BIOHYDROGEN PRODUCTION FROM PALM OIL MILL EFFLUENT VIA SEQUENTIAL DARK-PHOTO FERMENTATION

PURANJAN MISHRA

Doctor of Philosophy

UNIVERSITI MALAYSIA PAHANG
SUPERVISOR’S DECLARATION

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy.

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Position : PROFESSOR
Date : 16/07/2018
STUDENT’S DECLARATION

I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

_______________________________
(Student’s Signature)

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Date : JULY 2018
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PURANJAN MISHRA

Thesis submitted in fulfillment of the requirements for the award of the degree of Doctor of Philosophy

Faculty of Engineering & Technology
UNIVERSITI MALAYSIA PAHANG

JULY 2018
“Specially dedicated to my beloved grandfather Late Shri Gangadhar Mishra, my parents and my lovable sisters Priyanka and Monika Mishra, who constantly encouraged supported me all the way since the beginning of the studies”.
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Penghasilan hidrogen secara fermentasi daripada biomass (iaitu produk yang terhasil memalui fotosintesis) menjanjikan peluang ke arah penghasilan tenaga yang lestari. Konsep baru berbekaikan dua peringkat penembusan gelap dan fotografi (TSDPF) telah dicadangkan untuk meningkatkan penghasilan hydrogen (H<sub>2</sub>) dan COD<sub>removal</sub> menggunakan efluen minyak kelapa sawit (POME) dari kilang sebagai substrat bagi proses fermentasi dalam kajian ini. Objektif utama kajian ini adalah penghasilan H<sub>2</sub> dari POME menggunakan sistem TSDPF melalui penghasilan secara berkelompok. Pada permulaan kajian, pengasingan bakteria dari POME telah dilakukan bagi memperoleh bakteria penghasil H<sub>2</sub> yang dinamakan ‘Bacillus strain PUNAJAN1’. Melalu beberapa data analysis parameter fiziko-kimia yang dicadangkan dalam kajian ini, parameter optimum yang diperoleh adalah pada suhu 35°C, pH 6.5, 1.2 g L<sup>-1</sup> NH<sub>4</sub>Cl (sebagai sumber nitrogen) dan 10 g L<sup>-1</sup> mannose (sebagai sumber karbon) untuk pengeluaran bio-H<sub>2</sub> maksimum sebanyak 2.42 mol H<sub>2</sub> / mol heksosa. Disamping itu, bacteria PUNAJANI juga menunjukkan penghasilan H<sub>2</sub> yang berkesan pada 0.23 L-H<sub>2</sub>/g-CODremoved apabila POME digunakan sebagai sumber karbon. Selain itu, nanopartikel nikel dan kobalt oksida yang dihasilkan secara ‘hidrothermal’ telah ditambah kepada POME dengan jutil 0.25 hingga 3.0 mg L<sup>-1</sup> POME. Keputusan menunjukkan bahawa POME dengan penambahan 1.5 mg L<sup>-1</sup> NiO NPs dan 1.0 mg L<sup>-1</sup> CoO NPs mempunyai ciri-ciri sebagai pemangkin dan ianya dapat meningkatkan hasil H<sub>2</sub> sebanyak 1.51 dan 1.67 kali ganda, jika dibandingkan dengan kawalan. Tambahan pula, kajian parameter terhadap pengoptimuman foto-fermentasi H<sub>2</sub> dari POME gelap (DPOME) telah dijalankan menggunakan metodologi statistic pengeluaran Box-Behnken. Hasil eksperimen tindak balas Box-Behnken terhadap permukaan telah menunjukkan kesan positif diantara pencairan DPOME, pH awal dan rejim agitasi) terhadap pengeluaran foto-H<sub>2</sub>. Hasil maksimum H<sub>2</sub> yang diperoleh adalah pada keadaan optimum 40% pencairan DPOME, pH awal 6.0 dan kadar pengadukan sebanyak 140 rev/min. Melalui penggunaan strategi pengoptimuman ini, peningkatan hasil H<sub>2</sub> yang ketara dari 0.79 hingga 3.11 telah dicapai. Peningkatan pengeluaran H<sub>2</sub> dari DPOME di bawah keadaan optimum telah mencapai peningkatan hampir lima kali ganda. Akhirnya, kebarangkalian sistem TSDPF telah berjaya dilaksanakan menggunakan POME sebagai substrat. Penapaian tahap pertama dilakukan dengan menggunakan PUNAJAN1 yang terisolasi mempunyai hasil H<sub>2</sub> maksimum 37.11 mlH<sub>2</sub>/g-COD dan 41% COD<sub>removal</sub>. Lebih 40% dicairkan DPOME dengan air paip yang disterilkan untuk kegunaan fermentasi tahap kedua (foto-penapaian). Hasil keseluruhan H<sub>2</sub> dari sistem TSDPF meningkat dari 37.11 sehingga ke 130.89 ml H<sub>2</sub>/g-COD, sementara peratusan COD<sub>removal</sub> secara serentak meningkat dari 41 hingga 93%. Peningkatan pengeluaran H<sub>2</sub> ini lebih tinggi daripada fermentasi POME gelap berperingkat tunggal. Hasil ini memberi kesimpulan terhadap keberkesanan penggunaan POME fermentasi gelap ke arah pengeluaran H<sub>2</sub> yang maksimum dan juga pengurangan kepekatan COD.
ABSTRACT

Fermentative hydrogen production using biomass (a product of photosynthesis) is a promising route toward the sustainable bioenergy production. A novel concept of two stage-sequential dark-photo fermentation (TSDPF) system was proposed for enhanced biohydrogen production and COD\textsubscript{removed} using palm oil mill effluent (POME) as fermentative substrate. The main objective of this study comprises the hydrogen production in batch mode from POME using TSDPF system. In the initial stage of the study, isolation of an indigenous hydrogen producing strain, ‘Bacillus strain PUNAJAN1’ was done using POME sludge. The analytical data of various physicochemical parameters indicated the maximum biohydrogen production of 2.42 mol H\textsubscript{2}/mol hexose at optimal temperature of 35°C, pH 6.5, 1.2 g L\textsuperscript{-1} of NH\textsubscript{4}Cl (as a nitrogen source) and 10 g L\textsuperscript{-1} of mannose (as carbon source). Besides, the strain PUNAJAN1 has also shown the efficient hydrogen production ability of 0.23 L-H\textsubscript{2}/g-COD\textsubscript{removed}, when POME was subjected as a carbon source. Further, hydrothermally prepared nickel (NiO NPs) and cobalt oxide nanoparticles (CoO NPs) were added to POME with the range of 0.25 to 3.0 mg L\textsuperscript{-1} POME. Results demonstrated 1.51 and 1.67 folds of noticeable enhancement in biohydrogen production from POME supplemented with 1.5 mg L\textsuperscript{-1} NiO NPs and 1.0 mg L\textsuperscript{-1} CoO NPs respectively, in comparison to the control. Furthermore, a statistical approach to optimize the production of photo-fermentative H\textsubscript{2} from dark fermented POME using Box–Behnken response surface methodology. Experimental data has shown a positive correlation between interdependence among various parameters (such as dilution of DPOME, initial pH and agitation regime) with improved photo-H\textsubscript{2} production, as significant enhancement of hydrogen yield from 0.79 to 3.11 mol-H\textsubscript{2}/mol-acetate was observed under the optimal condition of 40% of dilution of DPOME; pH 6.0; and agitation rate of 140 rev/min. The observed enhancement in photohydrogen production from DPOME under optimized conditions was almost fivefold. Finally, feasibility of TSDPF system in enhancing photo-H\textsubscript{2} production using POME has been successfully validated, where first stage fermentation was carried out using PUNAJAN1 strain (resulted 41% of COD\textsubscript{removed} along with hydrogen yield of 37.11 ml H\textsubscript{2}/g-COD) followed by second stage fermentation using 40% diluted DPOME with sterilized tap water (photo-fermentation). Applicability of using TSDPF system in increasing hydrogen yield (from 37.11 to 130.89 ml H\textsubscript{2}/g-COD) and COD\textsubscript{removed} rate (from 41 to 93%) has been implicated in this study which is reportedly far superior to single stage dark fermentation of POME. So, these results confirmed an effectual utilization of sequential dark-photo fermentation using dark POME can result in substantial hydrogen production and COD\textsubscript{removed}.
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<tr>
<td>$H_{(Ac)}$</td>
<td>ACETIC ACID</td>
</tr>
<tr>
<td>$H_{(Bu)}$</td>
<td>Butyric acid</td>
</tr>
<tr>
<td>$H_{(Pr)}$</td>
<td>Propionic acid</td>
</tr>
<tr>
<td>FeCl$_2$</td>
<td>Ferric chloride</td>
</tr>
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<td>Sodium hydroxide</td>
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<tr>
<td>w/v</td>
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<tr>
<td>min</td>
<td>Minute</td>
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<tr>
<td>g/L or gL$^{-1}$</td>
<td>Gram per litre</td>
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<td>Kilo-joule</td>
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<tr>
<td>MJ</td>
<td>Mega joule</td>
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<tr>
<td>H$_2$</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>L-H$_2$/g-</td>
<td>Litre hydrogen per gram of cod removal</td>
</tr>
<tr>
<td>g-CODL$^{-1}$</td>
<td>Gram COD per litre</td>
</tr>
<tr>
<td>CFU/ml</td>
<td>Colony forming unit per millilitre</td>
</tr>
<tr>
<td>W/m$^2$</td>
<td>Watt per meter square</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>ºC</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>COD$_{removed}$</td>
<td>COD removal from substrate</td>
</tr>
</tbody>
</table>
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>Anaerobic Digestion</td>
</tr>
<tr>
<td>BLAST</td>
<td>Basic local alignment search tool</td>
</tr>
<tr>
<td>BOD</td>
<td>Biochemical oxygen demand</td>
</tr>
<tr>
<td>CCD</td>
<td>Central composite design</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
</tr>
<tr>
<td>CSTR</td>
<td>Continuous stirrer tank reactor</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DPOME</td>
<td>Dark fermented palm oil mill effluent</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
</tr>
<tr>
<td>DoE</td>
<td>Design of experiment</td>
</tr>
<tr>
<td>EIA</td>
<td>Energy information administration</td>
</tr>
<tr>
<td>H2</td>
<td>Hydrogen</td>
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<tr>
<td>HPP</td>
<td>Hydrogen production potential</td>
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<tr>
<td>HPR</td>
<td>Hydrogen production rate</td>
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<tr>
<td>HY</td>
<td>Hydrogen yield</td>
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<tr>
<td>N</td>
<td>Normality</td>
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<tr>
<td>NPs</td>
<td>Nano-particles</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>POME</td>
<td>Palm oil mill effluent</td>
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<tr>
<td>RSM</td>
<td>Response surface methodology</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>TSS</td>
<td>Total suspended solids</td>
</tr>
<tr>
<td>UASB</td>
<td>Up-flow anaerobic sludge blanket</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile fatty acid</td>
</tr>
<tr>
<td>VSS</td>
<td>Volatile suspended solids</td>
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</tbody>
</table>
REFERENCES


production from organic matter. *Environmental science & technology*, 42(23), 8630-8640.


sugar beet thick juice in outdoor conditions. *international journal of hydrogen energy, 37*(2), 2044-2049.


