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Biohydrogen Production from Starch Residues

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***Abstract*—**This review summarizes the potential of starch agroindustrial residues as substrate for biohydrogen production. Types of potential starch agroindustrial residues, recent developments and bio-processing conditions for biohydrogen production will be discussed. Biohydrogen is a clean energy source with great potential to be an alternative fuel, because it releases energy explosively in heat engines or generates electricity in fuel cells producing water as only by-product. Anaerobic hydrogen fermentation or dark fermentation seems to be more favorable, since hydrogen is yielded at high rates and various organic waste enriched with carbohydrates as substrate result in low cost for hydrogen production. Abundant biomass from various industries could be source for biohydrogen production where combination of waste treatment and energy production would be an advantage. Carbohydrate-rich nitrogen-deficient solid wastes such as starch residues can be used for hydrogen production by using suitable bioprocess technologies. Alternatively, converting biomass into gaseous fuels, such as biohydrogen is possibly the most efficient way to use these agroindustrial residues.

***Keywords*—**Biofuel, dark fermentation, starch residues, food waste.

I. Introduction

DIRECT utilization of fossil fuels results in considerable environmental problems due to CO_2, NO_X, SO_X, volatile organic compounds and other pollutant emissions causing air pollution, global warming and acid rain [1], [2]. The need for alternative energy sources has increased in recent years due to the rapid depletion of fossil fuels.

Bioenergy production, i.e. biohydrogen, methane and bioethanol, through low-cost carbohydrate rich in substrates via microbial growth, is an emerging alternative energy resource [3]-[7]. Biohydrogen is a green energy carrier with great potential to replace fossil fuels in the future due to its high energy content and ability to produce it from various agroindustrial residues [8]-[10]. Fermentative pathways of hydrogen production using biomass or carbohydrate-based substrates represent a more promising route for biological hydrogen production when compared to photosynthetic or chemical routes.

Current studies have been focused on many factors on biohydrogen production from agroindustrial residues. These factors include substrate concentration, inoculums, pH [5], [11]-[13], nitrogen source [2], metal ion [13], temperature, hydraulic retention time, organic loading rate and reactor type [14]. Since 2002, there has been an increase in the number of publications in peer reviewed journals per year dealing with biohydrogen production (Fig. 1). Approximately 60% of all biohydrogen papers were published from 2008 to 2010 (Fig. 1 (b)).

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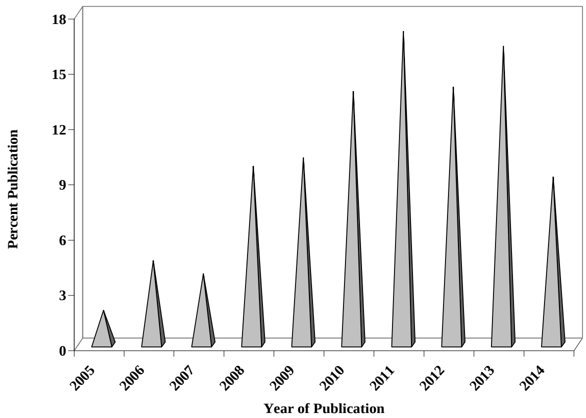
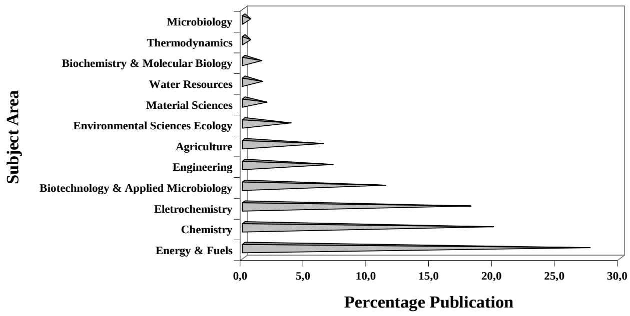


(a)



(b)

Fig. 1 R&D publications with the word “biohydrogen” or “bio-hydrogen” in the title (a) in relation to subject-wise percentage and (b) year-wise [15]

A major obstacle for the commercialization of biohydrogen is the high production cost, and thus there is an urgent need to develop strategies that could make it more economically feasible. Food wastes from industry and household contain high carbohydrate and protein levels. The organic constituent especially carbohydrate in food wastes could be a potential substrate for anaerobic hydrogen production. Utilization of wastes to generate hydrogen energy could reduce the production cost, making hydrogen gas more available and cheaper [16], [17]. However, ethanol, organic acids, biocompounds, animal feed and energy from food wastes would be a value-added strategy for the treatment of food wastes [18]-[20].

Despite the low hydrogen production from biomass in the present study (Table I) biohydrogen production processes have become important mainly due to three reasons: zero carbon emission, utilization of renewable energy resources and operation at room temperature and atmospheric pressure.

TABLE I
HYDROGEN PRODUCTION BY DIFFERENT GENERATION SOURCES

H_2 production source	(%)
Natural gases	40
Heavy oil and naphtha	30
Coal	18
Electrolysis	4
Biomass	1

Many researchers have used a variety of carbohydrates and food wastes for fermentation, from simple sugars, including glucose [21]-[23], sucrose [24]-[26], and xylose [21], [26], to more complex structures such as starch [23], [27]-[31], carbohydrate-rich agricultural products such as rice [23], [32], corn [33], [34], cassava [35]-[37], wheat [38]-[40], sorghum [41]-[43], potato [44], [45], sago [46], and others residues such as cellulose [9], sugar cane [47], [48], whey [47], [49], molasses [50]-[52], winery wastewater [53], alcohols [12], [54], oil [55], paper [56] and glycerol from biodiesel production [57].

This paper aims to give an overview of biohydrogen production from starch residues by dark fermentation process. Several biohydrogen systems and their hydrogen production rates are described.

II. BIOHYDROGEN PRODUCTION

Biohydrogen has higher energy content per weight unit (122 kJ g^{-1}) and energy yield, which is about 2.8 times higher than gasoline and diesel and zero carbon emission comparable to 0.46 to 0.9 kg of carbon kg^{-1} of fuels (Table II).

The major criteria for substrate selection are biodegradability, cost, carbohydrate content and availability. Commercially produced food products, such as corn and sugar, are not yet economically feasible for hydrogen production. Agroindustrial residues, wastewaters, and other substrates, which are generally rich in carbohydrates, can provide essential nutrients required for biohydrogen.

Fermentative hydrogen production has the advantage of high production rate and simple operation in comparison to photosynthetic hydrogen production. In addition, it is of great significance to produce hydrogen from organic wastes by fermentative route, because this way of hydrogen production not only treat organic wastes but also produce very clean energy carrier [58]. Anaerobic bacteria, fermentation bacteria, aerobic bacteria, photosynthetic bacteria, and algae can be used alone or in combination, depending on the substrate to be used [59]-[63].

Hydrogen can be produced by anaerobic bacteria and grow in the dark on carbohydrate-rich substrates. Bacteria known to produce hydrogen include *Enterobacter*, *Bacillus*, and *Clostridium* species. Glucose, hexose isomers or polymers in the form of starch or cellulose yield different amounts of hydrogen per mole of glucose, depending on the fermentation pathway and end-product. The bioconversion of substrates on biohydrogen generation is rapid and processes do not require solar radiation, making them useful for treating large amounts of wastewater using a large fermenter.

TABLE II
COMPARISON OF ENERGY, CARBON EMISSIONS AND LOW HEATING VALUE OF FUELS

Fuel type	Energy (MJ kg^{-1})	CE (Kg C kg^{-1})	LHV (MJ kg^{-1})
Bio-diesel	37	0.50	-
Coal (anthracite)	15-19	0.50	33.3
Diesel	42.8	0.90	43.0
Ethanol	21	0.50	27.0
Gasoline	42-45	0.84	42.5
Hydrogen gas	122	0.00	120.1
Natural gas	33-50	0.46	38.1
Petrol (naptha)	40-43	0.86	44.9

CE: Carbon emission; LHV: Lower Heating Value.

Major disadvantage of this fermentative broth is the disposal. On the other hand, fermentative broth can be treated as a carbon source for photo-fermentation process [64], [65]. Hydrogen production by these bacteria is highly dependent on the process conditions such as inoculum preparation and stat-up, pH, hydraulic retention time, temperature, substrate concentration, nutrients and gas partial pressure, which affect metabolic balance.

Sequential dark and photo-fermentation of organic compounds is a promising method for producing renewable hydrogen. The effluent of the dark fermentation is used as substrate for photosynthesizing bacteria during the second photo-fermentative step, in which short-chain organic acids are assimilated to hydrogen when light is present, producing up to 4 to 6 moles of hydrogen per mole of acetate and lactate, respectively [66], [67].

III. BIOHYDROGEN FROM STARCH RESIDUES

Essentially, starch is composed of two related polymers in different proportions according to its source: amylose 16 to 30% and amylopectin 65 to 85%. Amylose is a glucose polymer linked by α-1,4 bonds, mainly in linear chains and amylopectin is a large highly branched glucose polymer including α-1,6 bonds at the branch points.

Within the plant, cell starch is stored in the form of granules located in amyloplasts, intracellular organelles surrounded by lipoprotein membrane. Starch granules are highly variable in size and shape depending on the plant material. During the gelatinization process, starch granules swell when heated in the presence of water, which involves the breaking of hydrogen bonds, especially in the crystalline regions [68]. Starch materials require a reaction of starch with water to break down the starch into fermentable sugars [69].

Starch is the second most abundant organic compound on earth [70] and could act as the effective low-cost substrate for biohydrogen production. Starch containing biomass such as agricultural wastes offer special advantages for biohydrogen production since these raw materials are readily available, inexpensive and starch can be easily hydrolyzed into simple sugars such as glucose and maltose by enzymatic or acid saccharification followed by conversion of carbohydrates into organic acids and then to hydrogen [2], [7]. The use of these by-products for hydrogen generation prevents direct competition with primary food production [71]. Table III summarizes the microorganism and yields of biohydrogen production for bath, feed-bath and continuous operations from different starch as substrate.

TABLE III
YIELDS AND MICROORGANISMS OF BIOHYDROGEN PRODUCTION FROM DIFFERENT STARCH AS SUBSTRATE

Agroindustrial residue	Microorganism	Yield	Ref.
Bread waste	Microflora of rice	1.30 mol H_2/mol hexose.	[72]
Cassava starch	Mixed cultures	249.3 mL H_2/g starch	[6]
Cassava starch	*Clostridium acetobutylicum*	2.41 mol H_2/mol glucose	[36]
Corn starch	Dairy manure compost	346 mL H_2/g TVS	[34]
Corn starch	*Clostridium pasteurianum*	194 mL H_2/g starch	[33]
Potato starch	*Thermotoga neapolitana*	3.8 mol H_2/mol glucose	[71]
Potato starch	*Clostridium butyricum*	2.4 mol H_2/mol glucose	[44]
Rice starch	Mixed cultures	0.98 mol H_2/mol hexose	[73]
Sago starch	Thermophilic mixed culture	422 mL H_2/g starch	[46]
Starch	Anaerobic mixed cultures	274 mL H_2/g starch	[74]
Starch	Anaerobic sludge	2.32 mol H_2/mol glucose	[75]
Starch	Anaerobic sludge	1.7 mol H_2/mol glucose.	[28]
Starch	Mesophilic and thermophilic cultures	2.8 mol H_2/mol glucose	[76]
Starch	*Clostridium butyricum*	1.9 mol H_2/mol glucose	[77]
Starch	*Rhodobacter* sp M-19	3.6 mol H_2/mol glucose	[77]
Starch	*Clostridium amygdalinum* C9	390 mL H_2/g starch	[78]
Starch	Mixed cultures	5.28 mmol H_2/g starch	[70]
Starch	*Clostridium butyricum*	2.6 mol H_2/mol glucose	[79]
Starch	*Escherichia coli* NCIMB 11943	1.8 mol H_2/mol hexose	[80]
Starch	*Enterobacter aerogenes*	1.1 mol H_2/mol hexose	[80]
Starch	Mixed cultures	10.66 mmol H_2/g glucose	[70]
Starch wastewater	Mixed cultures	110 mL H_2/g COD,	[81]
Starch wastewater	*Clostridium butyricum*	2.7 mol H_2/mol glucose	[82]
Starch wastewater	Mixed cultures	2.1 mmol H_2/g COD	[83]
Starch wastewater	Anaerobic sludge	186 mL H_2/g starch	[84]
Sweet sorghum	*Ruminococcus albus*	2.6 mol H_2/mol glucose	[85]
Sweet sorghum	Mixed cultures	127.2 mL H_2/g TVS	[43]
Wheat starch	Mixed cultures	1.3 mol H_2/mol hexose	[38]
Wheat starch	Mixed cultures	1.30 mol H_2/mol hexose	[73]
Wheat Starch	Anaerobic sludge	96 mL H_2/g starch	[86]
Wheat Starch	Anaerobic sludge	65.2 mL H_2/g starch	[87]
Wheat Starch	Anaerobic sludge	2.40 mol H_2/mol glucose	[88]
Wheat Starch	Anaerobic sludge	465 mL H_2/g starch	[89]
Wheat Starch	Anaerobic sludge	201 mL H_2/g starch	[87]
Wheat Starch	*Rhodobacter sphaeroides*	1200 mL H_2/g TVFA[a]	[90]
Wheat Starch	*Clostridium beijerinkii*	90 mL H_2/g starch	[39]

[a]TVFA: total volatile fatty acids; TVS: total volatile solids; COD: Chemical oxygen demand.

A. Sorghum

Sweet sorghum is an annual C4 plant of tropical origin, well-adapted to sub-tropical and temperate regions and highly productive in biomass. It is an extraordinary promising multifunctional crop not only due to its high economic value but also due to its capacity to provide a very wide range of renewable energy products, industrial commodities, food and animal feed products [64]. Sorghum biomass is rich in readily fermentable sugars and thus can be considered an excellent raw material for fermentative hydrogen production [85].

Antonopoulou et al. [85] used sorghum as substrate for biohydrogen production in continuous and batch fermentations cultures. The highest biohydrogen yield obtained from sorghum extract was 0.86 mol H_2 mol^{-1} of glucose. Panagiotopoulos et al. [41] obtained an optimal condition for alkaline pretreatment of sweet sorghum bagasse were achieved at 10% NaOH with delignification of 46%. The maximum hydrogen production observed in batch experiments under controlled conditions was 2.6 mol H_2 mol^{-1} hexose.

B. Potato

During potato chip processing, large amounts of residue streams are generated [69]. This implies that potato residues can be used as carbon source and, probably, many other fermentations instead of sugars, and similar expensive carbon sources for biohydrogen production.

Yokoi et al. [44] performed a repeated batch culture using a mixed culture of *Clostidium butyricum* and *Enterobacter aerogene*. The yield hydrogen production was 2.4 mol H_2 mol^{-1} glucose. Zhu et al. [91] studied the co-production of methane and biohydrogen from potato waste using a two-stage anaerobic digestion process. The hydrogen concentration in the gas was 45% (v/v), on average. Overall, 70% of volatile solids and 64% of total chemical oxygen demand were removed from the substrate. Mars et al. [71] studied biohydrogen production by extreme thermophiles *Caldicellulosiruptor saccharolyticus* and *Thermotoga neapolitana* from glucose, and hydrolyzed and untreated potato steam peels as carbon source. When hydrolyzed and untreated potato steam peels were added of 10 to 14 g L^{-1} of glucose units, both strains grew well and produced biohydrogen with reasonable to high molar yields of 2.4 to 3.8 mol H_2 mol^{-1} glucose.

C. Rice

A large amount of rice residue is annually produced in rice growing countries. Grains, agroindustrial residues and wastewater from rice processing are another potential starch-based waste from rice industry, since rice is the most common dietary food.

Fang et al. [32] carried out an experiment on biohydrogen production by *Clostridium* sp. using rice slurry containing 5.5 g carbohydrate L^{-1}. After a 36-h acclimation period, the sludge showed maximum specific hydrogen production rate of 2.1 L g^{-1} VSS d^{-1} and hydrogen production of 346 mL H_2 g^{-1} carbohydrate, corresponding to 62.6% of stoichiometric yield. Based on the 16S rDNA analysis, the 28 clones developed from this acidophilic hydrogen-producing sludge may be classified into nine operational taxonomy units, all of which are affiliated with genus *Clostridium*.

D. Wheat

Starch containing biomass such as wheat waste is rich of carbohydrates and deficient in other nutrients such as nitrogen, phosphorous and minerals. Therefore, when used as substrate for biohydrogen production, wheat powder may not provide all nutrients required for microbial metabolism and hydrogen production [2]. Wheat waste is an inexpensive and reliable renewable resource for biohydrogen production due to its high starch and gluten content above 95% [87]. Biohydrogen production from ground wheat starch by dark fermentation has been extensively studied by our research group using heat-treated anaerobic sludge.

Hussy et al. [38] used a particulate co-product from wheat flour industry for biohydrogen production. In continuous operation, hydrogen yield was 1.3 mol H_2 mol^{-1} hexose. Cakir et al. [88] studied biohydrogen production from hydrolyzed wheat starch by mesophilic and thermophilic dark fermentation. The highest cumulative hydrogen gas production of 0.7 L was obtained from acid-hydrolyzed ground wheat starch at 55°C. Argun et al. [86] produced biohydrogen from fermentation of powder wheat using heat-treated anaerobic sludge. Cumulative biohydrogen, hydrogen yield and formation rate were maximum at powder wheat concentration of 20 g L^{-1}. Kargi and Pamukoglu [89] produced biohydrogen from wheat starch by dark fermentation. High feed powder wheat at concentrations of 30 g L^{-1} resulted in the formation of high concentrations of volatile fatty acids, causing inhibition on hydrogen production rate and yield.

E. Corn

Corn, as a common starch-rich substrate, is a valuable and vast renewable biomass resource. An alternative approach is to convert aging corn to biohydrogen as a high value-added clean energy carrier.

Arooj et al. [3] studied continuous biohydrogen production in an anaerobic continuous stirred-tank reactor using corn starch as substrate for 158 days. The volatile fatty acids profile supported the fact that the butyrate/acetate ratio was the most important parameter to justify hydrogen production at various hydraulic retention times. Liu and Shen [33] investigated the influences of initial pH, nitrogen, iron and starch concentrations on biohydrogen production. Optimum pH, iron and nitrogen concentrations for hydrogen production at starch concentration of 15 g L^{-1} were 7 to 8 mg of Fe^{2+} L^{-1} and NH_4HCO_3 of 5.64 g L^{-1}, respectively. Fan et al. [34] produced biohydrogen by anaerobic culture from aging corn as substrate. The bio-pretreatment of aging corn with solid microorganism additives was essential for adequate conversion of the substrate into biohydrogen. The maximum hydrogen yield and hydrogen production rate were 346 mL H_2 g^{-1} of total volatile solids and 11.8 mL H_2 h^{-1}, respectively, at substrate concentration of 10 g L^{-1} and initial pH of 6.0. Hydrogen yield was approximately 2.5 times values obtained from raw aging corn.

F. Cassava

Cassava is considered an important source of food and dietary calorie for a large population in tropical countries of Latin America, Africa and Asia. Cassava starch residue is a solid fibrous dry (moisture 12-13%) by-product from starch industries [92]. The dry residue has starch composition of 56-60%, cellulose 15-18%, hemicellulose 4-5%, lignin 2-3%, protein 1.5-2.0% and low contents of reducing sugars 0.4-0.5% [92], [93].

Su et al. [35] studied the combination of dark and photo fermentation on biohydrogen production from cassava starch as substrate. When cassava starch, which is gelatinized by heating or hydrolyzed with α-amylase and glucoamylase, was used as substrate, the maximum hydrogen production, respectively, increases to 258.5 and 276.1 mL H_2 g^{-1} starch, and the maximum hydrogen production rate increases to 172 and 262.4 mL H_2 L^{-1} h^{-1}. Cappelletti et al. [36] studied the effect of initial substrate concentration, pH, and biohydrogen production from cassava wastewater by *Clostridium acetobutylicum*. The results showed that higher substrate concentrations of 30 and 15 g COD L^{-1} led to lower hydrogen production and lower and less effective substrate conversion. O-Thong et al. [6] used a natural microbial consortium from hot spring to developed thermophilic mixed cultures for biohydrogen production from cassava starch processing wastewater. Significant hydrogen production potentials were obtained from three thermophilic mixed cultures with maximum hydrogen production of 249.3, 180 and 124.9 mL H_2 g^{-1} starch, respectively from raw cassava starch and 252.4, 224.4 and 165.4 mL H_2 g^{-1} starch, respectively from gelatinized cassava starch. Wang et al. [37] studied biohydrogen and methane production by co-digestion of cassava stillage and excess sludge under thermophilic condition. Compared with one-phase anaerobic digestion, two-phase anaerobic digestion offered an attractive alternative with higher biogas and energy production. Results from continuous experiments demonstrated that the ratio of 3:1 was optimal for hydrogen production with the highest hydrogen production of 74 mL H_2 g^{-1} total volatile solids.

G. Others Sources

Bread, brewery and sago residues have been used for biohydrogen production. Cui et al. [94] produced biohydrogen from beer lees using anaerobic mixed bacteria. Optimal environmental factors of substrate pretreated with 2% HCl, pH 7.0 and 113.67 mg L^{-1} Fe^{2+} were observed. Fan et al. [95] produced biohydrogen from beer lees biomass by cow dung compost. The hydrogen content in the biogas was more than 45% and the maximum H_2 yield of 68.6 mL H_2 g^{-1} of total volatile solids was observed.

Krishan et al. [96] produced biohydrogen from beer brewery wastewater. Chemical oxygen demand was reduced to 30% at hydraulic retention time of 1 day with biogas volume of 6.7 L and hydrogen content of 60%.

Hasyim et al. [46] studied the feasibility of producing biohydrogen from sago starch residues. The methane-free biogas contained up to 55% hydrogen, with the remainder comprising carbon dioxide. Gelatinized dry starch at initial pH of 6.5 and initial starch concentration of 2.5 g L^{-1} showed maximum hydrogen yield of 422 mL H_2 g^{-1} starch; representing 80% of the theoretical value.

IV. Conclusion

Renewable energy sources, such as starch containing biomass are an abundant, inexpensive and reliable substrate for biohydrogen production and have considerable advantages. The microbial hydrogen production will be an alternative way to produce hydrogen. The use of agroindustrial residues and food wastes to generate hydrogen energy could reduce the production cost, making the hydrogen gas more available and cheaper. The ability of hydrogen to be produced from a wide variety of raw materials and using a wide variety of processes makes it so that every region of the world may be able to produce much of their own energy. However, several biological and engineering challenges must be overcome before this promising technology becomes a practical reality. Foremost, cellular metabolism and basic biochemistry that support this process must be well understood and much fundamental research on the mechanism of biohydrogen production must be carried out.

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