EFFECT OF ULTRASOUND ON ANAEROBIC FERMENTATION BY *SACCHAROMYCES CEREVISIAE*

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SUPERVISOR DECLARATION

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering

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Date

STUDENT DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. This thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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LIST OF ABBREVIATIONS AND SYMBOLS

A	Area
P	Power
°C	Degree Celcius
mL	Millileter
g/L	Gram per militer
gL^{-1}	Gram per liter
h	Hour
s	Second
W	Watt
W cm^{-2}	Watt per Centimeter Squared
%	Percentage
m	Meter
µ	Micro
w/w	Weight per weight

KESAN UTRASOUND TERHADAP PENAPAIAN ANAEROBIK OLEH *SACCHAROMYCES CEREVISIAE*

ABSTRAK

Kajian ini menerangkan tentang kesan ultrasound terhadap penapaian anaerobik *Saccharomyces cerevisiae*. Pengeluaran biojisim yang kurang dan hasil etanol yang lebih tinggi dalam penghasilan etanol menyebabkan ultrasound diperkenalkan dalam proses ini. Pengasilan etanol yang tinggi telah menghalang penapaian dan memperlahankan pertumbuhan biojisim. Kaedah ultrasound digunakan untuk menentukan hasil pengeluaran etanol, pertumbuhan biojisim dan untuk menentukan parameter kinetik. Penapaian telah dijalankan dengan menggunakan ultrasound dan tanpa ultrasound yang digunakan sebagai kawalan. Penapaian telah dijalankan selama 24 jam dan setiap 2 jam sampel diambil. Kemudian sampel yang telah diambil dikenakan kepada ultrasound dengan dua kitar tugas yang berbeza iaitu 10% dan 30% kitaran. Kepekatan biojisim, kepekatan glukosa, kepekatan etanol telah diperolehi daripada kajian ini. Parameter kinetik juga dapat ditentukan. Daripada kajaian ini, rejimen sonication yang terbaik adalah 10% kitaran berbanding 30% kitaran. Nadi ultrasound telah memberi kesan kepada pertumbuhan biojisim yang berkaitan dengan bilangan pertumbuhan sel yang berjaya. Apabila nadi ultrasound rendah, pertumbuhan biojisim meningkat, serta kepekatan etanol juga meningkat. Hasil etanol terakhir adalah $12.64gL^{-1}$. Kajian ini perlu diteruskan lagi dengan menggunakan komposisi yang berbeza untuk glukosa dan jenis yis atau kulat yang berbeza. Selain itu, kajian ini juga boleh diteruskan dengan menggunakan kuasa ultrasound yang berbeza atau intensiti yang berbeza untuk menentukan kesan ultrsound terhadap penapaian anaerobik.

EFFECT OF ULTRASOUND ON ANAEROBIC FERMENTATION BY *SACCHAROMYCES CEREVISIAE*

ABSTRACT

This research describe about the study on the effect of ultrasound on anaerobic fermentation of *saccharomyces cerevisiae*. The less biomass production and higher ethanol produce in the production of ethanol causes the ultrasound to apply for this process. The high ethanol inhibits the fermentation and slowed the biomass growth. The ultrasound method is applied to determine the production yield of ethanol, the growth of biomass and to determine the kinetic parameter. The fermentation was conducted with the ultrasound and without the ultrasound which is used as a control. The fermentation was conducted for 24 hour and every 2 hour the sample was collected. Then the sample was attached to the ultrasound with two different duty cycles which is 10% and 30% duty cycle. After that, the sample was analysed. The biomass concentration, glucose concentration, ethanol concentration was obtained from this research. The kinetic parameter also was determined. From this research, the best sonication regiments are 10% duty cycle rather than 30% duty cycle. The pulse ultrasound effect the biomass growth which is related to the number of successive growth of cell. The lower the pulse ultrasound, the biomass growth increase, as well as the ethanol concentration also increase. The final ethanol yield is $12.64gL^{-1}$. This research is reaccommodated to use a different composition of glucose and different type of yeast or fungus. Besides, use a different power or different sonication intensity to determine the effect of ultrasound towards the fermentation.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Recently, fermentation is widely used in order to produce a wine. Product from the fermentation is in form of alcohol. In wine fermentation, the *Saccharomyces cerevisiae* play an important role which is to change the grape sugar into the ethanol, (Parapouli, 2010). *Saccharomyces cerevisiae* can growth well in aerobic and anaerobic conditions. Aerobic fermentation is condition which is oxygen is supplied to the fermentation. Meanwhile, an anaerobic fermentation is the condition in which oxygen is absent or not present and other gas is used such as nitrogen.

Some of studies use an ultrasound effect to the fermentation is reported. A study carried out by Sulaiman et al., (2011) state that ultrasound had been used in laboratory scale for ruptured the cell walls in order to release intracellular products. Ultrasound also related with the damage to cell. Ultrasound wave could be divided into two powers which is high power and low power, (Jomdecha & Prateepasen, 2006). Both powers give a different effect towards the reaction or process.

1.2 Problem Statement

Increasing demand in alcohol and wine fermentation makes the production of ethanol also increase. In the production of ethanol, less biomass is produce while higher ethanol produces in anaerobic fermentation of *Saccharomyce cerevisiae*. Due to the high ethanol production, ethanol inhibits the fermentation reaction and slowed the biomass growth (cell). A study by Hoppe and Hansford (1982) stated that the ethanol inhibit the biomass yeast growth in anaerobic fermentation. Then the study is carried out in order to optimise the ethanol production by depend on the biomass growth. *Saccharomyce cerevisiae* yeast being used in production of ethanol because in previous study stated that, this yeast can generate and produce more ethanol than other type of yeast. The ultrasound is applied to increase the biomass growth.

1.3 Research Objective

The main objective of this research is to study the effect of ultrasound to anaerobic fermentation of *Saccharomyces cerevisiae.*

The measurable objective for this research are:

1.3.1 To examine the effect of sonication regiments on anaerobic fermentation of *Saccharomyces cerevisiae.*

1.3.2 To determine the kinetic parameters of the fermentation with and without ultrasound.

1.4 Research Questions/Hypothesis

1.4.1 What is the production yield of ethanol in anaerobic fermentation?

1.4.2 What is the effect of ultrasound on growth of Saccharomyces cerevisae in anaerobic fermentation?

1.4.3 What is the kinetic parameter of anaerobic fermentation of Saccharomyces cerevisae?

1.5 Scope of Study

In order to gain the objective, scope of study is needed as follows:

a) To study the effect of sonication regiments for improving productivity of a live cell in *Saccharomyces cerevisae* fermentation.

b) To determine the kinetic parameters of the fermentation with and without ultrasound.

1.6 Significance of Study

Ethanol fuel gives benefits toward human because it acts as a bioenergy. The ethanol production is having an environmental friendly because use a biological instead of using the chemical production. The ethanol also can serve as fuel to the mobile transportation. Beside that, ethanol will be used as a medical treatment to the patient. Ultrasound method will be used into the industry to enhance the bioprocess performance and to increasing the production of ethanol. Ultrasounds also can enhance the fermentation time. By introduce ultrasound to industry, the ethanol production will increase, and increasing the economy of industry in which the time of fermentation decrease so that it will save the cost for the production and raw materials.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

A literature review was introduced to classified studies that related to the topic. There are four themes in this literature review which is *saccharomyces cerevisiae* yeast, application of *saccharomyces cerevisiae* yeast, substrate**,** ultrasound, effect of ultrasound, application of ultrasound in fermentation, application of ultrasound in biotechnology, alcohol fermentation and anaerobic fermentation of *saccharomyces cerevisiae* yeast that have been studies in this chapter.

2.2 *Saccharomyces Cerevisiae* Yeast

Saccharomyces cerevisae is atype of yeast. Study by Dequina and Casaregol (2011) stated that there are eight of different type of this yeast that mostly valuable in food and beverage fermentation. For the type of yeast that related to antrophic environments is *Saccharomyces cerevisiae*, *Saccharomyces bayanus and Saccharomyces pastorianus.* Meanwhile, *Saccharomyces paradoxus, Saccharomyces kudriavzevii, Saccharomyces cariocanus, Saccharomyces mikatae* and the lately express *Saccharomyces arboricolus* are mainly isolated from natural environments.

Industrial of Saccharomyces yeasts are not commonly in the form of interspecific hybrids, (Dequina & Casaregol, 2011). The hybrid nature of these yeast genomes is an benefits that bring together the properties from each of the parental stains. Their features as hybrids, including type of species that given rises to them and the difficulty of their genomes, differences of their specialization and industrial environment (Figure 2.1).

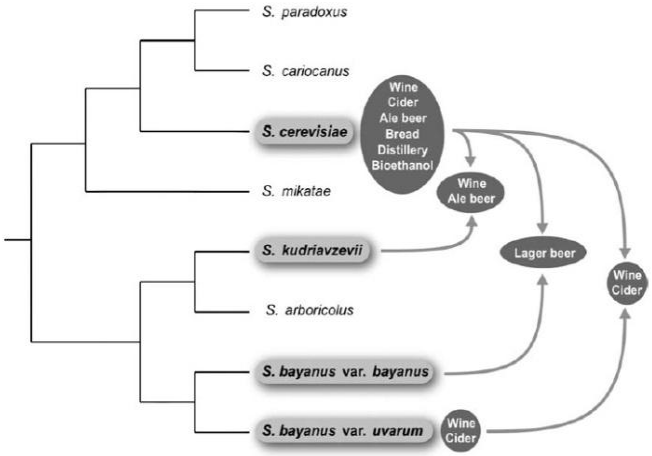

Figure 2.1 Phylogenetics relationship between saccharomyces species and their industrial application. Light grey for industial process or in hybrids. Dark grey for products of hybrids and non-hybrids.Arrows shown as hybrids.

(Source: Naumov, 1994)

Variety of mono-, di- and oligosaccharides and respiratory substrate can be used by *Saccharomyces cerevisae* such as ethanol, glycerol, acetic acid, lactate and pyruvate, (Papagianni, Boonpooh, Mattey & Kristiansen, 2007). Another study by Deken (1996) stated that *Saccharomyces cerevisae* is the type of prototypical Crab-tree-positive yeast for aerobic fermentation. Crab-effect is regulatory system which is including the repression of energy source by another type of energy source.

A study by Papagianni et al. (2007) is reported that *Sacchromyces Cerevisae* fermentated in anaerobic conditions and acts as a facultative anaerobe. *Sacchromyces Cerevisae* is able to respire or breathe on low concentration of sugar or in the respirator substrate. This yeast can growth on simple sugar which is glucose also in dissacharide sucrose, (Lin & Tanaka, 2006).

2.3 Application of *Saccharomyces Cerevisiae* Yeast

Saccharomyces cerevisiae yeast is applicable to fermentation in anaerobic growth, (Liden et al., 1995). Beside that, it also can display fermentative metabolism in aerobic culture state, (Cortassa & Aon, 1998). *Saccharomyces cerevisiae* is widely used in some industrial application in the production of beer, baker's yeast, alcohol, wine, heterologous proteins and ethanol fermentation, (Olsson & Nielsen, 2000). This is because it can manipulate growth and have relatively similar structure of human cells.

According to Parapouli (2010) the wine fermentation have been use a *Saccharomyces cerevisiae* yeast and it play most important role to changing the grape sugar into the ethanol. For wine industries, it utilizes a commercial yeast

strains as a stater culture. *Saccharomyces cerevisiae* yeast also used in ethanol production on anaerobic fermentation by applied the ultrasound and without ultrasound in order to determine the differences of productivity between these two condition. A study by Sulaiman et al., (2011) has reported that this yeast is the only yeast that consider to some level in ultrasound fermentation. Besides that, the *Saccharomyce cerevisiae* yeast is used in fermentation because when the ultrasound is applied the yeast will assess to some level.

2.4 Substrate

Glucose is some type of the substrate that use in fermentation of *Saccharomyces Cerevisae*. According to Papagianni et al. (2007) the glucose is used as a carbon source in fermentation and as a suitable metabolism in EMP pathway which give a product of ethanol. Another type of sugar substrates to the fermentation of *Saccharomyces Cerevisae* is the α-glucoside which is including the maltose, sucrose and maltotriose, (Dequin, 2001). Maltose is chosen as the most use in the fermentation of *Saccharomyces Cerevisae.*

2.5 Ultrasound

Ultrasound is some kind of mechanical elastic energy or wave. The power of ultrasound is divided by two which is high power and low power, (Jomdecha & Prateepasen, 2006). Ultrasound has been use in research to produce, collect or

measure sound wave in medium. Beside that, the effect to an irradiation medium also had been study by using ultrasound wave which is produce from ultrasound equipment. According to Lanchun et al., (2003) ultrasound has the transmission in material and the phenomena of calefaction and cavitation when it passes through in medium.

A cavitation phenomenon is a major mechanism that causing change of biological tissue which mainly increasing the membrane permeability during the fermentation process, (Bommannan et al., 1992). Membrane permeability is referring to the diffusion of molecule through the membrane. In this phenomenon, the bubbles will exits until it stable and then will collapse at critical state then produce high temperature and pressure.

In addition, the ultrasound can enhance the yeast growth, (Matsuura, 1994). The mass transfer around the cell and inside the cell is increasing when ultrasound is applied in direction of diffusion. (Laugier, 2008). Low frequency level of ultrasound will speed up the movement of liquid medium, increase mass transfer and reaction rates for homogeneous and multiphase system, (Liu et al., 2006). Ekaterina (2009) stated that productivity of fermentation process also can be improve by applied the ultrasound towards to the process.

2.6 Effects of Ultrasound

Ultrasound gives an effect towards the fermentation and cell. Several studies reported the ultrasound give effect to the some factor. The factor is divided to three parts which is cavitation, mass transfer enhancement and thermal effect.

2.6.1 Cavitation

Cavitation is a process of a bubbles form when the ultrasound is being applied to the medium. Cavitations enhance the membrane permeability of the cell. There two type of cavitation which is non-internal and internal cavitation. Non-internal cavitation is a stable cavitation when bubble form with a constant number of acoustic cycle meanwhile internal cavitation is unstable when the bubbles oscillate on unstable cycle, (Riesz & Kondo, 1992). Figure 2 shows the bubble form when ultrasound is applied.

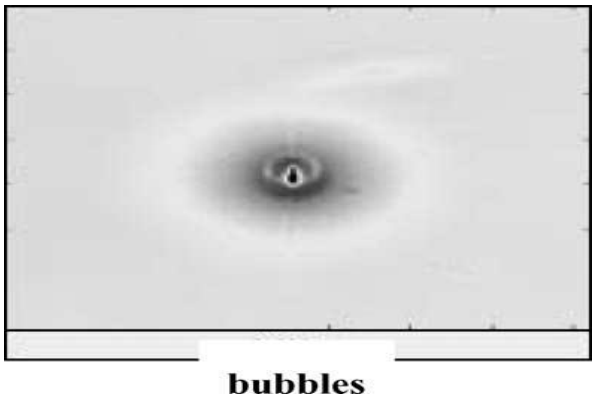

Figure 2.2 The formation of cavitation bubble induced by ultrasound,

(Source: Liu et al., 2006)

2.6.2 Mass Transfer Enhancement

Study by Bar (1988) stated that mass transfer and reaction rate increasing when the low intensity of ultrasound is applied. The gas bubbles generate along the circulation liquid was known as microstreaming. Microstreaming will lead the flux of reagents to the cell and thus increasing the reaction rate. Ultrasound also increases the mass transfer for both artificial and biological membrane, (Liu et al., 2006).

2.6.3 Thermal Effect

The use of ultrasound to the medium will increase the temperature of the medium when the medium absorb the energy from the ultrasound. This phenomenon does not make as a main activation of the fermentation. But can use a important part or factor to the fermentation, (Liu et al., 2006).

2.7 Application of Ultrasound in Fermentation

Applications of ultrasound towards fermentation process have been widely use in food industry and alcohol fermentation nowadays. Study by Lamberti et al., (2009) uses an ultrasound to monitor the wine alcohol fermentation. The ultrasound also been applied in biorenewables as a new concept and have a function to improve the enzymatic hydrolysis and consequent to the ethanol fermentation. Ultrasonic is use in the process to produce and determine the sound wave in medium of cells then study it characteristic and properties. In addition, ultrasonic wave is being applied to investigate the energy effect to an irradiation medium from the generated ultrasonic equipment, (Jomdecha & Prateepasen, 2006).

Jomdecha and Prateepasen (2006) said that the power of ultrasound have some applications. For high power ultrasonic involve in cleaning, welding and biological or chemical process while low power used for testing a material, measuring and communications are depend to the properties or character of the

received ultrasonic waves. Beside that, the ultrasound range about 1-10MHz is used in medical imaging, (Chisti, 2003). High power in ultrasound treatment for aqueous media has been used to reduce hatch times offish eggs and germination times of seeds. In one study, intermittent ultrasound treatment for accumulative period of 150 s using a 25 kHz tube resonator (constant 80 W effective output) caused 76% increase in the release of intracellular gentamicin during production by *Micromonospora echinospora.*

A study by Lamberti et al., (2009) said that an ultrasonic method is used to investigate the alcohol and extract contains in wines, even though they not have go through enough in fermentation process itself, an relationship between ultrasonic parameters and the concentrations of alcohol and soluble solids was proposed, indicating that these concentrations mainly determine the speed of the sound in fermenting media.

2.8 Application of Ultrasound in Biotechnology

The varieties of method have been researched to improve the biotechnology process. Ultrasound is the new method to improve the bioprocess performance. This ultrasound method can reduce the process time compare to the conventional method. For biotechnology, ultrasound is use in the production of biofuel from triglycerides, (Rokhina, 2009). The ultrasound will used to optimize the change of triglycerides to biofuel. The low frequency of ultrasound (28 and 40 kHz) will give influence towards the production of biofuel from triglycerides.

Ultrasound method also used in enzyme biocatalysts process. For the last few years, ultrasound has been used to improve or increase the enzyme-catalyzed waste treatment, (Rokhina, 2009). Biosensor also used ultrasound to aggregate and drive in order to enhance the sensitivity and efficiency of biosensors, (Zourob, 2005).

2.9 Alcohol Fermentation

In fermentation, the energy is required to produce the ethanol. This energy is needed for the growth of yeast cell. Glucose acts as a carbon sources then will produce an energy to the fermentation process. The chemical equation when the alcohol fermentation occurs is illustrated in Figure 2. Based on this reaction, the theoretical yield of ethanol will produce is 0.511 and for carbon dioxide is 0.489 on a mass basis of glucose metabolized, (Bai et al., 2008).

$$\text{Glucose (carbon source)} \longrightarrow 2 \text{ ethanol} + 2 \text{ carbon dioxide} \quad (2.1)$$

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2 \quad (2.2)$$

Figure 2.3 Chemical reaction of ethanol production,

(Source : Teh & Lutz, 2010)

2.10 Anaerobic Fermentation of *Saccharomyces Cerevisiae* Yeast

According to the Dake et. al (2010) common definition for the fermentation in chemical term is the conversion of carbohydrate to the acids or alcohols. Another definition is the breakdown of carbohydrates to ethanol, carbon dioxide and water by

using micoorganism, (Osunkoya & Okwudinka, 2011). Meanwhile, anaerobic fermentation is the fermentation in absence of oxygen and nitrogen is being used instead off oxygen. For *Saccharomyces cerevisiae* yeast can growth well under the aerobic and anaerobic conditions. Aerobic condition is in the present of oxygen.

A study by Brandberg, Gustafsson & Franzen (2007) reported that the limitation of nitrogen can cause a biosynthesis to be reduced. Complete starvation for nitrogen give many benefit towards *Saccharomyces cerevisiae*. A change to the macromolecular composition was occurred and transfer of glucose also will inactivate. Nitrogen will acts as a limiting factor for cell growth when it is being suppling continuously.

CHAPTER 3

METHODOLOGY

3.1 Introduction

Fermentation is carried out in order to determine the effect of sonication. The fermentation converted the glucose into the ethanol by using yeast *saccharomyces cerevisiae*. The fermentation was accomplished with and without sonication. The step involve to do this research is micoorganism, preparation of agar plate, culture, preparation of inocolumn, fermentation process and ultrasound procedure. There three step to analysis this research which is ethanol concentration analysis, biomass concentration and glucose concentration.

3.2 Micoorganism

Baker's yeast *(Saccharomyces cerevisiae)* was obtained from the University Malaysia Pahang. The yeast was provided in the form of solid.

3.3 Preparation of Agar Plate

The maintenance agar medium was prepared by mixing with deionized water and the composition of 50gL^{-1} glucose, 2gL^{-1} yeast extract, 6.25gL^{-1} $(NH_4)_2SO_4$, 2gL^{-1} $MgSO_4.7H_2O$, 4 gL^{-1} KH_2PO_4 and 15gL^{-1} agar and stir using magnetic stirrer with the heat on and poured into the 1L Schotte bottle. After that, the solution was sterile by autoclaving at 121°C for 20 min. The solution are left to cool at 50°C after sterilize. About 15-20ml of the sterilize solution per plate was poured into the plate under laminar flow hood then left it to cool to solidify (agar plate) and keep it about 4°C in chiller until it use.

3.4 Culture

One scoop of solid yeast *Saccharomyces cerevisiae* was poured in sterile medium with previous composition without the agar and incubates in orbital shaking incubator at 30°C and 180rpm for 24 hour and then inoculated the cell to the agar plate. The agar plates then incubate in incubator at 30°C for 24hr. After the

incubation period, the agar plate was stored at 4°C in chiller. The culture of yeast is shown in Figure 3.1.

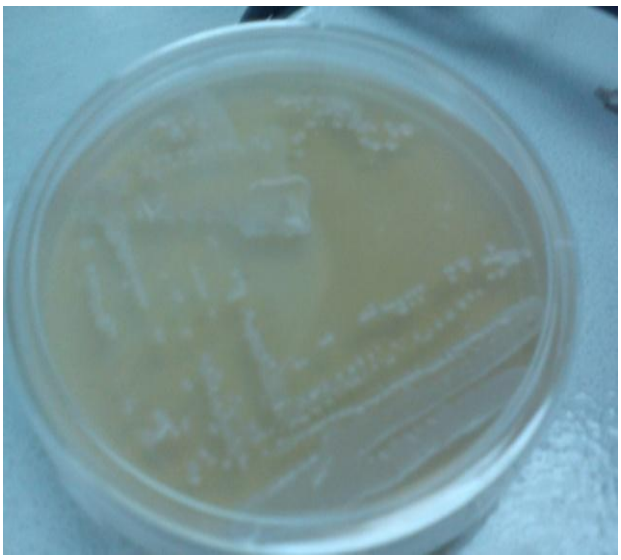

Figure 3.1 Culture of yeast *Saccharomyces cerevisiae* in agar plate.

3.5 Preparation of Inoculum

The inoculum was prepared by inoculate the cell from the agar plate by using inoculating loop towards the 80 ml of sterile medium with previous composition without the agar under laminar flow. Then, incubate the inoculum in orbital shaking at 30°C and 180rpm for 24 hour. After 24 hour the inoculum was stored at 4°C.

3.6 Fermentation

About 80 ml of inoculum solutions were averagely transferred into two Erlenmeyer flasks, in which 800ml of medium contained. After being shaken homogenously, one of the solutions will stimulated by ultrasonic under the optimum parameters, the other cultured under the same conditions was acts as a control. Fermentation was carried out at 30°C and 180rpm for 24 h. Optical density (OD) values of the samples were measured at intervals of time. Under the conditions mentioned as above, the time of lag phase of *S.cerevisiae* was about 1- 2 h, and the time of the exponential phase and the stationary phase are at 2-22 and 24 h, respectively. Figure 3.2 shown of anaerobic fermentation after several hours.

Figure 3.2 Anaerobic Fermentation in Erlenmeyer flask.

3.7 Ultrasound Procedure

Power of ultrasound is very important in the interaction between ultrasonic and the biology system. There were reported that the shape of cell could be easily changed if power of ultrasonic was excessively big. The amplitude setting of sonotrode was set at position 22% to get the power input of 15W or sonication intensity of 11.77W cm^{-2}. The sonication intensity was determined by using the following equation:

$$I=\frac{P}{A}$$

where A was the area of sonotrode tip which is 1.274 cm^2. The duty cycle of sonication were represented to the energy imparted to the sample, (Sulaiman et al., 2011). The duty cycle determine the time of sonication was on. Duty cycle of 10% was represented as a 1s and rest period (no sonication) at 9s for about 1 minute. A 10% (1s sonication, 9s rest period) and 30% (3s sonication, 7s rest period) was applied to the sample shown in Figure 3.3.

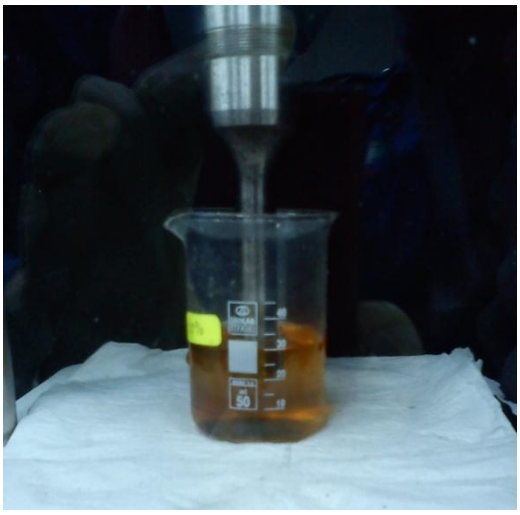

Figure 3.3 Ultrasound attach to the sample

Table 3.1 illustrate summarize of the procedure for applying the ultrasound to the sample.

Table 3.1 Sonication regiments used to anaerobic fermentation

Duty cycle (%)	Pulse ratio
10	1s sonication, 9s rest period (no sonication)
30	3s sonication, 7s rest period.

3.8 Analysis Method

3.8.1 Ethanol Concentration

Ethanol concentration was analysed by using a Gas Chromatography (GC) with a HP innowax column (15mmx0.53mm) and helium as a carrier gas with flow rate of 20Lmin^{-1}. The temperatures of the injection unit and flame ionization detector (FID) were set at 175°C and 250°C. The temperature of 45°C was used to be heated of the oven for 2.5 minutes and the temperature was raised to 110°C at rate of 20°C/min and then held at 110°C for 2 minutes. Standard ethanol was prepared by diluting absolute ethanol with butanol (0.5g/L) in the concentration range of 2-8gL^{-1} Butanol act as a function of internal standard. The sample volume was injected about 2μL. The sample was prepared by pre filtered through 0.45μm membrane filter and shown in Figure 3.4. The ethanol concentration of supernatant was determined by

calculating the relative area under the ethanol peak and comparing it with standard curve which is prepared from standard solution.

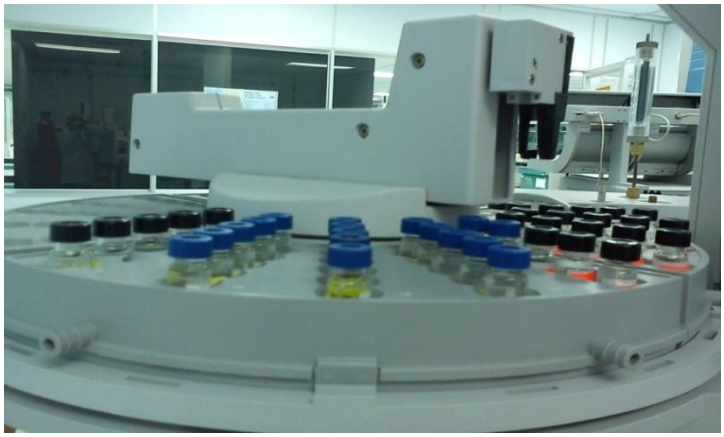

Figure 3.4 Standard ethanol and sample ready to analysis by GC.

3.8.2 Biomass Concentration

Biomass concentration was analysed by using spectrophotometry. The absorbance or optical density was measured by using a UV/Visible spectrophotometer at 620nm and used a sterile medium as a blank. The samples were diluted with sterile medium in ratio 1:24ml, (Sulaiman et al., (2011).

For dry cell weight analysis, 20ml of sample was centrifuged at 4°C and 10000 rpm for 5 minute. The supernatant was discarded and then cell washing was applied to the cell that precipitated in centrifuge tube. The cells were washed by using the deionized water and then use a vortex to mix first before centrifuge again. The produce of cell washing were repeated but not centrifuged meanwhile filtered through membrane filter under suction, (Sulaiman et al., (2011). The membrane filter with the wet biomass was dried at 80°C for overnight. After drying period, the membrane was cooled first to the room temperature and weighed. The dry weight of

biomass can be determined by subtracting the weight of membrane filter with biomass and weight of membrane filter. The biomass concentration was obtained from the standard calibration curve of biomass (Appendix B.3).

3.8.3 Glucose Concentration

Glucose concentration was determined by using modified dinitrosalicylic acid (DNS) by Miller (1959). About 1% (w/v) solution of DNS reagent was prepared by diluted between 10g DNS and 2g of phenol in 1000ml of a solution of sodium hydroxide ($10gL^{-1}$) and sodium sulfite ($0.5gL^{-1}$). The sample containing the glucose was diluted first with deionized water. The 3ml of dilute sample was mix with 3ml of DNS reagent and leaved at boiling water bath at 100°C for 15min. A 1ml of potassium-sodium tartrate ($400gL^{-1}$) was added to the mixture and cooled down to the room temperature as shown in Figure 3.6. The absorbance or optical density was measured by using UV/Vis spectrophotometer at 575nm. Deionized water was used as blank. The glucose concentration was obtained by comparing with the standard curve of glucose (Appendix B.4).

Figure 3.5 DNS method for determination of glucose concentration.

CHAPTER 4

RESULT AND DISCUSSION

This chapter were discussed about the effect of ultrasound towards the fermentation growth of cells, effect of ultrasound to the glucose concentration and effect the ultrasound to the ethanol concentration and kinetic parameter when the difference duty cycle and constant power intensity was applied.

4.1 Effect of Ultrasound to Biomass Concentration

The biomass concentration was determined by using the standard curve of dry cell weight and optical density. Biomass concentration is referring to the concentration of cell that growth after several hours of fermentation. The

fermentation was started by applying the same procedure of control experiment then applied ultrasound with specific duty cycle and constant power intensity. The 10% and 30% was used to the fermentation and 11.78 wcm^{-2} of power intensity. The result obtained every 2 hours for 24 hours of fermentation. Figure 4.1 was shown as a difference of biomass concentration between the control or no sonication, 10% of duty cycle and 30% of duty cycle.

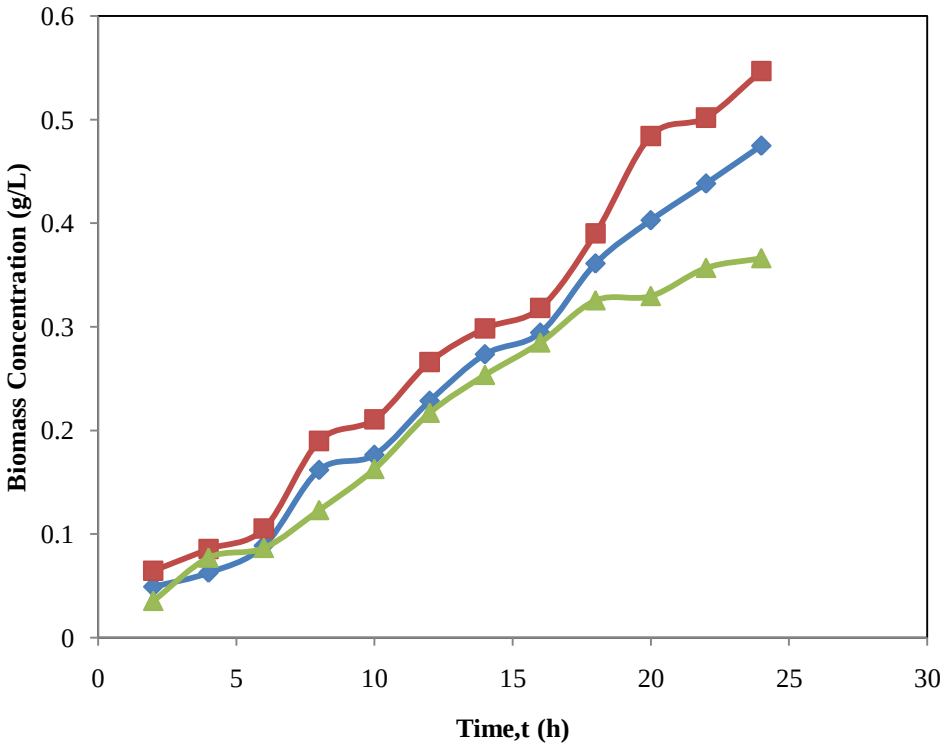

Figure 4.1 Effect of ultrasound to biomass concentration for difference sonication regiments. . control, 10% duty cycle, 30% duty cycle

From the graph, the biomass concentration is difference when the difference sonication was applied. The biomass concentration for 10% duty cycle was more than the other two parameters. The cells is continue to growth without having any failure and at 30% duty cycle the growth of cell is slowly than the 10% of duty cycle. The low of sonication regiment which is 10% duty is the best sonication toward the biomass concentration. The lower growth rate and biomass concentration was related

to the glucose consumption. High pulse ultrasound limits the number of successive growth of cell which is related to the excessive energy accumulation.

Study by Lanchun et. al (2003) stated that the low intensity of ultrasound could increase the biomass growth for the fermentation of *S.Cerevisae*. In this experiment.11.72 wcm^{-2} were used. This proven that low intensity of ultrasound increases the biomass growth.

The biomass or cell can return at the original size at the off period. When, the duty cycle is 30% the off period is short and make the stable cavitation cannot happen because of the bubble expand and growing to the critical size. The biomass concentration of 10% duty cycle is 0.55 g/L while at 30% duty cycle is 0.37 g/L. The value for control experiment is 0.479 g/L. Therefore, 10% duty cycle is more suitable sonication regiments towards the fermentation.

4.2 Effect of Ultrasound to Glucose Concentration

Glucose concentrations were obtained by measuring the absorbance or optical density which of the standard curve of the glucose and compare the result to get the glucose concentration. Difference sonication regiments would effect the glucose concentration of the fermentation. Glucose was used as a food or nutrient to the cell in order to make the cell growth. Bubbles were form as the time fermentation is increase. This means the cells was live and consume the glucose. Figure 4.2 was shown the bubble and foaming form.

Figure 4.2 Bubble or foaming form after several hour of fermentation

The glucose concentration for the difference sonication regiments which is 10% duty cycle and 30 % duty cycle was shown in Figure 4.3.

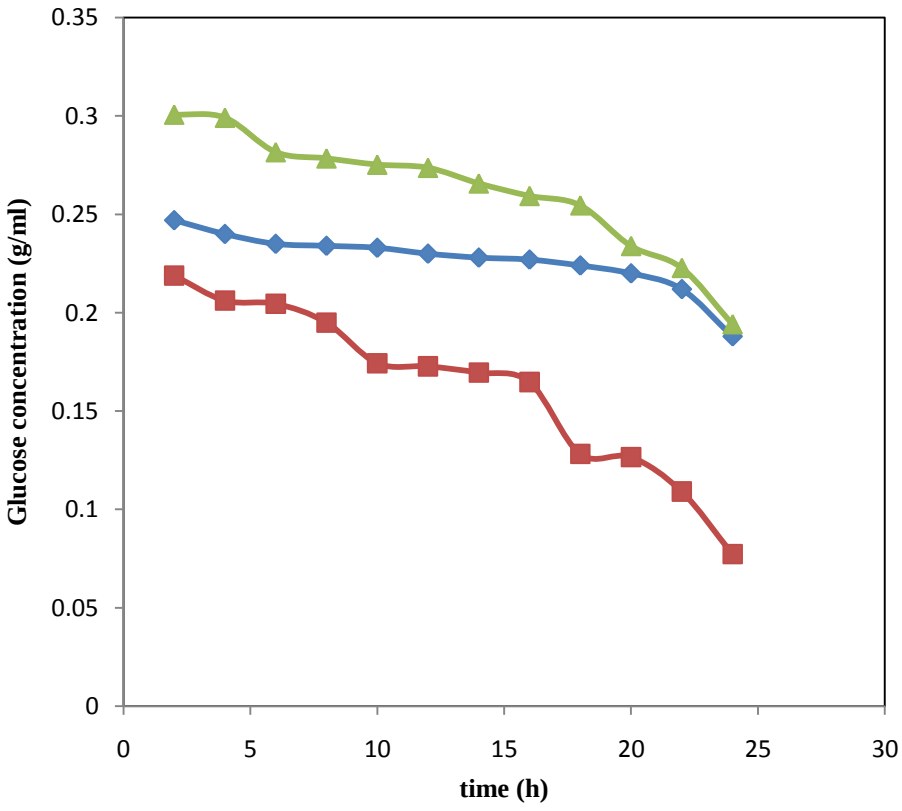

Figure 4.3 Effect of ultrasound to glucose concentration for difference sonication regiments. . control, 10% duty cycle, 30% duty cycle

The graph shows that, the concentration of glucose is decreasing for all condition even the control also decrease. The glucose was used by the cell to growth further and to make the cell live. Therefore, as the time of fermentation increase, the concentrations of glucose become decrease. This glucose consumption was related to the biomass concentration.

According to Lanchun et.al (2003), low intensity of ultrasound increase the cell metabolism and speed up the material transport. Therefore, the cell is active and consume the glucose rapidly and this proven in 10% duty cycle. The value of 10% duty cycle is more suitable regiments to the glucose reduction. The value for the glucose reduces after 24 hr at 10% duty cycle is 0.077g/ml. The glucose reduce at 30% duty cycle is less than the control which the control value is 0.188g/ml meanwhile for 30% is 0.194g/ml.

4.3 Effect Ultrasound to Ethanol Concentration

An alcohol was produce from the anaerobic fermentation of *S.Cerevisae* which is ethanol. Ethanol was determined by using the gas chromatography. The concentration of ethanol was obtained from comparing the standard ethanol with the sample. The ethanol concentration was shown in Figure 4.4 with comparison to the control. Different sonication parameter was applied which is 10% duty cycle and 30% duty cycle.

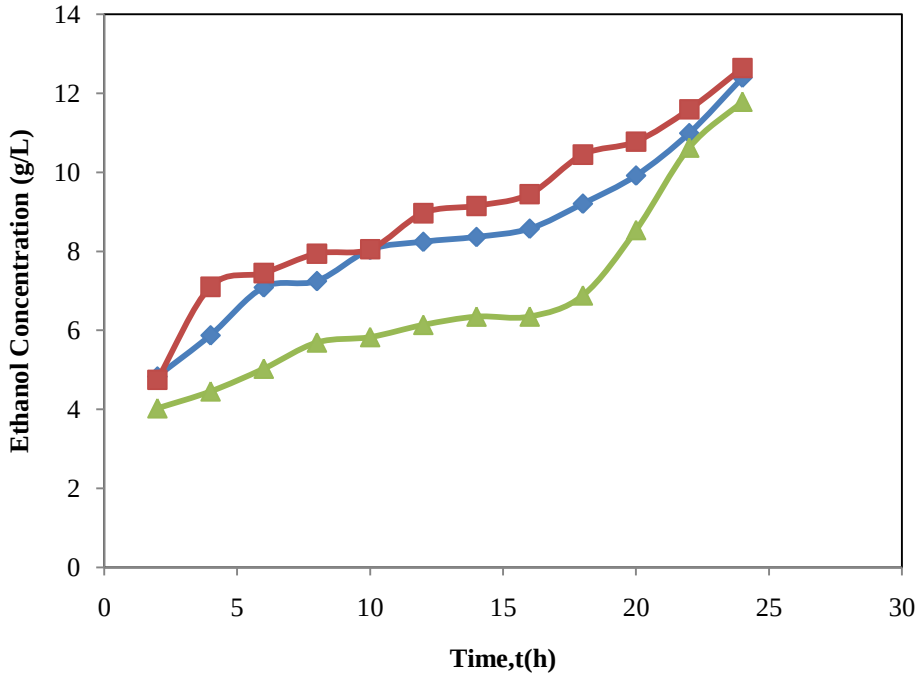

Figure 4.4 Effect of ultrasound to ethanol concentration for different sonication regiments. . control, 10% duty cycle, 30% duty cycle

From the graph, the ethanol concentration increase as the time increase. The graph show the 10% duty cycle is more effective than the 30% duty cycle. About 12.64 g/L ethanol concentration was gained from the 10% duty cycle whereas 30% is 12.40 g/L. This is related to the biomass growth. When biomass growth increases, the cell will consume more and glucose then produce more ethanol. Therefore, the ethanol increases when the biomass increases by applying the sonication about 10% duty cycles.

4.4 Kinetic Parameter

Kinetic parameter was obtained from the biomass concentration and ethanol concentration. The maximum biomass yield, maximum biomass concentration, maximum ethanol yield and maximum ethanol concentration was the kinetic parameter for this experiment. All this kinetic parameter was compared between the control which no sonication applied and with different sonication applied. The kinetic parameter of this fermentation was shown in the Table 4.1.

Table 4.1: Comparison of fermentation kinetic

Kinetic parameter	Control (no sonication)	Sonication regiments (duty cycle)	
		10%	30%
Maximum biomass yield on glucose, $Y_{x/s}$ (g g^{-1})	$1.9x10^{-4}$	$2.0x10^{-4}$	$1.4x10^{-4}$
Maximum biomass concentration, X max (gL^{-1})	$9.5x10^{-3}$	$10.9x10^{-3}$	$7.10x10^{-3}$
Final ethanol yield on substrate, $Y_{p/s}$ (g g^{-1})	$2.47x10^{-1}$	$2.53x10^{-1}$	$2.24x10^{-1}$
Final ethanol concentration (gL^{-1})	12.40	12.64	11.79

The maximum biomass was calculated from the biomass weight over the substrate or glucose concentration. From Table 4.1, the best duty cycle or the best sonication regiment is 10% duty cycle rather than the 30% duty cycle. The duty cycle 10% which gave the maximum biomass yield is $2.0x10^{-4}$ g g^{-1}. The maximum biomass concentration at 10% duty cycle is $1.9x10^{-4}gL^{-1}$ and the maximum ethanol concentration is $12.64gL^{-1}$. The final ethanol yield on substrate at the best sonication is $2.53x10^{-1}$ g g^{-1} .

Study by Bai et.al (2008) claimed that the ethanol yield is 0.511. However, the result gate from the result at 10% duty cycle is 0.253. This is happen maybe due to the concentration of substrate that used in this research. Another concentration of substrate could produce the high yield of ethanol.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Based on the study the main objective was achieved. The best sonication regiment was determined through the experiment. 10% duty cycle for sonication is the best sonication regiment instead of the 30% duty cycle. By applying the ultrasound, the ethanol not inhibits the biomass growth. The ethanol concentration increase as well as the biomass concentration also increases. This is proven by comparing the experiment with and without ultrasound. Beside that, the kinetic parameter also was obtained form the experiment. The final ethanol yield for 10% duty cycle is 12.64 g L^{-1} whereas without ultrasound is 12.40 g L^{-1}. Pulse ultrasounds are limiting the number of successive growth of biomass. Then, the less pulse ultrasound is good to the growth of biomass. Therefore, ultrasound is the best method to increase the ethanol concentration and increase the membrane permeability as well increase the biomass growth.

5.2 Recommendation

Several recommendations are being introduced in order to obtain the effect of ultrasound to the ethanol production and biomass growth and being listed in below:

a) Determination of other parameter

Ultrasound with the different sonication regiment gave an effect to the fermentation. The productivity of variety of bioreaction system also increases when applying the ultrasound. The other parameter for further study is added more duty cycle such as 20% duty cycle and 40% duty in order to determine the effect of ultrasound towards the fermentation of biomass growth and ethanol concentration. Beside that, the different power of ultrasound and different sonication intensity also can be added to determine the effect.

b) Substrate

In addition the substrate use in this research also can be changed in order to get the effect to the fermentation. Maltose or lactose can be used to further this research. The different concentration of glucose also can introduce to further this research and determine the best concentration to the fermentation. Sucrose also can be used as substrate to the fermentation.

c) Use another micoorganism

Another micoorganism can be different effect towards the anaerobic fermentation when the ultrasound is applied. The fungi also can be used to determine the effect. *K.marximus* is example of micoorganism that can be used in this fermentation. Another type of strains of S.Cerevisae also can replace this yeast in order to gain the best sonication regiments towards the fermentation and the yield of ethanol.

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APPENDIX A

a) Calculation of kinetic parameter (Doran, 1995).

i. Maximum biomass yield on substrate, $Y_{x/s}$ (g g^{-1})

$$Y_{x/s} = -\frac{\Delta X}{\Delta S}$$

where $Y_{x/s}$ at maximum biomass concentration, Xmax

ii. Final ethanol yield on substrate, $Y_{p/s}$ (g g^{-1})

$$Y_{p/s} = -\frac{\Delta P}{\Delta S}$$

where ΔP is the change of ethanol concentration during fermentation which is the final concentration.

APPENDIX B

APPENDIX B.1

The graph correlation between optical density (620nm) and time was demonstrated in Figure B.1 using the gravimetry method to obtain the biomass and Table B.1 is the data of optical density for 24 hour of fermentation.

Table B.1 Optical density (620nm) at 24hour of fermentation

Time,t (h)	Optical Density (620nm)
2	0.041
4	0.071
6	0.100
8	0.139
10	0.166
12	0.210
14	0.219
16	0.259
18	0.289
20	0.341
22	0.359
24	0.383

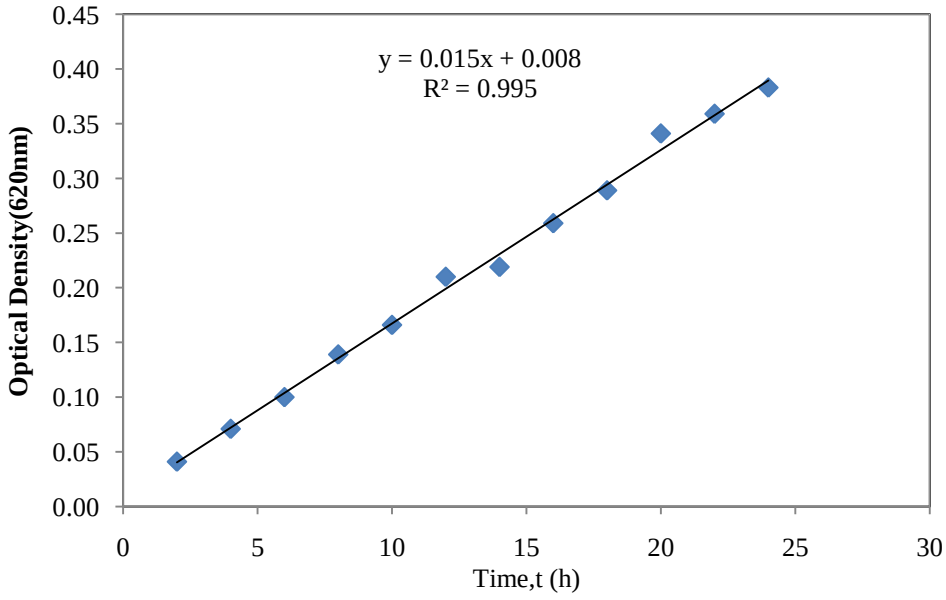

Figure B.1 Graph correlation between Optical density at 620nm and time

APPENDIX B.2

Figure B.2 illustrated the standard calibration curve of biomass that was analysed using UV-Vis U-1800 spectrophotometer (Hitachi) at wavelength of 620 nm and Table B.2 for the data of biomass for 24 hpur fermentation.

Table B.2 Biomass (g) for 24hour of fermentation

Time,t (h)	Biomass (g)
2	0.0010
4	0.0013
6	0.0021
8	0.0035
10	0.0039
12	0.0044
14	0.0057
16	0.0059
18	0.0062
20	0.0066
22	0.0067
24	0.0084

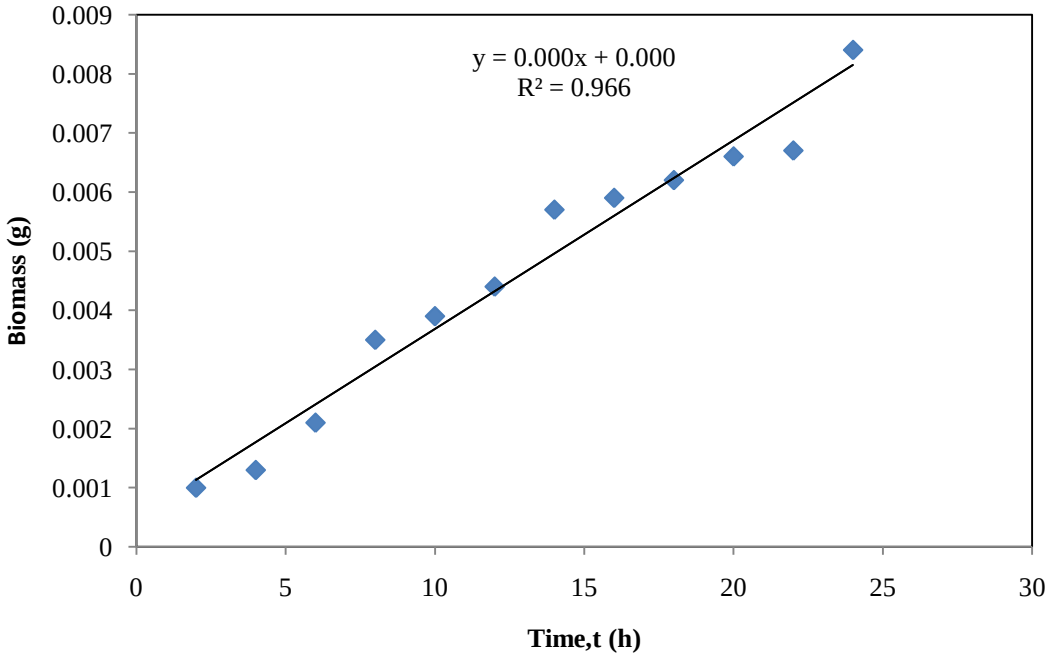

Figure B.2 Graph correlation between biomass and time

APPENDIX B.3

The standard calibration curve of biomass was demonstrated in Figure B.3 using correlation between optical density at 620 nm and Table B.3 was shown as the data for the standard calibration of biomass. From the biomass(g) convert to g/L by dividing with the volume of the sample.

Table B.3 Data correlation between biomass and optical density (620nm)

Biomass (g)	Optical Density (620nm)
0.0010	0.0410
0.0013	0.0710
0.0021	0.1000
0.0035	0.1390
0.0039	0.1660
0.0044	0.2100
0.0057	0.2190
0.0059	0.2590
0.0062	0.2890
0.0066	0.3410
0.0067	0.3590
0.0084	0.3830

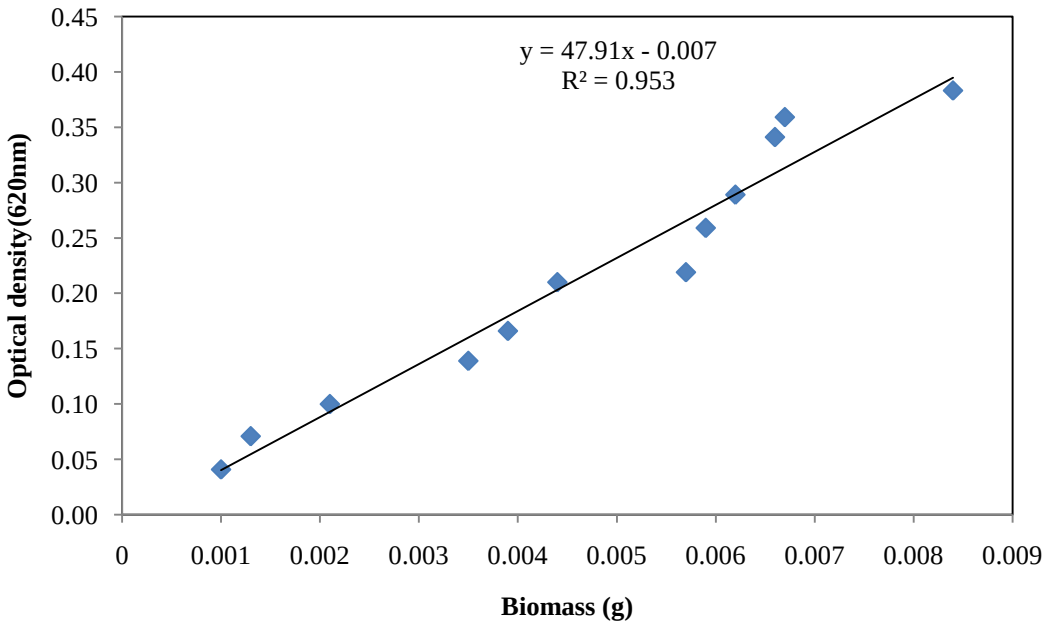

Figure B.3 Calibration curve of biomass and optical density

APPENDIX B.4

The standard calibration curve of glucose concentration was demonstrated in Figure B.4 using correlation between optical density at 575 nm and Table B.3 was shown as the data for the standard calibration of glucose concentration.

Table B.4 Data correlation concentration of glucose and optical density (575nm)

Concentration of glucose (g/ml)	Optical density (575nm)
0.00	0.000
0.02	0.134
0.04	0.392
0.06	0.631
0.08	0.913
0.10	1.245

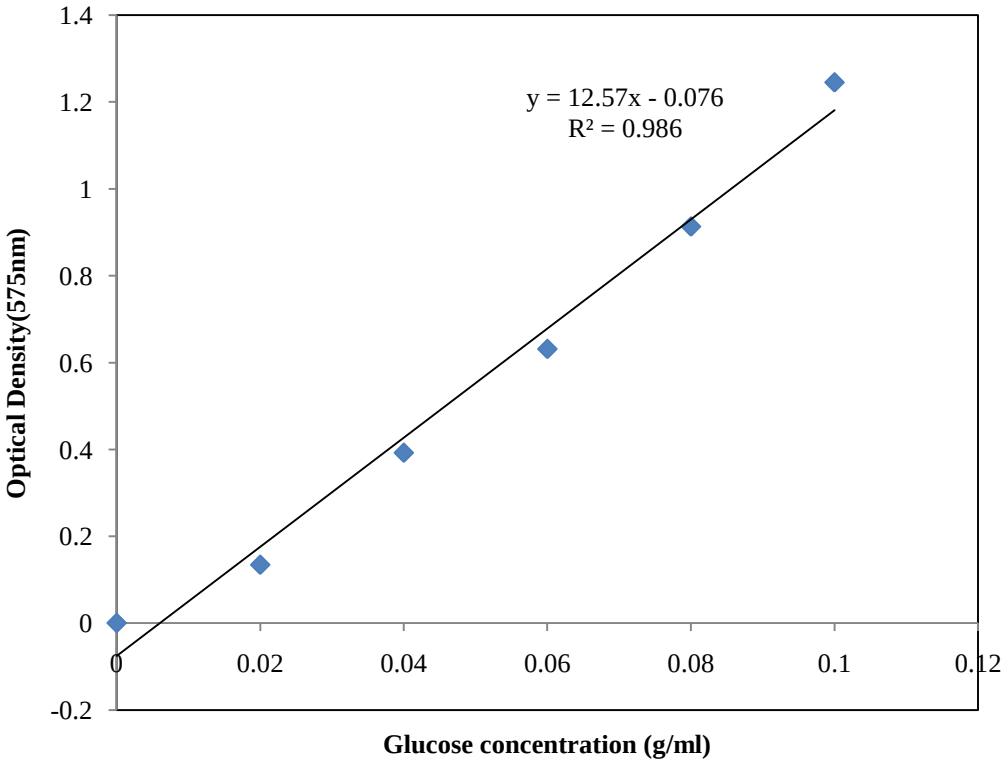

Figure B.4 Calibration curve for glucose concentration

APPENDIX B.5

The standard calibration curve of ethanol concentration was demonstrated in Figure B.5 using relative area of standard ethanol and Table B.5 was shown as the data for the standard calibration of ethanol concentration.

Table B.5 Data correlation between concentration of ethanol and area

Concentration of ethanol (g/L)	Area (A*s)
2	2057.27490
4	2226.70020
6	2555.30835
8	2672.04686
10	2783.44995

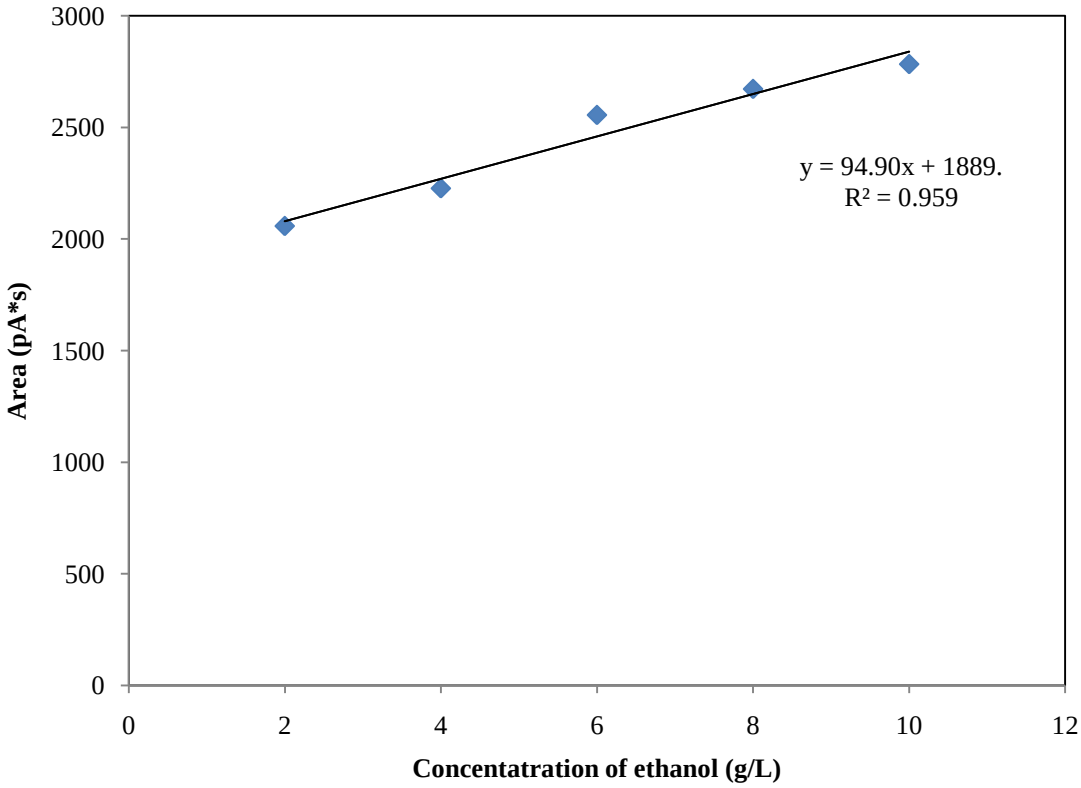

Figure B.5 Calibration curve for standard concentration of ethanol.

APPENDIX C

Table C.1 and C.2 demonstrated the data of biomass concentration from determination of optical density and compared to the standard calibration curve of biomass at different sonication regiments.

Table C.1 Result of optical density (620nm) between control and different sonication regiments

Time, t (h)	Control (no sonication)	Sonication regiments 10%	30%
2	0.040	0.055	0.027
4	0.053	0.075	0.067
6	0.078	0.094	0.076
8	0.148	0.175	0.111
10	0.162	0.195	0.149
12	0.212	0.248	0.201
14	0.255	0.279	0.236
16	0.275	0.298	0.266
18	0.339	0.367	0.305
20	0.379	0.457	0.309
22	0.413	0.474	0.335
24	0.448	0.517	0.344

Table C.2 Result of biomass concentration between control and different sonication regiments

Time, t (h)	Control (no sonication)	Sonication regiments 10%	30%
2	0.049	0.065	0.035
4	0.063	0.086	0.077
6	0.089	0.105	0.087
8	0.162	0.190	0.123
10	0.176	0.211	0.163
12	0.229	0.266	0.217
14	0.273	0.298	0.254
16	0.294	0.318	0.285
18	0.361	0.390	0.326
20	0.403	0.484	0.330
22	0.438	0.502	0.357
24	0.479	0.547	0.366

Table C.3 and C.4 demonstrated the data of glucose concentration from determination of optical density and compared to the standard calibration curve of glucose concentration at different sonication regiments.

Table C.3 Result of optical density (575nm) of fermentation with different sonication regiments

Time, t (h)	Sonication regiments	
	10%	30%
2	0.065	0.035
4	0.086	0.077
6	0.105	0.087
8	0.190	0.123
10	0.211	0.163
12	0.266	0.217
14	0.298	0.254
16	0.318	0.285
18	0.390	0.326
20	0.484	0.330
22	0.502	0.357
24	0.547	0.366

Table C.4 Result of glucose concentration of fermentation with different sonication regiments

Time, t (h)	Control (no sonication)	Sonication regiments	
		10%	30%
2	0.247	0.219	0.301
4	0.240	0.206	0.299
6	0.235	0.205	0.282
8	0.234	0.195	0.278
10	0.233	0.174	0.275
12	0.230	0.173	0.274
14	0.228	0.170	0.266
16	0.227	0.165	0.259
18	0.224	0.128	0.255
20	0.220	0.127	0.234
22	0.212	0.109	0.223
24	0.188	0.077	0.194

Table C.5 and C.6 demonstrated the data of ethanol concentration from determination of relative area and compared to the standard calibration curve of ethanol concentration at different sonication regiments.

Table C.5 Result of relative area of ethanol concentration between control and different sonication regiments

Time, t (h)	Control (no sonication)	Sonication regiments 10%	30%
2	2347.710	2340.047	2271.331
4	2446.567	2563.114	2311.927
6	2561.667	2596.141	2366.495
8	2576.834	2642.646	2429.378
10	2651.710	2653.279	2442.350
12	2671.041	2740.032	2471.686
14	2682.640	2757.489	2491.561
16	2702.567	2785.670	2491.678
18	2762.594	2880.549	2542.441
20	2830.113	2911.923	2699.369
22	2931.861	2989.243	2897.471
24	3065.082	3088.383	3007.658

Table C.6 Result of ethanol concentration between control and different sonication regiments

Time, t (h)	Control (no sonication)	Sonication regiments 10%	30%
2	4.835	4.754	4.030
4	5.877	7.105	4.457
6	7.090	7.453	5.033
8	7.250	7.943	5.695
10	8.039	8.055	5.832
12	8.242	8.970	6.141
14	8.365	9.153	6.351
16	8.575	9.451	6.352
18	9.207	10.451	6.887
20	9.919	10.781	8.541
22	10.991	11.596	10.629
24	12.395	12.641	11.790

APPENDIX D

Figure D.1, D.2, D.3, D.4, D.5 was shown as equipment for the fermentation and analysis of the sample.

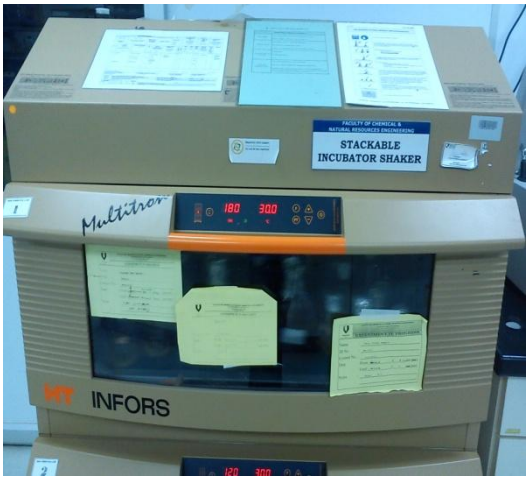

Figure D.1 Incubater shaker Inforis

Figure D.2 UV-Vis U-1800 (Hitachi)

Figure D.3 Gas Chromatography

Figure D.5 Centrifuge 5810 R

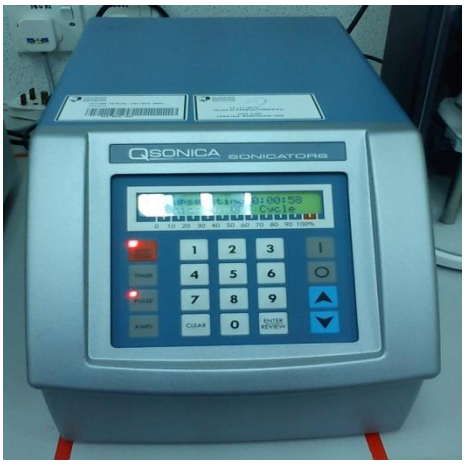

Figure D.4 Sonicators (Qsonica)