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Biohydrogen Production from Pineapple Biomass Residue using Immobilized Co-culture of *Clostridium sporogenes* and *Enterobacter aerogenes*

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Abstract

In this study, the co-culture bacteria of *Clostridium sporogenes* and *Enterobacter aerogenes* were immobilized onto two different support materials: loofah sponge and activated carbon (AC) sponge. Both immobilized co-cultures were used in the batch fermentation of pineapple residues for biohydrogen production. The performance of both immobilized loofah and AC sponge was compared with free cell (FC) co-culture in terms of biohydrogen cumulative production and production rate within 48 hr fermentation time. It was found that the immobilized co-culture on AC sponge produced the highest rate of biohydrogen of 35.9 mmol/hr/$L_{substrate}$ compared to loofah and FC co-culture after 24 hr fermentation. However, in terms of preservation of biohydrogen production rate, loofah as a support showed better consistency in terms of performance for 48 hr fermentation time compared to AC. This study also showed that the pH of substrate has a relation to the optical density (OD_{600}) reduction of the bacteria, which could affect biohydrogen production rate.

Keywords: Batch fermentation, bacteria co-culture, immobilization, biohydrogen production.

1.0 INTRODUCTION

Biohydrogen is an alternative energy that can be produced by biological routes such as fermentation from renewable feedstock like agricultural waste, food waste, industrial waste, and municipal waste. Biological process might have a lower production rate than other processes but it is worthy as the operational cost is much lower with better environmental benefits. As fermentation process can be carried out at ambient pressure and temperature conditions, energy consumption is not intensive; hence, the process holds high potential to be commercialized in future energy production.

There are two main routes of fermentation: aerobic (presence of oxygen) and anaerobic (absence of oxygen). Compared to aerobic, anaerobic fermentation commonly has better performance of organic compound conversion to hydrogen with the utilization of bacteria through acidogenesis and acetogenesis processes. Anaerobic microorganisms such as anaerobic fermentative bacteria are commonly used in anaerobic fermentation because they are able to utilize carbon substrates to release hydrogen and also byproducts [1]. Hydrogen-producing bacteria such as *Clostridium sp.* is the most well-known bacteria that is suitable for higher biohydrogen production [2]. In order to maximize the production in short fermentation time, co-culture technique is the best option as it may improve culturing and cell behavior [3, 4].

Co-culture bacteria can be used either in a free or an immobilized form on suitable support materials. Currently, immobilized cell has been widely used for hydrogen production either in laboratory or industrial scale as an alternative to enhance microorganisms' activity in a fermentation system [2–17]. The most applied method for immobilization is cell entrapment [4–8, 16, 18, 20–23], in which microorganisms are enclosed in a porous matrix to allow cell-substrate diffusion and cells-products away from the material utilized. The advantages of immobilized cultures include reducing risk of contamination [21], higher metabolic activity [4], reusable [4, 5], and easier separation of solids and liquids [22].

In this study of the production of biohydrogen, immobilized method was applied to sustain the bacteria for the maximum utilization of pineapple substrate. Two types of support materials, loofah sponge and activated carbon (AC) sponge, were used for the new combination co-culture of *Enterobacter aerogenes* (*E. aerogenes*) and *Clostridium sporogenes* (*C. sporogenes*). *E. aerogenes* was selected because it is a facultative anaerobe that can survive if oxygen is present. *E. aerogenes* commonly utilizes oxygen and simultaneously provides anaerobic condition to a strict anaerobe, *C. sporogenes*. The main objective of this work is to compare biohydrogen production of the immobilized co-culture on both support materials with free co-culture. The optical density of bacteria and pH condition of substrate were monitored to relate both factors to biohydrogen performance.

2.0 METHODOLOGY

2.1 Pineapple Substrate Preparation

Fresh pineapple waste was obtained from fruit stalls at a local market. In this experiment, only pineapple peels were used and subjected to steam heat pretreatment (autoclaved). The pineapple peels were chopped into small pieces. Afterwards, the chopped pineapple waste was crushed using a steel blender (Waring Commercial Blender) with distilled water in the ratio of 1:2. Next, the mixture was filtrated to obtain the hydrolysate or extract for characterization analysis. After characterization, the hydrolysate was stored in a refrigerator at 4 °C and restored to ambient temperature (25 °C) prior to use. The substrate was neutralized to pH 7 every time before it was mixed with inoculum for fermentation.

2.2 Co-culture and Immobilization Preparation

Facultative anaerobe (*E. aerogenes*–ATCC 13048) and a strict anaerobe (*C. sporogenes* – ATCC 19404) purchased from Microbiologics (Saint Cloud, USA) were utilized as the co-culture to perform fermentation process. Commercial loofah sponge and AC sponge were used as the support materials to retain the co-culture bacteria. For inoculum (co-culture) preparation, both bacteria were activated onto the agar before cultivated into nutrient broth. Next, approximately 0.2 ± 0.1 (OD_{600}) of *E. aerogenes* and *C. sporogenes* were mixed carefully and aseptically, and then incubated for 24 hr (overnight) before immersion of the support materials. Meanwhile, loofah and activated carbon (AC) sponges were cut into pieces (1 ± 0.2 cm × 1 ± 0.2 cm) and soaked in boiling water for 30 min. After that, the sponges were washed under tap water before left in distilled water for 24 hr (changed 3 to 4 times). This is essential to remove all fine suspended particles [8]. Next, the sponges were dried in an oven at 70 °C overnight before dried uniformly in a desiccator. The sponges were then soaked inside 90 ml of nutrient broth (30% of working volume) and incubated for another 24 hr at 130 rpm and 37 °C prior to use. The sponges were mixed with the substrate for further fermentation. The inoculum was directly used or mixed with pineapple substrate for mobilize co-culture set-up without soaking any sponge for immobilization.

2.3 Experimental Condition (Batch Set-Up)

The schematic diagram of experimental setup is shown in Figure 1. The fermentation of collected pineapple waste was carried out in a 500 ml Dreschel bottle with the working volume of 300 ml. 210 ml of pineapple waste was first added to a 500 ml Dreschel bottle and the inoculum or/and immobilized sponges were then added to the substrate. The initial pH of the substrate was adjusted using 0.5 M sodium hydroxide (NaOH) to achieve the initial pH of 7. Nitrogen sparging was applied to provide anaerobic condition for the fermentation process and the bottles were sealed and placed in a water bath to maintain the culture medium at 33 ± 1 °C. Mixing was provided by a stirring magnetic bar in the bottle. In this experiment, gas purging was only applied at the early stage of the set-up and manual mixing was applied at every 12 hr interval. The volume of biogas collected was read from the scales on the gas collection measuring cylinder through water displacement method at 12 hr interval. The volume of water displaced in the measuring cylinder was determined as the volume of biohydrogen produced whereas the gas captured in polyvinylidene fluoride (PVDF) gas bag was analyzed using gas chromatography (Agilent Technologies, 6890N, Network GC System) equipped with a thermal conductivity detector (TCD) to obtain the composition and amount of biohydrogen produced.

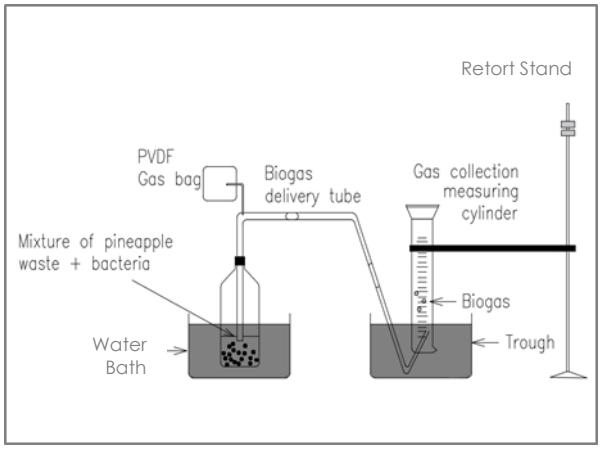


Figure 1. Schematic diagram of the experimental set up

3.0 RESULTS AND DISCUSSION

Figure 2 presents cumulative biohydrogen production of immobilized activated carbon (AC), loofah sponge (LS), and free cell (FC) throughout the experiment. AC and LS reached the maximum production during 48 hr fermentation time while FC reached the maximum cumulative biohydrogen at 36 hr and decreased slightly at 48 hr fermentation time. For FC, during 48 hr fermentation time, the volume of biogas captured increased but the biohydrogen percentage was low due to high content of carbon dioxide (CO_2). The decrement of biohydrogen production might be due to exhausted nutrient that potentially occurred after a certain extended period. In this stage, a metabolic shift of biohydrogen-producing pathways to biohydrogen-inhibiting biochemical reaction occurred [24]. Biohydrogen formation could be reduced by the formation of fatty acid with high CO_2 content in biogas composition.

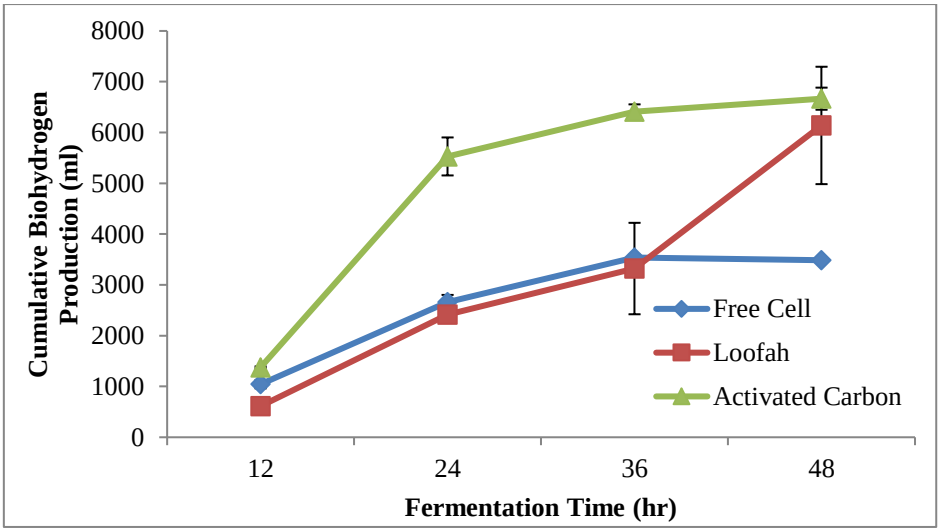


Figure 2. Cumulative biohydrogen production for different immobilized support materials

Figure 3 shows the biohydrogen production rate obtained for FC co-culture, as well as immobilized co-culture onto LS and AC sponge. As can be seen in Fig. 3, the highest rate obtained using immobilized AC sponge was obtained at 35 mmol/hr/$L_{substrate}$ after 24 hr fermentation time. However, the production rate dropped approximately 22% after 36 hr and further decreased by approximately 16% towards the end of fermentation. Meanwhile, immobilized LS facilitated a consistent increase of biohydrogen production rate from only 7.86 mmol/hr/$L_{substrate}$ at the early stage and reached 21.54 mmol/hr/$L_{substrate}$ after 48 hr fermentation time, which is up to 60% increment. For FC co-culture, the highest rate was achieved at 12 hr fermentation time and dropped at every interval until the end of observation. The different production

trends by immobilized co-culture on the support materials of AC and LS might be due to the retention on the sponge [8]. It could also be due to imperfect immobilized cell loading amount that resulted in poor mass transfer and led to poor biohydrogen production [25].

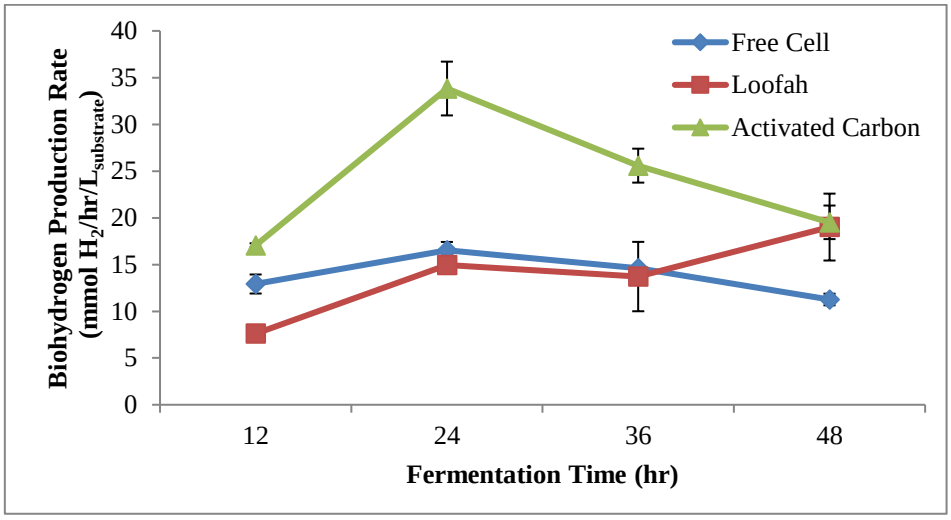


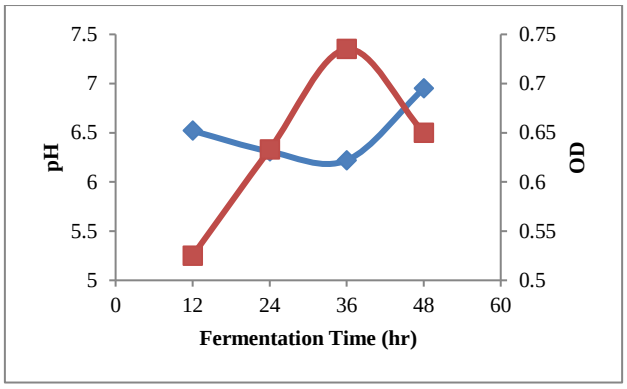
Figure 3. Biohydrogen production rate for different immobilized support materials

The relation of biohydrogen production rate with optical density (OD_{600}) of bacteria and pH changes of the substrate was studied, and this is shown in Figure 4. The graphs showed that the highest OD concentration of the co-culture was mostly achieved after 24 hr fermentation time and afterwards they started to reach the stationary phase. This indicated the growth of the bacteria during the middle of exponential stage and started to utilize the pineapple substrate, which produced high amount of biohydrogen. Slightly lower biohydrogen started to be produced as the inoculum grew slightly before reaching the stationary phase.

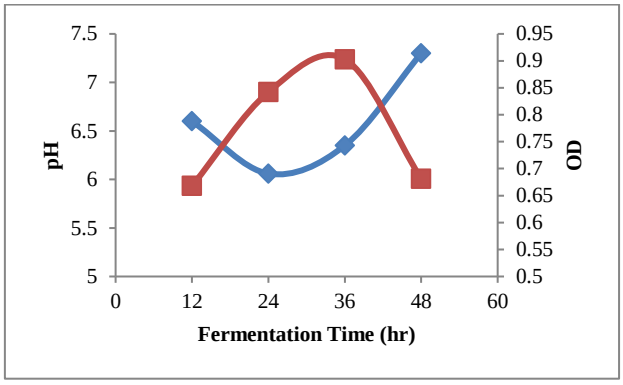
In addition, the production also remained high during the acidogenesis stage for all conditions based on the reduction of pH from the initial pH value. The reduction of pH occurred due to the production of organic acid as the byproduct instead of biohydrogen. Clearly, methanogenic hydrogen consumers are absent in this fermentation system because no methane was detected in this study and biohydrogen reached the maximum production at a certain interval.

The pH reduction started when the inoculum was mixed with the substrate and it is in contrast with the OD_{600} of the bacteria, which increased gradually until 36 hr fermentation time. Initial pH is a very important parameter as it will determine the survival of the inoculum introduced. As it is introduced at right or suitable pH, it will grow and acidogenesis will start. All samples showed that the pH dropped at only moderately acidic pH around 5.8 to 6 and the cumulative biohydrogen produced was higher, which is similar to a previous discussion that moderately acidic pH is good for inhabiting methanogenic activity and enhancing hydrogen-producing bacteria to produce biohydrogen [20, 25]. In this study, the pH dropped during 12 hr and 24 hr fermentation time, in which the production of CO_2 is high or maximum. It suggests that the reduction of pH happened due to the production of soluble metabolites [16] and also due to the increase of CO_2 content in biogas.

Overall, it could be emphasized that in this experiment, the co-culture inoculum seems to have taken a short period of time (within 24 hr) to survive in the substrates, despite that inoculum usually takes some time to adapt to the environment before starting to produce biohydrogen [20, 26, 27]. The initial OD_{600} of every co-culture used was in the range of 0.2–0.3. After 12 hr, the OD_{600} increased and doubled at 24 hr and started to decrease after 36 hr.



(a)



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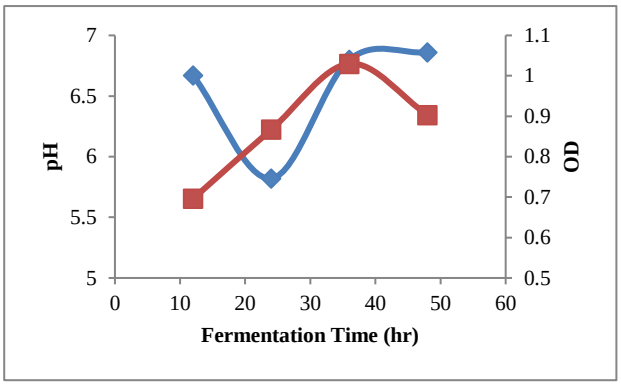

(c)

Figure 4. Relationship of biohydrogen production rate with optical density and pH changes for (a) free cell, (b) immobilized loofah sponge, and (c) activated carbon sponge

4.0 CONCLUSION

The biohydrogen production rate using co-culture by immobilization reached up to 39 mmol/hr/$L_{substrate}$. The combination of co-culture *E. aerogenes* and *C. sporogenes* worked well with the utilization pineapple substrate. Immobilization using AC sponge was determined as a better support material compared to LS, which could enhance approximately 67% of the production rate compared to FC co-culture.

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References

[1] P. Sivagurunathan, P. Anburajan, J. H. Park, G. Kumar, H. D. Park, and S. H. Kim (2017). Mesophilic biogenic H2 production using galactose in a fixed bed reactor. *Int. J. Hydrogen Energy*, vol. 42, no. 6, pp. 3658–3666.

[2] K. Bru *et al.* (2012). "nnovative CO2 pretreatment for enhancing biohydrogen production from the organic fraction of municipal solid waste (OFMSW). *Int. J. Hydrogen Energy*, vol. 37, no. 19, pp. 14062–14071.

[3] L. Goers, P. Freemont, and K. M. Polizzi. (2014). Co-culture systems and technologies: taking synthetic biology to the next level. *J. R. Soc. Interface*, vol. 11, no. 96.

[4] P.M. Kao, B.-M. Hsu, K.-H. Huang, C.-W. Tao, C.-M. Chang, and W.-T. Ji. (2014). Biohydrogen Production by Immobilized Co-culture of Clostridium Butyricum and Rhodopseudomonas Palustris. *Energy Procedia*, vol. 61, pp. 834–837.

[5] G. Balachandar, N. Khanna, and D. Das. (2013). *Chapter 6 - Biohydrogen Production from Organic Wastes by Dark Fermentation*, 1st ed., Elsevier B.V.

[6] T. Keskin, E. Aksöyek, and N. Azbar. (2011). Comparative analysis of thermophilic immobilized biohydrogen production using packed materials of ceramic ring and pumice stone. *Int. J. Hydrogen Energy*, vol. 36, no. 23, pp. 15160–15167.

[7] T. Keskin, L. Giusti, and N. Azbar, "Continuous biohydrogen production in immobilized biofilm system versus suspended cell culture," *Int. J. Hydrogen Energy*, vol. 37, no. 2, pp. 1418–1424, 2012.

[8] A. A. Rahma,. (2013). Biohydrogen Production by Modified Anaerobic Fluidized Bed Reactor (AFBR) Using Mixed Bacterial Cultures in Thermophilic Condition . Master Thesis. Retrieved from http://library.iugaza.edu.ps/thesis/111749.pdf.

[9] L. Singh, M. F. Siddiqui, A. Ahmad, M. H. A. Rahim, M. Sakinah, and Z. A. Wahid. (2013). Biohydrogen production from palm oil mill effluent using immobilized mixed culture, *J. Ind. Eng. Chem.*, vol. 19, no. 2, pp. 659–664.

[10] L. Singh, Z. A. Wahid, M. F. Siddiqui, A. Ahmad, M. H. A. Rahim, and M. Sakinah. (2013). Biohydrogen production from palm oil mill effluent using immobilized Clostridium butyricum EB6 in polyethylene glycol, *Process Biochem.*, vol. 48, no. 2, pp. 294–298.

[11] B. Hu. (2013). Biological Hydrogen Production via Self Immobilized Bacteria, *J. Chem. Inf. Model.*, vol. 53, no. 9, pp. 1689–1699.

[12] a. Tenca, a. Schievano, F. Perazzolo, F. Adani, and R. Oberti. (2011). Biohydrogen from thermophilic co-fermentation of swine manure with fruit and vegetable waste: Maximizing stable production without pH control, *Bioresour. Technol.*, vol. 102, no. 18, pp. 8582–8588.

[13] N. Basak, A. K. Jana, D. Das, and D. Saikia. (2014). Photofermentative molecular biohydrogen production by purple-non-sulfur (PNS) bacteria in various modes: The present progress and future perspective, *Int. J. Hydrogen Energy*, vol. 39, no. 13, pp. 6853–6871.

[14] P. Koskinen. (2008). *The Development and Microbiology of Bioprocesses for the Production of Hydrogen and Ethanol by Dark Fermentation*.

[15] H. Argun and F. Kargi. (2011). Bio-hydrogen production by different operational modes of dark and photo-fermentation : An overview," *Int. J. Hydrogen Energy*, vol. 36, no. 13, pp. 7443–7459.

[16] P. T. Sekoai and E. B. Gueguim Kana. (2013). A two-stage modelling and optimization of biohydrogen production from a mixture of agro-municipal waste, *Int. J. Hydrogen Energy*, vol. 38, no. 21, pp. 8657–8663.

[17] L. Xiao, Z. Deng, K. Y. Fung, and K. M. Ng. (2013). Biohydrogen generation from anaerobic digestion of food waste," *Int. J. Hydrogen Energy*, vol. 38, no. 32, pp. 13907–13913.

[18] W. Han, Z. Wang, H. Chen, X. Yao, and Y. Li. (2011). Simultaneous Biohydrogen and Bioethanol Production from Anaerobic Fermentation with Immobilized Sludge, vol. 2011, pp. 1–6.

[19] L. Zhao *et al.* (2011). Enhanced bio-hydrogen production by immobilized Clostridium sp. T2 on a new biological carrier, *Int. J. Hydrogen Energy*, vol. 37, no. 1, pp. 162–166.

[20] M. S. de Graaff, H. Temmink, G. Zeeman, and C. J. N. Buisman. (2010). Anaerobic Treatment of Concentrated Black Water in a UASB Reactor at a Short HRT, *Water*, vol. 2, pp. 101–119.

[21] E. Topics. (2007). Immobilisation of yeasts for continuous fermentation," pp. 418–425.

[22] L. Singh, Z. A. Wahid, M. F. Siddiqui, A. Ahmad, M. H. A. Rahim, and M. Sakinah.(2013). Application of immobilized upflow anaerobic sludge blanket reactor using Clostridium LS2 for enhanced biohydrogen production and treatment efficiency of palm oil mill effluent, *Int. J. Hydrogen Energy*, vol. 38, no. 5, pp. 2221–2229.

[23] P. Sekoai, K. Yoro, and M. Daramola. (2016). Batch Fermentative Biohydrogen Production Process Using Immobilized Anaerobic Sludge from Organic Solid Waste, *Environments*, vol. 3, no. 4, p. 38.

[24] "Immobilization Studies Using Nanomagnetite to Enhance Biohydrogen Production Trevor Seelert Department of Chemical Engineering McGill University , Montreal," no. September, 2013.

[25] C. Mao, Y. Feng, X. Wang, and G. Ren. (2015). Review on research achievements of biogas from anaerobic digestion, *Renew. Sustain. Energy Rev.*, vol. 45, pp. 540–555.

[26] A. Babaee and J. Shayegan. (2011). Effect of Organic Loading Rates (OLR) on Production of Methane from Anaerobic Digestion of Vegetables Waste, *World Renew. energy Congr.*, no. November, pp. 411–417.

[27] N. H. M. Yasin, T. Mumtaz, M. A. Hassan, and N. Abd Rahman (2013). Food waste and food processing waste for biohydrogen production: A review, *J. Environ. Manage.*, vol. 130, no. June 2011, pp. 375–385.