS standard data

CONCENTRATION (g L^{-1})	OD
0	0.000
0.2	0.092
0.4	0.368
0.6	0.524
0.8	0.710
1	0.931

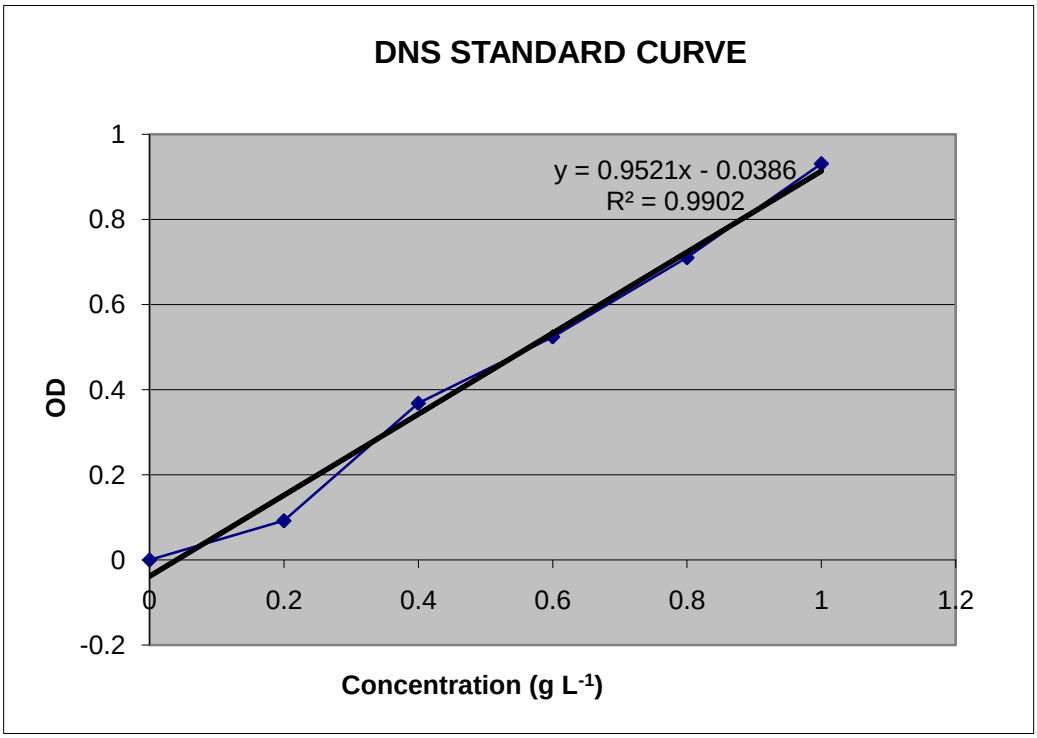

Figure A.1 DNS standard curve

APPENDIX B

Table B.1 Peak area of butanol standards

RETENTION TIME = 4.465 min	PEAK AREA
Butanol 1.0	197.7136
Butanol 2.0	397.1444
Butanol 3.0	614.6014
Butanol 4.0	839.5170
Butanol 5.0	990.3093

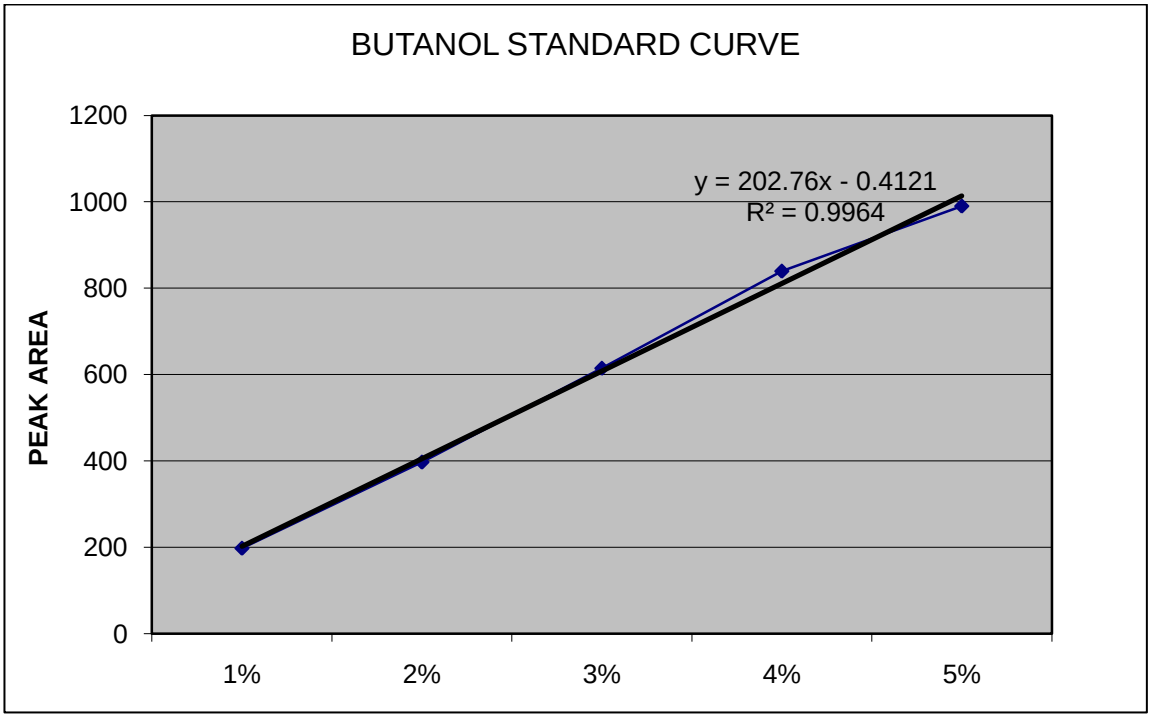

Figure B.1 Butanol standard curve

Table B.2 Peak area of acetone standards

RETENTION TIME = 2.518 min	PEAK AREA
Acetone 0.5	64.1879
Acetone 1.0	139.3209
Acetone 1.5	209.9058
Acetone 2.0	273.5171
Acetone 2.5	348.2100

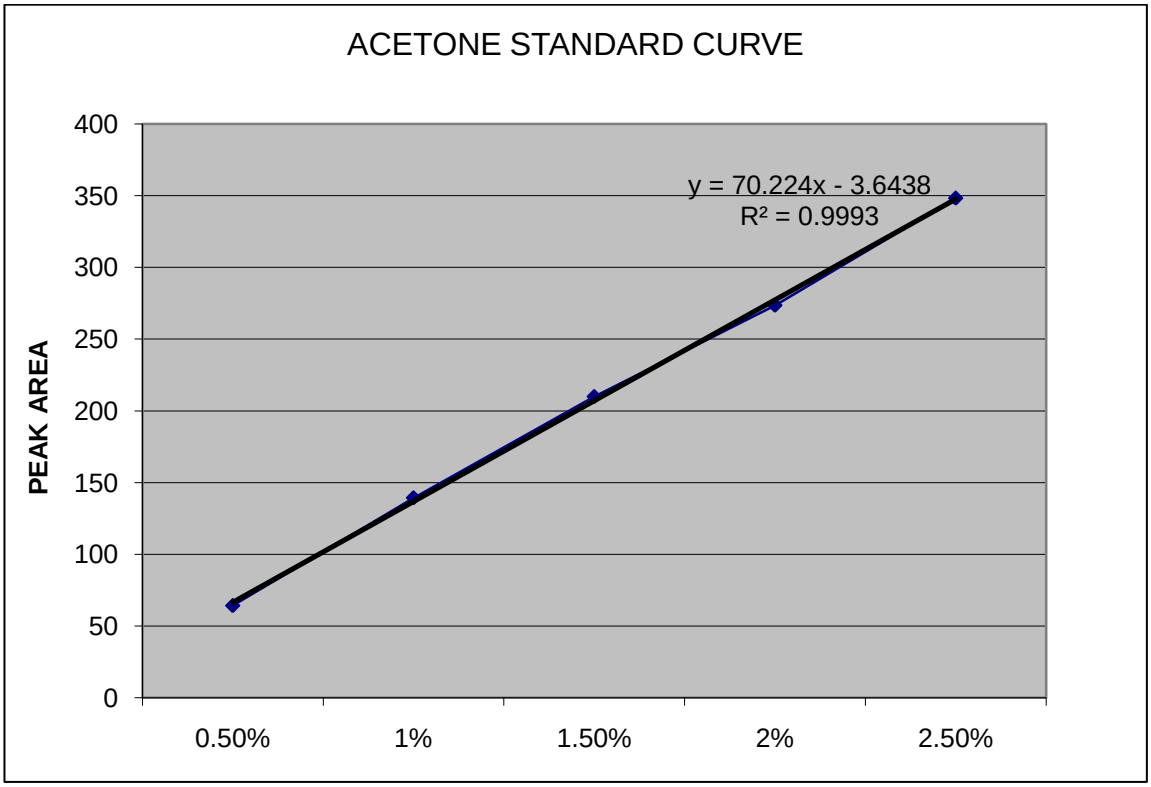

Figure B.2 Acetone standard curve

Table B.3 Peak area of ethanol standards

RETENTION TIME = 2.933 min	PEAK AREA
Standard	24.9483
Ethanol 0.4	54.7450
Ethanol 0.6	81.0866
Ethanol 0.8	111.0166
Ethanol 1.0	137.1377

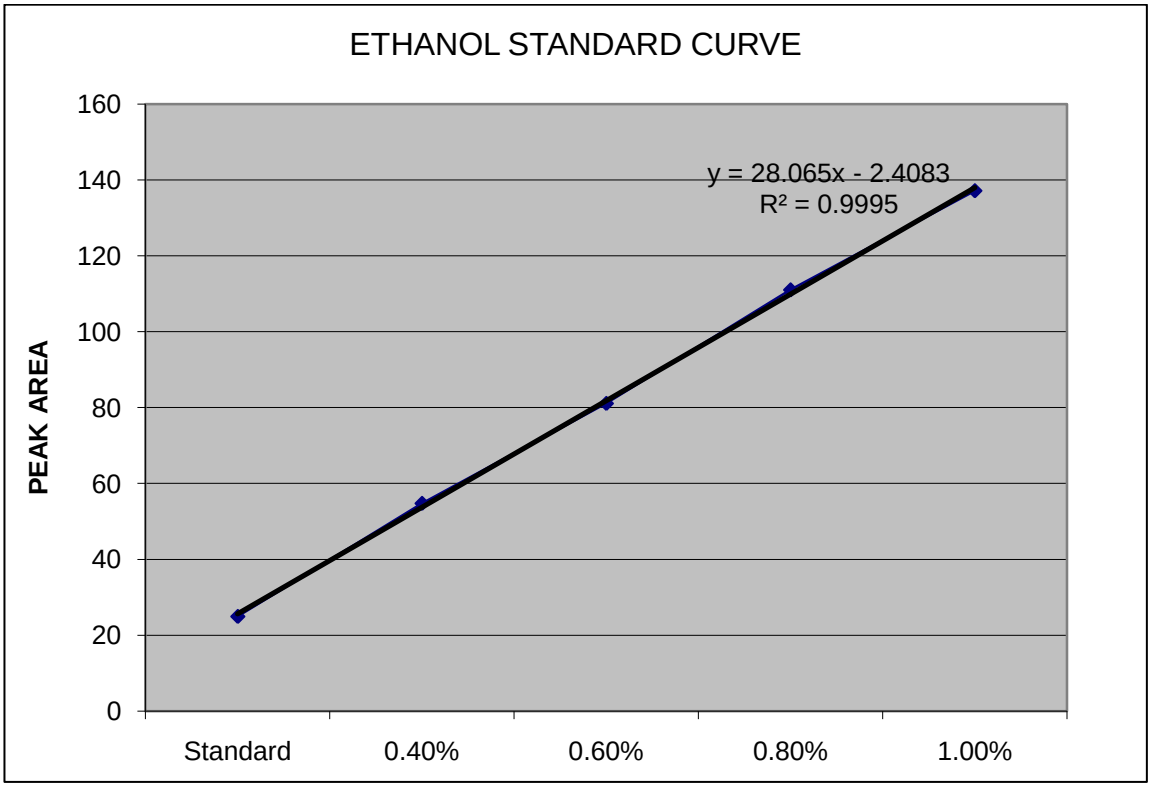

Figure B.3 Ethanol standard curve

APPENDIX C

Table C.1 Cell concentration every 6 hours intervals in POME

	FERMENTATION RUNS					
	35°C		40°C		45°C	
Interval (hours)	48 hours	72 hours	48 hours	72 hours	48 hours	72 hours
0	0.770	0.708	0.157	0.215	0.222	0.477
6	0.805	0.725	0.25	0.331	0.494	0.505
12	0.938	0.871	0.368	0.398	0.567	0.622
18	1.000	0.923	0.426	0.336	0.505	0.675
24	0.991	1.232	0.578	0.412	0.543	0.772
30	1.211	1.223	0.637	0.477	0.547	0.861
36	1.391	1.432	0.938	0.542	0.573	0.871
42	1.542	1.529	0.935	0.863	0.589	0.867
48	1.565	1.522	0.956	0.899	0.599	0.891
54		1.589		0.963		0.953
60		1.721		1.041		0.967
66		1.342		0.992		0.874
72		0.831		0.863		0.853

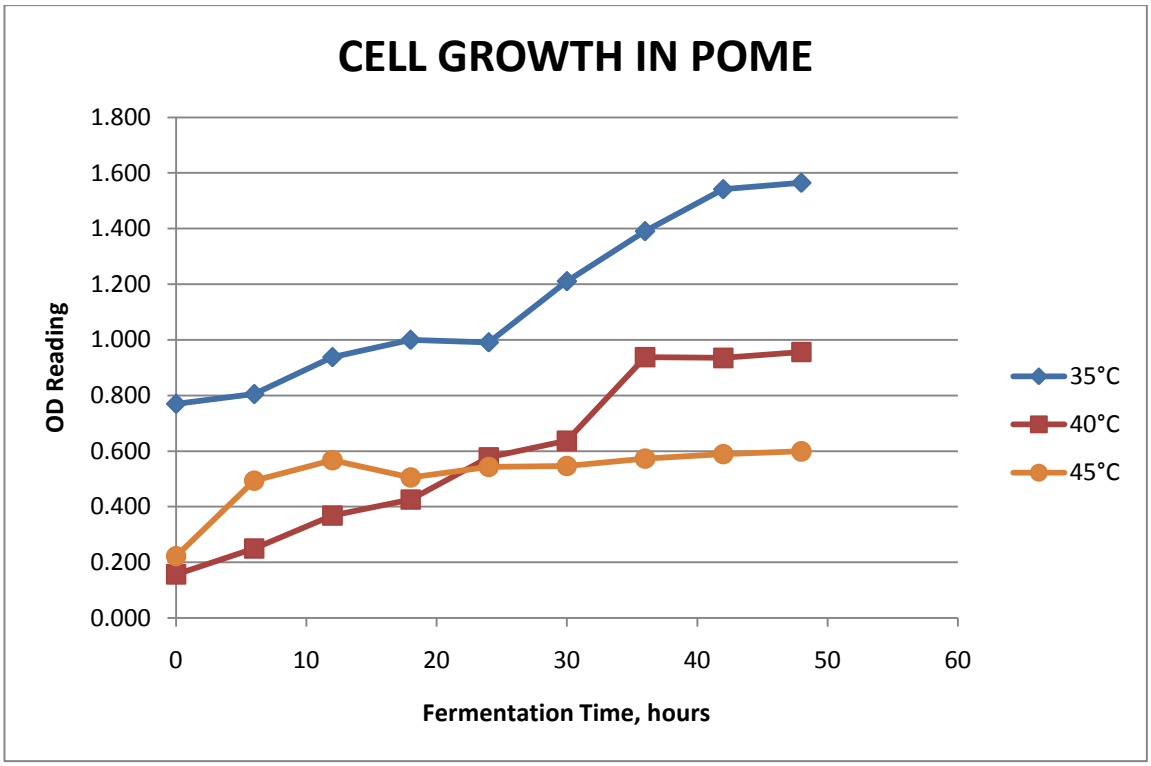

Figure C.1 Cell concentration in POME through 48 hours fermentation

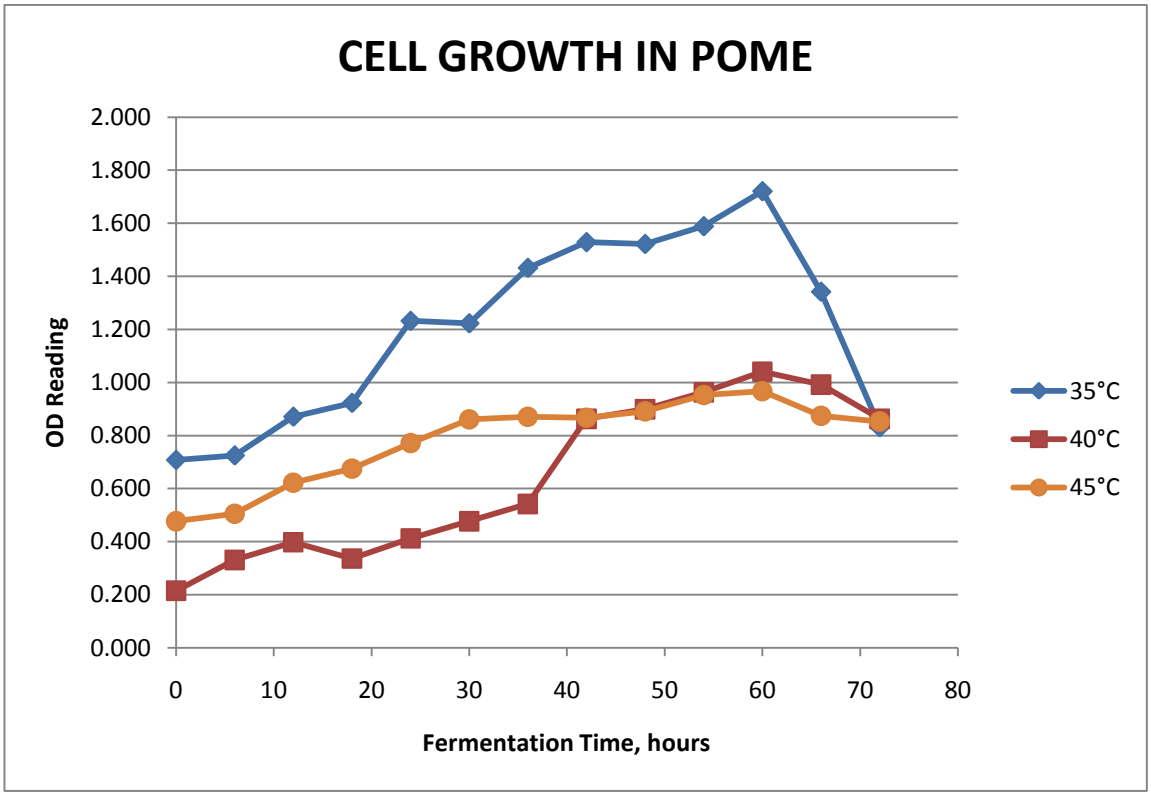

Figure C.2 Cell concentration in POME through 72 hours fermentation

Table C.2 Cell concentration every 6 hours intervals in RCM

	FERMENTATION RUNS					
	35°C		40°C		45°C	
Interval (hours)	48 hours	72 hours	48 hours	72 hours	48 hours	72 hours
0	1.252	1.005	0.913	1.322	0.778	0.821
6	1.499	1.303	1.361	1.609	0.857	0.878
12	1.520	1.532	1.593	1.867	0.924	0.912
18	1.821	1.652	1.703	2.102	1.034	0.934
24	2.203	2.004	1.943	2.312	1.101	0.989
30	2.312	2.000	2.071	2.345	1.156	1.034
36	2.317	2.009	2.198	2.355	1.166	1.265
42	2.512	2.076	2.2	2.4	1.182	1.321
48	2.529	2.115	2.21	2.201	1.189	1.347
54		2.145		2.199		1.657
60		2.200		2.05		1.691
66		2.000		2.003		1.534
72		1.834		1.945		1.389

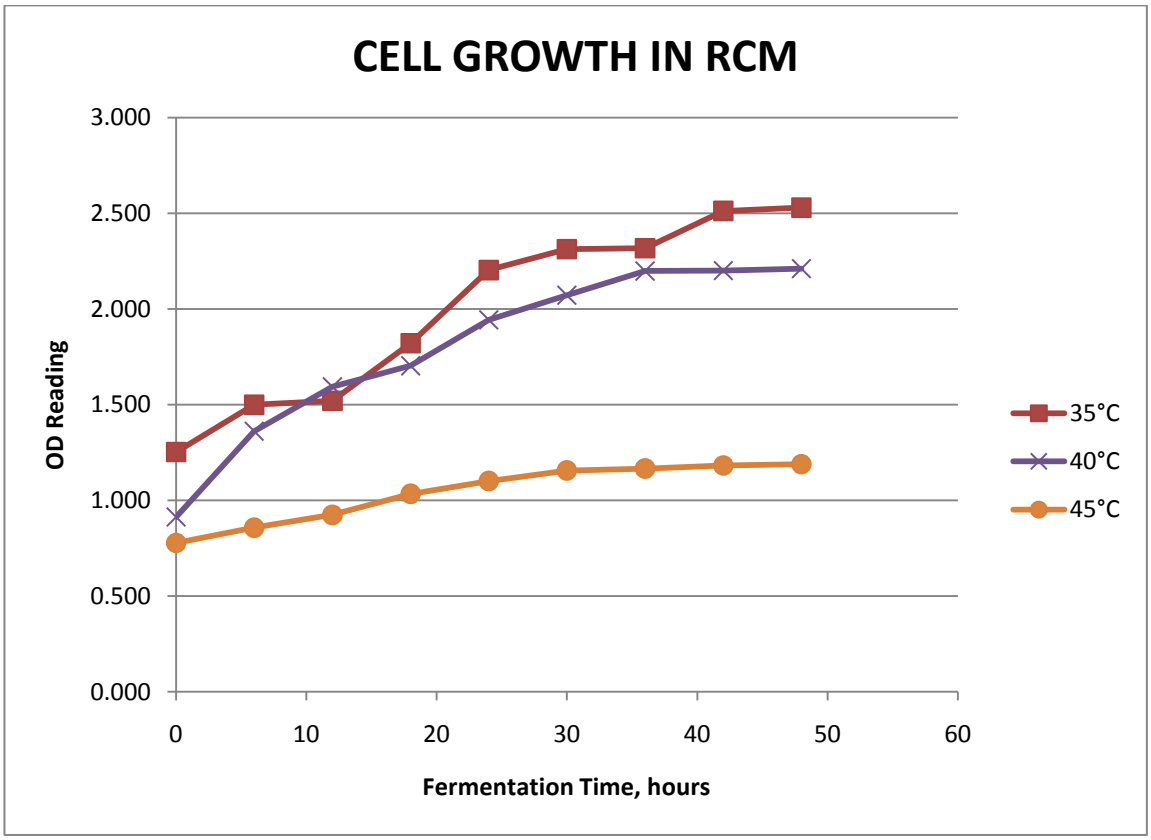

Figure C.3 Cell concentration in RCM through 48 hours fermentation

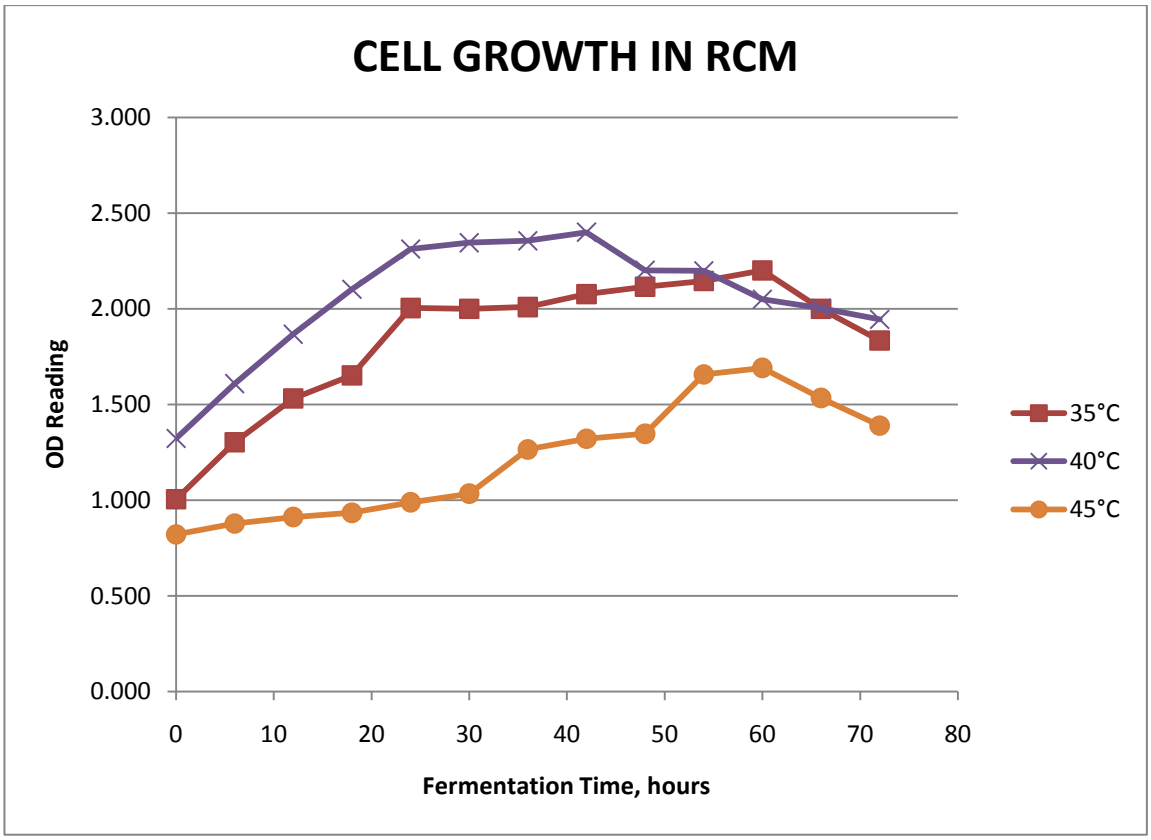

Figure C.4 Cell concentration in RCM through 72 hours fermentation