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BIOELECTRICITY GENERATION FROM SAGO HAMPAS BY Clostridium beijerinckii SR1 USING MICROBIAL FUEL CELLS

MOHD AZWAN JENOL

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By

MOHD AZWAN JENOL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Chair : Professor Suraini Abd Aziz, PhD
Faculty : Biotechnology and Biomolecular Sciences

Sago hampas is a starch-based biomass resulted from the sago starch extraction process that has potential to be a substrate for bioenergy production. Microbial fuel cells (MFCs) is a promising technology that employ the microorganisms to utilize the substrate, which then generate the electrical power. Therefore, through the concept of biomass fuel cells, this study was aimed to utilize sago hampas as substrate in microbial fuel cells to generate the bioelectricity by *Clostridium beijerinckii* SR1, via sugar and volatile fatty acids (VFAs) platform and direct biomass utilization system. A single culture, *C. beijerinckii* SR1 has been employed to produce VFAs from 40 g/L of sago hampas (containing 20 g/L of starch content), resulted 6.71 g/L of total VFAs with the VFAs yield of 0.30 g/g. The enhancement of VFAs from sago hampas was conducted using one-factor-at-a-time (OFAT) with the variables of carbon sources concentration, nitrogen (yeast extract) concentration and addition of inorganic nitrogen sources. The production of VFA has successfully enhanced by 14.6%, with the OFAT condition obtained was 3% (w/v) of sago hampas, 3 g/L of yeast extract and the additional 2 g/L of NH_4NO_3, resulted the production of VFAs (7.69 g/L) with 0.45 g/g of VFAs yield. Furthermore, the effect of sugar and VFAs platform from sago hampas on the bioelectricity generation using microbial fuel cells were studied. The results showed the VFAs platform of sago hampas has better bioelectricity generation, in term of maximum power density (max PD) as compared to sugar platform. The highest max PD obtained was 72.62 mW/m^2 from 2 g/L of VFAs hydrolysate. At the final stage, direct biomass fuel cells was performed using sago hampas directly as a substrate in MFCs. Result shown that 73.78 mW/m^2 of maximum power density was obtained from direct biomass fuel cells by *C. beijerinckii* SR1. This result shows a comparable maximum power density as compared to VFAs platform of sago hampas. Overall, the generation of bioelectricity from sago

hampas has been successfully studied using microbial fuel cells system by *C. beijerinckii* SR1.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENJANAAN BIO ELEKTRIK DARIPADA HAMPAS SAGU OLEH *Clostridium beijerinckii* SR1 MENGGUNAKAN SEL BAHAN API MIKROB

Oleh

MOHD AZWAN JENOL

November 2020

Pengerusi : Profesor Suraini Abd Aziz, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Hampas sagu adalah biojisim berasaskan kanji terhasil daripada proses pengekstrakkan kanji sagu yang mempunyai potensi sebagai substrat untuk penghasilan biotenaga. Sel bahan api mikrob adalah teknologi boleh percaya yang memanfaatkan mikroorganisma untuk menggunakan substrat, dan kemudian menjana tenaga elektrik.Justeru, melalui konsep sel bahan api biojisim, kajian ini telah menetapkan untuk menggunakan hampas sagu sebagai substrat di dalam sel bahan api mikrob untuk menjana bioelektrik oleh *Clostridium beijerinckii* SR1, melalui gula dan asid lemak meruap (ALM), dan sistem penggunaan biojisim langsung. Kultur tunggal, *C. beijerinckii* SR1 telah digunakan untuk menghasilkan ALM daripada 40 g/L hampas sagu (mempunyai 20 g/L kandungan kanji), menghasilkan 6.71 g/L keseluruhan ALM dengan hasil ALM 0.30 g/g. Peningkatan penghasilan ALM daripada hampas sagu telah dijalankan menggunakan *one-factor-at-time (OFAT)* dengan parameter kepekatan sumber karbon, kepekatan submer nitrogen (esktrak yis) dan penambahan sumber tak organik. Penghasilan ALM telah berjaya ditingkatkan sebanyak 14.6%, dengan kondisi *OFAT* yang telah didapatkan ialah 3% (w/v) kepekatan hampas sagu, 3 g/L kepekatan esktrak yis dan penambahan 2 g/L NH_4NO_3, menghasilkan 7.69 g/L ALM dengan hasil ALM 0.30 g/g. Tambahan pula, kesan platfom gula dan ALM daripada hampas sagu ke atas penjanaan bioeletrik menggunakan sel bahan api mikrob telah dikaji. Keputusan menunjukkan platfom ALM hampas sagu mempunyai penjanaan bioelektrik yang lebih baik, dari segi ketumpatan kuasa maksima berbanding platfom gula. Ketumpatan kuasa maksima tertinggi telah dicatatkan ialah 72.62 mW/m^2 daripada 2 g/L hydrolisat ALM. Pada peringkat akhir, sel bahan api biojisim langsung telah dijalankan menggunakan hampas sagu secara langsung sebagai substrat di dalam sel bahan api mikrob. Keputusan menunjukkan ketumpatan kuasa maksima yang telah dicatatkan adalah 73.78 mW/m^2 daripada sel bahan api biojisim langsung oleh *C. beijerinckii* SR1. Keputusan ini menunjukkan ketumpatan kuasa maksima yang setara dicatatkan oleh sel bahan api biojisim langsung berbanding platfom ALM hampas sagu. Keseluruhannya, penjanaan

bioelektrik daripada hampas sagu telah berjaya dikaji menggunakan sistem sel bahan api mikrob oleh *C. beijerinckii* SR1.

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I certify that a Thesis Examination Committee has met on 11th August 2020 to conduct the final examination of Mohd Azwan Jenol on his thesis entitled "Bioelectricity generation from sago hampas by *Clostridium beijerinckii* SR1 using microbial fuel cells" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the (insert the name of relevant degree).

Members of the Thesis Examination Committee were as follows:

Nor'aini binti Abdul Rahman, PhD
Associate Professor
Department of Bioprocess Technology
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Norhafizah bt Abdullah, PhD
Associate Professor
Department of Chemistry and Environmental Engineering
Faculty of Engineering
Universiti Putra Malaysia
(Internal Examiner)

Phang Lai Yee, PhD
Associate Professor
Department of Bioprocess Technology
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Misri Gozan, PhD Ing
Professor
Faculty of Engineering
Universitas Indonesia
Indonesia
(External Examiner)

ZURIATI AHMAD ZUKARNAIN, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Suraini Abd Aziz, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohamad Faizal Ibrahim, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Ezyana Kamal Bahrin, PhD
Senior lecturer
Centre of Foundation Studies for Agricultural Science
Universiti Putra Malaysia
(Member)

____
ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Signature:	
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Signature:	
Name of Member of Supervisory Committee:	Assoc. Prof. Dr Mohamad Faizal Ibrahim

Signature:	
Name of Member of Supervisory Committee:	Dr Ezyana Kamal Bahrin

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LIST OF ABBREVIATIONS

AD	Anaerobic digestion
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BOD	Biological oxygen demand
BTAC	Biomass Technical Advisory Committee
Btu	British thermal unit
C.	*Clostridium*
C/N	Carbon to Nitrogen
CCR	Carbon catabolite repression
CE	Coulombic efficiency
COD	Chemical oxygen demand
CV	Cyclic voltammetry
DCW	Dry cell weight
DET	Direct electron transfer
dH_2O	Distilled water
DNS	Dinitrosalicyclic acid
DP	Degree of polymerization
EC	Enzyme Commission
G.	*Geobacter*
g/g	Gram per gram
g/L	Gram per litre
GNI	Gross national income
h	Hour
ha	Hectare
HCl	Hydrochloric acid
I	Current
kJ	Kilojoule

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Since the late 1800, where the massive consumption of fossil fuel is started, human societies are deemed to insatiable towards fossil fuel. In fact, the consumption of fossil fuel by human has evolved from the long preserved era to the short-lived expend. On the other hand, the high dependency on fossil fuel has significantly contributed to environmental deterioration and climate change (Levin *et al.*, 2004; Perera, 2017, 2018). In fact, the by-products from fossil fuel combustion such as emissions of toxic particles and gases and carbon dioxide (CO_2) are the most significant threat to child's health and growth (Perera, 2017, 2018). Therefore, the shift towards sustainable bioenergy is needed in order to decrease the reliance on fossil fuel. Electricity is one of the most importance form of energy that behests the quality life of human society. The demand for electricity will be kept increasing in the future due to the fact that needs for global population and third-world nations development (Evans *et al.*, 2010; Gude, 2016; Owusu & Asumadu-Sarkodie, 2016; World Nuclear Association, 2019). As a matter of fact, fossil fuels are known to be the primary source of the production of electricity. According to U.S. Energy Information Administration (2017), in 2015, about 66% of electricity was generated from fossil fuel, including 33% from coal and 33% from natural gas in the United States. In particular, out of 343 615 MWh produced from renewable resources, biomass is accounted as 18% of total production in 2016.

Nature is a sustainable production chain of chemicals, fuels and materials (Athaley *et al.*, 2019; Badgujar *et al.*, 2019; Kamm & Kamm, 2006). In fact, there are many biobased industry products used nowadays are resulted from processing of natural cycle by-products such as biomass, including starch, cellulosic materials, protein as well as lignin. The term "biomass" is referring to the raw organic material used for various processes such as the generation of energy resources, including heat, fuels and electricity. Biomass is an organic material since it comes from living organisms, including animals and plants. Plants, woods and other agricultural wastes are the most common biomass that have been used as feedstock of energy. Biomass contain energy from the sun, in which it converts carbon dioxide and water to nutrients through photosynthesis. The stored energy in biomass can be converted into useable energy via two different approaches; direct and indirect. Biomass can be burned to generate heat and further converted to electricity (direct); or processed into fuel (indirect). In our available technology nowadays, there are many development have been made in order to optimize the utilization of biomass for the production of energy. Fuel cells are one of the most promising technologies that can utilize biomass for the production of energy, such as electricity.

In recent years, the attention of researchers are appealing towards the development of fuel cells for green, portable and better suits for a variety of application. The concept of fuel cells is transforming the chemical reactivity into electricity by oxidizing the electron donor in the anode and reducing the electron acceptor in the cathode (Logan, 2009; Toczyłowska-Mamińska *et al.*, 2018), which provides the electrical power outputs as long as the availability of sufficient fuels and oxidants. Microbial fuel cells (MFCs) is a bio-electrochemical device that can generate the electrical voltage from many types of the chemical through the catalytic oxidation of electrolyte/fuel at the anode compartment and chemical reduction at the cathode compartment as a synergically process. In nature, the bacteria have gained the metabolic energy by transmitting the electron from the donor, such as organic matter, to the acceptor, such as oxygen. The larger the potential between the electron donor and acceptor, the bigger the gains of energy by bacteria. MFCs have a unique feature, whereby they do not require the utilization of metal-based catalyst in the anode, instead of the employment of microorganisms that can biologically oxidize the organic matter and provide the free electron in the anode. Microorganisms that can oxidize the organic matter and capable to transfer the electron extracellular are known as exoelectrogens (Logan, 2009). Several microorganisms have been identified as exoelectrogens, including *Geobacter* sp. (Okamoto *et al.*, 2014), *Shewanella* sp. (El-Naggar *et al.*, 2010; Logan, 2009), *Escherichia coli* (Miroliaei *et al.*, 2015; Nandy *et al.*, 2016; Nguyen & Taguchi, 2019), as well as *Clostridium* sp. (Finch *et al.*, 2011; Niessen *et al.*, 2004, 2005; Poincare & Nancy, 1976) . Based on the reviews, acetate (Chae *et al.*, 2009; Liu *et al.*, 2005) and glucose (Catal *et al.*, 2008; Liu *et al.*, 2010) are the most types of substrate used in the production of bioelectricity using MFCs. Up to date, there are many recent development of MFCs which leads to the variety of applications, including biohydrogen (Holzman, 2005; Liu *et al.*, 2005), biosensor (Kim *et al.*, 1999) and wastewater treatment (Ren *et al.*, 2014; Sonawane *et al.*, 2014).

Sago palm is one of the important industries in Malaysia, which the plantation is mainly focused in the state of Sarawak. According to the Department of Agriculture Sarawak, (2013), the total sago palm plantation was recorded in 2013 is 54,087 ha, occupied over three-quarter of the peat land of Sarawak. It is being said as the only plant that has the ability to grow well in the swampy area (Bujang, 2008). Malaysia has been considered as one of the most crucial sago starch exporters. In 2013, the export value recorded was MYR 71 million equivalent to 48,000 mt, subsequently in 2014, the increment to MYR 81 million at the volume of 46,900 mt. (Ming *et al.*, 2011). The export value is expected to increase by 15 – 20% per year (Department of Agriculture Sarawak, 2013).

Up to date, there are many available technologies that have been developed in bioconversion of biomass into value-added products, especially biofuels and bioenergy. According to Chang *et al.* (2010), the typical platform employed in several of the research are including sugars, carbon-rich chain, thermochemical (syngas) and biogas platform. These available platforms have their respective unique advantages and disadvantages. Each platform is explained as follows:

1. The sugars platform: uses pentose and hexose and sugars extracted and/or produced from the agricultural residues;
2. The carbon-rich chain platform: uses the long-chain fatty acids or glycerides to produce biofuel such as biodiesel;
3. The thermochemical platform: includes the conversion of biological or chemical processes using gasification or pyrolysis of plant mass in order to produce biofuels;
4. Lastly, the biogas platform: uses the municipal solid wastes to produce biogas such as methane gas through anaerobic digestion (AD) process that exploits acidogenesis and methanogenesis process.

Interestingly, the biogas platform is considered as an economical process since it is suitable for organic waste treatment and unrequired a high-cost pretreatment and additional hydrolysis enzymes. The AD consists of four stages, which are hydrolysis, acidogenesis, acetogenesis and methanogenesis done by the natural consortia of mixed anaerobic bacteria (Chang *et al.*, 2010; Chen *et al.*, 2017). In general, the acidogenesis phase is the production of short-chain fatty acids, known as volatile fatty acids (VFAs), including acetate and butyrate. These VFAs are rapidly produced from all parts of biomass (carbohydrates, lipids, proteins) except for lignin, which potential to be used as a carbon source for bioenergy production (Huang *et al.*, 2016; Jin *et al.*, 2017; Sawatdeenarunat *et al.*, 2017)

1.2 Significant of study

The problems associated to the conventional fossil-fuel-driven power plant, in which leads to increment in the pollutants emissions and greenhouse gases. Thus, it is crucial to shift the focus on the fossil fuel based power plant towards an alternative electricity generation by using renewable resources for better sustainability and environmentally friendly approaches. With the advances in technology available, the application of microbial fuel cells covered the utilization of wastewater as substrate. However, there are only a few information related to the utilization of biomass, specifically agricultural biomass in bioelectricity generation using microbial fuel cells. Even so, the available topics related to the utilization of agricultural biomass in bioelectricity generation using microbial fuel cells have encountered several limitations. The problems associated with current agricultural biomass are the pretreatment process required as well as low power density obtained. Thus, other potential agricultural biomass is needed to be discovered in order to further extend the application of microbial fuel cells in bioelectricity generation.

Sago hampas is the by-product resulted after the extraction of sago starch, which contains the residue of starch and lignocellulosic materials. Sago hampas (dry basis) is made up of 58% starch, 23% cellulose, 9.2% hemicellulose and 3.9% lignin (Awg-Adeni *et al.*, 2013; Jenol *et al.*, 2014; Ozawa *et al.*, 1998). Sago hampas could be considered as a great potential substrate to be used as a feedstock for the production of fermentable sugars due to the fact that it has high total carbohydrates content. In addition, since the composition of lignin is considerably low in sago hampas, no pretreatment is needed. There are several studies have been done on the utilization of sago hampas as a feedstock for the production of value-added products, including fermentable sugar mainly glucose, subsequently further use in biofuels, such as bioethanol (Awg-Adeni *et al.*, 2013), and bioenergy, such as biohydrogen (Jenol *et al.*, 2014). Note to these findings, it is suggested that the sago hampas has great value as a feedstock for the production of fermentable sugar, which further utilize in the production of value-added products.

The VFAs platform is deliberated as a new emerging technology approach, thus the true potential is not fully understood yet. Supplementary, a baseline of comparative study between VFAs platform and sugars platform is necessary to give additional knowledge in the utilization of biomass. In addition, there is very little information regarding the utilization of biomass in the production of bioelectricity using the MFCs system. Furthermore, the utilization of sago biomass would be an advantage due to the fact that no pretreatment is required.

On the other hand, the employment of *Clostridium beijerinckii* SR1 in this study was due to the advantages of this anaerobic bacteria. *Clostridium* sp. is known to be the microorganism that able to secrete glucoamylase, in which essential enzyme in hydrolysing the starch (Md Salleh *et al*., 2001). On the other hand, since *Clostridium* sp. has two distinguish metabolic pathways, which are acidogenesis and solventogenesis, it able to produce organic acids, including VFAs from biomass. During acidogenesis, *Clostridium* sp. is reported to produce acetic acid and butyric acid (Oh *et al*., 2002; Pattra *et al*., 2008; Cubillos *et al*., 2010). Thus, this study is focused on the utilization of acetic acid and butyric acid, as VFAs platform in bioelectricity generation using microbial fuel cells. In bioelectricity generation, *Clostridium* sp. is reported that able to generate the bioelectricity from wide range of substrate, including simple organic, such as VFA and glucose, up to complex carbohydrate, such as starch (Liu *et al*., 2015). It also interesting to mention that, *Clostridium* sp. is required no mediator in order to facilitate the electron transfer mechanism in bioelectricity generation (Gottwald *et al*., 1975; Finch *et al*., 2011).

1.3 Research questions

There are several research questions are needed to be addressed in order to utilize sago hampas as a substrate in bioelectricity generation using microbial fuel cells by *Clostridium beijerinckii* SR1. These questions are as followed:

1. Can VFA be produced and enhanced from sago hampas by *C. beijerinckii* SR1?
2. How the utilization and performance of VFA and sugar hydrolysate from sago hampas in bioelectricity generation using microbial fuel cells system?
3. Can sago hampas be directly used as a substrate in microbial fuel cells for bioelectricity generation by *Clostridium beijerinckii* SR1?

1.4 Objectives

Therefore, the overall objective of this study is to produce the bioelectricity from sago biomass by *Clostridium beijerinckii* SR1 using a microbial fuel cell system. The specific objectives are:

1. To enhance the production of VFA from sago hampas by *Clostridium beijerinckii* SR1
2. To compare the sugar-based hydrolysate and VFA-based hydrolysate on the production of bioelectricity by *Clostridium beijerinckii* SR1 in microbial fuel cell system
3. To generate the bioelectricity using direct biomass fuel cell from sago hampas by *Clostridium beijerinckii* SR1

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BIODATA OF STUDENT

Mohd Azwan Jenol was born on 04th Jan 1990 and later on, grown up in Kota tinggi, Johor. He received his early education at Sekolah Kebangsaan Sungai Papan, Bandar Penawar, Johor from 1997 – 2011. Further, the author was pursued his secondary study at Sekolah Menengah Kebangsaan Tanjung Datuk, Pengerang, Johor and sat for Sijil Pelajaran Malaysia in 2006. In 2007, He went to Johor Mactriculation College, Tangkak, Johor for one year matriculation program organized by the Malaysia Education Ministry (MOE).

After completed his matriculation program, he was promoted to continue his First Degree in Bachelor Science of Biotechnology, a three years program at Universiti Putra Malaysia, Serdang, Selangor. During his final semester of Bachelor's Degree, he was managed to complete a final year project, entitled "Optimization of biobutanol production from oil palm empty fruit bunch by *Clostridium butyricum* A1 using response surface methodology approach". The author also awarded with *Anugerah Naib Canselor* (ANC) by Universiti Putra Malaysia. He started his Master's Degree in the field of Environmental Biotechnology under supervision of Professor Dr. Suraini Abd-Aziz. He managed to complete his Master's Degree with thesis entitled "Biohydrogen production from sago hampas by *Clostridium butyricum* A1" in 2014. During his Master's Degree, he managed to publish one journal paper as main author and one journal paper as co-author. In December 2014, he was offered to pursue his PhD Degree (Environmental Biotechnology) at UPM under supervision Professor Dr. Suraini Abd Aziz. His current PhD Degree project was presented in this thesis. During his study, the author managed to publish one journal paper as main author and one book chapter as co-author.

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LIST OF PUBLICATIONS

Jenol, Mohd Azwan, Ibrahim, Mohamad Faizal, Kamal Bahrin, Ezyana, Kim, Seung Wook, & Abd-Aziz, Suraini (2019). Direct bioelectricity generation from sago hampas by *Clostridium beijerinckii* SR1 using microbial fuel cell. *Molecules*, *24*(13), 2397.

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