Faculty of Engineering

HEAT EVOLUTION OF ETHANOL FERMENTATION USING *SACCHAROMYCES CEREVISIAE CSI-1*

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Bachelor of Engineering with Honours
(Chemical Engineering)
2018

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Final Year Project Report	☐
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This declaration is made on the 12th of June 2018.

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This final year projects report which entitled “Heat Evolution of Ethanol Fermentation Using *Saccharomyces Cerevisiae CSI-1*” was prepared by Stanley anak Anhtony Nyoel (46049) as a partial KNC 4344 Final Year Project 2 course fulfilment for the Bachelor of Engineering with Honours (Chemical Engineering) is hereby read and approved by:

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(Supervisor)

12th June 2018
Date

HEAT EVOLUTION OF ETHANOL FERMENTATION USING *SACCHAROMYCES CEREVISIAE CSI-1*

STANLEY ANAK ANTHONY NYOEL (46049)

This project is submitted in partial fulfilment

of the requirement for the Degree of

Bachelor of Engineering with Honours

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Dedicated to my beloved parents and siblings who always been supporting me with unconditional love, motivations and encouragements. All glory to God in the highest!

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ABSTRACT

The exothermic reaction during the ethanol fermentation has been a major problem in the industry as high cost was required for heat control especially in tropical countries. Hence, the ethanol fermentation was studied through the heat evolution using *Saccharomyces cerevisiae CSI*-1 yeast and also the relationship between small scale and pilot scale fermentations. The small-scale fermentation was started at room temperature in a 3-L bioreactor and using 200 g/L glucose with 5g/L yeast extract as fermentation medium. Temperature, Optical density and pH were monitored and recorded during the fermentation. The fermentation gave a yield of 0.511 g glucose/g ethanol with final ethanol concentration of 102.26 g/L after 26 hours. The same parameters were observed for the pilot scale fermentation in a 100 L open Stirred Tank Reactor (STR) where the yield is 0.508 g glucose/g ethanol and final ethanol concentration of 101.655 g/L after 120 hours. This showed that the small-scale and pilot scale has the same amount of product produced and that they differed in terms of fermentation time. Lastly, simulation on the fermentation using SuperPro software was conducted based on the data obtained from the pilot scale fermentation where the simulated fermentation time was reduced to 78 hours.

Keywords: Exothermic reactions, heat evolution, *Saccharomyces cerevisiae CSI*-1, ethanol fermentation, pilot scale, simulation

ABSTRAK

Reaksi eksotermik semasa penapaian etanol menjadi masalah utama dalam industri ini kerana kos yang tinggi diperlukan untuk kawalan haba terutama di negara-negara tropika. Oleh itu, penapaian etanol dikaji melalui evolusi haba menggunakan yis *Saccharomyces cerevisiae CSI-1* dan juga hubungan antara penapaian skala kecil dan skala besar. Penapaian skala kecil bermula pada suhu bilik dalam bioreaktor 3 L dengan menggunakan 200 g/L glukosa dengan 5 g/L ekstrak yis sebagai medium penapaian. Suhu, ketumpatan optik dan pH dipantau dan direkodkan semasa penapaian. Penapaian memberikan hasil 0.511 g glukosa/g etanol dengan kepekatan etanol akhir sebanyak 102.26 g/L selepas 26 jam. Parameter yang sama diperhatikan untuk penapaian skala besar dalam tangka reaktor berkapasiti 100 L di mana hasilnya ialah 0.508 g glukosa/g etanol dan kepekatan etanol akhir 101.655 g/L selepas 120 jam. Ini menunjukkan bahawa skala kecil dan skala besar mempunyai jumlah produk yang sama dan mereka berbeza dari segi masa penapaian. Akhir sekali, simulasi penapaian menggunakan perisian SuperPro dijalankan berdasarkan data yang diperoleh daripada penapaian skala perintis di mana masa penapaian simulasi dikurangkan kepada 78 jam.

Kata kunci: Tindak balas eksoterma, evolusi haba, *Saccharomyces cerevisiae CSI-1*, penapaian etanol, skala besar, simulasi

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
B.C.	Before Christ
CO_2	Carbon dioxide
EtOH	Ethanol
GRAS	Generally Recognized as Safe
Ks	Half-velocity constant
NADH	Nicotinamide adenine dinucleotide
pH	Potential hydrogen
rpm	Revolution per minute
S. cerevisiae	*Saccharomyces cerevisiae*
STR	Stirred tank reactor

NOMENCLATURE

%	Percentage
˚C	Degree Celsius
µm	Micrometre
g	Gram
g g^{-1}	Gram per gram
g g^{-1} h^{-1}	Gram per gram per hour
g/L	Gram per litre
g/L·h	Gram per litre per hour
g/mL	Gram per milli-litre
g/mol	Gram per mole
h	Hour
J/g·K	Joule per gram per Kelvin
J/h	Joule per hour
J/mol·˚C	Joule per mole per Degree Celsius
kJ/h	Kilo-Joule per hour
kJ/mol	Kilo-Joule per mole
L	Litre
M	Molar
ml	Milli-litre
mmol L^{-1}	Milli-mole per litre
mPa·s	Millipascal-second
N	Normality
wt %	Weightage percentage
$\boldsymbol{\rho}$	Density

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Ethanol or commercially known as ethyl alcohol is a type of chemical substance that exists in the form of clear and colorless liquid. The chemical structure of ethanol is CH_3CH_2OH or C_2H_6O and it has molecular weight of 46.069 g/mol. There are mainly two types of ethanol namely bioethanol and synthetic ethanol and these two are differentiated through the way that they are produced. Bio-ethanol is a renewable ethanol that produced through the fermentation of sugars, starches and cellulosic biomass (Demirbaş, 2005). Meanwhile, synthetic ethanol is a petroleum-derived product produced from the hydrolysis of the ethylene (Ritslaid et al., 2014). Ethanol is typically used as main ingredients in alcoholic beverages and it is also used to fuel up vehicles.

In the last few decades, attention has been turned to the renewable energy sources as an alternative to the crude oil (Demirbas, 2009). One of the most studied alternative is the use of the biofuel which is liquid fuel produced from biomass. There are two most common biofuels namely biodiesel and bioethanol. Biodiesel is produced from the transesterification of vegetable oil with alcohol with the help of a catalyst while bioethanol is produced from fermentation and distillation of starch and sugars from sugar-rich plants such as corn and sugar cane plants (Dufey, 2006).

Molasses and by products of sugar industries from sugar cane and sugar beet are widely-used as substrates for bioethanol production while yeast are the most commonly used microorganism for the fermentation of bio-ethanol (El-Gendy et al., 2013). However, the yield of the bioethanol varied according to variables such as strains, growth factors and fermentation conditions (Fakruddin et al., 2012). The word ‘fermentation’ came from the Latin verb *fervere* (to boil) which indirectly described the appearance of the action of yeast on extracts of fruit or malted grain (Stanbury at al., 2013). Fermentation is one of the oldest methods known to mankind where evidence has shown that ancient Egyptians and Sumerians had learned the techniques in converting starchy grains to alcohol which is known today as fermentation (McNeil & Harvey, 2008). In today's term, fermentation has a wider scope which is basically the conversion of organic substance into simpler compounds by the enzymatic actions (Shurtleff & Aoyagi, 2017).

In this study, the kinetics of heat evolution for ethanol fermentation was studied in which the amount of heat evolved during the fermentation was obtained through enthalpy changes due to heat of formations from reactant consumed and products formed. The heat evolution obtained along with the concentration of substrate used were then used to determine the performance of fermentation using *Saccharomyces cerevisiae* CSI-1 under tropical climate temperature. The study was carried out in a lab-scale batch fermentation using *Saccharomyces cerevisiae* CSI-1 as the microorganism for the fermentation. The process was then scaled-up to a pilot scale fermenter using the parameters obtained from laboratory scale fermentation to study the relationship between the laboratory scale and scale-up fermentations in terms of the yield and productivity. In addition, a simulation was also conducted to simulate the ethanol fermentation for commercial scale also using the parameters obtained from the laboratory scale fermentation.

1.2 Problem Statement

Ethanol fermentation is a biological process in which ethanol and carbon dioxide are produced from the conversion of simple sugars using yeast under anaerobic condition (Khan Academy, 2017). The ethanol produced from this process were mainly used as biofuel, beverages raw material and pharmaceutical use. For the ethanol fermentation yo generate maximum yield of ethanol, the operating condition of the process must be at the most optimum level. One of the parameters that must be considered in achieving optimum operating condition is the temperature of the fermentation. As the fermentation itself is an exothermic reaction, the effect of heat evolution from the reaction will affect the productivity and yield of ethanol.

The exothermic reaction generated from fermentation will raise the temperature of the fermentation, and temperature will keep rising even after cell has reached its maximum growth. Increase in temperature beyond the maximum growth temperature causes slowing down of fermentation and finally stops when the medium becomes intolerable to the temperature. However, temperature keeps increasing after fermentation stops in addition to surrounding with high temperature such as in temperate countries making it hard to control the temperature of the fermenter and increases the operating cost of production (Rajoka at al., 2005).

In addition, there has not been any research conducted to study the heat kinetics and thermodynamics for ethanol fermentation using *S. cerevisiae* CSI-1 yeast. Most of the researches related to ethanol fermentation have been carried out in the laboratory scale and that there is very limited research done on the scaling up of the ethanol fermentation especially using the *S. cerevisiae* CSI-1 yeast. Thus, there is a need to conduct a study on relationship between the laboratory scale and pilot scale fermentations so that the parameters from the laboratory scale can be applied in the commercial scale fermentation.

1.3 Aim and Objectives of Study

In order to overcome the problem, the main aim of this study was to fully understand the kinetics of heat evolution during the fermentative process using *S. cerevisiae CSI-1*. There were also a few objectives required in order to achieve the aim of the study and they were as follows:

i. To determine the performance of batch ethanol fermentation using *S. cerevisiae CSI-1* both in small and pilot scales.
ii. To determine the relationship between small scale fermentation and pilot scale fermentation.
iii. To make a process simulation using SuperPro Designer v10 based on the parameter determined in a pilot scale fermentation.

1.4 Hypothesis

The *S. cerevisiae* strain can perform well under high temperature and tropical climate condition where the lower heat energy released during fermentation could reduce the cooling requirement in industrial fermentation. In addition, the production rate of ethanol for the laboratory scale is also similar to the pilot scale fermentation.

1.5 Scope of Study

This research was conducted to study the characteristics of the ethanol fermentation where the yeast used for this study was the Saccharomyces cerevisiae CSI-1 strain obtained from the Biochemistry Laboratory of Faculty of Resources and Science Technology (FRST) Unimas. The fermentation medium used in both of the small and pilot scale fermentation included glucose and also yeast extract that were prepared in 3 L bioreactor for small-scale and in 100 L Stirred Tank Reactor (STR) for pilot scale. Batch fermentation were used for both the small and pilot scale fermentations in which medium and broth were inserted prior to fermentation and were extracted at the end of fermentation. Both the small and pilot scale fermentation was started at room temperature and no temperature controls were applied throughout the fermentation. The performance of the fermentations was analysed based on the yield and final concentration of ethanol while the heat evolution was studied based on the enthalpy during the fermentation.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In this chapter, the detailed information needed to carry out the study was discussed to get an in-depth knowledge on the study.

2.2 Ethanol

2.2.1 General Properties of Ethanol

Ethanol which is also commonly known as ethyl alcohol or absolute alcohol, is a chemical compound that is made up of ethyl group and hydroxyl group which gave molecular weight of 46.07 g/mol and the molecular formula of CH_3CH_2OH as shown in **Figure 2.1** (Ritslaid et al., 2014). In normal room condition, ethanol is present in liquid state which is in the form of clear and colorless liquid with agreeable odor (Mariam et al., 2009). Ethanol has a sweet flavor when it is in dilute form but it will be turned into burning taste as it gets more concentrated. At a temperature of 20ºC, the ethanol has a density of 0.789 g/ml while the melting point and boiling point are –114.1 ºC and 78.5 ºC respectively. It is also soluble in water due to the presence of hydroxyl group in its chemical compound composition (Shakhashiri, 2017). The earliest form of ethanol is produced using fermentation of sugar which is one of the earliest organic reactions carried out by mankind. The ethanol produced back then are used as alcoholic beverage in which archeologists in China had discovered dried ethanol residue on a 9000 years old pottery (Roach, 2005).

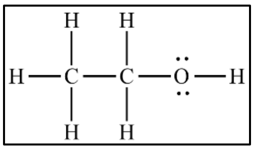

Figure 2.1 Molecular Structure of ethanol. (UCLA, 2017)

2.2.2 Production of Ethanol

There were a few methods that can be used in producing ethanol. The first method involved the production of ethanol derived from petroleum and called synthetic ethanol. In this method, ethylene obtained through oil cracking undergoes hydration with the presence of steam and phosphoric acid catalyst (Chemical Industry Education Centre, 2017). The second method involved the production of ethanol from sugar and starches through fermentation with the aid of yeast. The fermentation method is one of oldest method known by mankind in producing alcohol where it used varieties of crops as raw material such as sugar cane, corn and maize. Ethanol fermentation consisted of a series of chemical reactions that will convert carbohydrates into ethanol and carbon hydroxide (Schwietzke et al., 2009). The third method involved the production of ethanol using biomass waste with the help of modified bacteria. The best example for this method is where genetically engineered *E-coli* bacteria is used to convert plant sugars into ethanol instead of acids and this has allow the bagasse to be converted into ethanol (Chemical Industry Education Centre, 2017).

2.2.3 Applications and Market of Ethanol

Ethanol has a lot of applications apart from being used in the manufacturing of alcohol beverages. For the industrial applications, it is used in the medical and pharmaceutical fields as antiseptics, detergents and medicinal solvent (Brinker, 2017). The main reason is that ethanol possesses antimicrobial property that enabled it to kill bacteria, fungus and viruses (McDonnell & Russell, 1999). In addition, it is also used in the chemical processing industry as feedstock whereby it is used as a precursor for other organic compounds such as ethyl esters, ethyl halides and acetic acid (Beltrán et al., 2015). The largest use of ethanol meanwhile comes from the application as biofuel which substituted the usage of conventional petroleum fuel. This biofuel which is also known as bioethanol has been widely used to fuel up vehicles around the world especially America and Brazil due to the fact that it is biodegradable and environmental-friendly and also can be produced from common biomass sources that can be easily obtained (Ritslaid et al., 2014). The domination of application of ethanol as biofuel can be clearly seen in **Figure 2.2**. Ethanol production for biofuel has drastically increased for the past four decades which indicated the increased in awareness among human population on searching alternatives for fossil fuels.

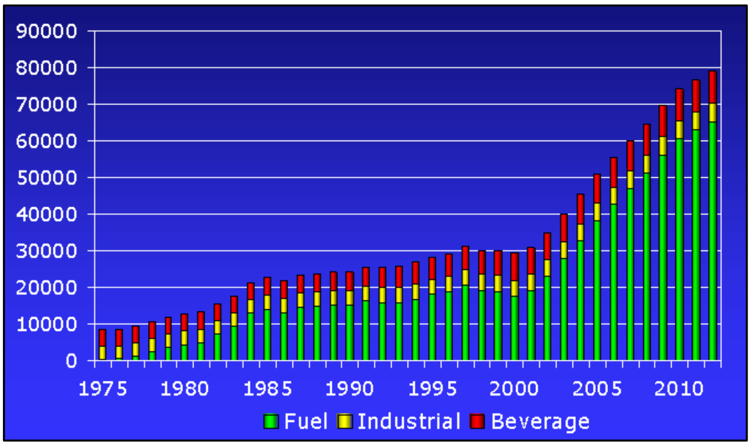

Figure 2.2 Graph of ethanol production by type in million litres (Berg, 2015)

In terms of the ethanol global market, the two major producer of ethanol are America and Brazil based on the data illustrated in **Table 2.1**. The reason is that both the countries have been widely using bioethanol as an alternative to fossil fuel. As can be seen in **Figure 2.3**, America has the largest production of ethanol in 2015 amounting to 14.7 billion gallons of ethanol. This makes America to dominate 57% of the global ethanol production followed by Brazil which contributes to 28% of the global production. For Brazil, the country has started using ethanol from sugarcanes since the 1970s to replace the petroleum derived gasoline (Torres et al., 2011). The reduction in petroleum reserves, the tendency of supply disruptions, price volatility and environmental issues has led the Brazilian government to promote the usage of bioethanol which till this day has already replaced 40% of the petroleum-based gasoline that would otherwise be consumed in Brazil (Belincanta et al., 2016).

Table 2.1 Global Ethanol Fuel Production for 2015

Country	Millions of Gallons
United States	14,807
Brazil	7,093
European Union	1,387
China	813
Canada	436
Thailand	334
Argentina	211
India	211
Rest of World	391

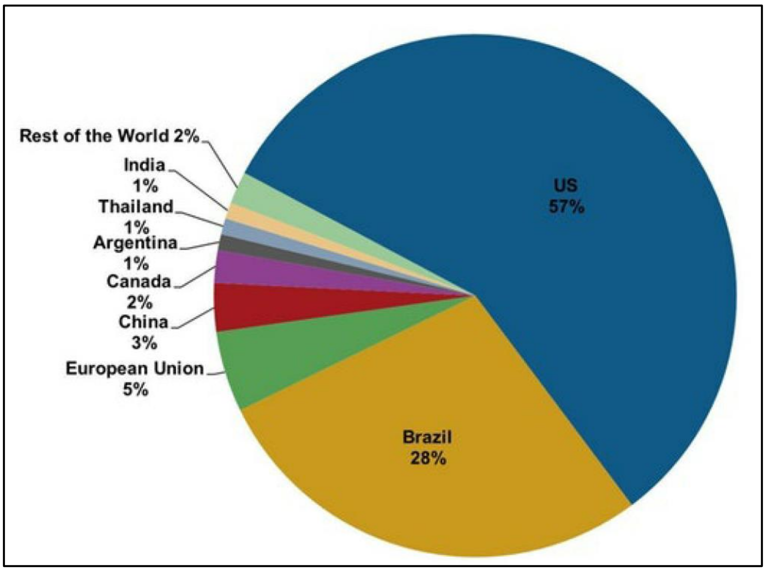

Figure 2.3 Global ethanol production in 2015 (Jones, 2016)

2.3 Ethanol Fermentation

2.3.1 Operating Mechanism

Fermentation has been one of the earliest form of scientific activities known and practiced by humankind since to the prehistoric times which was even before they understood the scientific principles (Drake & McKillip, 2000). Fermentation is a type of metabolic process in which partial degradation of sugars is carried out without the presence of oxygen (Tortora, 2015). Fermentation involved the reaction between NADH and organic electron acceptor in the form of pyruvates. These pyruvates are then metabolized into varieties of compounds through several processes (Stanbury et al., 2013). Among the most common processes that are widely applied in the industry and daily lives are lactic acid fermentation and ethanol fermentation. Lactic acid fermentation occurred when glucose was converted into pyruvate with the help of Lactic Acid Bacteria (LAB) through glycolysis followed by reaction with NADH which produced lactate before being purified into lactic acid (El-Enshasy et al., 2013).

Meanwhile, ethanol fermentation used the same pathway as lactic acid fermentation up till the pyruvate formation. For ethanol fermentation, pyruvate is then converted to acetaldehyde and carbon dioxide. The acetaldehyde is subsequently reduced to ethanol by the NADH from the previous glycolysis, which is returned to NAD+ as shown in **Figure 2.4** (KhanAcademy, 2017). According to Ritslaid at al. (2014), one molecule of glucose is able to produce 2 molecules of ethanol and 2 molecules of carbon dioxide. The typical ethanol fermentation under anaerobic condition is able to convert 90% (w/w) of glucose to ethanol (McGhee, Julian, Detroy, & Bothast, 1982). Thus, the chemical equation for the ethanol fermentation is as follows:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5O \qquad \textbf{eq. 2.1}$$

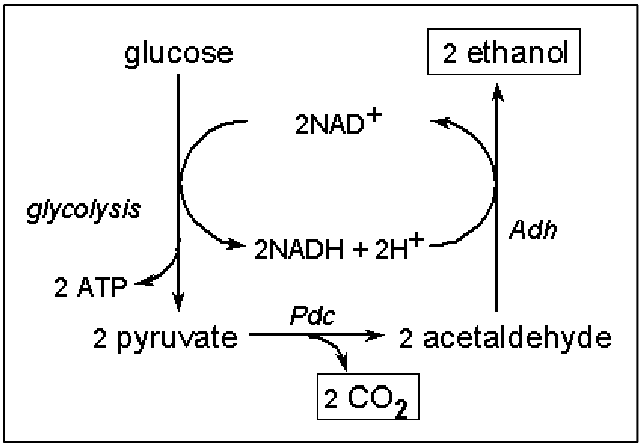

Figure 2.4 Ethanol fermentation mechanism (iGEM, 2017)

Ethanol can be derived from either sugars, starchy materials or lignocellulosic materials. Among the common feedstock for ethanol fermentation were corn, sugar cane, molasses and crop residues (US Department of Energy, 2017). For an anaerobic ethanol fermentation, yeast played a vital role in catabolizing the glucose to produce the ATP and ethanol. Ritslaid at al. (2014) mentioned that among the advantages of ethanol fermentation using yeast were that it has a high selectivity, low by-products accumulation, high ethanol yield and good tolerance towards both increased ethanol and substrate concentrations. There are a few species of yeasts that were commonly used in the fermentation due to their high productivity rate. These included *Saccharomyces cerevisiae, Candida utilis* and *S. uvarum* (Ritslaid et al., 2014).

Meanwhile, there are varieties of substrates that can be used in the ethanol fermentation and that mostly were from nonfood source and cheap. Among the sources for these substrates include wastes namely molasses, sugar beet pulp, cassava starch production wastes, food waste leachate and others. The benefits gained by using these wastes were that this application is able to eliminate or reduce the need for waste disposal in addition to being able to greatly reduce the cost of the ethanol production since these wastes were cheap to be obtained (Tesfaw & Assefa, 2014). Reports from past studies also discovered that nonfood extracts such as sweet sorghum and cashew apple are suitable to be applied as substrates for production of ethanol under high gravity