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REVIEW ARTICLE

BIOHYDROGEN PRODUCTION USING ANAEROBIC DECOMPOSITION AND CLASSFYING THE BIOMASSES BASED ON THE EMPLOYMENT OF THE PRETREATMENT METHODS

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ABSTRACT

Hydrogen has the highest energy content per unit mass when compared to fuel types. When used as fuel, the product released to the atmosphere is only water or water vapor. Hydrogen gas can be produced by solar energy, wind, wave and biomass. Although biohydrogen studies help determine the biomass types that can serve as an alternative to fossil fuels while having a lower environmental impact, the selected biohydrogen production methods should also be cost-effective and sustainable at an industrial scale. Since pretreatments and their supply and some high-cost microorganism species affect the cost of hydrogen production, they should be taken into account to determine the economic applicability of a given biomass. The paper reviews the current gaps in the related information by classifying the biomass (according to whether pretreatment was used or not) used in the biohydrogen production through dark fermentation and anaerobic decomposition contains the following: (i) synthetic wastewaters. (ii) non-synthetic wastes/wastewaters; (a) Biohydrogen production using non-pretreated wastes/wastewaters and mixed microorganisms, (b) Biohydrogen production using non-pretreated wastes/wastewaters and certain microorganism species, (c) Biohydrogen production using pretreated wastes/wastewaters and mixed microorganisms, (d) Biohydrogen production using pretreated wastes/wastewaters and certain microorganism species.

INTRODUCTION

Energy resources gained further importance worldwide as a result of the depletion of fossil fuel reserves, climate change, and global warming. Clean, sustainable, and renewable biofuel resources that can reduce the dependence on fossil fuels lead to the need for the utilization of alternative biomasses. Organic matter-rich wastewaters, agricultural and agriculture industry wastes, animal wastes, and energy plants are suitable biomass resources for biohydrogen production. Biohydrogen is proposed as a promising renewable energy alternative to fossil fuels, which are on the verge of extinction. High-density hydrogen (123 kJ / g 2.75) has a high electrical energy conversion efficiency and does not have any negative environmental impact. Among the hydrogen production methods, biological methods can convert various biomass types of organic origin in addition to being environmentally friendly and low-cost. Dark fermentation, in particular, allows the use of sustainable substrates, yields high amounts of hydrogen, and facilitates the operation of the process.

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Although biological hydrogen production with dark fermentation and anaerobic decomposition is an alternative energy resource that involves clean combustion products, it necessitates further advancements in the relevant technology as a result of the challenges faced in its storage and transport (Dias *et al.,* 2014; Udomsirichakorn and Salam, 2014; Dareioti *et al.,* 2015; Palomo-Briones *et al.,* 2017; Khan *et al.,* 2018).

The biomass resources of the future can be categorized as solid, liquid, and gas resources and listed as: bio-pellets; biodiesel, bioethanol, and bio-methanol; and biohydrogen and biogas, respectively. In this review paper, the effects of dark fermentation and anaerobic decomposition on biological hydrogen production was discussed by considering whether pretreatment (thermal, chemical, etc.) was applied to biomass-based synthetic wastewaters and non-synthetic wastes and wastewaters (Figure 1). Biological hydrogen production processes overcome a series of disadvantages encountered in the hydrogen production processes, offer the use of clean, sustainable, cheap, and easily operable organic matters, and eliminate the risk of bacterial contamination. Furthermore, the biohydrogen production process is also favorable for allowing the use of wastes from biomasses of organic origin. Among these processes, dark fermentation is the most advantageous

and effective process due to not requiring the use of sunlight or artificial light.

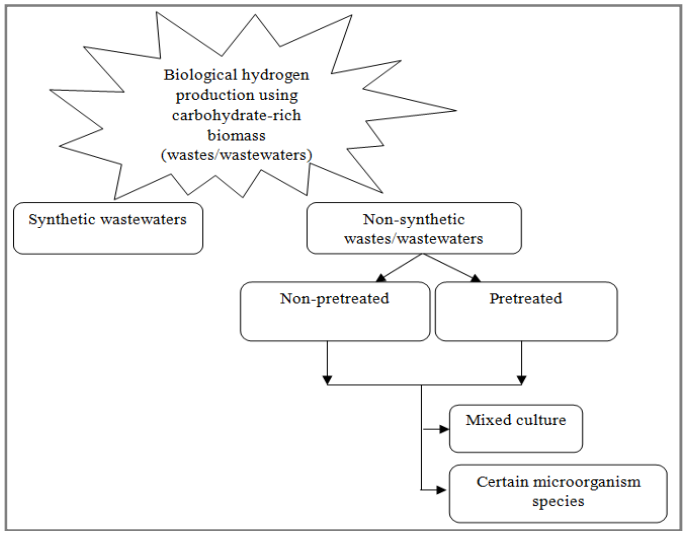

Figure 1. Schematic presentation of the treatment applied to biomass-based synthetic wastewaters and non-synthetic wastes/wastewaters

Biological hydrogen production using synthetic wastewaters: There are various biological hydrogen production studies in which synthetic wastewaters were used as substrates. In some of these studies, synthetic glucose (Cavalcante de Amorim *et al.,* 2009), synthetic sucrose (Munoz-Paez *et al.,* 2013), and synthetic saccharose (Lin *et al.,* 2006) were tested for their use as substrates. In general, heat pretreatments at temperatures ranging from 90 ^{0}C to 105 ^{0}C were applied to the inoculum sludges for durations ranging from 45 minutes to 50 minutes and the studies were carried out using anaerobic fluidized bed reactors. In the study conducted by Cavalcante de Amorim *et al.* (2009), the reactor was operated at 37 ^{0}C and an optimum pH of 4.0 with hydraulic retention times ranging from 1 hour to 8 hours; the reduction of the hydraulic retention time from 8 hours to 2 hours resulted in an increase in the hydrogen yield from 1.41 mol H_2/mol glucose to 2.49 mol H_2/mol glucose. In their study, Munoz-Paez *et al.* (2013) observed that, while the reactor was operated for 20 days with a hydraulic retention time of 1 day, the initial operating conditions were at room temperature with an organic loading rate of 5 g sucrose/L.day and pH 5.0-5.2; then, an increase in the performance was observed when pH was decreased to 4.3 and temperature and organic loading rate were increased to 35 ^{0}C and 8 g sucrose/L.day, respectively. The optimum hydrogen production at room temperature was 35 mmolH_2/L.day and the optimum hydrogen production at 35 ^{0}C was 920 mmolH_2/L.day. This led to the conclusion that the ideal temperature was 35 °C and ideal pH value was 4 for biohydrogen production. In another study conducted by Lin *et al.* (2006), the process was carried out with saccharose concentrations ranging from 5 g COD/L to 40 g COD/L (COD: Chemical Oxygen Demand) and hydraulic retention times between 2.2 hours and 8.9 hours. The optimal biogas hydrogen content was 40 g COD/L and obtained with a production rate of 2.27 L/h and a hydraulic retention time of 2.2 hours. In their study in which recycled polyethylene was used as a support material to attach the biomass, Fontes Lima *et al.* (2013) investigated hydrogen production in anaerobic fixed-bed bioreactors with glucose and saccharose-containing synthetic wastewater. With a hydraulic retention time of 2 hours, two reactors were operated at 25 ^{0}C for 60 days. The optimum hydrogen yield was 1.51 mol H_2/mol glucose with 2 gr/L sucrose concentration and 3.22 mol H_2/mol sucrose and 2 gr/L glucose concentrations. In another study, Cavalcante de Amorim *et al.* (2012) investigated biohydrogen production using four fluidized bed reactors that were operated at different conditions and fed glucose-containing synthetic wastewater. Expanded clay was used as a support material to attach the biomass and the anaerobic sludge from the wastewater of the up-flow sludge blanket reactor of a swine farm was used as inoculum. With a hydraulic retention time of 2 hours, the reactors showed a good H_2 production performance at glucose concentrations of 2, 4, and 10 g/L, while the reactors failed to yield a good H_2 production performance at 25 g/L glucose concentration. Under conditions in which hydraulic retention time was reduced from 8 hours to 2 hours and 2, 4, 10, and 25 g/L glucose concentrations were used, the maximum hydrogen yields were 2.49, 1.78, 1.26, and 0.60 mol/H_2mol. The highest hydrogen yield was achieved with a 2-hour hydraulic retention time and 2 g/L glucose concentration. In another study, Guwy *et al.* (1997) carried out hydrogen production under conditions in which synthetic baker's yeast wastewater was fed to a laboratory-scale fluidized bed anaerobic digester, high pollution ratios were used, the temperature was set to 37 ^{0}C, and hydraulic retention times were between 10.2 hours and 8.7 hours. The wastewater was batch-fed to the feed tank. The overload step resulted in a sharp peak in the online-measured biogas hydrogen level and by increasing the organic loading rate from 40 kg COD /m^{3}day to 63 kg COD /m^{3}day, the hydrogen concentration was increased from 290 ppm to 640 ppm.

Biological hydrogen production using non-synthetic wastes/wastewaters: The biohydrogen production studies in which non-synthetic wastes/wastewaters were used as substrates were discussed by classifying the studies according to whether pretreatment was used and to the microorganism species used in the studies.

Biohydrogen Production Using Non-pretreated Wastes/wastewaters and Mixed Microorganisms: Since pretreatments affect the cost during the production process, they should be taken into account when considering the economic applicability of the substrates. There are various optimization studies on biological hydrogen production in which pretreatment methods were not applied to wastes/wastewaters. Among such studies, in the study conducted by Tawfik *et al.* (2015), municipal food wastes and kitchen wastes were fed to an anaerobic baffled reactor at varying concentrations to investigate their applicability in biological hydrogen production. The hydrogen yield during the degradation of the waste mixture was significantly affected by pH (6.2 – 9.0), COD (12 - 24.1 g COD/L day), and hydraulic retention time (1.82 – 4.6 hours). The increase in hydraulic retention time positively affected the degradation efficiencies of carbohydrates, lipids, and proteins. The maximum hydrogen production rate was 141 ml/hour (at pH 5). In another study by Kim *et al.* (2004), sewage sludges that were collected at the same amount from the primary and secondary sludge thickeners and the food wastes collected from a dining hall to produce hydrogen were crushed in a blender and hydrogen production was carried out in serum bottles. The researchers determined that the properties of heat-pretreated sludge were

Table 1. Some studies according to whether pretreatment was applied or not and the waste/wastewater type

Type of waste/wastewater	Reactor type	Inoculum	The status of pretreatment to inoculum or certain microorganism species	Operating conditions	H_2 production	Reference
cellulose	continuous stirred tank reactor	anaerobic sludge	non-pretreated	at an organic loading rate of 10 g cellulose/L, 10-day hydraulic retention time, at pH 5.5-6.0, temperature 70 ± 1 ^{0}C	7.07 mmol H_2/g cellulose	Gadow *et al.,* 2013
sucrose	continuous stirred tank reactor	heat-pretreated seacoast sludge	heat pretreatment (60 min. at 95-100 ^{0}C)	at an organic loading rate of 17.8 g sucrose/L, 6-hour hydraulic retention time, at pH 5.5, temperature 37 ^{0}C	0.937 mol/L/day	Lee *et al.,* 2012
dissolved and condensed molasses wastewater was heat treated 20 minutes at 40 °C	continuous stirred tank reactor	*Clostridium* species-rich activated sludge	non-pretreated	at an organic loading rate of 320 g COD/L/day and 3-hour hydraulic retention time	390.0 mmol H_2/L-day	Lay *et al.,* 2010
enzymatic hydrolysate-pretreated cornstalk	batch reactor	after fungal pretreatment *Thermoanaerobacterium thermosaccharolyticum W16*	fungal pretreatment (15 days at 29 ^{0}C)	at pH 6.5-7.0, temperature 60 ^{0}C	80.3 ml /g	Zhao *et al.,* 2012
whey	continuous stirred tank reactor	heat-pretreated anaerobic sludge	heat pretreatment	at organic loading rates of 92.4; 115.5; 138.6, and 184.4 g lactose/L/day, 6-hour hydraulic retention time, at pH 5.9, temperature 37 ^{0}C	23.32; 36.44; 46.61 mmol H_2/L/hour, respectively,and sharp fall in hydrogen production	Davila-Vazquez *et al.,* 2009
domestic solid waste organic part of which was diluted with water	continuous stirred tank reactor	digested anaerobic sludge	non-pretreated	at an organic loading rate of 64.4 kg COD/m^3/day, 1.3-day hydraulic retention time, temperature 55 ^{0}C	205 ml H_2/gVS	Chu *et al.,* 2008
sugarcane stillage and glucose mixture	anaerobic fluidized bed reactor	heat-pretreated anaerobic sludge	heat pretreatment	at a mean organic loading rate of 5000-5300 mg.COD.L^{-1},gradually-decreased hydraulic retention times of 8 hours to 1 hour, hydraulic retention time, at pH 4.0, temperature 55^{0}C	0.78 L.H_2.h^{-1}. L^{-1}	Christine Santos *et al.,* 2014a
fungal-pretreated cornstalk	batch reactor	white rot fungus (*Phanerochaete chrysosporium*) pretreatment, *Trichoderma viride* and *Thermoanaerobacterium thermosaccharolyticum W16*	fungal pretreatment (15 days at 29 ^{0}C)	at pH 6.5, temperature 60 ^{0}C	18.2 ml H_2/g-cornstalk/h	Zhao *et al.,* 2013
Liquid waste obtained from diluted and pure molasses	anaerobic fluidized bed reactors (AFBR1, AFBR2)	heat-pretreated anaerobic sludge	heat pretreatment (10 min. at 90 ^{0}C)	wastewater diluted in AFBR1 at an organic loading rate of 40.0 kg COD m^{-3}day^{-1}and pure wastewater in AFBR2 at an organic loading rate of 120.0 kg COD m^{-3}day^{-1}, in two reactors, 6-hour optimum hydraulic retention time, at pH 4.17-4.47, temperature 55 ^{0}C	AFBR1: 2.86 mmol H_2 g COD, AFBR2: 0.79 mmol H_2 g COD	Christine Santos *et al.,* 2014b
cassava wastewater	anaerobic fluidized bed reactor	heat-pretreated facultative sludge	heat pretreatment (10 min. at 90 ^{0}C)	at an organic loading rate of 28-161 kg COD/m^3-day, gradually-decreased hydraulic retention times of 8 hours to 2 hours, at pH 5.0, temperature 28 ± 2 °C	0.20-2.04 L/hour/L	Amorim *et al.,* 2014

similar to those of anaerobic spore-forming *Clostridium* bacteria and the heat-pretreated sludge was successful in organic matter fermentation. In the process, at different volatile soil concentrations ranging from 0.5% to 5.0%, two volatile solid-based substrate mixtures were tested at two mixing ratios of 0:100 and 100:0. The maximum hydrogen production efficiency was 122.9 ml/g carbohydrate COD and the maximum hydrogen production rate was 111.2 ml H_2/g VSS/h (VSS: volatile suspended solids); food waste and sludge were deemed to be a suitable main substrate and a useful auxiliary substrate, respectively.

In another study, Lay *et al.* (1999) investigated the feasibility of hydrogen production using heat pretreatment to the hydrogen-producing bacteria from domestic organic solid wastes and digested sludge from a soybean meal silo. High hydrogen production potentials of 140 ml H_2/g TVS and 180 ml H_2/g TVS (TVS: total volatile solids) were observed for pretreated sludge and hydrogen-producing bacteria, respectively. In both trials, hydrogen composition exceeded 60%. High hydrogenic activity was observed for the heat-pretreated sludge and the food-to-microorganism ratio (F/M) in the heat-pretreated sludge was 45 ml/g VSS.h, while a low F/M ratio of 36 ml/g VSS.h was obtained in the hydrogen-producing bacteria. The researchers determined that domestic organic solid wastes had an important biological hydrogen production potential. In another study, Van Ginkel *et al.* (2005) investigated the usability of highly concentrated and carbohydrate-rich wastewaters from the food processing industry and 25-fold concentrated domestic wastewaters in biological hydrogen production. The hydrogen gas produced from the microorganisms that were obtained from heat-treated (boiled at 100 ^{0}C for 2 hours) and sieved soil was measured in batch bottles. The CODs of the wastewaters collected from four different food processing industries were 9 g/L (apple processing), 21 g/l (potato processing), and 0.6 g/L and 20 g/L (confectionery A and confectionery B, respectively). The hydrogen gas conversions were 0.7–0.9 L-H_2/L-wastewater for apple processing, 0.1 L-H_2/L-wastewater for confectionery A, 0.4–2.0 L-H_2/L-wastewater for confectionery B, and 2.1–2.8 L-H_2/L-wastewater for potato processing. The estimated maximum amount of the gas produced from the 25-fold concentrated and undiluted domestic wastewater samples was 0.01 L-H_2/L-wastewater. Chen *et al.* (2006) conducted batch experiments to determine the growth kinetics of the hydrogen-producing bacteria with dark fermentation while using sucrose, dry skimmed milk powder, and food waste as substrates. The results revealed that hydrogen production potential and hydrogen production rate increased with increasing substrate concentrations. The maximum hydrogen yields for sucrose, dry skimmed milk powder, and food waste were 234 ml/g COD, 119 ml/g COD, and 101 ml/g COD, respectively. The researchers observed that low pH (pH<4) inhibited hydrogen production and low carbohydrate fermentation occurred at high substrate concentrations. Furthermore, the Michaelis-Menten equation was used to model the hydrogen production rates at different substrate concentrations. In other studies in the scientific literature, various wastes/wastewaters such as municipal solid wastes and the mixture of these wastes with the slaughterhouse wastes from a poultry processing facility (Gomez *et al.,* 2006), starch-containing wastewaters (Guo *et al.,* 2008), milk industry wastewaters (Mohan *et al.,* 2008), and molasse (Ren *et al.,* 2006) were tested as substrates. In general, the operating temperatures in the studies were between 30^{0}C and 35^{0}C and anaerobic activated sludge was used both as a reactor material and inoculum. Only in the study carried out by Mohan *et al.,* 2008, anaerobic activated sludge for use as inoculum was also tested by applying chemical pretreatment to the same sludge type (2 bromoethane sulfonic acid sodium salt (0.2 g / l); 24 hours) to compare the results according to the inoculums. Moreover, under the same conditions and with the same organic loadings, in the reactors that were inoculated with anaerobic sludge and chemically-pretreated anaerobic sludge, the maximum hydrogen production efficiencies were 0.0018 mmol/g COD and 0.0317 mmol/g COD, respectively. In other studies, with an organic loading rate of 0.5 g-starch/L.day, an optimum hydraulic retention time of 8 hours, and pH 3.95, the maximum hydrogen production efficiency was 0.11 L/g-COD (Guo *et al.,* 2008); in the study in which the optimum hydrogen production efficiencies of solid wastes and a mixture (solid waste + slaughterhouse) were compared to each other, the mixture (solid waste + slaughterhouse) had a higher efficiency than that of the solid waste (Gomez *et al.,* 2006); the biogas and hydrogen efficiencies increased with the organic loadings between 3.11 kg COD/m^{3}reactor/day and 68.21 kg COD/m^{3}reactor/day and decreased with the organic loadings between 68.21 kg COD/m^{3}reactor/day and 85.57 kg COD/m^{3}reactor/day, the ratios of hydrogen in biogas were between 40% and 52%, and the maximum hydrogen production rate was 0.75 m^3H_2/kg MLVSS/day (Ren *et al.,* 2006). Some studies aimed to inhibit the activity of methane-forming bacteria by heat pretreating the anaerobic wastewater sewage sludge for use as inoculum. In the studies, the effects of some operating parameters (pH, temperature, hydraulic retention time) on optimum hydrogen production were investigated.

In the study carried out by Jayalakshmi *et al.* (2009), using 95% biodegradable kitchen wastes, a maximum hydrogen production rate of 211.20 ml/kg TS h was tested in laboratory-scale batch reactor at an optimum pH of 6.0. In the study by Woo and Song (2010), with an optimum hydraulic retention time of 3 days, the maximum hydrogen production efficiency was 3.07 mmol H_2/g TS under thermophilic conditions (55^{o}C). In another study conducted by Lin *et al.* (2008), batch and continuous reactors at 35 ^{0}C were set up to evaluate hydrogen production from starch. The researchers carried out continuous experiments to determine the effect of hydraulic retention time on hydrogen production while using batch reactors at an optimal pH range of 5.0-7.0 and an optimal substrate concentration range of 5-60 g COD/L. In the batch system, the optimum pH was determined to be 5.5 and hydrogen production rate was 10.4 mmol-H_2/L/hour; in the continuous system, the optimum hydraulic retention time was determined to be 4 hours and hydrogen production rate was 450 mmol-H_2/L/hour. In their study, Lay *et al.,* 2013 used the sludge from the wastewater treatment plant of a swine farm as the inoculum. Using the powdered and pelleted mixtures of beverage wastewater and water hyacinth at varying ratios, batch fermentation was performed in serum bottles to investigate the hydrogen production potential. The pelleted water hyacinth: beverage wastewater mixture was obtained in the ratio of 1.6 g: 2.4 gand a biogas production of 105.5 mL and a hydrogen production of 55.6 mL were determined at pH 5.35. The powdered water hyacinth: beverage wastewater mixture was obtained in the ratio of 1.6 g: 2.4 g and a biogas

production of 82.5 mL and a hydrogen production of 41.9 mL were determined at pH 5.44. The pellet form of the mixture produced a higher amount of hydrogen than the powder form of the mixture. In the study by Shi *et al.* 2013, hydrogen production with dark fermentation at different temperatures (35, 50, and 65 °C) using non-pretreated *Laminaria japonica*was investigated. Anaerobic sludge was heat-pretreated (at 90 °C, 20 minutes) and used as an inoculum and the study was performed in batch reactors under mesophilic, thermophilic, and hyperthermophilic conditions. The highest hydrogen yield was 61.3 ± 2.1 mL H_2 /g TS and observed under mesophilic conditions (35 °C). The maximum hydrogen yields under mesophilic (pH:5.5; 3.4 g COD/L/d), thermophilic (pH:6.0; 3.4 g COD/L/d), and hyperthermophilic (pH:6.0; 3.4 g COD/L/d) conditions were 61.3 ± 2.0, 49.7 ± 2.8, and 48.1 ± 2.5 mL H_2 /g TS, respectively. The increase in the temperature resulted in a decreased microorganism diversity and the emergence of different dominant species.

Biohydrogen Production Using Non-pretreated Wastes/wastewaters and Certain Microorganism Species: In some studies, in which wastes/wastewaters were used as substrates, substrates were not pretreated and the hydrogen production potentials were investigated using certain microorganism species. Using non-pretreated switchgrass, microcrystalline cellulose, and glucose, Talluri *et al.* 2013 investigated anaerobic biological hydrogen production with the thermophilic (65 °C) *Caldicellulosiruptor saccharolyticus DSM 8903* bacteria. The study was performed with a shaker incubator using serum bottles. With the use of *Caldicellulosiruptor saccharolyticus DSM 8903*, switchgrass was fermented to produce 11.2 mmol H_2 /g.switchgrass without any physiochemical or biological pretreatment to switchgrass; microcrystalline cellulose was fermented to produce 9.4 mmol H_2 /g.cellulose at a 7-fold higher hydrogen production rate than in the case of switchgrass; using glucose, a theoretical yield of 4 mol H_2 / mol.glucose was achieved. In another study, Song *et al.* 2014 investigated the batch production of biohydrogen in serum bottles at 36 °C without the pretreatment of raw cornstalk. In the study, the isolate obtained from the cow dung compost-based anaerobic acclimatized sludge was identified as *Clostridium butyricum FS3* via a series of physiological and chemical experiments and 16S rDNA gene sequence. Under optimal conditions, the optimal hydrogen yield was 92.9 mL/g with a substrate concentration of 20 g /L raw cornstalk. The researchers reached to the conclusion that *Clostridium sp. FS3* was an ideal microorganism for use in biological hydrogen production from raw cellulosic biomass.

Biohydrogen Production Using Pretreated Wastes/wastewaters and Mixed Microorganisms: As an energy bearer of the future, hydrogen is advantageous due to its high conversion efficiency as well as being a clean fuel, its high energy content, and its usability as a fuel cell for electricity generation. However, compared to fossil fuels and liquidized natural gas, its lower availability in nature necessitates its production from biomass materials, municipal solid wastes, and agricultural wastes. To optimize biological hydrogen production, various pretreatments to wastes and wastewaters were reported. The pretreatment method usually involves the elimination of the undesired bacteria species through the heat treatment of inoculum sludge or substrate. In one of these studies, by considering hydrogen production from different wastes, hydrogen production from alkali-hydrolyzed rice straw was investigated. The anaerobic sludge for use as inoculum was heat-treated and the process was carried out at 35 ^{0}C in an anaerobic baffled reactor. For this purpose, with different organic loading rates (0.5 - 2.16 g COD/L), optimal parameters were adjusted so that pH was 6.8 and hydraulic retention time was 20 hours. The hydrogen production was 1.19 mol H_2/mol glucose. Through the phylogenetic analysis of the reactor samples, the dominance of the hydrogen-producing *Clostridium* bacteria was detected (El-Bery *et al.,* 2013). Azbar *et al.* (2009) investigated hydrogen production with dark fermentation using cheese processing wastewaters and a continuous stirred tank reactor under thermophilic conditions. The hydrogen-producing bacteria-rich anaerobic sludge was used as inoculum. Prior to its use, whey was heat-pretreated to eliminate lactic acid bacteria. By performing trials with different hydraulic retention times and organic loading rates, the reactor was operated for 274 days.

The maximum hydrogen production efficiency was obtained at pH 5.6 and with a hydraulic retention time of 3.5 days and 22 mmol/g COD. Xing *et al.* (2010) investigated biohydrogen production from dairy manure both in a batch system using serum bottles and in a continuous stirred anaerobic bioreactor. Pretreatment with HCl and NaOH and heat pretreatment to dairy manure were carried out, pH was adjusted to 7, and the manure was exposed to an infrared oven for 2 hours. The microflora was incubated with sucrose and used as inoculum. In the batch system, with the acidic pretreated manure and 70 g/L substrate concentration and at pH 5.0, the maximum hydrogen yield was 31.5 ml H_2/g-total volatile solid. In the continuously stirred system, at pH 5.0 and 8.5, the cumulative hydrogen production efficiencies of 32 and 16.5 ml/g-total volatile solid were obtained in the 40 and 75 hours. In some studies, heat or chemical pretreatment was applied both to the inoculum microorganisms and to wastes/wastewaters; in general, batch reactors were preferred. In these studies, to inhibit the bioactivity of the methanogens in the sludges collected from anaerobic wastewater treatment plants, the sludges were usually heat-pretreated at a temperature range of 90-100 ^{0}C and for durations from 30 minutes to 60 minutes. The investigation of the studies focusing on the pretreated (chemical, heat) wastes/wastewaters reveal that the treatments were tested:

- by chemically hydrolyzing heat-pretreated and cellulose-containing sugar cane bagasse residues (at 100 ^{0}C for 2 hours NaOH solution) (Chairattanamanokorn *et al.,* 2009),
- using the hydrolysate obtained by applying high-pressure steam to cornstalk and corn leaves after neutral and acidic pretreatments (Datar *et al.,* 2007),
- by gelatinizing raw cassava starch at 112 ^{0}C for 15 minutes and, then, prior to fermentation, applying pretreatment by successively adding alpha-amylase and glucoamylase for enzymatic hydrolysis (Su *et al.,* 2009),
- by sterilizing sweet sorghum syrup after heat treatment at 110 ^{0}C for 28 minutes and condensation (Saraphirom and Reungsang, 2010),
- by applying steam pretreatment at 100 ^{0}C for 30 minutes to rice slurry (Fang *et al.,* 2006),

The evaluation of the hydrogen production potentials at different operating conditions (pH, temperature, hydraulic retention time) of the substrates that were tested for their use in hydrogen production showed that:

- In the study in which pretreatments at different temperatures and pHs were applied to the cultures, for optimum hydrogen production performance, the temperature was 56.5 ^{0}C and the pH was 5.22; the maximum hydrogen production rate was 7.03 ml/L/hour (Chairattanamanokorn *et al.,* 2009),
- In the study in which neutral and acidic pretreatments were applied to the waste and, then, the mixed sugars that were present in the hydrolysate were used, the hydrogen yields in the reactor at 35 ^{o}C that was continuously stirred at 140 rpm were 2.84 and 3.0 mol H_2/mol glucose, respectively (Datar *et al.,* 2007),
- With the increase in starch concentration after applying gelatinization and enzymatic hydrolysis, the maximum hydrogen production rates reached 72.5 ml/l/h for raw starch, 146.2 ml/l/h for gelatinized starch, and 229.3 ml/l/h for hydrolyzed starch (Su *et al.,* 2009),
- In the study in which the system was at 30-32 ^{0}C and operated with 25 g /L total sugar, 4.78 initial pH, and 1.45 g/L $FeSO_4$ for maximum hydrogen production, the maximum hydrogen yield was 6897 ml H_2/L hexose (Saraphirom and Reungsang, 2010),
- The most effective pH for hydrogen production at 37 ^{0}C was determined to be pH 4.5 and hydrogen production occurred after a long lag phase of 36 hours. Following the 36-hour lag phase, maximum hydrogen yield was 346 ml/g-carbohydrate and the wastewater was made up of 28.3-43.0% acetate and 51.4-70.9% butyrate (Fang *et al.,* 2006).

In another study, Zhu *et al.* (2008) investigated hydrogen production in serum bottles by mixing a mixture of primary sludge and activated sludge and food wastes collected from a cafeteria at certain ratios. In the study, food waste (1:0), mixed sludge (0:1), and food waste: mixed sludge were tested at different volumetric ratios (3:1, 1:1, 1:3). An important amount of hydrogen production was obtained in a batch system with pH 7.0 and with the addition of phosphate to the food wastes. The best hydrogen production efficiency was 112 mL/g-volatile solid and obtained with the 1:1 volumetric ratio.

Biohydrogen Production Using Pretreated Wastes/wastewaters and Certain Microorganism Species: In other studies, in which wastes/wastewaters were used as substrates, the substrates were pretreated and hydrogen production potentials were investigated using certain microorganism species. In these studies, activated sludge from a wastewater treatment plant (Guo *et al.,* 2010), sugar cane bagasse waste (Pattra *et al.,* 2008), waste wheat powder solution (Argun *et al.,* 2009), and steam-cooked potato peels (Mars *et al.,* 2010) were used as substrates. The *Pseudomonas*species (Guo *et al.,* 2010), *Clostridium butyricum* (Pattra *et al.,* 2008), *Clostridium acetobutyicum*, *Clostridium butyricum*, *Enterobacter aerogenes*, heat-treated an aerobic sludge, and a mixed culture obtained by mixing these four cultures (Argun *et al.,* 2009), and *Thermotoga neapolitana* and *Caldicellulosiruptor saccharolyticus* (Mars *et al.,* 2010) were tested for their use as inoculums. In the study conducted by Guo *et al.* (2010), the filtrate obtained by the centrifugation of the sludge that was pretreated with sterilization (at 121 ^{0}C, 20 minutes) and sewage sludge were tested and the maximum hydrogen production efficiency at 35 ^{0}C was 4.44 mg H_2/g total COD for the filtrate and 1.34 mg H_2/g total COD for the sewage sludge. In their study, Pattra *et al.* (2008) used the supernatant obtained by applying H_2SO_4 acid hydrolysis to waste in an autoclave. In the system at 37 ^{0}C, with a pH value of 5.5 and a sugar concentration of 20 g-COD /L, the hydrogen production rate was 1611 mL H_2/L/day. In another study, Argun *et al.* (2009) boiled wheat particle-containing dissolved starch for 1.5 hours to obtain partial hydrolysis.

The hydrogen production yields depending on different inoculum resources were: 222.85 ml H_2/g starch for heat-treated anaerobic sludge; 125.53 ml H_2/g starch for *Clostridium acetobutyicum*; 118.98 ml H_2/g starch for *Clostridium butyricum*; 159.04 ml H_2/g starch for *Enterobacter aerogenes*; 133.09 ml H_2/g starch for the mixture of the cultures. The heat-treated anaerobic sludge was determined to be the most effective culture. In another study, under pH-controlled conditions, potato peels were used after the pretreatment by the hydrolysis and the subsequent centrifugation of a portion of the peels with alpha-amylase and the hydrolysis and the subsequent centrifugation of a portion of the peels with both alpha-amylase and amyloglucosidase. For the two bacteria species, the hydrogen productions that were carried out by using pure glucose, the peels that were hydrolyzed with alpha-amylase and with both alpha-amylase and amyloglucosidase, and untreated peels were investigated at different glucose loadings. The optimum hydrogen production rates for the *Thermotoga neapolitana* bacteria were 12.3 mmol/L/hour, 10.6 mmol/L/hour, 8.9 mmol/L/hour, and 12.5 mmol/L/hour, respectively; the optimum hydrogen production rates for the *Caldicellulosiruptor saccharolyticus*bacteria were 16.4 mmol/L/hour, 13.3 mmol/L/hour, and 13.1 mmol/L/hour, respectively. The researchers determined that the hydrolyzed peels were highly suitable for use as substrates in efficient hydrogen production (Mars *et al.,* 2010). Table 1 shows the studies that were sorted according to their employment of pretreatment and their choice of waste/wastewater types.

Conclusion

In the paper, the biomass types used in biohydrogen production were classified and the heat treatment methods were evaluated in terms of the approaches to dark fermentation and anaerobic decomposition. A general overview of the studies revealed that the most frequently used inoculum was mixed consortiums that were derived from different resources. This was mainly attributed to the high costs of the supply of certain microorganisms and the challenges arising in sterilization and working with pure species.

Future Perspectives: In conclusion, biohydrogen production has the potential to profoundly improve the future well being of the world by utilizing the wastes/wastewaters of organic origin and thus minimizing negative environmental impacts.

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