STARCH DEGRADATION AND BIOHYDROGEN GENERATION BY *Bacillus* sp. SSA

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This dissertation is dedicated to my beloved mother and father.

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ABSTRACT

Population increase has also increased the need for energy. The use of non-renewable fossil fuels as the main energy supply has decreased its resources and hydrogen become an attractive substitute for it. Besides, environmental pollution has always accompanied the use of fossil fuels. Hence, simultaneous treatment of wastes and energy generation is desirable. Starch-containing cassava wastewater has the potential to be used as the feedstock for biohydrogen production via dark fermentation process. In the present study, effective starch utilization and biohydrogen production by *Bacillus* sp. SSA was investigated. It was observed that *Bacillus* sp. SSA produced hydrogen as early as 12 hours after batch culture fermentation starts. In order to find the optimum conditions for biohydrogen production, batch experiments were conducted at various temperature (30–40ºC), initial medium pH (4.5–5.5) and initial substrate concentrations (0.5–1.5 g/L). The maximum biohydrogen production was obtained at temperature 30ºC, pH 5.5 and initial substrate concentration 1.0 g/L with a total value of 0.141 mL. The biohydrogen production rate, starch utilization, specific growth rate, maximum specific growth rate and yield of product on cell at this condition were the highest with a total of 0.008 mL/h, 23.9%, 0.013 h^{-1}, 0.026 h^{-1} and 2.104 mL/g, respectively. This study supports the potential of simultaneous cassava wastewater treatment and biohydrogen production using *Bacillus* sp. SSA strain as the biological agent.

ABSTRAK

Pertambahan penduduk telah meningkatkan keperluan untuk tenaga. Penggunaan bahan api fosil yang tidak boleh diperbaharui sebagai bekalan tenaga utama telah mengurangkan sumbernya dan hidrogen menjadi pengganti yang menarik untuk itu. Selain itu, pencemaran alam sekitar sentiasa disertai dengan penggunaan bahan api fosil. Oleh itu, rawatan sisa dan penjanaan tenaga secara serentak adalah wajar. Sisa air ubi kayu yang mengandungi kanji mempunyai potensi untuk digunakan sebagai bahan mentah bagi pengeluaran biohidrogen melalui proses penapaian gelap. Dalam kajian ini, penggunaan kanji yang berkesan dan pengeluaran biohidrogen oleh *Bacillus* sp. SSA telah dikaji. Dapat diperhatikan bahawa *Bacillus* sp. SSA menghasilkan hidrogen seawal 12 jam selepas penapaian kultur berkelompok bermula. Dalam usaha untuk mencari keadaan optimum untuk pengeluaran biohidrogen, eksperimen berkelompok telah dijalankan pada pelbagai suhu (30–40ºC), pH permulaan media (4.5–5.5) dan kepekatan permulaan substrat (0.5–1.5 g/L). Pengeluaran biohidrogen tertinggi diperolehi pada suhu 30ºC, pH 5.5 dan kepekatan permulaan substrat 1.0 g/L dengan jumlah sebanyak 0.141 mL. Kadar pengeluaran biohidrogen, penggunaan kanji, kadar petumbuhan spesifik, kadar petumbuhan spesifik maksimum dan hasil produk pada sel pada keadaan ini adalah yang tertinggi dengan jumlah 0.008 mL/h, 23.9%, 0.013 h^{-1}, 0.026 h^{-1} and 2.104 mL/g, mengikut turutan. Kajian ini menyokong potensi rawatan sisa air ubi kayu serentak dengan pengeluaran biohidrogen menggunakan keturunan *Bacillus* sp. SSA sebagai agen biologi.

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LIST OF ABBREVIATIONS

ATP	-	Adenosine triophosphate
BOD	-	Biochemical oxygen demand
$C_6H_{12}O_6$		Glucose
$CH_3CH_2CH_2COOH$		Butyrate
CH_3CH_2COOH		Propionate
CH_3CH_2OH		Ethanol
CH_3COOH		Acetate
CO_2	-	Carbon dioxide
COD	-	Chemical oxygen demand
CS	-	Cassava starch
H_2	-	Hydrogen
H_2O	-	Water
NAD^+/NADH	-	Nicotinamide adenine dinucleotide
N_2	-	Nitrogen
OD	-	Optical density
O_2	-	Oxygen
RPM	-	Round per minute
TVS	-	Total volatile solid

LIST OF SYMBOLS

^{o}C	-	Degree Celsius
µL	-	Microliter
cm	-	Centimeter
g	-	Gram
g/g	-	gram/gram
g/L	-	gram/Liter
kg	-	Kilogram
kJ/g	-	Kilojoules/gram
L	-	Liter
M	-	Molar
mL	-	milliliter
mL/g	-	milliliter/gram
mL/h	-	milliliter/hour
mL/min	-	milliliter/minute
nm	-	Nanometer
v/v	-	volume/volume
w/v	-	weight/volume

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Fossil fuels consist of coal, petroleum and natural gasses. They are the main resources for energy supply and widely used to provide electricity, run vehicles, making of medicines, plastics and many more. The higher requirement has results in the depletion number of fossil fuels in nature. It is estimated that oil, gas and coal supply will depletes completely around the year of 2040, 2042 and 2112, respectively. As fossil fuels are non-renewable energy, researches to find other reliable alternative energy resource have been done extensively (Shafiee and Topal, 2009).

Hydrogen is an attractive substitute for fossil fuels due to its clean, renewable, conversion efficient and high-energy yield. The presence of hydrogen is abundant in nature and its combustion yields water. There are many ways to produce hydrogen but the production from biological sources gain extra attention and more favored. This is because the hydrogen produced is more environmental friendly and requires less energy compare to thermochemical and electrochemical processes. Biohydrogen can be generated by either photosynthetic or fermentative microorganisms (Ni *et al*., 2006; Kotay and Das, 2008).

Production of biohydrogen by fermentation processes is more preferable than photosynthetic as such methods stimulate high amount of hydrogen and high percentage of bacterial growth at low energy. Dark fermentation processes emit mixed biogas that contains primarily hydrogen and carbon dioxide with small amount of methane, carbon monoxide and hydrogen sulphide. Carbohydrates such as glucose, cellulose or starch are the good and commonly used as substrate for hydrogen-producing fermentations. Different types of carbohydrate will yields different amount of hydrogen depending on the pathway of fermentation processes and the end products (Levin *et al*., 2004; Hasyim *et al*., 2011).

Starch-containing wastewater is one of the abundant wastes discharged by industrial activities such as textile and food factories. As the waste possesses high level of carbohydrate and organic contents, it can be a good feedstock for biohydrogen production. The use of waste to produce hydrogen will not only minimize the pollution but also provide a clean and readily usable energy in a sustainable way. Many fermentative microorganisms can be detected to colonize in this area. Thus, the study on the production of biohydrogen by starch-degrading fermentative bacteria is well known and has been done extensively (Sompong *et al*., 2011).

Fermentative microorganisms such as *Clostridium*, *Enterobacter*, *Lactobacillus* and *Streptococcus* are the common species used for the production of biohydrogen from starch-containing wastewater. The bacteria will take up the starch and hydrolysed it into glucose and maltose by acid or enzymatic means. Under anaerobic conditions, fermentative microorganisms will convert these organic wastes into organic acids and produce hydrogen as a by-product. Then, the organic acids will be used to generate methane. The ability of microorganisms to produce hydrogen can be affected by several factors such as working temperature, pH and initial starch concentration of the substrate (Kapdan and Kargi, 2006; Davila-Vazquez *et al*., 2009).

1.2 Statement of Problem

Nowadays, the global requirements for fossil fuels are increasing rapidly but the availability is restricted as they take millions of years to form. The present fossil fuels reserves are being consumed much faster than they are being made. In addition, the products emitted by the combustion of fossil fuels are dangerous which can affect the health of living organisms, causing global warming and the greenhouse effect. Thus, a major divergent of energy system from fossil fuels to hydrogen will be able to eliminate many problems and improve the quality of life (Bilgen, 2014).

There are many factories in Malaysia that make fried chips from cassava. This type of food manufacturing involves washing and cleaning processes of cassava from dirt and also after the peeling of skin by using running tap water. As a result, the wastewater released contains high level of starch and organic matters that can support the growth of fermentative microorganisms to produce biohydrogen. Uses of wastewater as substrate will also help to reduce water pollution as it contains high chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total solids (Du *et al*., 2007).

Identifying and isolating bacterial strains that are capable of utilising starch is the first step towards fermentation studies for biohydrogen production. Study by Goyal *et al*. has found a new strain, *Bacillus* sp. 1-3 that show a high performance on digesting raw potato starch (Goyal *et al*., 2005). However, less effort has been dedicated to study about the starch-degradation and biohydrogen generation by *Bacillus* sp. SSA on cassava starch-containing wastewater.

Besides that, the production of biohydrogen by fermentative bacteria is found to be affected by several environmental factors. Initial substrate concentration, pH and working temperature may influence the starch utilization, hydrogen production and distribution of microbial population. Understanding the effects of these factors can improve and enhance the biohydrogen production from starch-containing wastewater (Sinha and Pandey, 2011).

1.3 Objectives of Research

The objectives of this research are:

I. To measure the performance of *Bacillus* sp. SSA for biohydrogen production on cassava-containing wastewater in batch experiment.

II. To optimize the production of biohydrogen based on temperature, starch concentration and pH (OFAT).

III. To study the kinetic of biohydrogen production by *Bacillus* sp. SSA.

1.4 Scope of Study

In this study, the experiment was conducted to investigate the growth and ability of *Bacillus* sp. SSA to produce biohydrogen from cassava wastewater. Through fermentation process, the bacteria take up starch in the wastewater as a carbon source. At the same time, the effect of initial substrate concentration, working temperature and pH is also taken into consideration.

1.5 Significance of Research

Through this research, the optimum environmental conditions required for high biohydrogen yield from bacteria isolated in starch-rich wastewater can be achieved. Moreover, the research would also provide a potential solution in handling waste from food industries. Thus, with large amount of starch-containing wastewater produced, it can be treated in environmental friendly way and at the same time producing biohydrogen as the alternative solution to the depleted fossil fuels.

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