UNIVERSITI PUTRA MALAYSIA

USE OF METABOLIC ENGINEERED Escherichia coli STRAINS FOR ENHANCED BIOHYDROGEN PRODUCTION FROM PALM OIL MILL EFFLUENT

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USE OF METABOLIC ENGINEERED *Escherichia coli* STRAINS FOR ENHANCED BIOHYDROGEN PRODUCTION FROM PALM OIL MILL EFFLUENT

By

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USE OF METABOLIC ENGINEERED *Escherichia coli* STRAINS FOR ENHANCED BIOHYDROGEN PRODUCTION FROM PALM OIL MILL EFFLUENT

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November 2017

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Faculty : Biotechnology and Biomolecular Sciences

Biological hydrogen production offers a method through which biomass can be utilized for production of premium-energy carrier as an alternative energy for replacement of non-renewable fossil fuels. The present work aims to elucidate the substrate utilization in biohydrogen production from palm oil mill effluent (POME) by *E. coli* strains. The experiments were performed in 150 mL serum bottles and the cultures were supplemented with autoclaved-pretreated POME to investigate the potential use of various carbon sources and its preference towards biohydrogen production. The cultures conditions were maintained at 37 °C for 24 h with mild agitation at 120 rpm. The maximum hydrogen yield (MHY) of 0.68 mol H_2 / mol total sugars with 81% substrate consumption based on total sugar and hydrogen productivity of 3552 μmol / 10^{10} cfu were obtained from engineered *E. coli* BW25113 after 24 h of fermentation. The biohydrogen production by modified strain was enhanced by 3.5 fold compared to wild type strain. On the other hand, studies on the utilization of various substrates on the synthetic medium showed that the highest MHY of 0.77 mol H_2 / mol total sugar was obtained from fermentation of fructose by engineered *E. coli* BW25113. The preference of the substrates based on the synthetic medium for biohydrogen production was in the following order; fructose > glucose > formic acid. These findings indicated that the use of modified strain *E. coli* BW25113 has enhanced biohydrogen production from POME.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGGUNAAN BAKA *Escherichia coli* METABOLIK KEJURUTERAAN UNTUK PENINGKATAN PENGHASILAN BIOHIDROGEN DARIPADA EFLUEN KILANG MINYAK SAWIT

Oleh

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Penghasilan hidrogen secara biologi menawarkan satu kaedah di mana biomas boleh digunakan untuk pengeluaran tenaga premium sebagai tenaga alternatif untuk menggantikan bahan api fosil yang tidak boleh diperbaharui. Tujuan kajian ini adalah untuk menjelaskan penggunaan substrat daripada sisa buangan kilang kelapa sawit (POME) dalam penghasilan biohidrogen oleh bakteria *Esherichia coli*. Eksperimen ini dilakukan di dalam serum botol 150 mL dan dikulturkan bersama POME yang telah dirawat menggunakan autoklaf untuk menentukan potensi penggunaannya sebagai sumber karbon bagi penghasilan biohidrogen. Kultur bakteria *E. coli* BW25113 ditetapkan pada suhu 37° C selama 24 jam dengan kelajuan goncangan yang sederhana pada 120 rpm. Hasilan hidrogen maksimum (*MHY*) daripada *E. coli* terjurutera genetik adalah sebanyak 0.68 mol H_2/mol daripada jumlah gula dan produktiviti 3552 µmol / 10^{10} cfu bagi tempoh 24 jam fementasi, iaitu dengan kadar penggunaan substrat adalah sebanyak 81%. Justeru, penggunaan terjurutera genetik *E. coli* BW25113 telah meningkatkan penghasilan biohidrogen sebanyak 3.5 kali ganda berbanding *E. coli* BW25113 induk. Sebaliknya, kajian sampingan terhadap penggunaan kepelbagaian substrat ke atas medium sintetik mendapati (*MHY*) tertinggi adalah 0.77 mol H_2 / mol yang dihasilkan melalui fermentasi fruktosa daripada *E. coli* BW25113. Keutamaan substrat yang diperolehi untuk penghasilan biohidrogen adalah seperti susunan yang berikut; fruktosa > glukosa > asid formik. Penemuan ini menunjukkan bahawa pengunaan terjurutera genetik *E. Coli* BW25113 telah meningkatkan pengeluaran biohydrogen daripada POME

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I certify that a Thesis Examination Committee has met on 20 November 2017 to conduct the final examination of Azam Fikri Taifor on his thesis entitled "Use of Metabolic Engineered *Escherichia coli* Strains for Enhanced Biohydrogen Production from Palm Oil Mill Effluent" 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 Master of Science.

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Declaration by Members of Supervisory Committee

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

POME	Palm oil mill effluent
MHY	Maximum hydrogen yield
OPB	Oil palm biomass
OPF	Oil palm frond
GHG	Greenhouse gases
ATP	Aldose tyrosine pyruvate
PFL	Pyruvate formate lyse
FHL	Formate hydrogenase lyse
MPOB	Malaysia Palm Oil Board
DOE	Department of environment
PEMANDU	Performance Management and Delivery Unit
CPO	Crude palm oil
COD	Chemical oxygen demand
BOD	Biological oxygen demand
MSM	Mineral salt medium
OD	Optical density
CFU	Colony forming unit
TS	Total solid
TSS	Total suspended solid
VSS	Volatile suspended solid
HPLC	High performance liquid chromatography

CHAPTER 1

INTRODUCTION

1.1 Research overview

Hydrogen (H_2) is a very attractive energy resource and has potential to become an alternative energy to fossil fuels. It contains high energy content (2.75 times higher than known liquid fossil fuels) and its combustion produces only water. Hydrogen can be generated mainly from coal-gasification, water-electrolysis, gas reformation or biological process (Mohd Yusoff *et al.*, 2012). Biological activities such as photolysis and fermentation have been known as most cost-effective and environmentally friendly methods to produce biological hydrogen compared to other methods (Mohd Yusoff *et al.*, 2012). Intriguingly, biological process of hydrogen production through dark-fermentation has been widely studied due to its capability to utilize wide-range of carbohydrate-rich substrate (Ntaikou *et al.*, 2008; O-Thong *et al.*, 2016; Uyar *et al.*, 2009). Carbohydrate-rich substances such as agriculture waste, municipal food waste and wastewaters (including mills effluent) have been widely studied as fermentation feedstock for biohydrogen production (Kapdan and Kargi, 2006; Masset *et al.*, 2012; Tanikkul and Pisutpaisal, 2014).

Malaysia is the second largest palm oil producer in the world. Thus, high production of palm oil for market supply demand had produced abundance of oil palm biomass (OPB) in Malaysia. Approximately, nine tons of OPB is produced for each ton of crude palm oil (CPO) and palm kernel oil (PKO) (Basiron and Weng, 2004). Palm oil mill effluent (POME) is one of the carbohydrate-rich resources that are continuously produced in palm oil mills during the process of obtaining crude palm oil (CPO) of which known as a renewable and non-toxic resource (Chong *et al.*, 2009a). The disposal of POME requires treatment systems such as ponding treatment and open digesting tank (Poh and Chong, 2009). Alternatively, POME has been studied as a substrate for production of biohydrogen, which in the next decade is likely to see a considerable rise in biohydrogen production for energy generation (Atif *et al.*, 2005; Azman *et al.*, 2016; Mohd Yusoff *et al.*, 2009). However, there is still in a need for discussion on the two major bottlenecks of this approach which are the low yield of biohydrogen production and the low rate of carbon sources consumption (Das and Veziroglu, 2008). Therefore, recent studies in biohydrogen conversion from POME have been mainly aimed to elevate hydrogen yield and carbon sources conversion rate (Krishnan *et al.*, 2016; Singh *et al.*, 2012; Mishra *et al.*, 2016).

Based on literature search, limited information is available regarding the ability of hydrogen producing bacteria to utilize carbon sources from POME. Genetic modification is one of the molecular approaches used to access molecular activity for enhanced hydrogen yield (Kandasamy *et al.*, 2013; Vardar-Schara *et al.*, 2008; Yoshida *et al.*, 2005). Despite numerous successions of genetic engineering studies to enhance biohydrogen yield, its application is only limited to biohydrogen production based on synthetic media. Previously, Yasin and colleagues (2013) showed that the use of genetically modified strains on oil palm frond (OPF) juice has successfully enhanced biohydrogen yield. However, information regarding carbon sources consumed for biohydrogen production from biomass containing medium has not yet been discussed in particular detail. It is particularly important to determine the specific carbon sources that contribute to biohydrogen production and following that, discuss on the methods of enhancing hydrogen production yield. This would allow for a better understanding of interactions between variables involved in biohydrogen production from biomass containing medium using genetically modified strain.

Hence, the aim of the present works is to investigate the use of genetically engineered strain in order to increase biohydrogen yield and to elucidate the effect of carbon sources that are found in POME towards biohydrogen production. The genetically modified strain, which was previously constructed by Maeda *et al. (*2007b) for enhanced biohydrogen production from technical grade glucose in mesophilic batch fermentation was used as biohydrogen producer. In order to investigate the effectiveness of the organism and the use of carbon sources in this process, pure culture with sterile substrate condition has been used throughout the experiment. The information obtained throughout the study will deliver substantial fundamental evidence for the ability of genetically engineered *E. coli* from various carbon sources present in POME.

Objectives:

1. To identify potential carbon sources that are available in palm oil mill effluent, and its preferability for biohydrogen production by *E. coli* strains.

2. To produce biohydrogen using wild type and metabolically engineered *E. coli* strains in batch culture.

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