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INFLUENCE OF VARIOUS COMBINATIONS OF HEAT PRETREATMENT ON HYDROGEN FERMENTATION FROM DEOILED JATROPHA WASTE USING MIXED MICROFLORA

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Abstract

This study emphasis the hydrogen fermentation from de-oiled Jatropha waste (DJW), a secondary and promising waste from biodiesel industry via influence of various combination of heat pretreatment using sewage sludge as inoculum. Batch experiments were carried out at a DJW concentration of 50 g/L, temperature of 55°C and an initial cultivation pH of 7. The peak hydrogen production rate and hydrogen yield of 16.3 mmol H_2/L-d and 1.10 mmol H_2/g volatile solids (VS), respectively, were obtained when untreated DJW was fermented with heat-treated seed sludge. The peak methane production rate and methane yield of 6.10 mmol CH_4/L-d and 1.80 mmol CH_4/g VS were observed from the control experiment. In addition, the untreated sludge with heat-treated substrate produced methane revealed that heat-treatment is necessary to inhibit methane producers. Mixed acid type metabolic pathway was observed in all the pretreatment conditions and the soluble metabolic products mainly composed of butyrate and acetate with smaller quantities of other acids and alcohols. The peak energy generation rate and energy yield were calculated as 94 kJ/L-d and 1.8 kJ/g VS added, respectively.

Keywords: De-oiled Jatropha Waste (DJW), energy yield, heat pretreatment, hydrogen production rate, soluble metabolic products

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1. Introduction

Rapid fossil fuels consumption leads to an increased level of CO_2 into the atmosphere which causing global warming, acid rain and severe threats to the natural eco-systems. In order to alleviate the negative effects of CO_2 into the environment, utilization of non-carbonaceous fuels are an utmost important in this advanced modern world. In this spotlight, hydrogen seems to be an excellent potential fuel for the future fuel usage, because of its high energy content (122 kJ/g). Besides, while combustion it releases only the water vapor (Das and Veziroglu, 2000) as by product, meanwhile no other toxic greenhouse gases release shows a positive impact on the surroundings and promises cleaner earth. In current status, mostly hydrogen is derived from non-renewable resources like methane and oil/naphtha (Ewan and Allen, 2005, Petrone et al., 2017). All these processes are economically challenging and also the depletion of available non-renewable resources should be considered. Hence, an alternative way is need for the hydrogen production to make its economy as cheaper and affordable. Additionally, hydrogen fermentation of lignocellulose/solid waste aids in waste removal which helps in the growth of the sustainable biofuel development process (Kumar et al., 2015).

Biohydrogen production can be categorized as biophotolysis of water, dark fermentation and photo

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fermentation. Photo fermentation and biophotolysis are energy intensive and high cost processes (Hallenbeck and Benemann, 2002). Recently, dark fermentation is extensively studied over bio-photolysis and photo-fermentation for hydrogen production due to comparatively less maintenance, affordable operation cost and conversion of wide range of organic wastes like lignocellulosic wastes, industrial wastewaters, etc., into hydrogen and also provides an economical process from low cost feed stocks and ultimately reduction of pollutants (Kumar et al., 2015). However, an efficient pretreatment strategy is necessary for the development of better hydrogen-production in a fermentative bioprocess system. Avoiding the hydrogen consumers like methanogens (Danko et al., 2008) is a mandatory process for an efficient hydrogen generation systems. Hence, in regards to industrial scale applications, it may also require large quantities of readily available hydrogen-producing seeds in case of process upsets and sudden recovery.

The significant advances made in the biodiesel sector using Jatropha biomass resulted in the generation of enormous amount of solid waste as it's by product, which is called De-oiled Jatropha waste (DJW). This DJW could create major environmental problems due to its toxic (mainly due to the presence of phorbol esters) and recalcitrant nature, if not treated properly (Kumar et al., 2013; Kumar and Lin, 2013). Previously, this recalcitrant waste has been used for anaerobic digestion to generate methane (Chandra et al., 2012; Raheman and Mondal, 2012; Sen et al., 2013). But due to the high value of hydrogen, it would be meaningful and sustainable to develop a hydrogen fermentation process from DJW. Besides, it could be used for other purposes such as valorizing protein, microalgae growth support, co composting, and enzyme production. Furthermore, the application of mild acid (HCl) hydrolysis method to recover the sugars from DJW is more economical. In our previous study the enzyme and acid pretreated DJW showed a suitable feedstock for hydrogen fermentation (Kumar et al., 2013). Thus, here an attempt is made to utilize the DJW in hydrogen fermentation, which leads to dual benefit as the generation of clean energy (H_2), and the environmental friendly treatment of this toxic/recalcitrant waste.

2. Material and methods

2.1. Seed sludge and substrate

The seed sludge was obtained from a Municipal Wastewater Treatment Plant located in central Taiwan. The characteristics of the sludge were: pH 6.9, alkalinity 1.23 g/L as $CaCO_3$, volatile suspended solids (VSS) 32 g/L, total chemical oxygen demand (TCOD) 48.7 g/L, and total carbohydrate 7.1 g/L as glucose. The collected sludge was stored in a refrigerator at 7°C prior to use. DJW was collected from a processing unit of biodiesel industry in Chunghwa (central Taiwan). It was dried at 105°C for 24 h to remove the moisture and then powdered using blender. The cellulosic characteristics of DJW was analyzed as 42.3 % of its total solid content and the analytical methods were described elsewhere (Kumar et al., 2012).

2.2. Experimental setup for selection of efficient combination

The batch serum vials (holding capacity- 225 mL) with a working volume of 150 mL were used for H_2 fermentation. The contents included are 30 mL of various combinations of heat pretreated sewage sludge seed, 7.5 g of dried substrate (making the initial substrate concentration to 50g/L), 5 mL solution for adjusting pH to 7.0 using either HCl or NaOH. Tap water is used to make the final working volume. Three combinations (II, III, and IV) of heat treatments were employed and they are as follows, as in case II, only the seed sludge was heat-treated at 95°C for 30 minutes. In case of experiment III, substrate was heat-treated for 30 minutes at 95°C and untreated sludge was added.

In the case of IV, seed sludge and substrate were both heat-treated for 30 minutes at 95°C (III) and control experiment (I) was kept without any treatment. The conditions and purpose of the combinations are represented in Table 1. Argon gas was purged in the headspace for 5 minutes to create an anaerobic environment. These batch bottles were kept in a reciprocal air-bath shaker at 150 rpm with the temperature controlled at 55°C for fermentation. The volume and composition of gas were analyzed every day. The soluble metabolic product (SMP) analysis was done at the end of fermentation. Fermentation was carried out till the gas production was observed as zero.

2.3. Solid, liquid and gas analysis

The characteristics of seed sludge such as pH, total solid (TS), chemical oxygen demand (COD) and volatile suspended solids (VSS) were analyzed as mentioned is APHA procedure (APHA, 1995). The alcohols and volatile fatty acids (VFAs) were analyzed with a gas chromatograph having a flame ionization detector (Shimadzu GC-14, Japan).

Table 1. Heat pretreatment combinations

S. No	*Symbol*	*Description*	*Purpose*
1	I	Control	Act as a blank
2	II	HT-S+UT-DJW	To eliminate the methanogens
3	III	HT-DJW+UT-S	To know the Possible contribution from the substrate
4	IV	HT-DJW+HT-S	Logical combination

Note: HT- heat treated, DJW- Deoiled Jatropha Waste, UT- untreated

The volume of biogas was determined by a glass syringe and composition was analyzed with a gas chromatograph equipped with a thermal conductivity detector (China Chromatograph 8700T) as indicated in our earlier studies (Chen et al., 2002; Lin and Chang, 1999). To measure total carbohydrate concentration Anthrone-sulphuric acid method was used (Koehler, 1952). Cellulose, hemicelluloses and lignin were determined by FIBERTEC^{TM} 1020 (M6).

2.4. Model simulation

The hydrogen production potential (P), maximum hydrogen production rate (R_m) and lag phase time (λ) under each experimental condition were obtained from the modified Gompertz equation (Eq. 1) and estimated using Sigma plot software 10.0 (Systat Software Inc., USA) (Kumar et al., 2012).

$$H(t) = P \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

where: $H(t)$ is the cumulative hydrogen production (mL); P is the hydrogen production potential (mL); Rm is the maximum hydrogen production rate (mL/h); e is 2.71828; λ is the lag phase time (h) and t is the cultivation time (h).

Hydrogen production rate (HPR L H_2/L-d) was defined as R_m value divided by the reactor volume (0.15 L) and multiplied by a day (24 h), Hydrogen yield (HY mL H_2/g VS) was defined as hydrogen production per gram of dried DJW added (7.5 g in 150 mL). Methane production was also calculated in the similar way.

3. Results and discussion

3.1. Hydrogen production profile

The effect of various combinations of heat pretreatment methods on the enrichment of H_2 evolving bacterial population and their H_2 production efficiency was studied by using DJW as substrate. It is evident from the Fig. 1 that the heat pretreatment applied on the seed sludge and substrate has significant influence on the H_2 generation and biogas evolution rate. The results obtained under various heat pretreatment conditions were listed in Table 2, where it can be seen that the heat treatment of sludge alone (II) was shown to be the most efficient method by providing the peak HPR (16.3 mmol H_2/L-d) and HY (1.10 mmol H_2/g VS) values compared to the other tested combinations and the control experiment.

The treatment efficiency for hydrogen production was followed as II > III > IV > I. The kinetic analysis data also showed that the batch experiments significantly influenced by the bacterial lag phase time. The lag phase variations of the experimented conditions were followed as IV, II, III and I and the values were as 23.2, 12.8 5.3 and 1.6 h respectively. On the other hand the methane production was observed in control and the case III (untreated sludge+ heat treated substrate combination).

The peak MPR (6.10 mmol CH_4/L-d) and MY (1.80 mmol CH_4/g VS) were observed in the control experiment. This would be indicating that there is a significant microbial activity of methanogens in sludge when it is not heat treated. Hence, these results proved that heat treatment of sewage sludge is necessary for hydrogen formation.

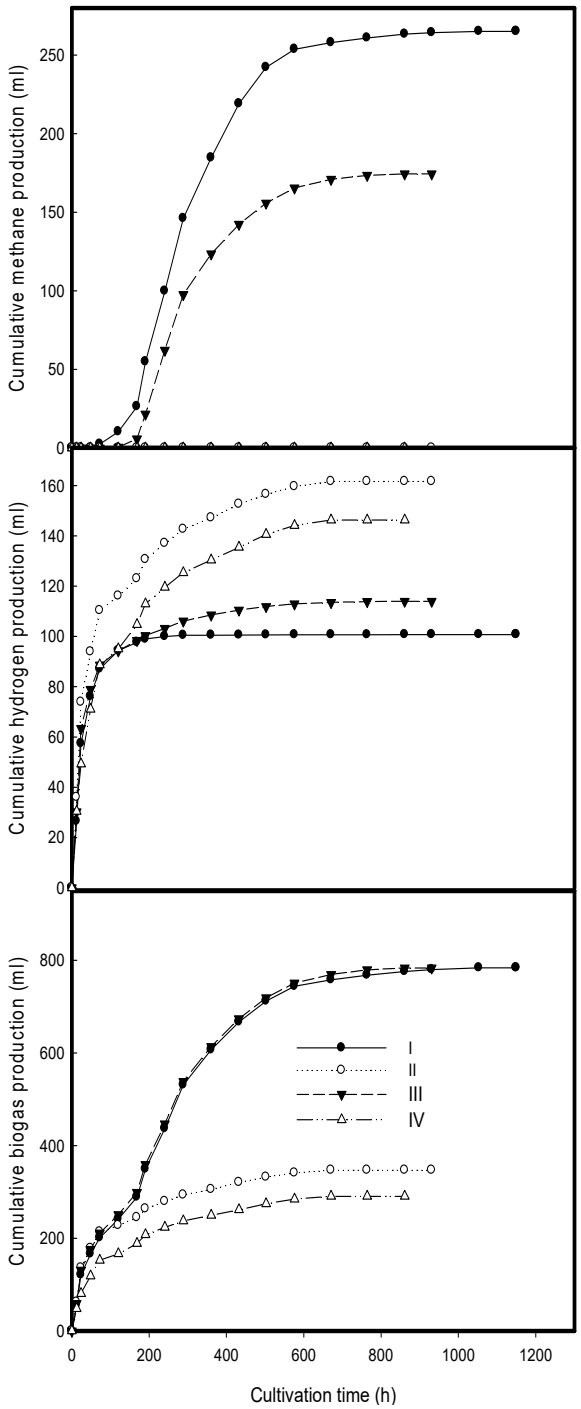

Fig. 1. Cumulative hydrogen and biogas production profile of batch experiments

Table 2. Hydrogen and biogas production of various experimental conditions

Experiment	Final pH	Total biogas (mL)	H_2 (mL)	CH_4 (mL)	Modified Gompertz equation parameter values for H_2 production				Modified Gompertz equation parameter values for CH_4 production				HPR_{max} (mmol H_2/L-d)	HY_{max} (mmol H_2/g VS)	MPR_{max} (mmol CH_4/L-d)	MY_{max} (mmol CH_4/g VS)
					P (mL)	R_m (mL/h)	Λ (h)	R^2	P (mL)	R_m (mL/h)	λ (h)	R^2				
I	6.8	784	101	265	99.4	2.1	1.6	1	263.3	0.91	136	1	13.9	0.7	6.1	1.8
II	5.7	347	162	ND	152	2.48	13	0.9	ND	ND	ND	ND	16.3	1.1	ND	ND
III	6.7	783.5	114	174	107.4	1.72	5.3	0.9	171.3	0.69	159	1	11.4	0.8	4.5	1.2
IV	5.9	290.5	146	ND	139.6	1.61	23	0.9	ND	ND	ND	ND	10.6	1	ND	ND

I-CTRL, II-HT sludge+ untreated substrate, III-HT substrate+ untreated sludge, IV-HT sludge+ HT substrate

3.2. Impact of heat pretreatment combinations on SMP distribution

The production of VFAs or solvents during the anaerobic fermentation process is often a crucial signal in monitoring the efficiency of hydrogen producing cultures (Dabrock et al., 1992; Khanal et al., 2004; Monti et al., 2013).

While glucose has been used as a substrate the maximum theoretical yields of 4 mol and 2 mol hydrogen were produced via acetic and butyric acid pathways as shown in the Eqs. (2) and (3). In contrast, propionic acid is considered as an undesirable product of hydrogen fermentation. As shown in Fig. 2, butyric acid and acetic acid were the dominant soluble metabolites observed in all the experiments. Monitoring the acetate/butyrate ratio is also an essential factor for the process control of the anaerobic bio-reactor system (Lin and Lay, 2004).

According to the soluble metabolic product distribution the efficiency of the fermentation system could be determined. The available H_2 from substrate utilization, during H_2 fermentation could be determined using the butyrate/acetate (B/A ratio) as an indicator for evaluating the effectiveness of H_2 production on various heat pretreatment conditions. In all the conditions the ABE (Acetone-Butanol-Ethanol) fermentation was observed and this might be due to the presence of the solventogenic clostridia present in the sludge.

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (2)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (3)$$

Theoretically the metabolic pathways of the three main products; acetic, propionic and n-butyric acid from xylose (hemi cellulose component of DJW) are as follows (Eqs. 4-6):

$$C_5H_{10}O_5 + 1.67H_2O \rightarrow 1.67CH_3COOH + 3.33H_2 + 1.67CO_2$$
$$\Delta G^o = \textit{-195.5 kJ/mol} \quad (4)$$

$$C_5H_{10}O_5 + 1.67H_2 \rightarrow 1.67CH_3CH_2COOH + 1.67H_2O$$
$$\Delta G^o = \textit{-315:1 kJ/mol} \quad (5)$$

$$C_5H_{10}O_5 \rightarrow 0.83CH_3(CH_2)_2COOH + 1.67H_2 + 1.67CO_2$$
$$\Delta G^o = \textit{-233.9 kJ/mol} \quad (6)$$

Theoretically the metabolic pathways of the acetic acid, propionic acid and n-butyric acid, ethanol from glucose (cellulose component of DJW) are as follows (Eqs. 7-11):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2$$
$$\Delta G^o = \textit{-215.7 kJ/mol} \quad (7)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3CH_2COOH + 2H_2O$$
$$\Delta G^o = \textit{-357.9 kJ/mol} \quad (8)$$

$$C_6H_{12}O_6 \rightarrow CH_3(CH_2)_2COOH + 2H + 2CO_2$$

ΔG^o= - *261.5 kJ/mol* (9)

$4H_2 + 2HCO^{-3} + H^+ \rightarrow CH_3COO^- + 2H_2O$

ΔG^o= - *70.3 kJ/mol* (10)

$CH_3COOH + H_2 \rightarrow C_2H_5OH + 4H_2O$

ΔG^o= *-49.51 kJ/mol* (11)

According to Eqs. (2)-(11), the production of acetic and n-butyric acids favors the production of hydrogen, and in contrast, the production of propionic acid results in less hydrogen turn out (Kumar et al., 2012). Eqs. (2) and (3) show the major metabolic pathway involved in hydrogen production. From these equations it might be concluded that the optimum pH range of 6.0~7.0 is favorable for acetate production. At this pH the reaction is driven predominantly towards acetate production (Khanna et al., 2011), while lower pH of 5.5~6.0 seemed to favor butyric acid production resulting in higher production of hydrogen.

Hallenbeck (2005) stated that acidic conditions favor the increased conversion of pyruvate to lactic acid. Since production of lactic acid reduces NAD+ to NADH+ and this diverts the reductants from the hydrogen production reactions. From our studies it is also clearly shown that less pH drop from initial (final pH 6.4~6.8) favors the acetate mediated methanogenesis pathway in the conditions I and III. While the lower final pH (5.4~5.8) was observed in the pretreated conditions of heat shock of sludge alone (II) and their combinations such as sludge + substrate.

Fig. 2 summarize the production of soluble metabolites obtained in this study. It shows that the experimental condition produces acetone, ethanol, butanol, acetic acid, propionic acid, butyric acid and valerate. The major VFA produced were butyrate (HBu) and acetate (HAc). The HAc/HBu ratio calculated for the combinational and control experiments are shown in Fig. 2. The highest HBu/HAc ratio was observed as 3.1 in experiment II, followed by 1.6 (experiment III), and 1.3 (experiment IV). The lowest B/A ratio of 1.0 was observed for control (I) experiment. These results indicated that the difference in soluble metabolic products might be due to the divergence of microbial communities by the initial stimulation of different combination of heat pretreatment conditions. Most of the studies reveal that heat treatment is a better treatment mainly due to its easy operation and effectiveness in enrichment of hydrogen producing bacteria (Lin et al., 2012).

Heat treatment of the lignocellulose biomass without any chemical agents is used to increase the hydrolysis rate of the complex polysaccharides, which can be further accessed by the anaerobic microorganisms to improve the fermentation ability (Kumar et al., 2009). However, the major limitation of this method is the formation of the fermentation inhibitors usually at higher temperatures, thus in this study the heat-treatment was conducted at lower temperature range of 95^{0}C for 30 min and to examine their effects of fermentation ability of the DJW. The results showed that thermal treatment enhanced hydrogen production in the conditions III and IV than the control without heat treatment. However, when both sludge and the substrate was pretreated by heat treatment there is a drop in cumulative biogas production and hydrogen production was noted, however the HY was not significantly affected with approximately over ~10% value of 1.0 mmol/gVS. The VFA pattern also affected significantly in case of heat treated substrate and sludge rather than heat treated sludge alone.

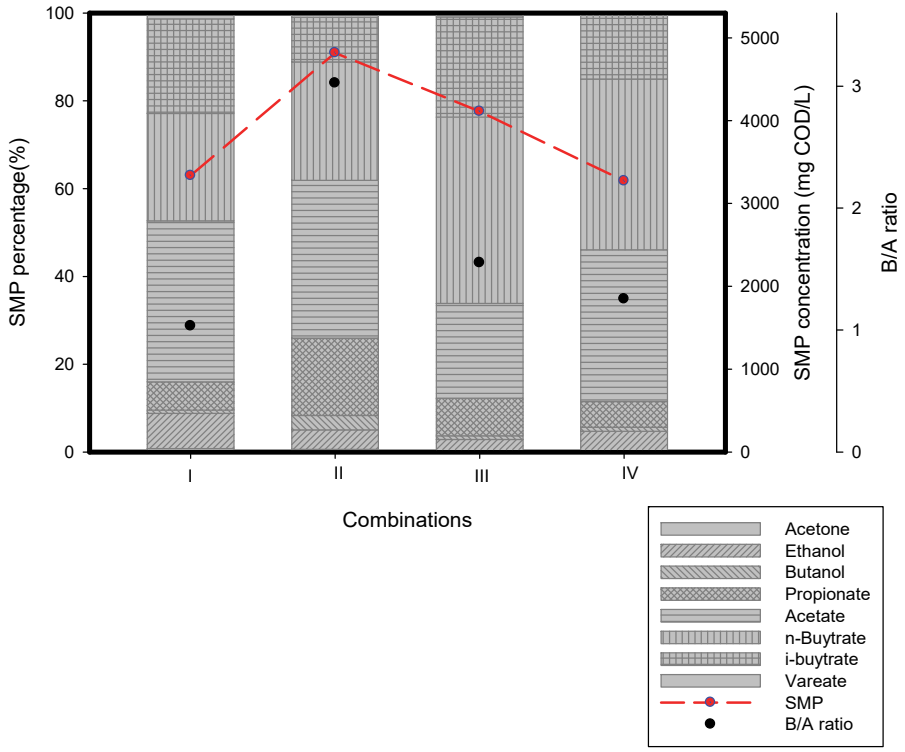

Fig. 2. SMP productions in the combinations of heat pretreatment batch experiments

The observed variance in the HPR and HY might be attributed by the generation of by-products during the heat treatment of the substrate and also the changes in the microbial community pattern under various substrate availability natures. Thus, heat-treatment of the sludge alone is recommended for efficient hydrogen production from DJW.

3.3. Energy production analysis

The details of the total energy production values (Kumar et al., 2012; US DOE, 2001) at all heat pretreatment combination conditions for the H_2 fermentation are given in Fig. 3. The peak total energy production rate (TEPR) and total energy yield (TEY) were obtained as 9405 J/L-d and 1800 J/g VS, while the untreated sludge and untreated substrate (control) employed on hydrogen production. The main reason for the high energy value from the control experiment is the significant contribution from methane which is bearing high energy value. The effluent of this fermentation contains various high energy solvents such as ethanol and butanol. The downstream process of these solvents could remarkably increase the energy values. Besides, the effluent could be used to produce methane by anaerobic digestion in order to increase the overall energy production values of DJW via dark fermentation.

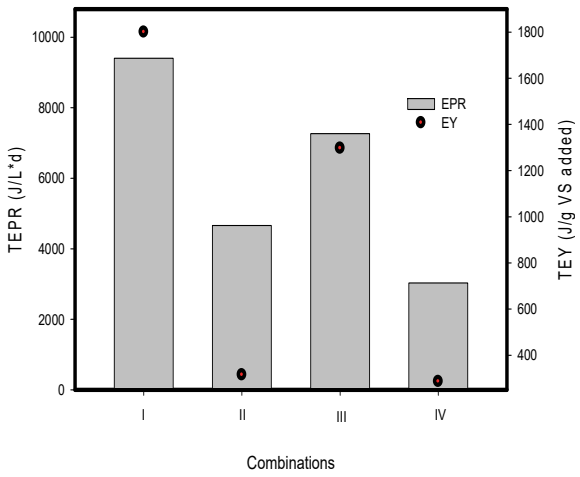

Fig. 3. Total energy production values of various pretreatment combinations

4. Conclusions

The hydrogen production potential of DJW via various heat pretreatment combinations of sewage sludge and substrate has been demonstrated. The heat pretreatment is not much significant to the substrate used in the fermentation, however crucial to the seed source to enrich the hydrogen producers. The maximal hydrogen production potential (P) and maximal hydrogen production rate (Rm) were attained as 152 mL, 2.48 mL/h respectively. The energy values revealed that hydrogen fermentation using lignocellulose based DJW is a feasible bioenergy production process.

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References

APHA, (1995), *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Nineteenth Edition, New York, USA.

Chen C.C., Lin C.Y., Lin M.C., (2002), Acid-base enrichment enhances anaerobic hydrogen production process, *Applied Microbiology and Biotechnology*, **58**, 224-228.

Chandra R., Vijay V.K., Subbarao P.M.V., Khura T.K., (2012), Production of methane from anaerobic digestion of jatrophaand pongamia oil cakes, *Applied Energy*, **93**, 148-159.

Dabrock B., Bahl H., Gottschalk G., (1992), Parameters affecting solvent production by *Clostridium pasteurianum*, *Applied and Environmental Microbiology*, **58**, 1233-1239.

Das D., Veziroglu T.N., (2001), Hydrogen production by biological process: a survey of literature, *International Journal of Hydrogen Energy*, **26**, 13-28

Danko A.S., Pinheiro F., Abreu A.A., Alves M.M., (2008), Effect of methanogenic inhibitors, inocula type, and temperature on biohydrogen production from food components, *Environmental Engineering and Management Journal*, **7**, 531-536.

Ewan B.C.R., Allen R.W.K., (2005), A figure of merit assessment of the routes to hydrogen, *International Journal of Hydrogen Energy*, **30**, 809-819.

Hallenbeck P.C., Benemann J.R., (2002), Biological hydrogen production fundamentals and limiting processes, *International Journal of Hydrogen Energy*, **27**, 1185-1193.

Hallenbeck P.C., (2005), Fundamentals of the fermentative production of hydrogen, *Water Science and Technology*, **27**, 21-29.

Koehler L.H., (1952), Differentiation of carbohydrate by enthrones reaction rate and colour intensity, *Analytical Chemistry*, **24**, 1576-1579.

Khanal S.K., Li L., Sung S., (2004), Biological hydrogen production: effects of pH and intermediate products, *International Journal of Hydrogen Energy*, **29**, 1123-1131.

Khanna N., Kotay S.M., Gilbert J.J., Das D., (2011), Improvement of biohydrogen production by *Enterobacter cloacae* IIT-BT 08 under regulated pH, *Journal of Biotechnology*, **152**, 9-15.

Kumar P., Barrett D.M., Delwiche M.J., Stroeve P., (2009) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production, *Industrial and Engineering Chemistry Research*, **48**, 3713-3729.

Kumar G., Lay C.H., Chu C.Y., Wu J.H., Lee S.C., Lin C.Y., (2012), Seed inocula for biohydrogen production from

biodiesel solid residues, *International Journal of Hydrogen Energy*, **37**, 15489-15495.

Kumar G., Lin C.Y., (2013), Bioconversion of de-oiled Jatropha Waste (DJW) to hydrogen and methane gas by anaerobic fermentation: Influence of substrate concentration, temperature and pH, *International Journal of Hydrogen Energy*, **38**, 63-72.

Kumar G., Sen B., Lin C.Y., (2013), Pretreatment and hydrolysis methods for recovery of fermentable sugars from de-oiled Jatropha waste, *Bioresource Technology*, **145**, 275-279.

Kumar G., Baknoyi P., Sivagurunathan P., Nemosthy N., Belafi-bako K., Lin C.Y., (2015), Improved microbial conversion of de-oiled Jatropha waste into biohydrogen via inoculum pretreatment: process optimization by experimental design approach, *Biofuels Research Journal*, **5**, 209-214.

Lin C.Y., Chang R.C., (1999), Hydrogen production during the anaerobic acidogenic conversion of glucose, *Journal of Chemical Technology and Biotechnology*, **74**, 498-500.

Lin C.Y., Lay C.H., (2004), Effects of carbonate and phosphate concentration on hydrogen production using anaerobic sewage sludge microflora, *International Journal of Hydrogen Energy*, **29**, 275-281.

Lin C.Y., Lay C.H., Sen B., Chu C.Y., Kumar G., Chen C.C., Chang J.C., (2012), Fermentative hydrogen production from wastewaters: A review and prognosis. *International Journal of Hydrogen Energy*, **37**, 15632-15642.

Monti M., Bertin L., Scoma A., Fava F., (2013), Production of biohydrogen and volatile fatty acids from dephenolized olive mill wastewaters in a sequential two-step anaerobic process, *Environmental Engineering and Management Journal*, **12**, 85-88.

Petrone M.T., Stoppiello G., De Bari I. (2017), Environmental impact of second-generation sugars production from cardoon residues, *Environmental Engineering and Management Journal*, **16**, 1769-1774.

Raheman H., Mondal S., (2012), Biogas production potential of jatropha seed cake, *Biomass and Bioenergy*, **37**, 25-30.

US DOE, (2001), US Department of Energy. Properties of hydrogen, On line at: http://www.eere.energy.gov/hydrogenandfuelcells/fuelcells/pdfs/ fcm01r0.pdf.

Sen K., Shanthi M., Sen B., (2013), Rapid and high yield biogas production from Jatropha seed cake by co-digestion with bagasse and addition of Fe^{2+}, *Environmental Technology*, **34**, 2989-2994.