# EFFECT OF HEAT PRE-TREATMENT ON BIOHYDROGEN PRODUCTION BY STARCH-DEGRADING BACTERIA

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This dissertation is dedicated to my beloved mother Hajah Fatimah binti Haji Bachik.

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# ABSTRACT

The combustion of fossil fuels emits majority parts of greenhouse gases thus contributing to global warming. In addition, global economic development leads to the increasing demand for fossil fuels as a major source of energy which causes this non-renewable resource to become depleted. Therefore, hydrogen was discovered as an alternative source of energy with carbon-neutral and renewable energy because it only produces water vapour and heat upon combustion and can be generated from waste materials. The low hydrogen yield is major challenges in biohydrogen production by the mixed culture via dark fermentation approach. Hence, this study was conducted to study the effect of heat pre-treatments on biohydrogen production of pre-treated cassava sludge at 80$^{o}$C, 90$^{o}$C and 100$^{o}$C for 60 minutes. The sludge was pre-treated to select spore-forming hydrogen-producing bacteria and at the same time inhibit non-spore hydrogen-consuming bacteria. Batch tests were conducted to investigate and compare biohydrogen formation of untreated and pre-treated sludge at 80$^{o}$C, 90$^{o}$C and 100$^{o}$C over 96 hours utilising cassava starch wastewater as substrate with an initial pH 5.5 at 30$^{o}$C. Based on the experimental results, only hydrogen and carbon dioxide was detected throughout the experiment without the presence of methane. No biohydrogen was recorded for untreated sludge, 80$^{o}$C and 100$^{o}$C pre-treated sludge but biohydrogen was detected only by 90$^{o}$C pre-treated sludge with 0.0883 mL of cumulative hydrogen formation. The biohydrogen production had the maximum biohydrogen production, maximum biohydrogen production rate, starch utilisation, the yield of biohydrogen per substrate and yield of biohydrogen per cell were 0.0012 mol $H_2$, 0.0002 mol $H_2$/h, 13.9%, 0.0050 mol $H_2$/g and 0.0002 mol $H_2$/g respectively. In conclusion, heat pre-treatment by boiling at 90$^{o}$C was suitable for enriching biohydrogen-producing bacteria from cassava-processing sludge.

# ABSTRAK

Pembakaran bahan api fosil menghasilkan sebahagian besar gas rumah hijau yang menyumbang kepada pemanasan global. Disamping itu, pembangunan ekonomi global menjadikan permintaan bahan api fosil sebagai sumber tenaga utama semakin meningkat lalu menyebabkan sumber tenaga yang tidak boleh diperbaharui ini menjadi semakin berkurang. Oleh itu, gas hidrogen telah dikenalpasti sebagai sumber tenaga alternatif dengan tenaga karbon neutral dan boleh diperbaharui kerana pembakarannya hanya menghasilkan wap air dan haba serta boleh dihasilkan daripada bahan buangan. Penghasilan hidrogen yang rendah adalah cabaran utama dalam penghasilan biohidrogen oleh kultur campuran melalui pendekatan penapaian gelap. Justeru, kajian ini dijalankan adalah untuk mengkaji kesan pra-rawatan terhadap pengeluaran biohidrogen oleh kultur campuran dari sisa mendapan ubi kayu pada suhu 80$^{o}$C, 90$^{o}$C dan 100$^{o}$C selama 60 minit. Tujuan pra-rawatan haba terhadap sisa mendapan ubi kayu adalah untuk memilih spora bakteria yang menghasilkan hidrogen dan pada masa yang sama membantutkan aktiviti bukan spora bakteria yang menggunakan hidrogen sebagai sumber tenaga. Pendekatan fermentasi kelompok telah dijalankan untuk menyelidik dan membandingkan pembentukan biohidrogen daripada sisa mendapan tanpa pra-rawatan dan pra-rawatan pada 80$^{o}$C, 90$^{o}$C dan 100$^{o}$C selama 96 jam dengan menggunakan air sisa buangan kanji ubi kayu sebagai substrat bermula dengan pH 5.5 pada suhu 30$^{o}$C. Berdasarkan keputusan eksperimen, hanya gas hidrogen dan karbon dioksida yang telah dikesan sepanjang eksperimen tanpa kehadiran gas metana. Tiada biohidrogen telah direkodkan dari sisa mendapan tanpa pra-rawatan dan pra-rawatan pada suhu 80$^{o}$C dan 100$^{o}$C tetapi hanya dikesan oleh pra-rawatan sisa mendapan pada 90$^{o}$C dengan kumulatif pembentukan hidrogen sebanyak 0.0883 mL. Pengeluaran biohidrogen ini mempunyai pengeluaran biohidrogen maksima, kadar pengeluaran biohidrogen maksima, penggunaan kanji, hasil biohidrogen pada substrat dan hasil biohidrogen pada sel adalah 0.0012 mol $H_2$, 0.0002 mol $H_2$/j, 13.9%, 0.0050 mol $H_2$/g and 0.0002 mol $H_2$/g mengikut turutan. Kesimpulannya, kaedah pra-rawatan haba pada suhu 90$^{o}$C adalah sesuai untuk memperkayakan bakteria yang menghasilkan biohidrogen dari sisa mendapan ubi kayu.

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| $CaCl_2$ | - | calcium chloride |
| CO | - | carbon monoxide |
| CoA | - | coenzyme A |
| $CO_2$ | - | carbon dioxide |
| $CH_3CH_2CH_2COOH$ | - | butyric acid |
| $CH_3CH_2COOH$ | - | propionic acid |
| $CH_3CH_2OH$ | - | ethanol |
| $CH_3CHOHCOOH$ | - | lactic acid |
| $CH_3COOH$ | - | acetatic acid |
| $C_6H_{12}O_6$ | - | glucose |
| DNS | - | dinitrosalicylic acid |
| $FeCl_3.5H_2O$ | - | iron (III) chloride pentahydrate |
| Fd | - | ferredoxins |
| GC-TCD | - | gas chromatography with thermal detector |
| $H_2$ | - | hydrogen |
| $H_2O$ | - | water |
| $H^+$ | - | hydrogen ion |
| $KH_2PO_4$ | - | potassium dihydrogen phosphate |
| $K_2HPO_4$ | - | potassium phosphate dibasic anhydrous |
| $MgSO_4.7H_2O$ | - | magnesium sulfate heptahydrate |
| $NH_4NO_3$ | - | ammonium nitrate |
| $NO_X$ | - | nitrogen oxides |
| ox | - | oxidation |
| red | - | reduction |
| rpm | - | rotation per minute |

| | | |
|---|---|---|
| $SO_X$ | - | sulfur oxides |
| VSS | - | volatile suspended solid |

# LIST OF SYMBOLS

| | | |
|---|---|---|
| % | - | percentage |
| $^{o}C$ | - | degree Celsius |
| µl | - | microliter |
| g | - | gram |
| g/g | - | gram/gram |
| g/L | - | gram/ litre |
| $h^{-1}$ | - | per hour |
| K | - | Kelvin |
| kJ/g | - | kilojoules/ gram |
| M | - | Molar |
| m | - | metre |
| mg/L | - | milligram/litre |
| mmol | - | millimoles |
| mL | - | millilitre |
| mL/h | - | millilitre/hour |
| mL/min | - | millilitre/minute |
| mol | - | molar |
| nm | - | nanometre |
| U/mL | - | unit/millilitre |
| w/v | - | weight/volume |

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# CHAPTER 1

# INTRODUCTION

## 1.1 Research Background

Globally, rapid increase in industrialization sector, urbanisation and human population growth causes high demand of energy and its resources (Mohanty *et al*., 2015). High dependency on fossil fuels as energy source especially for transportation causes depletion of the resources of fossil fuels to occur (Azwar *et al*., 2014). The combustion of it emits major parts of greenhouse gases (GHGs) and causes depletion of ozone layer to occur, thus contribute to global warming (Rachman *et al*., 2015). This GHGs emission phenomenon also occur in Malaysia as population growth rate increases and industries are developed gradually. Aside from that, this phenomenon not only give a negative impact on our environment and economic sector, but also affect human health (Hosseini *et al*., 2013).

Being a developing country with a growing economy, expected energy consumption in Malaysia will increase steadily at 2.8% per year from 65.9 5 million tonnes of oil equivalent (Mtoe) in the year 2005 until reaching 130.5 Mtoe in 2030 (Mustapa *et al*., 2010). Hence, effort to find greener, renewable and sustainable energy source gain mainstream interest. Hydrogen is suggested as a suitable candidate which is believed can provide a solution to this problem due to its environmentally friendly characteristics.

Over the last decades, the study of biohydrogen production has gained its momentum. Hydrogen has been identified as a promising green energy option because of its clean energy source with the highest energy content compared to other fuel (Kapdan and Kargi, 2006; Parihar and Upadhyay, 2015). This is due to the fact that hydrogen does not produce $CO_2$, CO, $SO_X$ and $NO_X$ but only produces water vapour and heat upon combustion (Moreno-Dávila *et al.*, 2011; Rachman *et al.*, 2015).

Being the most abundant element in the universe, pure hydrogen gas need to produce artificially such as through a biomass gasification process which is a conventional physicochemical method (Singh and Wahid, 2015). However, biological methods are favoured than conventional methods because it does not require high energy intensive and less potential to cause environmental problem throughout the process (Singh and Wahid, 2015; Bharathiraja *et al.*, 2016). Biological methods for biohydrogen production involved direct and indirect biophotolysis, photo-fermentation, dark fermentation and two-stage fermentation (Boodhun *et al.*, 2017).

Dark fermentation approach can manipulate a wide range of wastes on biohydrogen production such as municipal solid wastes, industrial waste and agricultural waste. Mixed microflora offers more benefit than pure culture in terms of efficiency in conversion of substrates due to the presence of more than one substrate-degrading bacteria within the mixed culture (Rafieenia *et al.*, 2018). Low biohydrogen production is a common problem when using mixed culture as inoculum via dark fermentation approach (Rafieenia *et al.*, 2018). This problem arises due to the production of hydrogen as an intermediate product and eventually reduced by hydrogen-consuming bacteria that is also present within the mixed culture (Singh and Wahid, 2015). Pre-treatment methods of inoculum or substrate are necessary to adopt for suppressing hydrogen-consuming bacteria while preserving biohydrogen-consuming bacteria (Wang and Yin, 2017).

Hydrogen have been utilised for transportation, buildings, utilities and industrial sectors. This type of gas is widely used as fuel in transportation and utilities sectors while it acts as a reactant in industrial sectors such as chemical industry (Elam *et al*., 2003). Furthermore, hydrogen also have been utilised as an energy source of electricity for buildings (Elam *et al*., 2003). Since hydrogen is an environmental friendly energy source, it is become an interesting option to replace fossil fuel in internal combustion engines (ICE) for transportation and power generation such as electricity (Tanksale *et al*., 2010; Moreno-Dávila *et al*., 2011). Pilot-scale studies on hydrogen production contribute to generation of hydrogen at high rate system thus ensuring more fuel cell cars could be fuelled (Fernandes *et al*., 2010).

## 1.2 Problem Statement

Nowadays, environmental and economical problems have been raised and gaining our main concerns from the usage of fossil fuels. In the economic sector, shortage of fossil fuel supply causes growing of oil price. This issue happens when consumption rate is faster than its natural regeneration due to its role as primary sources of energy (Cherubini and Strømman, 2011). In addition, products of combustion of fossil fuels give negative impacts to environment and human health (Hosseini *et al*., 2013). In response, hydrogen is foreseen as an alternative source of energy to achieve zero carbon emission energy. Hydrogen is a clean energy because it is only produce water as by-product and has high energy density (Carrieri *et al*., 2006).

Cassava wastes such as wastewater and sludge contain high organic content and suspended solids which must be treated properly before it is released into local waterways (Zhang *et al.,* 2016). Consequently, human and animal health problems and also our environment will be affected due to contamination of the wastes. However, utilising of these high levels of starch and rich organic matter of waste for bioconversion into value-added products could get rid of this problem. In this study, the cassava processing sludge was utilised as inoculum and wastewater as substrate for biohydrogen production via dark fermentation thus reducing the cost of treatment and disposal issues can be solved. This carbohydrate-rich waste is preferred as substrate compared to other waste due to biodegradability while the presence of natural microbial communities within the sludge may contribute in an efficiency of starch degradation. Hence, this research could contribute to a sustainable development of cassava industries.

Hydrogen gas is produced as an intermediate product during dark fermentation when using mixed culture. This problem causes low biohydrogen production yield and becomes a major challenge on biohydrogen production through dark fermentation approach (Rafieenia *et al.*, 2018). Furthermore, the biohydrogen-producing bacteria, hydrogen-consuming bacteria such as propionate-producing bacteria, homoacetogens and lactic acid-producing bacteria are also present in natural microbial flora. These hydrogen-consuming bacteria may result in low biohydrogen production when hydrogen is consumed and converted to other product. Hence, employment of inoculum pre-treatment methods is a strategy that could provide a solution to these problems to enrich the mixed microbial population with spore-forming biohydrogen-producing bacteria while simultaneously eliminating hydrogen-consuming bacteria.

It is quite difficult to select a suitable pre-treatment of inoculum because of the variability of the natural microbial community present in a sample which consequently affected the bacterial community that survives after a pre-treatment was applied. This factor results in different biohydrogen production even same pre-treatment method, duration and condition are conducted. Therefore, this study was conducted to suggest an appropriate heat pre-treatments for future reference from cassava-processing sludge using cassava wastewater as substrate.

## 1.3 Objectives of Research

In this study, starch-processing sludge was used as the source of inoculum. The aims of this research are as follows:

I. To apply heat pre-treatment methods on starch processing sludge to enrich spore-forming biohydrogen-producing bacteria in the mixed culture
II. To investigate biohydrogen production and starch utilisation using pre-treated sludge
III. To perform kinetic analysis of biohydrogen production and starch utilisation using pre-treated sludge

## 1.4 Scope of the Study

This study was conducted to assess the effects of heat pre-treatment methods of starch processing sludge on biohydrogen production. The starch processing sludge and wastewater samples were collected from a factory of Layang Food Sdn. Bhd. in Layang-Layang, Johor. Firstly, the sludge sample was pre-treated at different temperatures that were 80$^{o}$C, 90$^{o}$C and 100$^{o}$C. Secondly, a batch of the fermentation process was conducted for each pre-treated and untreated sample to test the ability to produce biohydrogen gas. Then, biohydrogen production by the mixed culture was analysed via Gas Chromatography-Thermal Conductivity Detector (GC-TCD). Lastly, the kinetic analysis of biohydrogen production was performed for observation of growth, utilisation of starch and formation of product throughout the fermentation process.

## 1.5 Significance of the Research

Rapid growth in the industrial sector over the years give rise to accumulation of effluents as it cannot be disposed to the environment. Disposal of wastewater from industrial effluent mainly starch processing sludge must go through a series of treatment before it is released into local waterways. This process requires a long time and high cost to treat this wastewater due to high organic content such as carbohydrate. As the wastewater may contain a potential energy source, it will be used in this project for biohydrogen production. Besides, the utilisation of this industrial effluent is beneficial because it is more economical for biohydrogen production and problems regarding accumulation and treatment of industrial effluent can be solved (Ntaikou *et al*., 2010).

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