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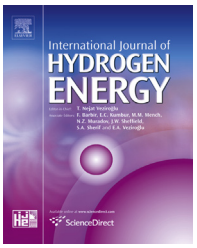

Elucidating substrate utilization in biohydrogen production from palm oil mill effluent by *Escherichia coli*

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ABSTRACT

The present work aims to elucidate substrate utilization from palm oil mill effluent (POME) for biohydrogen production. The experiment was performed in 150 mL serum bottles and the cultures were supplemented with autoclaved-pretreated POME or 0.05 M individual technical grade substrates to investigate the potential use of POME and substrates in preference towards biohydrogen production. The cultures were incubated at 37 °C for 24 h with mild agitation. The maximum hydrogen yield (MHY) obtained was 0.66 mol H_2/mol total monomeric sugars and productivity of 3551 μmol/10^{10} cfu were obtained from engineered *Escherichia coli*. The POME oligomeric sugars were not metabolized further, which render insignificant conversion of carbohydrates into hydrogen from POME. The yield of hydrogen production increased by 3.5 folds by engineered *E. coli* BW25113 compared to wild type *E. coli* BW25113. The preference of the substrates for biohydrogen production is in the following order; glucose > fructose > formic acid.

Introduction

Hydrogen 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 greater 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 [1]. The biological hydrogen production is the most cost-effective, environmental friendly and convenient production approach compared to other methods. The utilization of carbohydrate-rich substrate by dark-fermentation is considered as the best approach to produce hydrogen cost-

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effectively and in an environmental friendly way [2]. This approach has been extensively applied to the study of bioremediation (e.g. agriculture and municipal food waste) [3–5]. Palm oil mill effluent (POME) contains carbohydrate-rich renewable resources and non-toxic biomass that have been extensively researched for biological hydrogen production [6–11]. However, low hydrogen yield and limited utilization of carbon sources are two major drawbacks to produce hydrogen at a practical level through fermentation method [12].

There are two strategies suggested by Hallenbeck and Ghosh [13] in order to overcome these drawbacks; (i) the use of genetically modified organism to increase fermenting power of converting substrate into hydrogen, and (ii) the use of wide range of substrates for producing hydrogen. Numerous microorganisms are capable of hydrogen production with *Escherichia coli* (*E. coli*) one of the fermentative hydrogen-producing bacteria that has been well characterized and easily accessible for genetic engineering studies. Theoretically, *E. coli* produces 2 mol of hydrogen from 1 mol hexose [14] and 1.67 mol of hydrogen from 1 mol pentose [13]. However, maximum hydrogen yields (MHY) from single culture of wild type *E. coli* strains were far below its theoretical values. Previous genetic engineering studies have successfully constructed or modified *E. coli* strains to enhance MHY [14] and also to utilize various carbon sources for biohydrogen production [15]. However, studies on metabolically engineered *E. coli* strains were limited to synthetic media in which little information was obtained on its application on biomass as fermentation feedstock. Previously, Yasin and colleagues [16] have successfully elevated hydrogen production from oil palm frond (OPF) juice by using genetically modified *E. coli* strains. However, detailed information on specific substrate consumption was not reported in their study. The use of sole carbon source from lignocelullosic biomass by using pure culture microbes, *E. coli*, can be argued since many findings have reported that biohydrogen production was derived from various substrates [17]. Biohydrogen production from POME has been reported previously from various fermentation conditions such as temperatures, mix substrates, pure and mix cultures [5–11]. Furthermore, most of the reported works showed representable data on nutrient-rich substrates such as total carbohydrate, total nitrogen, oil and grease etc [5–11]. Therefore, no detailed work was ever performed on elucidating which carbon sources available in POME are channeled into biohydrogen production.

Thus, the present study aims to identify sources of carbon in POME and elucidating substrates preference by *E. coli* towards biohydrogen production. The total carbohydrate content determined was further characterized by high performance-liquid chromatography (HPLC) after hydrolyzing POME with 4% sulfuric acid (H_2SO_4) at 120 °C for 1 h. Monomeric and oligomeric sugars, formic acids and other by-products were detected from POME. Batch fermentation using POME as substrates followed by experiments using individual technical grade were performed on wild type *E. coli* BW25113 and metabolically engineered *E. coli* BW25113 *hyaB hybC hycA fdoG ldhA frdC aceE* to understand substrates preference by *E. coli* strains towards biohydrogen production. To the best of our knowledge there is no report thus far on fundamental studies on substrates available in POME for biohydrogen production and the information obtained throughout this study will deliver substantial evidence on the ability of genetically engineered *E. coli* in using various carbon sources present in POME.

Materials and methods

Bacterial strains and growth conditions

Parental strain of *E. coli* BW 25113 and engineered *E. coli* BW25113 *hyaB hybC hycA fdoG ldhA frdC aceE* were obtained from Kyushu Institute of Technology, Japan [19]. Throughout this manuscript, the *E. coli* BW25113 *hyaB hybC hycA fdoG ldhA frdC aceE* will be known as engineered *E. coli*. The strains were grown in Luria Bertani media containing 1% Bacto Tryptone, 0.5% yeast extract, and 0.5% NaCl with addition of antibiotic (100 μg/mL kanamycin) in engineered *E. coli*. The seed cultures were grown in incubator shaker at temperature of 37 °C and agitation speed of 120 rpm to reach cell turbidity of 0.5 at 600 nm prior to inoculation [16]. The incubation period of the cultures depend upon the growth rate of the strains. Cell growth was monitored by turbidity using spectrometer Genesys 20 (Thermo Scientific, USA).

Preparation of POME as a substrate

Palm oil mill effluent (POME) was obtained from Seri Ulu Langat palm oil mill, Dengkil, Selangor, Malaysia. The sample was collected at POME discharge point which is before entering treatment pond. It's temperature was recorded ranging from 70°C to 90 °C. POME was stored in a 25 L container at temperature less than 4 °C but above freezing point to prevent biodegradation by microbial activities. Prior to fermentation, the pH of POME was adjusted to pH 8.5 with 10 M NaOH and the POME was sterilized by autoclaving at 121 °C for 20 min to clear from presence of indigenous microorganism.

Preparation of technical grade substrates

Technical grade substrates such as glucose, fructose, arabinose, acetic acid and formic acid (0.05 M) were supplemented individually in the mineral salt medium (MSM) which consisted in g per liter of distilled water; 5.0 g yeast extract, 5.0 g tryptone, 7.0 g K_2HPO_4, 5.5 g KH_2PO_4, 0.5 g L-cysteine-HCl.H_2O, 1.0 g $(NH_4)_2SO_4$, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.021 g $CaCl_2$. $2H_2O$, 0.029 g $Co(NO_3)_2 \cdot 6H_2O$, 0.039 g, $Fe(NH_4)SO_4$. $6H_20$, 2.0 mg nicotinic acid, 0.172 mg Na_2Se0_3, 0.02 mg $NiCl_2$ and 10 mL of trace element solution containing 0.5 g $MnCl_2 \cdot 4H_2O$, 0.1 g H_3BO_3, 0.01 g $AlK(SO_4)_2 \cdot H_2O$, 0.001 g $CuCl_2H_2O$ and 0.5 g Na_2EDTA [20]. This work was performed to observe the preference and substrate consumption efficiency by both wild type *E. coli* BW2511and engineered *E. coli* strains for biohydrogen production. The selection of technical grade substrates used was based on the major substrates available in POME as determined by HPLC.

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