Kinetic Study of Acetic Acid Production from Banana Stem Waste Using Soil Bacteria; *Bacillus Thuringiensis*

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ABSTRACT

Nowadays, the demand of acetic acid in the world was about 7.8 million tons in 2005. The largest consumer of acetic acid was vinyl acetate with 42.4 % of consumption. Recently, most of the acetic acid in chemical industries was produced synthetically. The process required high chemical consumption. So, the anaerobic fermentation process was another alternative method to produce acetic acid. The anaerobic fermentation required raw material as substrates and microbes. In Malaysia, there are millions abandon lignocellulosic waste that can be use as substrates. Banana stem waste (BSW) is type of lignocelluloses waste contains cellulose, hemicelluloses and lignin. These renewable sources can be used in anerobic fermentation to produce acetic acid. *Bacillus Thuringiensis* is one of the soil bacteria that had ability to digest the banana stem waste. This research is about to study the kinetic parameter of acetic acid production from banana stem waste using soil bacteria; *Bacillus Thuringiensis*. This experiment was conducted in two different medium. First medium was banana stem medium. Second medium was glucose medium which later was used as references to compare with banana stem waste medium. First step was revival of the pure culture of *Bacillus Thuringiensis*. Second step was inoculums preparation. Third step was fermentation process. The anaerobic fermentation process was conducted in 72 hours at temperature of 30 ^{o}C. The biomass was separated using microcentrifuge. Glucose utilization concentration is measure using glucose analyzer. And acaetic acid produced concentrations were determined using High Performance Light Chromatography. After the data has been collected, the kinetic parameter will be determined using Kinetic Modeling Equation. The Runge-Kutte Fourth Order method is used to solve the model. The calculation is done by using Microsoft Excel Solver Software to determine the kinetic parameter in the kinetic modeling equation. The kinetic parameter obtained for both banana stem waste medium and glucose medium are slightly different. There are actually 7 kinetics

parameters from banana stem medium and glucose medium has been solved. Firstly, for BSW medium, k_1 is 0.5004 day^{-1}, k_2 is 0.0576 g L^{-1} day^{-1}, k_3 is 0.6197 g L^{-1}, k_4 is 0.1295 g L^{-1} day^{-1}, k_5 is 0.92897 g L^{-1}, k_6 is 0.0546 g L^{-1} day^{-1} and k_7 is 0.0001 g L^{-1}. For glucose medium, k_1 is 0.4667 day^{-1}, k_2 is 0.0671 g L^{-1} day^{-1}, k_3 is 0.023 g L^{-1}, k_4 is 0.2382 g L^{-1} day^{-1}, k_5 is 0.538 g L^{-1}, k_6 is 0.114 g L^{-1} day^{-1} and k_7 is 0.001 g L^{-1}.The percentage relative error of BSW medium was 0.44 and 0.77 in glucose medium. The yield of acetic acid produced from BSW medium was 2.98 g acetic acid/ g BSW and yield of acetic acid produce from glucose medium was 10.2 g Acetic Acid/ g glucose. The kinetic parameter is studied to predict the performance of the system such as stability of the system and the effluent of the system. So, the kinetic parameter will be used in scaling up the feed, biomass and production of the system.

ABSTRAK

Pada tahun 2005, permintaan dunia terhadap asid asetic adalah dalam lingkungan 7.8 juta tan. Penghasilan vinil asetat mencatat penggunaan asid asetik tertinggi dengan 42.4 %. Pada masa kini, kebanyakan asid asetik dalam industry kimia telah dihasilkan secara sintetik. Proses tersebut memerlukan kadar penggunaan bahan kimia yg tinggi. Jadi, proses penapaian anaerobik telah diperkenalkan dan ia merupakan salah satu kaedah alternatif untuk menghasilkan asid asetik. Proses ini memerlukan bahan mentah sebagai substrat dan kewujudan mikrob. Di Malaysia, terdapat berjuta-juta bahan buangan lignoselulosik yg tidak diproses dan terbiar yang boleh digunakan sebagai substrat. Batang pisang terbuang (BST) adalah salah satu daripada bahan buang liknosellulosik yang mengandungi lignin, sellulosa dan hemisellulosa. Sumber yang boleh diperbaharui ini boleh digunakan sebagai substrat dalam proses penapaian anaerobik untuk menghasilkan asid asetik. *Bacillus Thuringiensis* merupakan bakteria daripada tanah yang mempunyai keupayaan untuk mencerna batang pisang terbuang. Kajian ini adalah untuk mengkaji parameter kinetik dalam penghasilan asid asetik daripada batang pisang terbuang dengan menggunakan proses penapaian anaerobik oleh bakteria dari tanah iaitu *Bacillus Thuringiensis*. Eksperimen ini telah dijalankan dalam dua jenis media. Media pertama adalah media yang mengandungi batang pisang terbuang. Manakala media kedua adalah media yang mengandungi glukosa tiruan sahaja adalah digunakan sebagai media rujukan. Langkah pertama eksperimen adalah membangkitkan kultur tulen bacteria tanah iaitu *Bacillus Thuringiensis*. Lnagkah kedua adalah penyediaan inokulum. Langkah ketiga adalah proses penapaian. Proses penapaian anaerobic telah dijalankan selama 72 jam pada suhu 30 ^{o}C. Biojisim diasingkan menggunakan *microcentrifuge*. Manakala kepekatan penggunaan glukosa di ukur menggunakan *glucose analyzer*. Seterusnya, asid asetik yang terhasil diukur kepekatannya menggunakan *Performance Light Chromatography*. Setelah

kesemua data diperolehi, maka parameter kinetic dikenalpasti menggunakan persamaan model kinetic. Kaedah *Runge-Kutte Fourth Order* digunakan dalam menyelesaikan persamaan kinetic tersebut. Pengiraan parameter kinetik daripada persaaman model kinetic tersebut telah diselesaikan dengan menggunakan *Microsoft Excel Solver Software* Parameter kinetic yang diperolehi daripada media batang pisang terbuang dan media mengandungi glucose tiruan adalah berbeza sedikit. Terdapat 7 parameter kinetic daripada media batang pisang terbuang yang diperolehi. Pertamanya, untuk media batang pisang terbuang, k_1 adalah 0.5004 $hari^{-1}$, k_2 adalah 0.0576 g L^{-1} $hari^{-1}$, k_3 adalah 0.6197 g L^{-1}, k_4 adalah 0.1295 g L^{-1} $hari^{-1}$, k_5 adalah 0.92897 g L^{-1}, k_6 adalah 0.0546 g L^{-1} $hari^{-1}$ dan k_7 adalah 0.0001 g L^{-1}. Bagi media glukosa tiruan, k_1 adalah 0.4667 $hari^{-1}$, k_2 adalah 0.0671 g L^{-1} $hari^{-1}$, k_3 adalah 0.023 g L^{-1}, k_4 adalah 0.2382 g L^{-1} $hari^{-1}$, k_5 adalah 0.538 g L^{-1}, k_6 adalah 0.114 g L^{-1} $hari^{-1}$ dan k_7 adalah 0.001 g L^{-1}. Peratusan ralat relative untuk media batang pisang terbuang adalah 0.44 manakala untuk media glukosa tiruan adalah 0.77. Nisbah penghasilan asid asetik dripada media batang pisang terbuang 2.98 g acetic acid/ g BSW manakala bagi media glukosa tiruan adalah 10.2 g Acetic Acid/ g glucose.

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LIST OF ABREVIATION

k_1	**kinetic constant for non soluble organic matter degradation (1/day)**
k_2	**rate of soluble organic matter concentration (g/ l day)**
k_3	**maximum: saturation constant (g/ l)**
k_4	**maximum rate of soluble organic matter degradation (g/l day)**
k_5	**saturation constant (g/ l)**
k_6	**maximum rate of acetic acid consumption (g/ l day)**
k_7	**saturation constant (g/ l)**
BSW	**banana stem waste**
g/L	**concentration**
R^2	**coefficient of determination**
RK	**Runge-Kutta**

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Acetic Acid or commonly known as ethanoic acid is food product and commonly used in food industry, pharmaceutical industry and beverages industry. In food and beverages industry, it is use as food preservatives. In biogas production such as methane, acetic acid has been reported as precursor (Mountfort & Asher, 1978). Acetic acid is an important chemical reagent and industrial chemical, used in the production of soft drink bottles, photographic film as well as synthetic fibers and fabrics. In households, diluted acetic acid is often used in descaling agents. In the food industry, acetic acid is used as an acidity regulator and as a condiment. The global demand of acetic acid is around 6.5 Mt/a of which approximately 1.5 Mt/a is met by recycling; the remainder is manufactured from petrochemical feedstock or from biological sources.

There are many renewable sources that can be use to produce acetic acid such as lignocellulosic waste. Peninsular Malaysia approximately 4.2 million tons of crop residues were generated in 2006. These wastes contain very high concentrations of organic material, suspended solids, nitrogen and phosphorus.

From 1986 to 1990, agricultural waste contributed 13 percent of them total BOD pollution load. The preferable agricultural lignocellulosic waste is banana stem waste. Banana stem waste contains high holocellulose content but low lignin content. The monomeric content of holocellulose of banana stem waste consist of 71. 76% glucose, 11.20% xylose, 7.38% arabinose, 2.02% galactose, 0.58% mannose and followed by 7.09% of galacturonic acid (Li *et al.*, 2010).

In order to support green technology and preserve the land from polluted, anaerobic fermentation of BSW is used to produce acetic acid. The anaerobic bacteria; *Bacillus Thuringiensis* conducted fermentation to produced acetic acid from carbon sources contain in BSW. Even though, more time is required for the fermentation and the product is slightly less, it is proved that the process is environment friendly, lower cost and the product is much stable and high purity. It also includes the use of low-value substrates without the use of a sterile environment or the need for enzyme addition.

The kinetics parameters of this anaerobic fermentation of BSW were studied. The kinetic modeling based on Runge-Kutta Fourth Order was used for determination of kinetic parameters. Microsoft Excel Solver was used as software to calculate the kinetic parameters. It can contribute to basic process analysis, optimal design and operation, and maximum substrate utilization rate in anaerobic fermentation.

1.2 Problem Statement

Nowadays, the demand of acetic acid in the world was about 7.8 million tons in 2005. The largest consumer of acetic acid was vinyl acetate with 42.4 % of consumption. Recently, most of the acetic acid in chemical industries was produced synthetically. This process needed high temperature, pressure threat of explosion and high dependence of catalyst. Hence, biological process of fermentation was introduced as alternative method of producing acetic acid. This process required the usage of renewable sources such as lignocellulosic waste. In 2006, the annual global production of lignocellulosic waste from crops was about 4 billion tons of 60 % came from agricultural waste and another 40 % came from the forest. (Smith *et al.*, 2008). It is believed that the application of cellulose from banana stem waste (BSW) can give benefits as it is renewable and low cost. The cost of carbohydrate raw material influences the economy of many fermentation processes, hence the cost play a decisive role in future and scope of industries employing fermentation processes. Therefore, as an alternative way to produce acetic acid is by using soil bacteria in fermentation process. This procedure is much cheaper and can convert the useful product such as acetic acid. Hence, this research is about to identify the kinetic of anaerobic fermentation of BSW by soil bacteria.

1.3 Research Objective

The main purpose of this research is:

i. To study the production of acetic acid by anaerobic fermentation of banana stem waste (BSW) using soil bacteria.
ii. To study the kinetic of acetic acid production from banana stem waste (BSW) using soil bacteria.

1.4 Scope of Research

The scopes of this research

i. To study the production of acetic acid from BSW.
ii. To use anaerobic fermentation process in producing acetic acid using soil bacteria.
iii. To study the kinetic parameter of acetic acid production from BSW by using soil bacteria.
iv. To apply the kinetic modeling to determine the kinetic parameter by using Excel Solver Software.

1.5 Significant of Study

The acetic acid production from banana stem waste (BSW) brings several justifications. Firstly, the anaerobic fermentation by soil bacteria was used to convert the cellulose to glucose and directly into acetic acid in a single step. Secondly, the renewable organic compound was used to produce acetic acid. Since the banana stem waste (BSW) that was used as the raw material was cheap and widely found in Malaysia. Thirdly, the kinetic parameter was obtained from Kinetic Modeling suit for the banana stem waste. The application of Excel Solver Software in calculation was not complicated and a compatible software. Hence, this kinetic parameter can help to predict the performance of the system such as stability of the system, the effluent of the system.

CHAPTER 2

LITERATURE REVIEW

2.1 Acetic Acid

Acetic acid is one of the chemical compounds in carboxylic acid group that purposed pungent odor and sour taste. Sometimes it is called as ethanoic acid. It is the main component in vinegar besides water. Acetic acid is a colorless liquid with a pungent, vinegar-like odor. It is soluble in water, alcohols, ethyl ether, and other organic solvents. Acetic acid is very corrosive and may react quickly, under certain conditions, resulting in rapid evolution of heat. Acetic acid is stable under recommended storage conditions. Acetic acid will burn when heated or exposed to an ignition source. Vinegar is formed from dilute solutions of alcohol, by the action of certain bacteria in the presence of oxygen. In order words, it is known as fermentation. Vinegar that is the aqueous solution of acetic acid is a household product. The primary use of this acetic acid is in the manufacture of assorted acetate esters. Moreover, acetic acid is also used as a fungicide and as a solvent for many organic compounds. Acetic acid is also used in the pharmaceuticals industry.

Aspirin or scientifically, known acetylsalicylic acid is formed by the reaction between acetic acid and salicylic acid. Acetic acid is mainly used in the manufacture of Purified Terepthalic Acid (TPA), Vinyl Acetate Monomer, Acetic Anydride, Esters, Monochloroacetic acid.

Earlier acetic acid was obtained from natural carbohydrates by biochemical oxidation of carbohydrate and destructive distillation of wood. Today acetic acid is one of the fastest growing commercial organic chemical amongst the important aliphatic intermediates. The fermentation process of acetic acid was introduced by using bacteria. These bacteria require oxygen, and the overall chemical change is the reaction of ethanol with oxygen to form acetic acid and water (Shakhashiri, 2008). Here is the chemical reaction of alcohol to acetic acid:

$$CH_3CH_2OH + O_2 \longrightarrow CH_3\overset{\overset{O}{\|}}{C}OH + H_2O \qquad \textbf{(2.1)}$$

However, in order to reduce the course, the anaerobic fermentation is used with absent of oxygen. This fermentation process is much more cheaper and less energy consumption.

There are about 20 companies manufacturing acetic acid in India with a total installed capacity of approximately 150,000 TPA. The major concentration of units is in Western India followed by South and North India. In the Eastern region, there is only one plant with an installed capacity of 4,500 TPA. Malaysia produced large scale of agricultural waste every year. Actually, inside agricultural waste contained cellulose which can be degrade into sugar. Later on, the sugar can be degraded biologically to produce acetic acid, acetate and biogas. This method of acetic acid production is much cheaper and compatible.

2.2 Raw Material

Plants produce structural materials which can form cell walls, leaves, stems, stalks and woody parts of biomass. When the plant is died, the plant waste fibers can be described as lignocellulosic waste which consists of cellulose, hemicelluloses and lignin. Lignocellulosic included woods, agricultural wastes, grasses and other plant substances (Rowell *et al*., 2000).

Agricultural plant wastes such as pineapple, banana stem, leaf waste, sugarcane, groundnut shell, maize husk, rice husk and sorghum stalk are renewable because as a lignocellulosic, it can produce by- product such as acetic acid, methane, sugar and other industrial product (Reddy and Yang, 2005). According to A. Khalil *et al.* (2006), chemical composition, anatomy, lignin distribution and cell wall structure of Malaysian waste fibers were analyzed according to TAPPI Methods. Lignocellulose materials are resistant to physical, chemical and biological attack. But, it cellulose and hemicelluloses can be broken down into simple sugar through hydrolysis process.

2.2.1 Lignin

Actually, lignocellulosic plant is composed of many chemical components, primarily extractives, carbohydrates and lignin. Lignin has been described as a random, three-dimensional network polymer comprised of variously linked phenylpropane units (Sjöström, 1993).

Lignin is the second most abundant biological material on the planet, exceeded only by cellulose and hemicellulose, and comprises 15-25% of the dry weight of woody plants. This macromolecule plays a vital role in providing mechanical support to bind plant fibers together. Lignin plays an important function in a plant’s natural defense against degradation by impeding penetration of destructive enzymes through the cell wall (Sarkanen & Ludwig, 1971).

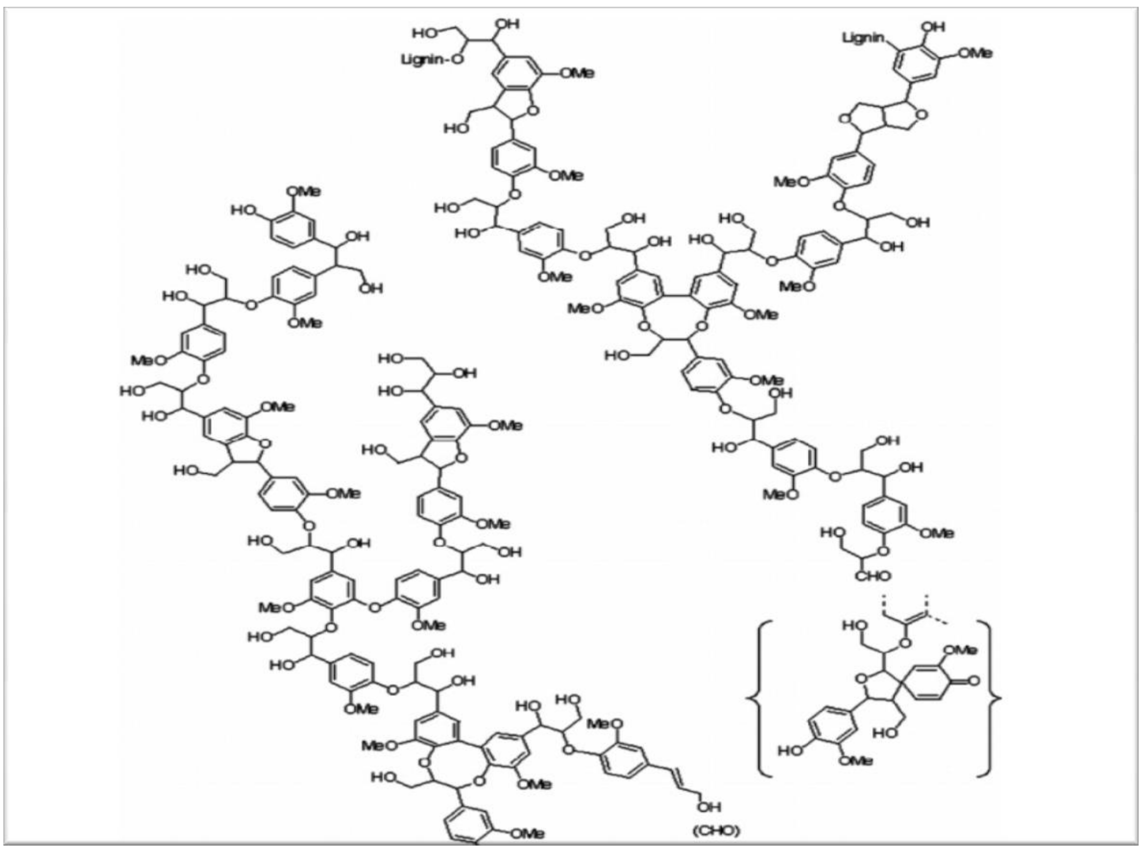

Figure 2.1: Structure of lignin