UNIVERSITI PUTRA MALAYSIA

ENHANCED MESOPHILIC BIO-HYDROGEN PRODUCTION BY CO-DIGESTION OF RAW RICE STRAW AND ACTIVATED SEWAGE SLUDGE

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By

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

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DEDICATION

To my dear husband, for his endless supports without which, accomplishments of this research were impossible.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

ENHANCED MESOPHILIC BIO-HYDROGEN PRODUCTION BY CO-DIGESTION OF RAW RICE STRAW AND ACTIVATED SEWAGE SLUDGE.

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NIKA ALEMAHDI

July 2015

Chair: Associate Professor Hasfalina Che Man ,PhD
Faculty: Institute of Advanced Technology

Huge amount of activated sewage sludge is being produced every year all over the world in wastewater treatment plants that if treated properly, has the potential to be used as inoculum and/or co-substrate for bio-hydrogen production. Activated sewage sludge and lignocellulosic waste material e.g rice straw have the potential to be used as a source for bio-hydrogen production. Pretreatment of lignocellulosic waste materials is often suggested by researchers to break the complex structure of these materials prior to dark fermentation. Pretreatment methods are costly, complicated and not environment-friendly due to high usage of acids, bases and energy. Therefore in this study try to find a way for bio-hydrogen production using raw materials. Batch bio-hydrogen production is carried out in serum bottles (150 mL) with maximum operation level of 120 mL and 30 mL headspace at mesophilic conditions (35°C). Activated sewage sludge (ASS) collected from inflow of a digestion tank in a wastewater treatment plant was used as the inoculum and co-substrate. Rice straw (RS) was used as the substrate. In order to achieve the highest bio-hydrogen yield and methanogens activity inhibition, heat treatment of inoculum was optimized at different exposure time and temperature ranges prior to the dark fermentation process. Three levels of time were defined (30, 45 & 60 min) and three levels of temperature (80, 90 and 100 °C) were chosen to conduct the experiments. Collected data was analysed using response surface methodology (RSM) as an analytical method. From the results, it was observed that the heat treatment of inoculum at 100 °C for 60 minutes produced the highest bio-hydrogen yield of 14.22 NmL H_2/g VS at concentration of 70.97 % in total produced biogas. The triplicate batch tests were conducted to verify the optimum condition introduced by RSM (100 °C for 60 minutes), resulted in 14.10 $\pm$ 0.2 NmL H_2/g VS at concentration of 69.45% in biogas. Production of 0.073 NmL CH_4/gVS at 0.17% concentration of total

biogas in these tests indicated a very successful achievement in inhibition of methanogens activity. The raw rice straw was also co-digested with heat-treated inoculum (100 °C for 60 minutes) for bio-hydrogen production at different operating process parameters. Three levels of volatile solids (VS) ratio (2:1, 4:1 and 6:1) and three levels of initial pH (4, 4.75 and 5.5) were defined as numerical variables. 4 categories of substrate size were tested to achieve the objective aforesaid: "Intact RS" [20-30 mm], "Intact RS ,Fine RS [0.25-0.5mm],Medium RS (0.5-2mm] and Large RS (2-20mm). Using RSM, the highest bio-hydrogen yield of 14.70 NmL /g VS was recognized at the optimum initial pH of 5.01 and VS ratio of 4.54 : 1 from the "Large RS" particles. Triplicate batch tests were done to produce bio-hydrogen applying optimized factors; these tests have average bio-hydrogen yield of 14.54 $\pm$ 0.29 NmL /g VS. By finding optimum VS ratio, pH and proper size of rice straw, we are able to achieve higher yield of bio-hydrogen production compared to other works in mesophilic conditions using mixed culture as inoculum and lignocellulosic waste materials as substrate. The overall results in this study indicated that co-digestion of raw rice straw as substrate and activated sewage sludge as both co-substrate and inoculum is a feasible approach for bio-hydrogen production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk ijazah Master sains

DIPERTINGKATKAN MESOPHILIC PENGELUARAN BIO-HIDROGEN OLEH RAKAN PENCERNAAN JERAMI PADI MENTAH DAN ENAP CEMAR KUMBAHAN DIAKTIFKAN.

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Sejumlah besar enapcemar kumbahan teraktif yang dihasilkan setiap tahun di seluruh dunia jika dirawat dengan betul mempunyai potensi untuk digunakan sebagai inokulum / substrat bersama untuk penghasilan bio-hidrogen. Enapcemar teraktif dan bahan buangan lignoselulosa contohnya jerami padi mempunyai potensi untuk digunakan sebagai sumber untuk penghasilan bio-hidrogen .Prarawatan bahan sisa lignoselulosa sering dicadangkan oleh penyelidik untuk memecahkan struktur kompleks bahan-bahan tersebut sebelum fermentasi gelap. Kaedah prarawatan adalah mahal, rumit dan tidak mesra alam kerana penggunaan asid, bes dan tenaga yang tinggi. Oleh itu di dalam kajian ini, percubaan dijalankan untuk mencari jalan untuk penghasilan bio-hidrogen menggunakan bahan-bahan mentah. Dalam kajian ini, penghasilan bio-hidrogen secara sesekelompok dijalankan dalam botol serum (150 mL) dengan tahap operasi maksimum 120 mL dan 30 mL ruang kepala pada keadaan mesofilik (35 ° C). Enap cemar kumbahan teraktif (ASS) yang dikumpul daripada aliran masuk tangki pencernaan dalam loji rawatan air kumbahan di Ibaraki, Jepun telah digunakan sebagai inokulum dan substrat-bersama. Jerami padi (RS) yang dituai dari ladang tempatan di Ibaraki, Jepun telah digunakan sebagai substrat. Untuk mencapai hasil tertinggi bio-hidrogen, perencatan aktiviti metanogen, rawatan haba inokulum telah dioptimumkan pada masa pendedahan dan sela-suhu yang berbeza sebelum proses fermentasi gelap. Tiga tahap masa telahditakrifkan (30, 45 & 60 min) dan tiga tahap suhu (80, 90 dan 100 ° C) telah dipilih untuk menjalankan eksperimen. Data yang diperolehi dianalisis dengan menggunakan kaedah tindakbalas permukaan (RSM) sebagai satu kaedah analisis.Daripadakeputusan, dapat diperhatikan bahawa rawatan haba inokulum pada suhu 100 ° C selama 60 minit menghasilkan bio-hidrogen yang tertinggi iaitu 14.22 mL H_2 / g VS pada kepekatan 70.97% daripada keseluruhan biogas dihasilkan. Tiga replikasi ujian sesekelompok telah dijalankan untuk mengesahkan keadaan optima yang diperkenalkan oleh RSM (100 ° C selama 60 minit), memghasilkan 14.10 ± 0.2 mL H_2 / g VS pada kepekatan 69.45% dalam biogas. Pengeluaran 0.073 mLCH4 / gVS padakepekatan 0.17% daripada jumlah biogas dalam ujian ini menunjukkan satu pencapaian yang baik dalam perencatan aktiviti metanogen.Jerami padi mentah juga diurai bersama dengan inokulum terawat haba (100 ° C selama 60 minit) untukpengeluaran bio-hidrogen pada parameter proses operasi yang berbeza; nisbah pepejal meruap (VS) substrat terhadap inokulum, pH awal dan saiz jerami padi .Tiga tahap nisbah VS (2: 1, 4: 1 dan 6: 1) dan tiga tahap pH awal (4, 4.75 dan 5.5) telah ditakrifkan sebagai pembolehubah berangka. 4 kategori saiz sustrat telah diuji untuk mencapai matlamat tersebut di atas.Jerami padi (RS) yang telah dipotong kepada kepingan bersaiz 20-30 mm dari hujung atas hingga kebahagian bawah batang telah dilabelkansebagai " RS Potong". Partikel jerami padi dengan saiz yang kurang dari pada atau sama dengan 500 mikron telah dilabelkan sebagai "RS Halus". Zarah jerami padi dengan saiz lebih dari 500 mikron dan kurang daripada 2 mm telah dilabelkan sebagai "RS Sederhana".Zarah jerami padi dengan saiz lebih daripada 2 mm dankurang daripada 20 mm telah dilabelkan sebagai "RS Besar". hasil bio-hidrogen tertinggi dikenalpasti diperolehi menggunakan RSM adalah 14.70 mL / g VS pada pH awal optimum 5.01, nisbah VS pada 4.54: 1 dan menggunakan "RS besar". Tiga replikasi ujian sesekelompok telah dilakukan untuk menghasilkan bio-hidrogen dengan mengaplikasikan faktor yang dioptimumkan ini. Ujian ini menghasilkan purata bio-hidrogen pada 14.54 ±0.29 ml /g VS. Dengan mencari nisbah pH optimum dan saiz jerami padi yang sesuai , penghasilan bio-hidrogen dapat ditingkatkan berbanding penyelidikan yang lain dalam keadaan mesofilik menggunakan kultur campuran sebagai inokulum dan bahan-bahan buangan lignoselulosa sebagai substrat. Keseluruhan keputusan dalam kajian ini menunjukkan bahawa menggunakan jerami padi mentah sebagai substrat dengan enapcemar kumbahan teraktif sebagai substrat bersama dan inokulum adalah pendekatan yang boleh dilaksanakan untuk penghasilan bio-hidrogen.

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APPROVAL

I certify that a Thesis Examination Committee has met on 8th July 2015 to conduct the final examination of Nika Alemahdi on her thesis entitled "Enhanced mesophilic bio-hydrogen production by co-digestion of raw rice straw and activated sewage sludge" 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 in Energy Engineering.
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LIST OF ABBRIVIATIONS

ASS	Activated Sewage Sludge
C	Carbon
CCD	Central composite design
CV	Coefficient of Variation
GC	Gas Chromatograph
HPY	Bio-hydrogen Production Yield
ha	Hectare
H_2	Hydrogen
RS	Rice Straw
NmL	Gas volume at Normal condition (0°C, 1 atm)
TS	Total solid
VS	Volatile solid

CHAPTER 1

INTRODUCTION

1.1 Overview of the study

Nowadays, environmental problems and increasing energy demand of people and industry push science and scientists to find a solution for energy shortage and global warming. One category of the alternative energies is biofuel which is popular because of its capacity to be produced from different kinds of biomass and wastes (Demirbas 2010). Producing energy from biomass is not just a key for energy shortage but a reliable solution to waste treatment and decrease environmental pollution.

Bio-hydrogen is known as the cleanest energy career, due to the product of its combustion which is only water. As the word "bio-hydrogen" suggests, this type of hydrogen is produced through some biological pathways. There are two different biological pathways to produce bio-hydrogen. The first category is "light-dependent" processes that can be either direct- or indirect-photolysis. The second category is named "light-independent". Dark fermentation, the most applied process, is classified as a dark independent process (Show et al., 2012). Compared to other biological pathways, dark fermentation has more potential for producing bio-hydrogen and it is more desirable. Since dark fermentation is not light dependent, there is no need to a vast area for production process and it is faster and operationally easier as well (Elsharnouby et al., 2013; Wang and Wan, 2009). On the other hand many different types of waste materials can be used as substrate in dark fermentation process (Chu et al., 2013; Gomez-Romero et al., 2014; Levin and Chahine, 2010; Panagiotopoulos et al., 2013).

Agricultural lignocellulosic waste materials are a promising source for biofuel production due to their abundance and low cost. Rice straw is one of the lignocellulosic waste materials that can be used as substrate. Although lignocellulosic materials are rich in carbon content, bio-hydrogen yield of direct digestion of them is quite low. Table 1.1 shows several studies of bio-hydrogen production from raw lignocellulosic waste materials in both thermophilic and mesophilic conditions. In all of these studies, substrates are raw and only size reductions in different levels is done. Bio-hydrogen yield is reported in Normal condition (273.15 °K, 1013.25 hPa).

Lignocellulosic materials have complex structures made from cellulose, hemicellulose, and lignin. Therefore, some scientists prefer pretreatments to obtain higher bio-hydrogen yield. Many different pretreatment methods have been used by researchers (Chang et al., 2011a; Lemée et al., 2012; Nasirian et al., 2011) to break the complex structure of lignocellulosic materials. Pretreatment methods are mostly classified as physical, biological, chemical and physiochemical. All types of pretreatment are costly due to high usage of energy and/or chemicals. Furthermore, Physical, chemical and physiochemical pretreatment processes are not environment-friendly due to the fact that plenty of energy, chemicals or both are used for these types of treatments (Agbor et al., 2011). Therefore it seems reasonable to find a way for high yield biohydrogen production from raw lignocellulosic waste material.

Biogas production is almost an ancient way to produce methane as an energy source (Bond and Templeton, 2011). Nowadays biogas production technology and recovery systems are well stablished. On the other hand, in some cases shifting from biogas to bio-hydrogen is considered. One of the most important reasons to replace bio-methane by bio-hydrogen is that bio-hydrogen is a carbon emission free energy career (Meher Kotay and Das, 2008; Show et al., 2012).

Table 1.1. Bio-hydrogen production from raw lignocellulosic materials

Substrate	Inoculum	T (°c)	H_2 yield (NmL H_2/g VS)	References
Wheat straw	Cow dung compost	36	1	(Fan et al., 2006b)
Corn straw	*Clostridium butyricum*	35	9	(Li and Chen, 2007)
Cornstalk	Anaerobic sludge	36	3.16	(Zhang et al., 2007)
Wheat straw	CSTR H2 sludge	36	6.4	(Nasirian et al., 2011)
Rice straw	Digested Sewage sludge	55	16.57	(Kim et al., 2012)
Sugarcane bagasse	*C. thermocellum or T. aotearoense*	55	8.4	(Cheng and Zhu, 2013)

Equation 1.1 shows chemical formulation of methane combustion. Combustion of one mole of methane releases one mole carbon dioxide and energy (Whiting and Azapagic, 2014) while combustion of one mole hydrogen releases only one mole water and energy. Equation 1.2 shows the chemical formulation of hydrogen combustion (Nasirian 2010).

$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$ + energy **Equation 1.1**

$H_2 + \frac{1}{2} O_2 \rightarrow H_2O$ + energy **Equation 1.2**

Although bio-hydrogen is a clean energy career, production of this gas rather than bio-methane will be irrational if production processes pollute environment more than bio-methane production and combustion processes.

Since bio-hydrogen is produced by certain bacteria, modifying bioreactor's environment condition for growth of organisms can significantly affect the yield of bio-hydrogen production. Factors such as inoculum, substrate, VS ratio, reactor type, nitrogen content, carbon content, temperature, pH etc... are some of the most important and controllable environmental factors for bio-hydrogen production (Wang and Wan, 2009).

1.2 Problem statement

Huge amount of wastewater is produced by urban areas every year. Wastewater treatment technology is used to manage this waste progressively. The volume of wastewater that produced by municipal and industrial units in Malaysia in 2011 was 2.97 billion cubic meters (IWK, 2011) that leads to production of activated sewage sludge (ASS) in most waste water treatment plants.

A well-established technology in wastewater treatment plants for energy production is methane production. Bio-methane is generated by digestion of activated sewage sludge (treated wastewater). Activated sewage sludge is rich with organic matters and the quantity of microorganism that is inhabitant in it is quite high (Kacprzak et al., 2005). These two characteristics give activated sewage sludge the potential to be used as both inoculum and co-substrate. Although activated sewage sludge is rich in nitrogen content which is necessary for microorganism activity, the yield of its bio-hydrogen production is low. One of the main reasons for this low bio-hydrogen production is natural existence of methanogen bacteria that consume hydrogen during the methane production process (Chang et al., 2011b). Different treatment methods can inhibit methanogens and increase yield of bio-hydrogen production using activated sewage sludge (Kim 2013; Penteado et al., 2013). These methods mostly are heat shock, alkali treatment, acid treatment, aeration, 2-bromoethanesulfonic acid (BESA) addition, sonication and long chain fatty acids (LCFA) addition (Chaganti et al., 2012).

Although a thermophilic fermentation process has higher yield of bio-hydrogen production, due to its required operation temperature it has a higher demand of energy compared to a mesophilic process. During a thermophilic fermentation process, temperature should be set to 40 - 80°C whereas for a mesophilic condition it could be set to 20 - 40 °C. Abundant amount of lignocellulosic materials and activated sewage sludge, huge and increasing rate of energy demand are known as the challenges in the century. Methane emits CO_2 to the atmosphere during its production and combustion processes. Lignocellulosic waste material pretreatment methods have high cost and are not environment-friendly ways of bio-hydrogen production. All these facts lead us to find a way for optimization of bio-hydrogen production from raw lignocellulosic waste materials and activated sewage sludge in mesophilic condition.

1.3 Objectives

The main objective of this study is to improve bio-hydrogen production yield in mesophilic condition using rice straw and activated sewage sludge. To achieve this goal two sub-objectives are defined as:

1. To determine the optimum heat treatment condition of activated sewage sludge as inoculum for bio-hydrogen production in existence of rice straw in batch bioreactor systems using "Response Surface Methodology (RSM)".

2. To determine the optimum VS ratio of substrate to inoculum, initial cultivation pH and substrate size for bio-hydrogen production in mesophillic condition by co-digestion of raw rice straw and sewage sludge in batch mode using Response Surface Methodology (RSM).

1.4 Scope of the study

In this study, activated sewage sludge from a wastewater treatment plant in Ibaraki, Japan, was collected as the Inoculum and co-substrate. Rice straw from a local farm in Ibaraki due to its abundance in East Asia was chosen as the substrate. Bio-hydrogen production using co-digestion of activated sewage sludge and rice straw was conducted in batch mode in lab scales. The highest yielding inoculum heat treatment condition for bio-hydrogen production in selected range of time and temperature was determined. The optimum initial cultivation pH and VS_{RS}: VS_{ASS} were assessed. Many different factors can affect bio-hydrogen production but these factors were chosen due to their importance, our time limitation and also limitation in number of running experiments and facilities.

1.5 Thesis layout

This thesis is comprised of five chapters. Chapter 1, is an introduction to the existing facts around the subject of energy shortage and potential of waste materials as a source. It also briefly mentions some of the challenges of bio-hydrogen production and suggests some promising solutions as research objectives. In Chapter 2, a detail review of other related works, an expanded explanation of problems and the benefit of suggested solution and methods are presented. Chapter 3, demonstrates the methodology of the research. It also explains materials' characteristics and preparation for the experiments. Chapter 4, presents the results of the experiments. It also discusses the effects of heat treatment condition of activated sewage sludge, VS ratio and initial cultivation pH on bio-hydrogen production. Suitable size of raw rice straw for biological hydrogen production is also discussed. Chapter 5, concludes the research and looks at the possible future works around the subject

REFERENCES

Ademark, P., Varga, A., Medve, J., Harjunpää, V., Torbjörn Drakenberg, Tjerneld, F., Stålbrand, H. (1998). Softwood Hemicellulose-Degrading Enzymes from Aspergillus Niger: Purification and Properties of a β-Mannanase. *Journal of Biotechnology*, 63(3), 199-210.

Agbor, V. B., Cicek, N., Sparling, R., Berlin, A., Levin, D. B. (2011). BiomassPretreatment: Fundamentals Toward Application. *Biotechnology Advances*, 29(6), 675-685.

Androga, D. D., Özgür, E., Eroglu, I., Gündüz, U., Yücel, M. (2011). Significance of Carbon to Nitrogen Ratio on the Long-Term Stability of Continuous Photofermentative Hydrogen Production. *International Journal of Hydrogen Energy*, 36(24), 15583-15594.

Argun, H., Kargi, F., Kapdan, I. K., Oztekin, R. (2008). Biohydrogen Production by Dark Fermentation of Wheat Powder Solution: Effects of C/N and C/P Ratio on Hydrogen Yield and Formation Rate. *International Journal of Hydrogen Energy*, 33(7), 1813-1819.

ASTM Standard. (2002). American Society for Testing and Materials , .

Azwar, M. Y., Hussain, M. A., Abdul-Wahab, A. K. (2014). Development of Biohydrogen Production by Photobiological, Fermentation and Electrochemical Processes: A Review. *Renewable and Sustainable Energy Reviews*, 31, 158-173.

Badiei, M., Asim, N., Jahim, J. M., Sopian, K. (2014). Comparison of Chemical Pretreatment Methods for Cellulosic Biomass. *APCBEE Procedia*, 9, 170-174.

Baghchehsaraee, B., Nakhla, G., Karamanev, D., Margaritis, A., Reid, G. (2008). The Effect of Heat Pretreatment Temperature on Fermentative Hydrogen Production using Mixed Cultures. *International Journal of Hydrogen Energy*, 33(15), 4064-4073.

Bao, M. D., Su, H. J., Tan, T. W. (2013). Dark Fermentative Bio-Hydrogen Production: Effects of Substrate Pre-Treatment and Addition of Metal Ions Or L-Cysteine. *Fuel*, 112, 38-44.

Barakat, A., Mayer-Laigle, C., Solhy, A., Arancon, R. A. D., de Vries, H., Luque, R. (2014). Mechanical Pretreatments of Lignocellulosic Biomass: Towards Facile and Environmentally Sound Technologies for Biofuels Production. *RSC Advances*, 4(89), 48109-48127.

Barakat, A., Monlau, F., Solhy, A., Carrere, H. (2015). Mechanical Dissociation and Fragmentation of Lignocellulosic Biomass: Effect of Initial Moisture, Biochemical and Structural Proprieties on Energy Requirement. Applied Energy, 142, 240-246.

Bisaillon, A., Turcot, J., Hallenbeck, P. C. (2006). The Effect of Nutrient Limitation on Hydrogen Production by Batch Cultures of Escherichia Coli. *International Journal of Hydrogen Energy*, 31(11), 1504-1508.

Bond, T., and Templeton, M. R. (2011). History and Future of Domestic Biogas Plants in the Developing World. *Energy for Sustainable Development*, 15(4), 347-354.

Chaganti, S. R., Kim, D., Lalman, J. A. (2012). Dark Fermentative Hydrogen Production by Mixed Anaerobic Cultures: Effect of Inoculum Treatment Methods on Hydrogen Yield. *Renewable Energy*, 48(0), 117-121.

Chang, A. C. C., Tu, Y., Huang, M., Lay, C., Lin, C. (2011a). Hydrogen Production by the Anaerobic Fermentation from Acid Hydrolyzed Rice Straw Hydrolysate. *International Journal of Hydrogen Energy*, 36(21), 14280-14288.

Chang, S., Li, J., Liu, F. (2011b). Evaluation of Different Pretreatment Methods for Preparing Hydrogen-Producing Seed Inocula from Waste Activated Sludge. *Renewable Energy*, 36(5), 1517-1522.

Cheng, J., and Zhu, M. (2013). A Novel Anaerobic Co-Culture System for Bio-Hydrogen Production from Sugarcane Bagasse. *Bioresource Technology*, 144, 623-631.

Chu, C., Tung, L., Lin, C. (2013). Effect of Substrate Concentration and pH on Biohydrogen Production Kinetics from Food Industry Wastewater by Mixed Culture. *International Journal of Hydrogen Energy*, 38(35), 15849-15855.

Datar, R., Huang, J., Maness, P., Mohagheghi, A., Czernik, S., Chornet, E. (2007). Hydrogen Production from the Fermentation of Corn Stover Biomass Pretreated with a Steam-Explosion Process. *International Journal of Hydrogen Energy*, 32(8), 932-939.

Davila-Vazquez, G., Alatriste-Mondragón, F., de León-Rodríguez, A., Razo-Flores, E. (2008). Fermentative Hydrogen Production in Batch Experiments using Lactose, Cheese Whey and Glucose: Influence of Initial Substrate Concentration and pH. *International Journal of Hydrogen Energy*, 33(19), 4989-4997.

de Vrije, T., Budde, M. A. W., Lips, S. J., Bakker, R. R., Mars, A. E., Claassen, P. A. M. (2010). Hydrogen Production from Carrot Pulp by the Extreme Thermophiles Caldicellulosiruptor Saccharolyticus and Thermotoga Neapolitana. *International Journal of Hydrogen Energy*, 35(24), 13206-13213.

Demirbas, A. (2010). Fuels from Biomass.*Biorefineries*, Springer London, 33-73.
Dincer, I., and Rosen, M. A. (2011). Sustainability Aspects of Hydrogen and Fuel Cell Systems. *Energy for Sustainable Development*, 15(2), 137-146.

Drapacho, C., Nhuam, N. P., Walker, T. H. (2008). *Biofuels Engineering Process Technology.* Mc Graw Hill, .

Elam, C. C., Padró, C. E. G., Sandrock, G., Luzzi, A., Lindblad, P., Hagen, E. F. (2003). Realizing the Hydrogen Future: The International Energy Agency's Efforts to Advance Hydrogen Energy Technologies. *International Journal of Hydrogen Energy*, 28(6), 601-607.

Elsharnouby, O., Hafez, H., Nakhla, G., El Naggar, M. H. (2013). A Critical Literature Review on Biohydrogen Production by Pure Cultures. *International Journal of Hydrogen Energy*, 38(12), 4945-4966.

Escobar, J. C., Lora, E. S., Venturini, O. J., Yáñez, E. E., Castillo, E. F., Almazan, O. (2009). Biofuels: Environment, Technology and Food Security. *Renewable and Sustainable Energy Reviews*, 13(6–7), 1275-1287.

Fan, L. T., Lee, Y., Beardmore, D. H. (1980). Mechanism of the Enzymatic Hydrolysis of Cellulose: Effects of Major Structural Features of Cellulose on Enzymatic Hydrolysis. *Biotechnology and Bioengineering*, 22(1), 177-199.

Fan, Y., Li, C., Lay, J., Hou, H., Zhang, G. (2004). Optimization of Initial Substrate and pH Levels for Germination of Sporing Hydrogen-Producing Anaerobes in Cow Dung Compost. *Bioresource Technology*, 91(2), 189-193.

Fan, Y., Zhang, G., Guo, X., Xing, Y., Fan, M. (2006a). Biohydrogen-Production from Beer Lees Biomass by Cow Dung Compost. *Biomass and Bioenergy*, 30(5), 493-496.

Fan, Y., Zhang, Y., Zhang, S., Hou, H., Ren, B. (2006b). Efficient Conversion of Wheat Straw Wastes into Biohydrogen Gas by Cow Dung Compost. *Bioresource Technology*, 97(3), 500-505.

Fan, Y., Zhang, Y., Zhang, S., Hou, H., Ren, B. (2006c). Efficient Conversion of Wheat Straw Wastes into Biohydrogen Gas by Cow Dung Compost. *Bioresource Technology*, 97(3), 500-505.

Fangkum, A., and Reungsang, A. (2011a). Biohydrogen Production from Sugarcane Bagasse Hydrolysate by Elephant Dung: Effects of Initial pH and Substrate Concentration. *International Journal of Hydrogen Energy*, 36(14), 8687-8696.

Fatih Demirbas, M., Balat, M., Balat, H. (2011). Biowastes-to-Biofuels. *Energy* Conversion and Management, 52(4), 1815-1828.

Gadow, S. I., Li, Y., Liu, Y. (2012). Effect of Temperature on Continuous Hydrogen Production of Cellulose. *International Journal of Hydrogen Energy*, 37(20), 15465-15472.

Garland, N. L., Papageorgopoulos, D. C., Stanford, J. M. (2012). Hydrogen and Fuel Cell Technology: Progress, Challenges, and Future Directions. *Energy Procedia*, 28, 2-11.

Gomez-Romero, J., Gonzalez-Garcia, A., Chairez, I., Torres, L., García-Peña, E. I. (2014). Selective Adaptation of an Anaerobic Microbial Community: Biohydrogen Production by Co-Digestion of Cheese Whey and Vegetables Fruit Waste. *International Journal of Hydrogen Energy*, 39(24), 12541-12550.

Greenwood, N. N., and Earnshaw, A. (1984). 2 - Chemical Periodicity and the Periodic Table.*Chemistry of the Elements*, Pergamon, 24-37.

Hakobyan, L., Gabrielyan, L., Trchounian, A. (2012). Bio-Hydrogen Production and the F0F1-ATPase Activity of Rhodobacter Sphaeroides: Effects of various Heavy Metal Ions. *International Journal of Hydrogen Energy*, 37(23), 17794-17800.

Hawkes, F. R., Dinsdale, R., Hawkes, D. L., Hussy, I. (2002). Sustainable Fermentative Hydrogen Production: Challenges for Process Optimisation. *International Journal of Hydrogen Energy*, 27(11–12), 1339-1347.

Infantes, D., González del Campo, A., Villaseñor, J., Fernández, F. J. (2011). Influence of pH, Temperature and Volatile Fatty Acids on Hydrogen Production by Acidogenic Fermentation. *International Journal of Hydrogen Energy*, 36(24), 15595-15601.

Ingram, T., Wörmeyer, K., Lima, J. C. I., Bockemühl, V., Antranikian, G., Brunner, G., Smirnova, I. (2011). Comparison of Different Pretreatment Methods for Lignocellulosic Materials. Part I: Conversion of Rye Straw to Valuable Products. *Bioresource Technology*, 102(8), 5221-5228.

Iranzo, M., Cañizares, J. V., Roca-Perez, L., Sainz-Pardo, I., Mormeneo, S., Boluda, R. (2004). Characteristics of Rice Straw and Sewage Sludge as Composting Materials in Valencia (Spain). *Bioresource Technology*, 95(1), 107-112.

Ivanova, G., Rákhely, G., Kovács, K. L. (2009). Thermophilic Biohydrogen Production from Energy Plants by Caldicellulosiruptor Saccharolyticus and Comparison with Related Studies. *International Journal of Hydrogen Energy*, 34(9), 3659-3670.

Izumi, K., Okishio, Y., Nagao, N., Niwa, C., Yamamoto, S., Toda, T. (2010). Effects of Particle Size on Anaerobic Digestion of Food Waste. *International Biodeterioration & Biodegradation*, 64(7), 601-608.

Jackson, R. S. (2014). 7 - Fermentation.*Wine Science (Fourth Edition)*, Academic Press, San Diego, 427-534.

Jia, X., Zhu, C., Li, M., Xi, B., Wang, L., Yang, X., Xia, X., Su, J. (2013). A Comparison of Treatment Techniques to Enhance Fermentative Hydrogen Production from Piggery Anaerobic Digested Residues. *International Journal of Hydrogen Energy*, 38(21), 8691-8698.

Junghare, M., Subudhi, S., Lal, B. (2012). Improvement of Hydrogen Production Under Decreased Partial Pressure by Newly Isolated Alkaline Tolerant Anaerobe, Clostridium Butyricum TM-9A: Optimization of Process Parameters. *International Journal of Hydrogen Energy*, 37(4), 3160-3168.

Kacprzak, M., Neczaj, E., Okoniewska, E. (2005). The Comparative Mycological Analysis of Wastewater and Sewage Sludges from Selected Wastewater Treatment Plants. *Desalination*, 185(1–3), 363-370.

Kamalaskar, L. B., Dhakephalkar, P. K., Meher, K. K., Ranade, D. R. (2010). High Biohydrogen Yielding Clostridium Sp. DMHC-10 Isolated from Sludge of Distillery Waste Treatment Plant. *International Journal of Hydrogen Energy*, 35(19), 10639-10644.

Kargi, F., Eren, N. S., Ozmihci, S. (2012). Bio-Hydrogen Production from Cheese Whey Powder (CWP) Solution: Comparison of Thermophilic and Mesophilic Dark Fermentations. *International Journal of Hydrogen Energy*, 37(10), 8338-8342.

Khandelwal, B., Karakurt, A., Sekaran, P. R., Sethi, V., Singh, R. (2013). Hydrogen Powered Aircraft : The Future of Air Transport. *Progress in Aerospace Sciences*, 60, 45-59.

Kim, M., Yang, Y., Morikawa-Sakura, M. S., Wang, Q., Lee, M. V., Lee, D., Feng, C., Zhou, Y., Zhang, Z. (2012). Hydrogen Production by Anaerobic Co-Digestion of Rice Straw and Sewage Sludge. *International Journal of Hydrogen Energy*, 37(4), 3142-3149.

Kim, S. (2013). Biological Hydrogen Production by Anaerobic Digestion of Food Waste and Sewage Sludge Treated using various Pretreatment Technologies.*Biodegradation*, 24(6), 753-764.

Lemée, L., Kpogbemabou, D., Pinard, L., Beauchet, R., Laduranty, J. (2012). Biological Pretreatment for Production of Lignocellulosic Biofuel. *Bioresource Technology*, 117, 234-241.

Levin, D. B., Carere, C. R., Cicek, N., Sparling, R. (2009). Challenges for Biohydrogen Production Via Direct Lignocellulose Fermentation. *International Journal of Hydrogen Energy*, 34(17), 7390-7403.

Levin, D. B., and Chahine, R. (2010). Challenges for Renewable Hydrogen Production from Biomass. *International Journal of Hydrogen Energy*, 35(10), 4962-4969.

Levin, D. B., Pitt, L., Love, M. (2004). Biohydrogen Production: Prospects and Limitations to Practical Application. *International Journal of Hydrogen Energy*, 29(2), 173-185.

Li, C., and Fang, H. H. P. (2007). Inhibition of Heavy Metals on Fermentative Hydrogen Production by Granular Sludge. *Chemosphere*, 67(4), 668-673.

Li, D., and Chen, H. (2007). Biological Hydrogen Production from Steam-Exploded Straw by Simultaneous Saccharification and Fermentation. *International Journal of Hydrogen Energy*, 32(12), 1742-1748.

Licht, S. (2014). Chapter 5 - Fermentation for Biofuels and Bio-Based Chemicals.*Fermentation and Biochemical Engineering Handbook (Third Edition)*, William Andrew Publishing, Boston, 59-82.

Limayem, A., and Ricke, S. C. (2012). Lignocellulosic Biomass for Bioethanol Production: Current Perspectives, Potential Issues and Future Prospects. *Progress in Energy and Combustion Science*, 38(4), 449-467.

Lin, C. Y., and Lay, C. H. (2005). A Nutrient Formulation for Fermentative Hydrogen Production using Anaerobic Sewage Sludge Microflora. *International Journal of Hydrogen Energy*, 30(3), 285-292.

Lin, C. Y., and Lay, C. H. (2004a). Carbon/nitrogen-Ratio Effect on Fermentative Hydrogen Production by Mixed Microflora. *International Journal of Hydrogen Energy*, 29(1), 41-45.

Lin, C. -., and Lay, C. H. (2004b). Effects of Carbonate and Phosphate Concentrations on Hydrogen Production using Anaerobic Sewage Sludge Microflora. *International Journal of Hydrogen Energy*, 29(3), 275-281.

Lin, C., and Cheng, C. (2006). Fermentative Hydrogen Production from Xylose using Anaerobic Mixed Microflora. *International Journal of Hydrogen Energy*, 31(7), 832-840.

Lin, P., Whang, L., Wu, Y., Ren, W., Hsiao, C., Li, S., Chang, J. (2007). Biological Hydrogen Production of the Genus Clostridium: Metabolic Study and Mathematical Model Simulation. *International Journal of Hydrogen Energy*, 32(12), 1728-1735.

Lo, Y., Su, Y., Cheng, C., Chang, J. (2011). Biohydrogen Production from Pure and Natural Lignocellulosic Feedstock with Chemical Pretreatment and Bacterial Hydrolysis. *International Journal of Hydrogen Energy*, 36(21), 13955-13963.

Luo, G., Xie, L., Zou, Z., Zhou, Q., Wang, J. (2010). Fermentative Hydrogen Production from Cassava Stillage by Mixed Anaerobic Microflora: Effects of Temperature and pH. *Applied Energy*, 87(12), 3710-3717.

Magnusson, L., Islam, R., Sparling, R., Levin, D., Cicek, N. (2008). Direct Hydrogen Production from Cellulosic Waste Materials with a Single-Step Dark Fermentation Process. *International Journal of Hydrogen Energy*, 33(20), 5398-5403.

Mars, A. E., Veuskens, T., Budde, M. A. W., van Doeveren, P. F. N. M., Lips, S. J., Bakker, R. R., de Vrije, T., Claassen, P. A. M. (2010). Biohydrogen Production from Untreated and Hydrolyzed Potato Steam Peels by the Extreme Thermophiles Caldicellulosiruptor Saccharolyticus and Thermotoga Neapolitana. *International Journal of Hydrogen Energy*, 35(15), 7730-7737.

Meher Kotay, S., and Das, D. (2008). Biohydrogen as a Renewable Energy resource—Prospects and Potentials. *International Journal of Hydrogen Energy*, 33(1), 258-263.

Mei, N., Zergane, N., Postec, A., Erauso, G., Ollier, A., Payri, C., Pelletier, B., Fardeau, M., Ollivier, B., Quéméneur, M. (2014). Fermentative Hydrogen Production by a New Alkaliphilic Clostridium Sp. (Strain PROH2) Isolated from a Shallow Submarine Hydrothermal Chimney in Prony Bay, New Caledonia. *International Journal of Hydrogen Energy*, 39(34), 19465-19473.

Meibom, P., and Karlsson, K. (2010). Role of Hydrogen in Future North European Power System in 2060. *International Journal of Hydrogen Energy*, 35(5), 1853-1863.

Mesa-Pérez, J. M., Rocha, J. D., Barbosa-Cortez, L. A., Penedo-Medina, M., Luengo, C. A., Cascarosa, E. (2013). Fast Oxidative Pyrolysis of Sugar Cane Straw in a Fluidized Bed Reactor. *Applied Thermal Engineering*, 56(1–2), 167-175.

Mohd Yasin, N. H., Rahman, N. A., Man, H. C., Mohd Yusoff, M. Z., Hassan, M. A. (2011). Microbial Characterization of Hydrogen-Producing Bacteria in Fermented Food Waste at Different pH Values. *International Journal of Hydrogen Energy*, 36(16), 9571-9580.

Motte, J. -., Escudié, R., Hamelin, J., Steyer, J. -., Bernet, N., Delgenes, J. -., Dumas, C. (2014). Substrate Milling Pretreatment as a Key Parameter for Solid-State Anaerobic Digestion Optimization. *Bioresource Technology*, 173, 185-192.

Mu, Y., Zheng, X., Yu, H. (2009). Determining Optimum Conditions for Hydrogen Production from Glucose by an Anaerobic Culture using Response Surface Methodology (RSM). *International Journal of Hydrogen Energy*, 34(19), 7959-7963.

Mu, Y., Zheng, X., Yu, H., Zhu, R. (2006). Biological Hydrogen Production by Anaerobic Sludge at various Temperatures. *International Journal of Hydrogen Energy*, 31(6), 780-785.

Muhamad, M. H., Sheikh Abdullah, S. R., Mohamad, A. B., Abdul Rahman, R., Hasan Kadhum, A. A. (2013). Application of Response Surface Methodology (RSM) for Optimisation of COD, NH3–N and 2,4-DCP Removal from Recycled Paper Wastewater in a Pilot-Scale Granular Activated Carbon Sequencing Batch Biofilm Reactor (GAC-SBBR). *Journal of Environmental Management*, 121, 179-190.

Nasirian, N. (2010). Biohydrogen Production from Agricultural Lignocellulosic Waste Material by Dark Fermentation- Method Development and Process Optimization. .

Nasirian, N., Almassi, M., Minaei, S., Widmann, R. (2011). Development of a Method for Biohydrogen Production from Wheat Straw by Dark Fermentation. *International Journal of Hydrogen Energy*, 36(1), 411-420.

Nissilä, M. E., Lay, C., Puhakka, J. A. (2014). Dark Fermentative Hydrogen Production from Lignocellulosic Hydrolyzates – A Review. *Biomass and Bioenergy*, 67(0), 145-159.

Pakarinen, O., Lehtomäki, A., Rintala, J. (2008). Batch Dark Fermentative Hydrogen Production from Grass Silage: The Effect of Inoculum, pH, Temperature and VS Ratio. *International Journal of Hydrogen Energy*, 33(2), 594-601.

Panagiotopoulos, I. A., Bakker, R. R., de Vrije, T., Claassen, P. A. M., Koukios, E. G. (2012). Dilute-Acid Pretreatment of Barley Straw for Biological Hydrogen Production using Caldicellulosiruptor Saccharolyticus. *International Journal of Hydrogen Energy*, 37(16), 11727-11734.

Panagiotopoulos, I. A., Pasias, S., Bakker, R. R., de Vrije, T., Papayannakos, N., Claassen, P. A. M., Koukios, E. G. (2013). Biodiesel and Biohydrogen Production from Cotton-Seed Cake in a Biorefinery Concept. *Bioresource Technology*, 136, 78-86.

Patterson, T., Esteves, S., Dinsdale, R., Guwy, A., Maddy, J. (2013). Life Cycle Assessment of Biohydrogen and Biomethane Production and Utilisation as a Vehicle Fuel. *Bioresource Technology*, 131, 235-245.

Penteado, E. D., Lazaro, C. Z., Sakamoto, I. K., Zaiat, M. (2013). Influence of Seed Sludge and Pretreatment Method on Hydrogen Production in Packed-Bed Anaerobic Reactors. *International Journal of Hydrogen Energy*, 38(14), 6137-6145.

Phowan, P., and Danvirutai, P. (2014). Hydrogen Production from Cassava Pulp Hydrolysate by Mixed Seed Cultures: Effects of Initial pH, Substrate and Biomass Concentrations. *Biomass and Bioenergy*, 64, 1-10.

Ramos, C., Buitrón, G., Moreno-Andrade, I., Chamy, R. (2012). Effect of the Initial Total Solids Concentration and Initial pH on the Bio-Hydrogen Production from Cafeteria Food Waste. *International Journal of Hydrogen Energy*, 37(18), 13288-13295.

Scoma, A., Bertin, L., Fava, F. (2013). Effect of Hydraulic Retention Time on Biohydrogen and Volatile Fatty Acids Production during Acidogenic Digestion of Dephenolized Olive Mill Wastewaters. *Biomass and Bioenergy*, 48(0), 51-58.

Shin, H., Youn, J., Kim, S. (2004). Hydrogen Production from Food Waste in Anaerobic Mesophilic and Thermophilic Acidogenesis. *International Journal of Hydrogen Energy*, 29(13), 1355-1363.

Show, K. Y., Lee, D. J., Tay, J. H., Lin, C. Y., Chang, J. S. (2012). Biohydrogen Production: Current Perspectives and the Way Forward. *International Journal of Hydrogen Energy*, 37(20), 15616-15631.

Silva, G. G. D., Couturier, M., Berrin, J., Buléon, A., Rouau, X. (2012). Effects of Grinding Processes on Enzymatic Degradation of Wheat Straw. *Bioresource Technology*, 103(1), 192-200.

Singhal, Y., and Singh, R. (2014). Effect of Microwave Pretreatment of Mixed Culture on Biohydrogen Production from Waste of Sweet Produced from Benincasa Hispida. *International Journal of Hydrogen Energy*, 39(14), 7534-7540.

Sørensen, B. (1991). A History of Renewable Energy Technology. *Energy Policy*, 19(1), 8-12.

Spear, B. (2014). Coal – Parent of the Industrial Revolution in Great Britain: The Early Patent History. *World Patent Information*, .

Taherzadeh, M. J., and Karimi, K. (2008). Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. *International Journal of Molecular Science.*, 9, 1621-1651.

Utama, N. A., Fathoni, A. M., Kristianto, M. A., McLellan, B. C. (2014). The End of Fossil Fuel Era: Supply-Demand Measures through Energy Efficiency. *Procedia Environmental Sciences*, 20, 40-45.

Vijayaraghavan, K., Ahmad, D., Khairil Bin Ibrahim, M. (2006). Biohydrogen Generation from Jackfruit Peel using Anaerobic Contact Filter. *International Journal of Hydrogen Energy*, 31(5), 569-579.

Walker, L. P., and Wilson, D. B. (1991). Enzymatic Hydrolysis of Cellulose: An Overview. *Bioresource Technology*, 36(1), 3-14.

Wang, J., and Wan, W. (2009). Factors Influencing Fermentative Hydrogen Production: A Review. *International Journal of Hydrogen Energy*, 34(2), 799-811.

Wang, J., and Wan, W. (2008a). Comparison of Different Pretreatment Methods for Enriching Hydrogen-Producing Bacteria from Digested Sludge. *International Journal of Hydrogen Energy*, 33(12), 2934-2941.

Wang, J., and Wan, W. (2008b). Effect of Temperature on Fermentative Hydrogen Production by Mixed Cultures. *International Journal of Hydrogen Energy*, 33(20), 5392-5397.

White, C. M., Steeper, R. R., Lutz, A. E. (2006). The Hydrogen-Fueled Internal Combustion Engine: A Technical Review. *International Journal of Hydrogen Energy*, 31(10), 1292-1305.

Whiting, A., and Azapagic, A. (2014). Life Cycle Environmental Impacts of Generating Electricity and Heat from Biogas Produced by Anaerobic Digestion. *Energy*, 70, 181-193.

Wong, Y. M., Wu, T. Y., Juan, J. C. (2014). A Review of Sustainable Hydrogen Production using Seed Sludge Via Dark Fermentation. *Renewable and Sustainable Energy Reviews*, 34, 471-482.

Xing, D., Ren, N., Wang, A., Li, Q., Feng, Y., Ma, F. (2008). Continuous Hydrogen Production of Auto-Aggregative Ethanoligenens Harbinense YUAN-3 Under Non-Sterile Condition. *International Journal of Hydrogen Energy*, 33(5), 1489-1495.

Yasin, N. H. M., Mumtaz, T., Hassan, M. A., Abd Rahman, N. (2013). Food Waste and Food Processing Waste for Biohydrogen Production: A Review. *Journal of Environmental Management*, 130, 375-385.

Yokoyama, H., Waki, M., Ogino, A., Ohmori, H., Tanaka, Y. (2007). Hydrogen Fermentation Properties of Undiluted Cow Dung. *Journal of Bioscience and Bioengineering*, 104(1), 82-85.

Yoo, C. G., Lee, C. W., Kim, T. H. (2011). Optimization of Two-Stage Fractionation Process for Lignocellulosic Biomass using Response Surface Methodology (RSM). *Biomass and Bioenergy*, 35(12), 4901-4909.

Yossan, S., O-Thong, S., Prasertsan, P. (2012). Effect of Initial pH, Nutrients and Temperature on Hydrogen Production from Palm Oil Mill Effluent using Thermotolerant Consortia and Corresponding Microbial Communities. *International Journal of Hydrogen Energy*, 37(18), 13806-13814.

Yue, D., You, F., Snyder, S. W. (2014). Biomass-to-Bioenergy and Biofuel Supply Chain Optimization: Overview, Key Issues and Challenges. *Computers & Chemical Engineering*, 66, 36-56.

Zecca, A., and Chiari, L. (2010). Fossil-Fuel Constraints on Global Warming. *Energy Policy*, 38(1), 1-3.

Zhang, J., Li, Y., Zheng, H., Fan, Y., Hou, H. (2015). Direct Degradation of Cellulosic Biomass to Bio-Hydrogen from a Newly Isolated Strain Clostridium Sartagoforme FZ11. *Bioresource Technology*, 192, 60-67.

Zhang, K., Ren, N., Guo, C., Wang, A., Cao, G. (2011). Effects of various Pretreatment Methods on Mixed Microflora to Enhance Biohydrogen Production from Corn Stover Hydrolysate. *Journal of Environmental Sciences*, 23(12), 1929-1936.

Zhang, M., Fan, Y., Xing, Y., Pan, C., Zhang, G., Lay, J. (2007). Enhanced Biohydrogen Production from Cornstalk Wastes with Acidification Pretreatment by Mixed Anaerobic Cultures. *Biomass and Bioenergy*, 31(4), 250-254.

Zhao, P., Shen, Y., Ge, S., Yoshikawa, K. (2014). Energy Recycling from Sewage Sludge by Producing Solid Biofuel with Hydrothermal Carbonization. *Energy Conversion and Management*, 78, 815-821.

Zheng, Y., Zhao, J., Xu, F., Li, Y. (2014). Pretreatment of Lignocellulosic Biomass for Enhanced Biogas Production. *Progress in Energy and Combustion Science*, 42, 35-53.