Wastewater: A Potential Bioenergy Resource

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Abstract Wastewaters are a rich source of nutrients for microorganisms. However, if left unattended the biodegradation may lead to severe environmental hazards. The wastewaters can thus be utilized for the production of various value added products including bioenergy (H_2 and CH_4). A number of studies have reported utilization of various wastewaters for energy production. Depending on the nature of the wastewater, different reactor configurations, wastewater and inoculum pretreatments, co-substrate utilizations along with other process parameters have been studied for efficient product formation. Only a few studies have reported sequential utilization of wastewaters for H_2 and CH_4 production despite its huge potential for complete waste degradation.

Keywords Bioenergy · Biohydrogen · Biomethane · Nanoparticles · Wastewater

Introduction

The two urgent problems faced by mankind today are energy crisis and waste management. The increasing pollution however requires a reduction of dependency on fossil fuels for energy and thus, encouraging focus on biological energy production [1]. The most economical

way to produce bioenergy would be to couple the production with waste utilization hence helping with the waste management [2]. An attractive approach would be to use a system wherein wastewater is used as a medium for the microbial community to utilize the substrate which is also a biowaste [3]. Such a system will reduce the cost and need of special medium for the bioenergy production as well as will drastically reduce the waste accumulation.

An important form of bioenergy that has attracted worldwide research in recent times is hydrogen (H_2) [1, 4–6]. H_2 being an environmental friendly fuel do not release notorious gases on combustion thus giving it a title of “clean fuel”. Apart from being “clean”, H_2 is also a high energy fuel with a heat value of 3042 cal/m^3 , which is higher than any other conventional fuels [7]. Biologically, H_2 can be produced through light and dark fermentation utilizing a diverse range of renewable resources. Dark fermentation on account of its moderate reaction conditions and high productivities seems to be a more attractive approach than light fermentation and thus has gained much research interest [8].

Apart from H_2 , bio-methane (CH_4) is also an environment friendly fuel that can utilize a variety of bio wastes through anaerobic digestion [9]. Since CH_4 is a greenhouse gas, its natural production through uncontrolled waste decomposition poses a risk to the environment through global warming. Therefore, a controlled system for waste to CH_4 conversion is highly appreciated. To make the process of waste utilization for energy production highly efficient, CH_4 can also be produced by microbial utilization of the leftover metabolites from H_2 producing stage [10, 11]. Apart from H_2 and CH_4 , algal bio-fuels production from wastewater has attracted immense research [12]. This algal oil can be converted into bio-diesel. Bioenergy in the form of electricity can be produced from wastewater

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using microbial electrolytic cells [13, 14]. Ethanol is another fuel that can be produced from wastewater. However, only a limited number of studies have been carried out in this area [15].

The current review aims to present a comprehensive information on wastewater to H_2 and CH_4 production through dark fermentation.

Wastewater to H_2

To address the problem of waste accumulation, an interesting strategy would be to use industrial and domestic wastewater for energy production. Wastewater contains high level of inorganic compounds which are easy to degrade but if left to decompose uncontrollably can deteriorate the ecosystem through its total solids and high biological and chemical oxygen demand [16]. Though, wastewater can be treated by both aerobic and anaerobic treatments, the later gives an added advantage of bioenergy production.

Effect of Process Parameters on H_2 Production

A wide range of wastewaters including food and beverage industry wastewaters to domestic wastewaters used for H_2 production have been listed in Table 1. The key process parameters: Reactor type, inoculum pretreatment, organic loading rate and hydraulic retention time (HRT) have been summarized.

Reactor Configuration

Different types of reactor configurations are used for bio- H_2 production with each configurations having its own pros and cons. The most inexpensive to operate are the batch reactors. However, these are very less efficient and results in lower productivities.

Batch Culture

H_2 production by batch cultures using Owen method and Respirometric methods were compared in 294 mL reactor bottles [17]. After a 20 h lag period the gas production was observed for 75 h. A 43% higher H_2 production was observed with respirometric method however the conversion efficiency was only 26% [17]. Similar reactors with working volume ranging from 100 to 294 mL were also used in food processing and domestic wastewater utilization for H_2 production [18–20]. Reactors with 1.0 L working capacity were also reported but required continuous stirring over a magnetic stirrer [21, 22]. Different

modifications of conventional batch reactors are also reported for anaerobic H_2 production utilizing wastewater. Packed bed reactors (PBR), Anaerobic fluidized bed reactors (AFBR), Anaerobic baffled reactors (ABR), and Upflow anaerobic sludge blanket (UASB) reactors are some of the commonly used high rate modifications of batch reactors [23–25].

The main idea behind a PBR is wastewater treatment using a bed of immobilized bacteria. A number of support materials such as polymers, activated charcoal, sand, granite, etc. can be used in a PBR [26]. Cheaper support materials are required to make the reactors function economically. Coupling of wastewater treatment with gas production provides an added advantage to the PBR. A plexiglass PBR with a working volume of 3 L produced 9.33 L H_2 /g VSS d at the optimized process parameters of 2 h HRT, COD of 34 g/L, pH at 5.5 and 55 °C [27]. A modified PBR configuration with four separate zones was used that assessed the effect of support materials on H_2 production [28]. Feed zone, packed bed zone, effluent collection and gas collection zones made a total working volume of 2.3 L using sugar vinasse as wastewater. With expanded clay and low density polyethylene as support materials, H_2 content of 39.1 and 46.3% respectively was produced while a very poor H_2 content of 26.4 and 4% was observed with charcoal and porous ceramic. The reactors were operated for 15 days [28]. A major disadvantage of PBRs is the blocking of the filter which can be counteracted on using AFBR. These reactors allow the fluidization of biomass through upward flow of liquid. Fine suspended solids are used for immobilization of biomass and to maintain a continuous upflow suspension [10, 29]. A 1.4 L AFBR consisting of expanded clay support material with a grain size of 2.8 and 3.35 mm produced 0.57 L H_2 /h L. The reactor was operated at a fluidization velocity of 1.24 cm/s [10]. The most widely used anaerobic reactors for wastewater treatment and bio- H_2 production are UASB. A 3.5 L working volume UASB reactor was run at an initial HRT of 8 h which was then reduced to 6 h. A H_2 production rate of 2.59 L/L h was observed which was higher than that produced by a CSTR [23]. A buffering system is required in UASB reactor since pH control is very difficult in these reactors [23, 30]. The heat killed methanogenic bacteria were used to form immobilization granules where the H_2 producing strain used was *Klebsiella* sp. TR17. The reactor volume of 1 L was used initially with pure glycerol as medium which was later replaced by crude glycerol, a biodiesel industry waste [4]. Although numerous works have been done with bench top reactors, a fewer studies have also reported H_2 production on a larger scale. A UASB reactor with 50 m³ working volume utilized citric acid wastewater for continuous H_2 production [31]. At an HRT of 12 h, 0.72 m³ H_2 /m³ d was produced by

Table 1 Utilization of waste water for hydrogen production

Feed	Reactor type	Inoculum	HRT (h)	Hydrogen		References
				%	Yield (mL H ₂ /g feed)	
Glucose and cheese whey	AFBR	Poultry slaughterhouse sludge	6	18.5	1.6 mmol/g COD	[21]
Cheese whey	Batch	Anaerobic sludge	–	48	3.3 mol/mol lactose	[15]
Cheese processing wastewater	CSTR	Anaerobic digester sludge	24–84	45	5–22 mmol/g COD	[31]
Sugary wastewater	CSTR	Municipal sewage treatment sludge	1	40	1.37 mol/mol hexose	[35]
Sugarbeet wastewater	CSTR-gs	Anaerobic digested sludge	15	57	231	[49]
Sugarcane vinasse	AFBR	Swine wastewater treatment sludge	1	–	14.74 mmol/g COD utilised	[10]
Sugarcane vinasse	^a APBR	Natural wastewater fermentation	24	46.3	3.2 mol/mol total carbohydrates	[24]
Molasses	Batch	Soil	–	–	109	[13]
Molasses wastewater	CMISR	Municipal wastewater treatment sludge	6	46.6	130.57 mmol/mol substrate	[36]
Dairy industry wastewater		<i>Enterobacter aerogenes</i> MTCC 8100		56.2	105 mL/g COD	[40]
Rice mill wastewater	Batch	<i>E. aerogenes</i> RM 08	–	–	1.97 mol H ₂ /mol sugar	[17]
Noodle Manufacturing wastewater	CSTR	Anaerobic digested sludge	18	–	200	[50]
Corn starch wastewater	Batch	<i>Bacillus cereus</i> , <i>Brevundimonas naejangsanensis</i>	–	–	1.88 mol/mol glucose	[18]
Cassava ethanol wastewater	Batch	Mixed H ₂ producing bacteria	–	–	386.6 mL/g COD	[16]
Cassava wastewater	ASBR	Sludge from cassava wastewater treatment anaerobic pond	16	–	186 mL/g COD removed	[28]
Cassava wastewater	AFBR	Swine wastewater treatment sludge	12	6	2 mmol/g COD	[25]
Cassava wastewater	UASB	Sludge from cassava wastewater treatment anaerobic pond	–	36.4	39.83 l H ₂ /kg COD removed	[27]
Cassava flour wastewater + Sucrose	CMTR	Natural fermentation of effluent	4	–	2.07 mol/mol substrate	[5]
Tapioca wastewater	ABR	Anaerobic mixed cultures	6	22.8	13.40 m L/g COD	[51]
Ethanol wastewater	UASB			1.5	1.66 mL/g COD removed	[26]
Rice winery wastewater	PBR	Acclimated sludge	2	61	291	[23]
Alcohol distillery wastewater	ASBR	Sludge from Red Bull Distillery anaerobic tank	16	34.7	172 mL/g COD removed	[29]
Biodiesel wastewater	ASBR	Activated sludge from biodiesel plant	–	33.4	75.15 mL/g glycerin removed	[30]
Crude glycerol	UASB	Seafood wastewater treatment sludge	4	42	44.27 mmol H ₂ /g glycerol	[4]
Coffee drink manufacturing wastewater	UASB	Anaerobic sludge	6	–	1.29 mol/mol hexose added	[19]
Beverage industry wastewater	CSABR	Municipal wastewater treatment sludge	1	30.1	0.30 mol/mol hexose	[52]
Beverage industry wastewater	CSTR	Compost	1.5	43.7	1.50 mol/mol hexose utilised	[34]

Table 1 continued

Feed	Reactor type	Inoculum	HRT (h)	Hydrogen		References
				%	Yield (mL H ₂ /g feed)	
Food processing and domestic wastewater	Batch	Soil	–	60	–	[14]
Paperboard mill wastewater	Continuous upflow anaerobic reactor	Mixed culture bacteria from activated sludge treatment plant	9.6	70	5.29 mmol/g COD	[1]

ABR anaerobic baffled reactor, AFBR anaerobic fluidized batch reactor, [@]APBR acidogenic packed bed reactor, APBR anaerobic packed bed reactor, ASBR anaerobic sequencing batch reactor, CMISR continuous mixed immobilized sludge reactor, CMTR continuous multiple tube reactor, CSABR continuously stirred anaerobic bioreactor, CSTR completely-stirred tank reactor, UASB upflow anaerobic sludge blanket reactor

Clostridium pasteurianum acclimatized in citric acid wastewater prior to inoculation [31]. Another UASB reactor with a working volume of 24 L produced 10.92 L H₂/kg MLVSS d with cassava wastewater. The resulting gas contained 56.4% H₂ [32]. An on-site microbial electrolytic cell of 120 L capacity used domestic wastewater and produced 0.015 L H₂/L d at an organic loading rate of 0.14 kg COD/m³ d [14].

Continuous Culture

For an efficient large scale operation it is advantageous to use reactors in continuous mode. ASBRs are one of the novel and high rate anaerobic reactors. ASBRs follow a cyclic sequence: feed, react, settle (requires a significant time) and decant. The working volumes of ASBRs is generally high and works best if the biomass is granulated. A work to analyze the effect of number of ASBR cycles per day on H₂ production was conducted. Feed and decant flow rates were optimized for the number of cycles (4 and 6) used per day. The working volume containing cassava wastewater used in this case was 4 L with an automated system to control the cyclic sequence of ASBR [33]. A uniform distribution of organic matter in the reactors with 6 cycles per day caused higher gas production of 388 mL H₂/g VSS d [33]. A very similar ASBR with cyclic time 15, 90, 120 and 15 min for feed, react, settle and decant stages (6 cycles per day) respectively was used to utilize distillery wastewater for H₂ production at a rate of 3.3 L H₂/L d [34]. The same reactor configuration was also used to treat glycerol containing biodiesel industry wastewater with a H₂ production of 75.15 mL/g glycerin removed [35].

Unlike batch reactors CSTRs require peristaltic pumps to continuous feed and decant the reactors. A 2 L working volume reactor initially operated at batch mode for 2 days was reported to produce a significant gas production. Lactose rich cheese whey wastewater was used at 1 day HRT to produce an average of 2.5 L H₂/L d [36]. The major drawback of CSTRs is the continuous washout of the

bacterial cells during medium replacement. This can be prevented by using immobilized cells [37]. Self-immobilization of *Bacillus amyloliquefaciens* CD16 cells in its biofilm was found to enhance bio-H₂ production. The technique utilized a novel biofilm forming media and a high yield of 165 L H₂/L crude glycerol was reported [37, 38]. A very high production rate of 55 L H₂/L d was reported with a CSTR utilizing beverage industry wastewater at 1.5 h HRT [39]. The reactor used immobilized cells to prevent cell washout and maintain high gas productivity. Alginate beads (5–6 mm) were used at a concentration of 10% w/v and 0.56 L reactor working volume. A batch period of 24 h was followed by continuous system at HRT 8 h which was then gradually decreased to 1.5 h. This allowed growth of biomass inside the reactor minimizing the washout [39]. High H₂ production rate of 88.73 L H₂/L d was observed using flex-matala packed bio filters as bacterial carriers. The unique thing about the reactor design was the placement of bio filters inside the reactor, which was installed in the middle of the reactor [40]. A novel modification in CSTR was reported that used continuous multiple tube reactors (CMTR) [5]. Cassava flour wastewater in a 1 L CMTR was used for bio-H₂ production. To prevent cell washout polypropylene rings were assembled at the outlet [5].

Substrate

The type of wastewater used has a strong impact on the amount of gas production depending upon the intrinsic microbial population. High amount of organic acids in some wastewaters or the presence of bacteriocins have a significant influence on H₂ production [36]. Wastewaters rich in lactic acid bacteria contain bacteriocins that might inhibit H₂ production. Cheese whey e.g. is also rich in lactic acid bacteria which may release bacteriocins causing lower H₂ productivities [36]. A very poor H₂ production has been observed from cassava wastewater and has been attributed to the high levels of propionic acid and butyric

acid produced during fermentation [5]. Some industrial wastewaters such as textile dye effluent contains a large fraction of non-biodegradable material and hence are difficult to utilize for bioenergy production. High COD of printing ink wastewater make its biodegradation difficult and thus is hard for bioenergy production [41]. Wastewater from leather and paper industries, forestry and plant medicines also find limited use for bioenergy production because of the tannins present in these wastewaters which are toxic to microbes [42].

To counter such limitations, wastewaters are often subjected to a pretreatment to improve H_2 production.

Other pretreatments include sieving of wastewater. In continuous culture reactors where peristaltic pumps are used to replace the medium, pump failure can be a problem when utilizing wastewater rich in solid matter. To counter such a problem, wastewater is sieved and allowed to settle for a few days as a pretreatment strategy [32, 35, 43]. Addition of nickel (Ni) containing nanoparticles (NPs) to the wastewater for increased H_2 production is yet another strategy [3]. The basic theory behind the strategy is to increase the catalytic activity of Ni-Fe hydrogenases present in a number of fermentative bacteria. A 129% improvement in H_2 production was achieved with 50 mg/L of Ni containing NPs during fermentation of industrial wastewater [3]. The effect of NPs on wastewater to H_2 is discussed in detail in later section.

Fortification of wastewater with a carbon and nitrogen source is also followed in some cases to improve gas production [7]. Distillery wastewaters for e.g. despite being rich in organic matter contain a number of compounds that inhibit biogas production. Addition of simple substrates to such wastewaters can help bacteria adapt and degrade the recalcitrant compounds [10]. Some industrial wastewaters however are very rich in sugars and can be utilized by microbes without any added carbon source for H_2 production. Corn starch wastewater e.g. is rich in sugars, starch, protein and also contain various inorganic salts [22]. Cassava starch wastewater which is rich in carbohydrates (16.12 g/L) is also a good source of nutrients for microbes [8, 33]. This wastewater has been used for H_2 as well as CH_4 production by mesophilic as well as thermophilic bacteria [44, 45]. The utilization however requires a pretreatment. Biodiesel industry wastewater is rich in glycerol which also acts as a rich substrate for H_2 producers [35, 46].

Inoculum

In most of the cases reported, mixed cultures are used for H_2 production for they are easy to control, robust and less prone to contamination. These cultures can be retrieved from a variety of sources and have the capability to utilize

diverse range of organic material in wastewater. Sludge from anaerobic digesters is a common source of mixed culture for H_2 production. A pretreatment is required to eliminate methanogens from the sludge and to enrich the H_2 producers. In heat treatment of the inoculum, the duration and temperature of pretreatment is a determining factor for H_2 production. A 15 min treatment at 100 °C to kill methanogens for continuous H_2 production resulted in 93.75 ± 8.9 mmol/day from paperboard mill wastewater at 9.6 h HRT [1]. A few heat resistant methanogens can survive heat treatment which causes some biogas production along with H_2 [16]. Acid treatments on the other hand causes lower H_2 production efficiency. Aeration of the sludge to inhibit methanogens is also reported with a H_2 production rate of 12.51 mmol/h L [43]. Different inoculum pretreatments including heat (90 °C for 15 min), ultrasonication (20 kHz, 750 W), alkaline treatment (pH 10 for 15 min) and a novel hydrodynamic cavitation (HC) pretreatment method were compared to treat anaerobic sludge [19]. The HC pretreatment along with alkaline treatment for 15 min resulted in a H_2 yield of 3.30 mol/mol lactose while with only HC treatment H_2 yield of 1.89 mol/mol lactose was observed [19].

Soil and compost are also a rich source of microbial community and are used as inoculum in various works after pretreatment [17, 18, 39]. Apart from mixed cultures a number of researchers also use pure cultures for H_2 production. Different strains of *Enterobacter aerogenes* are used as potent H_2 producers [21, 47]. Co-cultures to enhance the conversion efficiencies has also been used for e.g. a co-culture of *Clostridium butyricum* (strict anaerobe) and *E. aerogenes* (facultative anaerobe) reduced the need of expensive methods to maintain anaerobic conditions inside the reactor [48].

Organic Loading Rate

Organic loading rate (OLR) is the quantity of organic material subjected to biodegradation per unit reactor volume per unit time. An increase in OLR up to a certain threshold is known to increase bio- H_2 production rate and yield. However, its optimal concentration depends upon the reactor configuration, pH and temperature conditions and the type of substrate or wastewater. A very high OLR might inhibit the H_2 production due to substrate inhibition caused by an increase in VFAs [49]. In continuous systems however, the gas yield and production rate can be controlled by decreasing HRT or increasing OLR. In one of the studies, an increase in OLR from 8 to 24 kg COD/m³ d has been reported to increase H_2 yield in a CSTR using molasses wastewater. Beyond this the yield was observed to decrease [43]. The effect of increasing OLR (60–360 kg COD/m³ d and 80–480 kg COD/m³ d) and decreasing HRT

(6–1 h) on H_2 production was recorded in two AFBRs [50]. These reactors utilizing sugarcane stillage showed a decrease in H_2 yield from 2.23 to 1.62 mmol H_2 /g COD_{added} with an increase in OLR from 60 to 90 kg COD/m^3 d and 1.85–1.51 mmol H_2 /g COD_{added} with increasing OLR from 80 to 120 kg COD/m^3 d. The highest yield was obtained at OLR of 60 and 80 kg COD/m^3 d with an HRT of 6 h in both the reactors [50]. There are a number of works that have reported a higher OLR as optimum for efficient gas production, while a number of other studies have reported a lower OLR to be the optimum [51]. Thus depending upon the working conditions OLR has to be optimized for an efficient H_2 production.

Process parameters play an important role in modulating the production of any value added product from wastewater. A number of process parameters and their combinations are studied for bioenergy production. Earlier studies have reported use of synthetic wastewater for H_2 production. In most of the cases, carbohydrates such as glucose and sucrose were used as substrates. Use of starch and cellulose as substrate has also been reported in a few cases [52]. However, with substrates such as peptone and lactate H_2 yields were lower than that of carbohydrates containing wastewater. In case of batch reactors with carbohydrates as feed, the H_2 yield showed wide range from 0.4 mL H_2 /g hexose at 0.1% conversion efficiency with cellulose [17] to 333 mL H_2 /g hexose at 61% conversion efficiency with sucrose [53]. While, in case of continuous reactors the corresponding yields ranged from 64 mL H_2 /g hexose at 12% conversion efficiency [54] to 308 mL H_2 /g hexose at 56% conversion efficiency [55]. With other substrates such as lactate and peptone, the respective yields reported were 2.2 mL H_2 /g lactate [17] and 36.2 mL H_2 /g peptone [52].

However, when it comes to continuous supply of bioenergy, what seems important is the availability of a actual wastewater that has high conversion efficiency and gives easy and high rate of product formation. Wastewaters varying from simple sugary industrial effluent to complex lignocellulosic paperboard mill wastewater have been studied for their H_2 production efficiency (Table 1). Wastewaters rich in sugar such as sugarcane vinasse, sugarbeet wastewater and molasses wastewater are reported to produce a high yield of 3.2 mol/mol carbohydrate as well as a low 1.37 mol/mol carbohydrate under varying process parameters [10, 17, 28, 40, 43, 56]. With wastewaters rich in starch such as rice mill wastewater, corn starch wastewater and cassava wastewater, batch reactors are used in most cases to produce H_2 yields ranging from 1.88 to 1.97 mol/mol sugar [21, 22, 29, 32, 57, 58]. However, when fortified with sugars such as sucrose, the yield can increase up to 2.07 mol/mol substrate [5]. Beverage industry wastewaters rich in alcohol may not be as good as sugar or starch rich wastewaters. The bio- H_2 produced varied from 0.30 mol/mol substrate to

1.50 mol/mol substrate under different conditions and reactor designs [34, 39, 59].

Integrated H_2 and CH_4 Production from Wastewater

A single step bioconversion process is easy to operate and require simple reactor configuration. Industrial and domestic wastewaters rich in organic matter can be converted into H_2 , CH_4 , ethanol, bioplastic or even to some enzymes, proteins and fatty acids [60]. Continuous and batch digestion, UASB, CMST, anerobic upflow fluidized bed and filters, leach- bed process and multistage digestion are the anaerobic digestion technologies used for bioenergy production [60]. However, the problem arises when certain wastewaters contain a number of recalcitrant compounds. The bioconversion efficiencies in such cases can be quite low. To overcome this, an integrative bioconversion process can be designed. Such a process can convert unutilized metabolites from one stage of bioprocess to other valuable products in the following stage. Effluent from H_2 production stage contains some amount of organic acids as well as unutilized carbon source. This partially utilized effluent has the potential to either be used for biogas (CH_4) production or it can be recycled for further H_2 production. Waste glycerol containing media used for H_2 production has been recycled and reported to increase overall yield [32]. The effluent from the H_2 producing bioreactor when mixed with fresh media in 1:3 ratio and recycled for H_2 production did not show any adverse effect on bioenergy production. This improves waste utilization and total gas production [37].

Effluent from H_2 production can also be integrated with CH_4 production (Table 2). Generally, a short HRT is preferred for the first stage so as to increase the washout of slow growing methanogens. While, for the second stage a longer HRT makes sure the methanogens are retained inside the reactor.

A detailed analyses of food and vegetable waste utilization for H_2 , CH_4 and integrated H_2 – CH_4 production has been carried out [61]. The slurries of the waste was subjected to a microbial pretreatment by a hydrolytic consortia. At 3% total solids (TS), 85 L H_2 /kg TS, 63.3 L CH_4 /kg TS was produced from food waste slurry (FWS) and 17 L H_2 /kg TS, 61.7 L CH_4 /kg TS from vegetable waste slurry (VWS) was produced. However, with an integrated H_2 – CH_4 approach, the effluent of H_2 producing stage produced 31.7 L CH_4 /kg TS from FWS and 28.3 L CH_4 /kg TS from VWS. Thus, 70–75% of CH_4 yield produced by direct methanation could be recovered by the integrated approach [61]. The work shows significant gas production from food and vegetable wastes thus it may be possible to use a similar approach to treat wastes, the slurries of which can be prepared in wastewaters.

Table 2 Sequential hydrogen and methane production from wastewater

Feed	Reactor type	Inoculum	Hydrogen		Methane		References
			%	Yield (mL H ₂ /g feed)	Max. PR (L CH ₄ /L d)	Max. yield (L CH ₄ /kg COD)	
Sugarcane vinasse	AFBR	Swine wastewater treatment sludge		14.74 mmol/g COD utilised	–	–	[10]
Dairy industry wastewater	Batch	<i>Enterobacter aerogenes</i> MTCC 8100	56.2	105 mL/g COD	–	190 mL/g COD	[40]
Cassava ethanol wastewater	Batch	Mixed H ₂ producing bacteria		386.6 mL/g COD	0.88 mL/g COD/h	117.9 mL/g COD	[16]
Cassava wastewater	AFBR	Swine wastewater treatment sludge	6	2 mmol/g COD	0.9	–	[25]
Cassava wastewater	UASB	Sludge from cassava wastewater treatment anaerobic pond	36.4	39.83 L mL H ₂ /kg COD removed	0.91	115.23	[27]
Ethanol wastewater	UASB		1.5	1.66 mL/g COD removed	2081.4 mL CH ₄ /LRd	407 mL/g COD removed	[26]
Biodiesel wastewater	ASBR	Activated sludge from biodiesel plant	33.4	75.15 mL/g glycerin removed	672.84 mL/L d	128.4 mL/g COD removed	[30]

AFBR anaerobic fluidized batch reactor, ASBR anaerobic sequencing batch reactor, UASB upflow anaerobic sludge blanket reactor

A sequential dark and photo fermentation for H₂ production was coupled with CH₄ production [20]. The work reported treatment of effluent from dark fermentation to remove excessive NH₄⁺ before photo fermentation. These ions which are known to inhibit nitrogenase are removed by treating them with zeolite. A maximum H₂ yield of 1193 mL/g COD was achieved at OLR of 20 g COD/L after 72 h dark fermentation. While in photo fermentation, 375.8 mL H₂/g COD was achieved at a lower OLR of 5 g COD/L cassava ethanol wastewater. The effluent after these stages at OLR of 20 g COD/L resulted in 274 mL CH₄/g COD after a 432 h fermentation [20]. Various studies have investigated utilization of cassava wastewater for sequential H₂ and CH₄ production (Table 2). Thermophilic bacteria in a two stage UASB reactor produced 2.2 L H₂/d with a CH₄ yield of 183.31 mL CH₄/g COD_{removed} at an OLR of 90 kg/m³d [45]. Another UASB reactor reported H₂ and CH₄ yields of 15 mL/g COD_{removed} and 259 mL/g COD_{removed}, respectively under thermophilic conditions at OLR of 1200 mg/L cassava wastewater [44]. Under mesophilic conditions biodiesel industry effluent has been utilized at a high glycerol concentration to produce H₂ and CH₄ in a two stage ASBR. At an OLR of 11.3 kg/m³ d, H₂ yield was 75.15 mL/g glycerin removed [35].

Nano-particles in aid of bio-H₂ Production from Wastewater

The metabolism of bio- H₂ production through dark and photo fermentation involve two main enzymes: hydrogenases and nitrogenases [62]. The different types of hydrogenases: [Fe]-, [FeFe]- and [NiFe]- hydrogenases are strongly affected by the presence of iron and nickel ions in the medium thus, influencing the H₂ production process [62]. Hence, the NPs made from these ions as well as from other ions that can influence microbial metabolism have attracted a great attention for increased H₂ production [63–65].

Effectiveness of NPs varies with the source of industrial or domestic wastewater utilized for H₂ production (Table 3). With dairy wastewater, utilization of Fe₂O₃ NPs enhanced H₂ production by 24% while with molasses wastewater the enhancement was 44% but with a 4 times higher concentration of NPs [66, 67]. Fe₂O₃ NPs (25 mg/g VSS) when used with starch wastewater improved H₂ yield by 57.8% through inoculum immobilization [68]. Under thermophilic conditions, a 22.7% improvement in H₂ yield was seen when Ni NPs were used with monoethylene glycol containing wastewater [3].

With granular activated carbon (10 g/L), powdered activated carbon (5 g/L) and SiO₂ (120 mg/L) NPs the starch wastewater utilization showed significant improvement in H₂ yields [69]. An enhancement of 94.5 and 44% was observed with granular activated carbon and powdered activated carbon, respectively. While with SiO₂, 666% enhancement was observed [69].

Table 3 Nano-particles in aid of hydrogen production from wastewater

Feed	Nano-particles		Reactor type	Hydrogen		References
	Comp.	Conc. (mg/L)		Yield efficiency (%)	Yield (mmol/g COD)	
Sucrose containing wastewater	Au	10 nM	Batch	50	4.48 mol/mol sucrose	[65]
Dairy wastewater	Fe ₂ O ₃	50	Batch	24	16.75	[59]
Dairy wastewater	NiO	10	Batch	16	15.7	[59]
Molasses wastewater	Fe ₂ O ₃	200	Batch	44	7.85	[60]
Molasses wastewater	NiO	5	Batch	23.5	6.73	[60]
Starch wastewater	Fe ₂ O ₃	25 ^a	ABR	57.8	0.90 mol/mol hexose	[61]
Molasses wastewater	Fe ₃ O ₄	50	Batch	83.3	44.3 mL/g COD	[58]
Monoethylene glycol containing wastewater	Ni	60	Batch	23	24.7 mL/g COD	[3]
Wastewater	SiO ₂	120	CSTR	666	7.02 mol/Kg COD day	[2]
Sucrose wastewater	Fe ₂ O ₃		CSTR	75	0.3 L/g sucrose	[57]

ABR anaerobic baffled reactor, CSTR completely-stirred tank reactor

^amg/g VSS

Cu NPs unless encapsulated in some porous matrix or at concentration below 2.5 mg/L are inhibitory for H₂ production [63, 70]. Use of Ag NPs at 20 nM concentration can increase H₂ yield from glucose containing synthetic wastewater by 67.6%. The NPs have been shown to alter the production of metabolic intermediates such as acetate, butyrate, ethanol, propionate and valerate in H₂ producers [71]. Au NPs have also been used to enhance H₂ production from sucrose containing wastewater. At 5 nM concentration the NPs improved H₂ yield by 62.3% [72].

Although great success has been achieved in improving the biological H₂ production, however, to make the process of enhancement in H₂ yields more economical and effective focus on biologically synthesized NPs is required. This will allow better microbial tolerance than that in chemically synthesized NPs containing media [73].

Conclusion and Future Perspectives

A diverse range of wastewaters can be utilized to produce bioenergy, the yield and efficiency of which varies with the process parameters such as reactor configuration, operation mode, co-substrates, pH, temperature and hydraulic retention time. In wastewaters supplemented with cellulose results in poor conversion efficiencies whereas, with sucrose up to 61% conversion can be achieved. Wastewaters rich in sugar such as sugarcane vinasse, sugarbeet wastewater and molasses wastewater are reported to produce a high yield of 3.2 mol/mol carbohydrate. Wastewaters rich in starchy waste originating from diverse sources

results in around 1.9 mol H₂/mol sugar. However, addition of sucrose rich waste to starchy waste improves the yield up to 2.1 mol/mol substrate. With glycerol originating from biodiesel industry 3.3 mmol H₂ can be generated through ASBR and 44.4 mmol/g feed with UASB of 24 L capacity. In a continuous culture reactor 165 L H₂/L of CG has also been reported.

An integration of waste treatment plant with bioenergy production system can help with continuous supply of wastewater for H₂ production. The treated wastewater after collecting H₂ can be used as a substrate for biogas production. Thus, such a multi-channel system could save considerable energy required for waste treatment and lead to the production of cleaner fuels in the form of bioenergy.

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Compliance with Ethical Standards

Conflict of interest No conflict of interest declared.

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