Hydrogen production from agricultural waste by dark fermentation: A review

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Abstract

The degradation of the natural environment and the energy crisis are two vital issues for sustainable development worldwide. Hydrogen is considered as one of the most promising candidates as a substitute for fossil fuels. In this context, biological processes are considered as the most environmentally-friendly alternatives for satisfying future hydrogen demands. In particular, biohydrogen production from agricultural waste is very advantageous since agri-wastes are abundant, cheap, renewable and highly biodegradable. Considering that such wastes are complex substrates and can be degraded biologically by complex microbial ecosystems, the present paper focuses on dark fermentation as a key technology for producing hydrogen from crop residues, livestock waste and food waste. In this review, recent findings on biohydrogen production from agricultural wastes by dark fermentation are reported. Key operational parameters such as pH, partial pressure, temperature and microbial actors are discussed to facilitate further research in this domain.

Keywords

Agricultural waste, Anaerobic digestion, Biohydrogen production, Biological processes, Dark fermentation

Abbreviations

ASBR: Anaerobic Sequencing Batch Reactor
CSTR: Continuous Stirred Tank Reactor
COD: Chemical Oxygen Demand
HRT: Hydraulic Retention Time
HAB: Homo Acetogenic Bacteria

LCFA: Long Chain Fatty Acids
MPB: Methane-Producing Bacteria
SRB: Sulfate-Reducing Bacteria
UASB: Upflow Anaerobic Sludge Blanket
VS: Volatile solids
VFA: Volatile Fatty Acids

1. Introduction

The energy crisis and environmental degradation are currently two vital issues for global sustainable development. It is now accepted that the dependence on fossil fuels - over 80% of energy consumption - contributes not only to climate change and global warming, but also to a rapid exhaustion of natural energy sources [1]. Almost all countries worldwide are interested in the search for new, clean and renewable energy supplies. Over the last decades, research efforts have focused mainly on bioethanol and biodiesel production. These first generation biofuels made from food crops such as corn, sugar cane, and palm oil, have been seen as possible alternatives to ease the world's dependence on gasoline or diesel. However, they have indirectly caused an increase in food prices and thus contributed to the recent global food crisis. Hence, the production of second generation biofuels by the conversion to biofuels of whole plants, including agricultural residues, is now essential in the move towards renewable energy.

The original concept of "environmental biorefinery" consists of installations designed to produce a wide range of products to optimize the conversion of biomass. Alternative energy sources such as biogas from waste and especially biohydrogen need to be considered [2]. Biohydrogen can be used directly in combustion engines for transportation or, after purification, in fuel cells for producing electricity. Its high energy content per unit of weight (142 $kJ.g^{-1}$) and since water is the only by-product generated by oxidative combustion, makes hydrogen the ideal and most environmentally friendly alternative to fossil fuels [3]. To date, hydrogen is not commercialized as an energy source but it is widely used as a chemical reactant in the production of fertilizers, for refining diesel and for the industrial synthesis of ammonia. Schemes for the use of the hydrogen as energy resource have been restricted in large part by high production costs, technical storage requirements and distribution methods [4]. At present, 88% of commercial hydrogen derives from fossil fuels (natural gas, heavy oils or coal) [5]. Water electrolysis has extensively developed in recent years, and is now more widely used, supplying up to 4% of current total hydrogen production. However, all such techniques are highly energy-consuming and are unsustainable processes. One promising alternative is hydrogen produced biologically which requires much less energy. Regardless of the great interest in biohydrogen production from biomass at a laboratory research level,

substantial technical advances in the biological processes involved are still required if the biohydrogen market is to become economically viable. The most promising sources of biohydrogen involve direct water biophotolysis by green algae, indirect water biophotolysis by cyanobacteria, the photo-fermentation by photosynthetic bacteria, and dark-fermentation by strict or facultative anaerobic bacteria. Considering that agri-waste is made up of complex substrates and can be degraded biologically by complex microbial ecosystems, dark fermentation is a key technology for the production of hydrogen from crop residues, livestock waste and food waste.

The purpose of this paper is to present an up-to-date overview of current knowledge about biological dark fermentation processes producing hydrogen from agricultural and food waste.

2. Feedstock and hydrogen potential

Many studies investigating hydrogen production by dark fermentation have used simple sugars such as glucose or sucrose as model substrates. In contrast, fewer studies have looked into solid substrate conversion. For organic materials to be potentially useful as substrates for sustainable biohydrogen production, they must be not only abundant and readily available but, also, cheap and highly biodegradable. Agri-waste and food waste meet all these requirements. As to their abundance, about 0.7 billion tons of agricultural and forestry waste were generated in Western Europe between 1998 and 2001 [6]. In France, a survey of the years 1995 to 2006 showed that total annual waste production had increased to about 849 million tons by 2006, of which agricultural and forestry waste represented around 43%, *i.e.* 374 million tons [7]. In Germany, the second biggest agricultural country in Europe, agri-waste represented more than 175 million tons per year in 2000, including 25 million tons per year of agricultural biomass. By way of comparison, German municipal waste represented only 16 million tons per year and industrial waste 9 million tons [8].

Three categories of agricultural residues can be distinguished: (i) the waste generated from direct agricultural production, *i.e*. crop residues; (ii) livestock waste, *i.e*. animal manure, and (iii) food waste.

2.1. Crop Residues

Agricultural residues from harvested crops are the most abundant, cheapest and most readily available organic waste to be biologically transformed; they include straw, stover, peelings, cobs, stalks, bagasse, and other lignocellulosic residues [9]. The annual lignocellulosic biomass generated by the primary agricultural sector has been evaluated at approximately 200 billion tons worldwide [10]. All agricultural crops are biodegradable and, to varying degrees, may be converted biologically in anaerobic digestion processes to

biohydrogen and biomethane.

Hydrogen yields from various crop substrates, as recorded in the literature, are presented in Table 1. The origins of the organic substrates are quite similar, nevertheless, untreated raw material presents generally lower yields, ranging from 0.5 to 16 $mL_{H2}.g_{VS}^{-1}$. Under mesophilic conditions the lowest yield was reported from the conversion of wheat straw to hydrogen in a batch reactor [11], while the highest was obtained using cornstalks [12]. The yield of fermentative hydrogen from crop residues in thermophilic conditions at 70˚C was higher than that in mesophilic conditions indicating that temperature favors hydrolysis [13]. Indeed, the "cornstalks" category in Table 1 shows variable hydrogen yields, likely because of the varied composition of the carbohydrates, which include cellulose, hemicellulose and lignin [12][14]. Moreover, as reported in anaerobic digesters producing methane from agricultural waste, the crop species, the harvesting time and the variable silage period must all be considered as main factors impacting on biogas fermentation [15]. A recent review of the literature summarized the composition of different crops residues, *e.g.* wheat straw, corn stover and rice straw as containing cellulose, hemicelluloses and lignin in a range of approx. 32-47 %, 19-27% and 5-24%, respectively [16]. Although no trend was observed in the reported data, a reasonable hypothesis is that biohydrogen yields may be inversely correlated to the cellulose and lignin contents of the waste, as observed by Buffiere *et al.* [17] for methane production.

The production of biohydrogen from crop waste biomass is limited by the hydrolytic activity of the microorganisms involved in the biological attack of the heterogeneous and microcrystalline structure of lignocellulosic component, and in the decomposition of cellulose-like compounds to soluble sugars. Appropriate pretreatment steps for the raw material are often required in order to favor hydrolysis. The main pretreatments are based on mechanical, physical, chemical and biological techniques [9]. A mechanical shredding step is essential to reduce particle size and increase the surface area of the organic waste prior to fermentation. As a consequence, solubility and fermentation efficiency are both favored in the acidogenic fermentation process (Figure 1). In all studies reported in Table1, the crop residues were mechanically treated prior to the experiments and this technique should be further investigated to determine the influence of such pretreatment on overall performances. Chemical pretreatments methods using oxidizing agents, alkali, acids and salts are most frequently investigated because they require no direct energy input [9]. The biohydrogen yield from cornstalks treated by NaOH (0.5%) reached 57 $mL_{H2}.g_{VS}^{-1}$, *i.e.* 19-fold the initial value of raw material (3 $mL_{H2}.g_{VS}^{-1}$)[14]. Zhang *et al.* [14] also investigated biohydrogen production from cornstalk waste after an acidification pretreatment coupled to heat pretreatment. A maximum cumulative H_2 yield of 150 $ml_{H2}.g_{VS}^{-1}$ was obtained after a 0.2% HCl treatment, *i.e.* 50 times the initial value, thus proving the efficiency of the acidification pretreatment step [14]. Although this value is remarkable in the light of the average values reported in Table 1, such performances are within the range of the theoretical biohydrogen yield in mixed

cultures, *i.e.* 311 $mlH_2.g_{Hexose}^{-1}$, calculated from 2.5 $mol_{H2}.g_{Hexose}^{-1}$ according to Hawkes *et al.* [18]. Fan *et al.* [11] demonstrated that an acidic pretreatment of 2% HCl coupled to microwave heating led to the increase of soluble sugar content of wheat straw from 0.2% to 9.6% and to the decrease of cellulose and hemicellulose content from, respectively, 22% to 15% and 21% to 13%. The maximum hydrogen yield observed in this case was 68 $mL_{H2}.g_{VS}^{-1}$, which is 136 times the initial value (0.5 $mL_{H2}.g_{VS}^{-1}$) observed on untreated material [11]. Similar results were observed with steam explosion as pretreatment, with a yield increasing from 9 $mL_{H2}.g_{\text{raw corn straw}}^{-1}$ to 68 $mL_{H2}.g_{\text{treated corn straw}}^{-1}$ [12]. Given the present state of knowledge, further experimentation is required to better understand the impact on biohydrogen production performances of the compositions and characteristics of organic substrates. Pretreatment processes for crop residues also require specific investigation since the origins and compositions of the organic substrates determine which specific pretreatment is the most suitable.

2.2 Animal manure – livestock waste

Three main types of animal manure have been distinguished: urinary waste *i.e.* slurry or liquid manure from livestock or poultry; solid manure or farm yard manure; and wastewater which is a collection of process water in farms, feedlot runoff, silage juices, bedding, disinfectants and liquid manure [19]. More than 1500 million tons of animal manure is produced yearly, including 1284 million tons of cattle manure and 295 million tons of pig manure across the 27 member states of the European Union [20]. Where manure is not managed or treated, it represents a major risk of air and water pollution. On the one hand, nutrient leaching (primarily nitrogen and phosphorous) and pathogen contamination can lead to direct surface water damage and, on the other hand, manure can release up to 18% CO_2 equivalent and 37% CH_4, contributing to the green house effect [20].

On European farms, animal manure is usually treated in storage tanks, and then the liquid fraction is separated by centrifugation and finally spread on farmland. The solid fraction is subsequently treated by anaerobic digestion to be further used as fertilizer in agriculture [21]. Since agricultural biogas facilities have been extensively used to co-digest manure and other residues suitable for methane production, these large-scale farm installations provide the necessary equipment to readily implement biohydrogen bioprocesses [22].

Biohydrogen yields from livestock waste are presented in Table 1. Mainly, they are much lower than those observed from crop residues, with values ranging from 4 to 29 $ml_{H2}.g_{VS}^{-1}$. In most studies, either chemical or thermal pretreatment associated to thermophilic conditions are required to avoid methanogenic activity. Indeed, the indigenous methanogenic microflora will rapidly convert hydrogen to methane, as shown by Yokoyama *et al.* [23]. The highest yield (*i.e.* 65 $ml_{H2}.g_{VS}^{-1}$) was reported in a study investigating the potential for hydrogen production of cattle manure thermally pretreated (Table 1). This high yield was likely the

result of using fresh manure sampled directly at the cattle feedlot prior to the experiment. This assumption is supported by the study of Bonmati *et al.* [24] who observed a 3.5-fold decrease in methane production when the pig slurry was stored for several months. Meanwhile, the ammonium concentration increased 3-fold over the initial value because of the decomposition of organic matter [24]. A similar inhibition has been observed for biohydrogen production from animal slurry. Indeed, Kotsopoulos *et al.* [25] concluded that the low production yield of 4 $mL_{H2}.g_{VS}^{-1}$ from pig slurry was due to ammonium inhibition. Livestock manure from pork and poultry have been reported to contain up to 4g $N.L^{-1}$ and cattle manure about 1.5 g $N.L^{-1}$ [26]. Because of the high nitrogen content, shock loading of slurry can cause severe inhibition of the whole biological anaerobic and hydrogen fermentation processes [27] [28]. Additionally, it has also been observed that high sulfate concentrations in swine manure act as a strong inhibitor of biohydrogen production through the growth of highly competitive hydrogen-consuming sulfate-reducing bacteria [29]. With the aim of avoiding nitrogen inhibition, another study on liquid swine manure showed a high yield of 209 $mL_{H2}.g_{VS}^{-1}$ after the addition of glucose as an additional substrate in a semi-continuously-fed reactor [30]. This observation suggests the potential use of the co-digestion of animal manure and carbohydrate-rich feed to produce biohydrogen. In this case, the co-digestion process should even be envisaged locally, in the light of agricultural facilities to directly use local crop materials, in order to optimize the loading ratio C/N by dilution of other inhibiting factors. This should, consequently, increase the stability of the biological process. A recent study investigating the anaerobic co-digestion of cattle slurry with vegetable/fruit wastes and chicken manure showed a substantial 2-fold increase in the methane yield [31].

2.3. Food waste

Food waste has high energy content and is highly biodegradable, *e.g.* it contains 85-95% of volatile solids and 75-85% moisture, favoring microbial development [32]. Food waste is usually disposed as landfill which can lead to problems of putrid smells and leachates polluting underground water if not handled properly [22]. Anaerobic digestion is recommended for treating food wastes [33]. Over the last decades food waste has been the most studied feedstock for hydrogen production, including kitchen refuse [34], a part of municipal waste [36], food industry co-products such as oil mill [36] [37], cheese whey [38], and starch-manufacturing waste [39]. In Table1, several maximal biohydrogen production yields observed in anaerobic reactors are reported. As in the results obtained with crop residues and livestock waste, the performances display great variation, from 3 $mL_{H2}.g_{VS}^{-1}$ to more than 290 $mL_{H2}.g_{VS}^{-1}$, due to the different composition of the matter involved. The average production is substantially higher than the values obtained from crop residues and livestock. About ten years ago, individual food substrates *i.e.* rice, carrot, cabbage, chicken skin, egg and lean meat began to be sorted out from municipal waste for assessment [40]. In

the latter study, biohydrogen production was assessed from a range of relatively simple substrates for further assessment of the production potential with mixtures made up of such simple constituents. Later, other studies using food waste from institutional catering were carried out in batch tests and showed yields of 60 $mL_{H2}.g_{VS}^{-1}$ to 196 $mL_{H2}.g_{VS}^{-1}$ [32][41]. Studies of continuous fermentation systems have been reported more recently, showing no significantly higher yield, but they have proved the feasibility of using food waste in future continuous pilot or industrial-scale applications [13] [42]. Again, more recently, many studies have focused on agri-food industry waste as a source of substrates for producing biohydrogen [36] [37] [38] [43] [44]. Among them, carbohydrate-rich waste shows great promise for the intensive production of biohydrogen. For instance, biohydrogen yields from molasses and cheese whey approached a value of 2.5 $mol_{H2}.mol_{hexose}^{-1}$, which corresponds to the maximal expected yield in mixed culture [38] [44].

In addition, thermophilic conditions also favor biohydrogen production. Indeed, food waste from institutional catering generated around 81 $mL_{H2}.g_{VS}^{-1}$ under thermophilic conditions, compared to 63 $mL_{H2}.g_{VS}^{-1}$ under mesophilic conditions [45]. Other studies reported increasing yields from 13 $mL_{H2}.g_{VS}^{-1}$ to 65 $mL_{H2}.g_{VS}^{-1}$, respectively under mesophilic and thermophilic conditions [13] [42]. For the lowest values, *i.e.* 12.6 $mL_{H2}.g_{VS}^{-1}$, a mixture of slaughterhouse waste, food waste and manure was utilized as substrate. It included much protein and fat [13], which might well explain of the low hydrogen yield. Although thermophilic conditions are recommended, they are energy consuming. If the energy for heating the fermentation system could be generated through a biogas/thermal exchange system, thermophilic continuous processes could then be considered as sustainable.

In conclusion, crop residues, livestock, and food waste are potentially suitable substrates for hydrogen production by dark fermentation. Food waste gives the highest yield of hydrogen, followed by crop residues and animal manure. It is recommended that waste generated by agricultural activities such as crop residues, should be co-digested with animal manure using already existing biogas plants by implementing a dedicated biohydrogen production stage. By coupling with methane bioprocesses, the treated effluent could be finally used as fertilizer. In this scheme, the production of biohydrogen and biomethane might be used for heating and electricity generation or, in the case of biohydrogen, also as a chemical reactant. Although food waste offers great potential as a hydrogen resource, the performances of the biological processes are related not only to the operating conditions, but also, to the composition of the organic waste. Future research is recommended to better understand the influence of feedstock composition, to predict bioreactor performances and optimize the co-digestion system.

3. Biological reactor operation

The major limitation of biohydrogen production at an industrial scale concerns the low productivity and the low conversion yields of the fermentative biological processes. Based on current hydrogen productivity, industrial processes would require very large-volume reactors. Levin *et al.* [46] reported that the minimum size of a bioreactor required to power a small proton exchange membrane fuel cell installation of 1 kW was 198 L, when considering H_2 productivity of 2.7 $L.L^{-1}.h^{-1}$ using dark fermentation and mesophilic conditions [46]. The productivity of hydrogen-producing bioreactors treating agri-waste is substantially lower than the result cited above because of the use of complex and polymeric organic substrates and also the mixed cultures as inoculum. However, the optimization of the operating conditions of biological reactors remains a key parameter for the improvement of biohydrogen production. Specifically-optimized bioreactors could help to determine whether the use of agricultural waste *in situ* would be technically feasible and economically viable. To develop practical independent biohydrogen practical applications on farms, likely coupled with methane production, it is vital to consider concomitantly advances in biotechnology to enhance biohydrogen yield and biogas quality along with fuel cell development [46]. In order to meet these requirements, the following operating conditions must be considered.

3.1 Operating conditions

3.1.1 pH

pH is one of the most important factors to be regulated in anaerobic digestion processes [47][48]. Indeed pH affects not only the yields of hydrogen production in mixed cultures, but can also modify by-product spectrum and impacts the structure of the microbial communities [49][50][51]. Table 2 summarizes the operating parameters in reactors treating agricultural residues inoculated with naturally mixed microbial cultures. Optimal H_2 production appears to take place with a pH of 5.0 - 6.0 for food wastes [41][52][53], whereas a neutral pH is recommended for crop residues and animal manure [12][14][25][23]. Two different types of experimentation have been performed to determine the optimal pH : one involved adjusting different initial pHs in a series of batch tests while the other maintained the same pH in continuous reactors during the fermentation process [13] [54] [23]. Li *et al.* [12] investigated a large range of initial pHs, from 4 to 8, in batch tests. They showed that a pH of 7-7.5 as optimal for the conversion of corn straw to biohydrogen [12]. As the accumulation of by products, *i.e.* acetate and butyrate, lowered the pH of the medium, higher pH (*i.e.* around neutrality) led to better hydrogen yields. As suggested by Wang *et al.*[55], who reported that batch reactors with not regulated pH and treating sucrose are the systems most commonly studied, further investigations should focus rather on pH-controlled systems and on more complex organic wastes as substrates. In continuous reactors, in contrast, pH is usually controlled. A varied pH ranging from 4.5 to 6.5 was tested on tequila's vinasses in a semi-continuous CSTR reactor [48]. It was concluded that a pH of 5.5 was optimal for hydrogen

304 production. A similar value was proposed in another study devoted to brewery waste in a
305 CSTR with a pH ranging from 5.0-6.5 [56]. As a general rule, the optimal pH in terms of
306 biohydrogen production is within a range of 5.0 - 7.0 which probably favors the activity of the
307 hydrogenases and is also suitable for microbial development in dark fermentation [57].
308 In addition, the pattern of intermediate VFAs is different under variable pH conditions.
309 Butyrate and acetate are the two main products, but at low pHs butyrate is preferentially
310 produced. Hydrogen-producing butyrate-acetate pathways are favored at pH 4.5-6.0 while at
311 neutral or higher pH conditions, ethanol and propionate accumulate [18][41][58][59]. When
312 using brewery waste as a substrate, Fan *et al.* [56] observed that, at pH 6.0 or below, acetate
313 and butyrate were the major by-products whereas solventogenesis (propanol, butanol and
314 ethanol) occurred at pHs higher than 6.5 [56]. This was confirmed by Fang *et al.* [60] in a
315 study investigating the effect of pH from 4.0-7.0 on by-product formation. At low pH,
316 butyrate and acetate were dominant products while ethanol, lactate, propionate and caproate
317 appeared at higher pHs [60]. Temudo *et al.* [61] studied the impact of the pH on metabolic
318 activity and microbial diversity in fermentation processes with glucose, xylose, and glycerol
319 at 30°C. They showed that a low pH conditions (< 6), the product spectrum consisted mainly
320 of butyrate and acetate while at high pH, the spectrum shifted to acetate and ethanol. It is
321 noteworthy that under both high and low pH conditions, the fermentation pattern was clearly
322 associated with the dominance of *Clostridium* species, whereas at intermediate pHs,
323 metabolic shifts involved higher microbial diversity [61]. This suggests that pH effects result
324 not only from a shift in metabolic pathways but also in major changes in microbial
325 communities.
326

327 **3.1.2 Biohydrogen Partial Pressure**

328 Many studies have already reported that partial pressure of hydrogen is a restrictive factor
329 in the course of the fermentation of organic waste. The oxidation of reduced components such
330 as Long-Chain Fatty Acids to VFAs, concomitantly with hydrogen production, is the
331 consequence of a low biohydrogen concentration in the medium because reactions are
332 thermodynamically unfavorable [62]. The positive Gibbs energy of LCFA degradation (ΔG^0=
333 +48 mJ/mol) shows that the degradation of fat through the β-oxidation pathway is
334 thermodynamically unfavorable and therefore requires an extremely low level of hydrogen
335 partial pressure (see Equation 1) [62]
336

337 n-LCFA $\rightarrow$ (n-2) – LCFA + 2 Acetate +2 H_2 ΔG°= +48 kJ.mol^{-1} (1)
338

339 $CH_3COOH + 2H_2O \rightarrow 4H_2 + CO_2$ $\Delta G° = +104.6$ kJ.mol^{-1} (2)
340

341 Additional formation of hydrogen could also derive from the degradation of acetate (see

Equation 2) [63]. This conversion is thermodynamically unfavorable at moderate temperatures and the reaction is therefore extremely sensitive to biohydrogen concentration. Furthermore, the inverse reaction, called homoacetogenesis, is rather favored in the fermentation process and partly reduces the performance of bioreactors through the accumulation of acetate in the medium. By the increase of the hydrogen concentration in the medium due to microbial metabolism, not only biohydrogen production may be affected but also a shift of metabolic pathways towards solventogenesis has been observed, *i.e.* the accumulation of lactate, ethanol, acetone and butanol [46]. Recent research indicates, however, that the main factor leading to solventogenesis is the accumulation of volatile fatty acids rather than hydrogen partial pressure [64]. Especially when feeding with a high glucose concentration, the intermediate acids produced, particularly butyric acid, initiate solventogenesis [65].

To decrease pH_2 in the medium, especially in highly concentrated bioprocesses treating organic waste, agitation is the most usual technique. Chou *et al.* [66] studied the conversion of brewery grains to hydrogen in a 100 L pilot bioreactor. Experiments showed that the rate as well as the yield of biohydrogen production increased from 1.8 $mL.L_{reactor}^{-1}$ to 6.1 $mL.L_{reactor}^{-1}$ while the stirring was speeded up from 20 to 100 rpm [66]. Several other alternatives exist to improve gas extraction, including gas sparging and biohydrogen stripping from reactor headspace by membrane absorption. Mizuno *et al.* [67] showed that sparging nitrogen gas into a fermentor fed with simple sugars led to double the biohydrogen yield from 86.76 $mL_{H2}.g_{VS}^{-1}$ to 187.86 $mL_{H2}.g_{VS}^{-1}$. Others gases such as argon or a mixture of recirculation gases have also been used [67] [68]. The main disadvantage of these techniques is that, regardless of the significant biohydrogen removal, the sparging gas dilutes the biohydrogen content and creates a further reduction in separation efficiency. In the event of upscaling to an industrial level, the high energy consumption in sparging processes and H_2 purification would raise the production costs, and the fluctuation in gas prices would impact directly on the economic viability of the process. Membrane-absorption techniques offer other energy-effective alternatives for hydrogen removal from a gas mixture. Liang *et al.* [69] reported a reduced biogas partial pressure by introducing a submerged hollow-fiber silicone membrane into the reactor. A Pd-Ag membrane reactor [70] and a synthetic polyvinyltrimethyl silane membrane reactor [71] exhibited the highest hydrogen selectivity. The main disadvantage of using membrane-absorption techniques is the presence and the development of a biofilm over time which may favor the emergence of methanogenic bacteria.

Despite the different techniques available for reducing the partial hydrogen pressure, more research is still required to develop efficient and low cost gas purification systems aiming at the direct use of hydrogen from biogas to fuel cells at industrial scale.

3.1.3 Temperature

Temperature is often considered as one of the most important parameters affecting both biohydrogen production yields and microbial metabolisms in mixed cultures [57]. Because of the complexity of the agri-waste and the variable operating conditions, no optimal temperature for hydrogen fermentation can be assessed from the data in the literature. Most studies on fermentative hydrogen production have been based on mesophilic temperatures. Li *et al*. [57] reported that 73 of 101 case studies were carried out at mesophilic temperatures. Crop residues usually present higher yields at thermophilic temperatures due to a better hydrolysis of the lignocellulosic compounds. For instance, the highest amounts of hydrogen from grass were obtained at 70°C using a heat-treated inoculum from a dairy farm digester, *i.e.* 16 $mL_{H2}.g_{VS}^{-1}$ [58]. Regarding food waste, thermophilic temperatures seem more suitable to hydrogen production despite significantly different observations reported in the literature. These differences might be due to the origin of the inoculum, the quantity of readily-biodegradable compounds as well as the operating conditions. At 55°C, acetate was the dominant by-product while a propionate production pathway was favored at 20°C [13]. To examine the effect of the fermentation temperature on biohydrogen production, dairy cow waste slurry was cultured at 37°C, 50°C, 55°C, 60°C, 67°C, 75°C and 85°C [23]. Although two optima of production were observed at 60°C and 75°C, with yields of 29.25 $mL_{H2}.g_{VS}^{-1}$ and 18.5 $mL_{H2}.g_{VS}^{-1}$, the increase in hydrogen production globally correlated with higher operating temperatures. Performances were also influenced by changes in the microbial community structure. The structure of the microflora was significantly different at the two optimal fermentation temperatures. At 60°C, the predominant bacteria were affiliated to *Bacteroides xylanolyticus,Clostridium stercorarium, and Clostridium thermocellum*, while at 75°C three strains of the extremophilic thermophilic bacterium *Caldanaerobacter subterraneus* were dominant [23]. Without pretreatment of the initial inoculum, temperatures higher than 60°C are recommended in order to reduce hydrogen-consuming activity [59]. In any event, the main disadvantage of thermophilic anaerobic fermentation processes is the energy requirement for heating and maintenance.

3.2 Bioreactor configuration

At laboratory-scale, most studies dealing with dark fermentation from solid substrates have been performed in batch reactors [58] [72]. Batch-mode reactors possess the advantage of being easily operated and flexible. This has resulted in the wide utilization of batch reactors for determining the biohydrogen potential of organic substrates. However, in an industrial context, for practical reasons of waste stock management and for economic considerations, continuous bioprocesses are recommended. To date, no biohydrogen industrial-scale reactor has been set up, but it is expected that bioreactor design and system configuration will be

similar to methane biogas plants: only the operational parameters may vary between these two anaerobic applications. In view of the extensive the experience acquired in biogas plants treating agricultural organic waste, especially in Germany, the most probable reactor for biohydrogen production would be a vertical, continuously-stirred tank reactor with different types of mixers [73]. More than half of this type of reactor is covered with a single or double-membrane roof to store the biogas (see Figure 2) [73]. Within the one-stage fermentation concept at laboratory-scale, continuous stirred tank reactors (CSTR) are the most common continuous system used for anaerobic digestion [74][25] in hydrogen production research on substrates such as pig slurry [25], swine manure [30] for food waste [42][75](see Table 1). Other studies have reported successful use of ASBR, rather than CSTR, for food waste conversion [76]. Only a few studies have concerned the processes for treating high-solid-content agricultural waste [57]. The reasons could well be the instability of such systems in the course of hydrogen fermentation due to the highly variable composition of the feed and the metabolic instability of the microbial consortia. A remarkable reactor design was set up by Jayalakshmi *et al*. [34] to investigate kitchen waste in hydrogen conversion. This was a pilot-scale, inclined, plug-flow reactor, cylindrical in shape and kept at a 20˚ angle to the horizontal to facilitate movement of the waste. A screw arrangement inside the reactor, serving to push the material from the inlet at the bottom to the outlet at the top was designed with 14 leads to maintain seven days retention time, which was important for the solid waste to have sufficient hydrolysis time [34]. Additionally, a start-up in batch mode favored the formation of stable microflora granule, and consequently enhanced seed source activity [34] [66].

In order to complete the degradation of organic substrates, a two-stage systems coupling hydrogen fermentation with methane production is recommended for treating substrates such as livestock waste and food waste [38] [42] [77]. Such a two-phase anaerobic digestion system was first proposed by Pohland and Ghosh in 1971 [78]. In this system, only fast-growing acidogens are dominant in the first step and produce mainly VFAs, whereas slow-growing acetogens and methanogens are the main microorganisms present in the second step in which VFAs are converted to methane and carbon dioxide. This combination of fermentation systems greatly enhances the energy conversion compared to the one-stage process. A study estimated that only 5.78% of the influent COD was converted to hydrogen in the first stage, compared to 82.18% of COD converted to methane in the second stage [42]. Nevertheless, a maximum hydrogen yield of 65 $mL_{H2}.g_{VS}^{-1}$ and a H_2 production rate of 22.65 $kg_{VS}.m^{-3}.d^{-1}$ were observed using food waste and with an inoculum derived from the indigenous microbial cultures contained in this substrate [42]. Chu *et al.* [47] reported the successful association of reactors for hydrogen and methane production from food waste, under specific conditions of fermentation for each: respectively, 55˚C, pH 5.5, 31h HRT and 35˚C, neutral pH, 120h HRT. They demonstrated that a short HRT and acidic pH prevent methanogenic activity in the acidogenic stage. After optimization of the reactor association system, higher biogas yield (464 $ml_{CH4}.g_{VS}^{-1}$, 70%-80%) was observed thanks to the

hydrolytic activity in the first step; but treatment time was also reduced. An HRT of 5 days was already enough for the methane stage instead of a more usual HRT of 10 - 15 days in thermophilic and mesophilic conditions, respectively [79].

Another suggested two-stage system consists of the combination of dark and photo-fermentation. Nath *et al.* [86] described one sort of process associating dark and photo-fermentation in a sequential batch reactor. A glucose-based media was inoculated with *Enterobacter cloacae* DM11 to produce H_2, CO_2 and VFAs in dark fermentation. Then, in a second reactor, acetate was subsequently used by *Rhodobacter sphaeroides* O.U.001 to form hydrogen. The yield of hydrogen in the first stage was about 3.31 $mol_{H_2}.mol_{glucose^{-1}}$ and in the second stage in the range of 1.5-1.72 $mol_{H_2}.mol_{acetic\ acid^{-1}}$, equivalent to 3-3.4 $mol_{H_2}.mol_{glucose^{-1}}$. Thus, the overall yield exceeded 6 $mol_{H_2}.mol_{glucose^{-1}}$, which is higher than of the maximum 4 $mol_{H_2}.mol_{glucose^{-1}}$ obtained with the dark fermentation process alone. The use of agri-waste as a substrate in these types of association remains to be tested.

4. Microbiology of biohydrogen production from agricultural waste

Anaerobic digestion (AD) is a ubiquitous phenomenon found in nature under anaerobic conditions. The first stages in AD are hydrolysis and acidogenesis, in which dark fermentation is involved, with hydrogen-producers. Then, hydrogen as a key intermediate can be rapidly consumed by others microorganisms in mixed culture, mainly by homoacetogens, methanogens, and sulfate-reducing bacteria (Figure 1) [81] [29] [82]. The metabolic network of carbohydrates has been the most widely investigated. Among the large range of end products generated by the various microbial metabolisms, acetic acid accumulates from acetic fermentation as sole end product with a theoretical production of 4 $mol_{H_2}.mol_{hexose^{-1}}$, equivalent to 498 $mol_{H_2}.mol_{hexose^{-1}}$ (0˚C, 1atm.); while in the butyrate pathway, a lower molar hydrogen yield is observed with 2 $mol_{H_2}.mol_{hexose^{-1}}$, equivalent to 249 $mol_{H_2}.mol_{hexose^{-1}}$ (0˚C, 1atm.) (Eqs. (3) and (4) below) [18].

$$C_6H_{12}O_6 + 2\ H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (3)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (4)$$

However, the accumulation of acetate in the medium does not necessarily imply higher biohydrogen production since several microbial species can convert hydrogen and carbon dioxide to acetate (Eqs. (5)) [83].

$$2CO_2 + 4\ H_2 \rightarrow CH_3COOH + 2H_2O \quad (5)$$

In mixed cultures, a ratio of 3:2 of butyrate / acetate is usually observed, resulting in a theoretical average hydrogen yield of 2.5 mol $_{H2}$.mol$_{hexose}^{-1}$ [18]. In mixed cultures, propionate, ethanol, and lactic acid may also accumulate. Propionate is a metabolite of a hydrogen-consuming pathway, while ethanol and lactic acid are involved in a zero-hydrogen-balance pathway (Eqs. (6), (7) and (8)).

$C_6H_{12}O_6 + 2\ H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$ (6)

$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$ (7)

$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH + 2CO_2$ (8)

In a previous review paper, Nandi and Sengupta [84] listed the major hydrogen-producing bacteria related to strict anaerobic genera (*Clostridia*, methylotrophs, rumen bacteria, methanogenic bacteria, archaea), to facultative anaerobic genera (*Escherichia coli*, *Enterobacter*, *Citrobacter*) and to aerobic genera (*Alcaligenes*, *Bacillus*). In relation to biohydrogen production from agricultural waste, *i.e.* in mixed cultures, three classes of microorganisms could be distinguished: hydrogen producers, hydrogen consumers and metabolic competitors.

4.1. The biohydrogen producers

Although pure cultures have been intensively investigated over the past years, involving amongst of others *Bacillus coagulans*[85], *Thermoanaerobacterium* spp.[86], *Enterobacter aerogenes* [87], *Clostridium butyricum* [88], few studies refer to the characterization of mixed cultures. A large range of microbial sources has been used to obtain inocula for biohydrogen production, including anaerobic sludge from municipal wastewater plants and cow dung composts [47] [86] [42] [89], cattle or dairy residue composts [90] [11] , sludge from palm oil mill effluent [91] [92], soil, rice straw compost, fermented soy bean meal [93] as well as landfill lixiviates [13] [32]. Akutsu *et al.* [94] showed that the origin of the inoculum affects the overall performance of the bioreactor. In another study, four natural mixed-microflora seed sources (sludge from sewage treatment; cow dung compost; chicken manure compost; and river sludge) were tested for fermentation in a hydrogen reactor treating cattle wastewater, and sewage sludge showed the highest hydrogen-producing potential [89].

Another investigation of the effect on grass silage fermentation of the inoculum source, *i.e.* sludge from a dairy farm digester and from a wastewater treatment plant, showed only significant biohydrogen production for bioreactors inoculated with the dairy farm digester sludge [58]. This suggests that acclimation of the seed source is a major parameter that needs to be taken into account for biohydrogen fermentation.

From hydrogen-producing mixed cultures, a wide range of species have been isolated, more specifically from the genera *Clostridium* (*Clos. pasteurianum*, *Clos. saccharobutylicum*, *Clos. butyricum*), *Enterobacter (Ent. aerogenes)* and *Bacillus* under mesophilic conditions; and from the genera *Thermoanaerobacterium* (*Thermoanaerobacterium thermosacchatolyticum) Caldicellulosiruptor (C. saccharolyticus), Clostridium thermocellum, Bacillus thermozeamaize* under thermophilic or extremophilic temperatures [95][96][97][98][99]. Under mesophilic conditions, mainly sporulating bacteria of the *Clostridium* genus have been found in mixed mixtures, in all likelihood because of the systematic use of heat shock treatment on the inoculum. In thermophilic conditions, *Thermoanaerobacterium spp.* is preferentially selected by the operating conditions in mixed cultures [99].

As to microbial performances, a biohydrogen yield of 3.8 $mol_{H2}.mol_{glucose}^{-1}$, at 70˚C very close to the theoretical maximum, was reported for *Caldicellulosiruptor saccharolyticus* [98]. Maximum hydrogen production of 2.53 $mol_{H2}.mol_{hexose}^{-1}$ was observed for *Thermoanaerobacterium thermosaccharolyticum* at a temperature of 60˚C [99]. Other thermophilic hydrogen producers reach maximum hydrogen yields ranging from 1.5 to 3.3 $mol_{H2}.mol_{hexose}^{-1}$ for *Thermotoga elfii, Caldicellulosituptor saccharolyticus, Clostridium thermocellum, Clostridium thermolacticum. Clostridium thermobutyricum, and Clostridium thermosaccharolyticum* [100] [101][102][103][104][105]. Higher conversion yields were observed at high temperature for such microbes. This may partly explain the higher performances observed in bioreactors treating organic waste as well as the fact that hydrolysis is favored at thermophilic temperatures.

4.2. H_2 consumers and metabolic competitors

Three groups of bacteria are known to interfere directly or indirectly, by diversion of the biohydrogen potential from carbohydrates, *i.e.* the Sulfate-reducing bacteria (SRB), the Methane-producing Bacteria (MPB), and the Homoacetogenic Bacteria (HAB) (Figure 1).

4.2.1 Homoacetogenic bacteria

Homoacetogenic bacteria are strictly anaerobic microorganisms which catalyze the formation of acetate from H_2 and CO_2. They were first observed by Fischer *et al.* (1932) [108]. *Clostridium aceticum* and *Clostridium thermoaceticum* were the model species used to elucidate the metabolic pathway [106] [107]. They possess special enzymes which catalyze the formation of acetyl-CoA that is converted either to acetate in catabolism or to cell carbon in anabolism. The homoacetogens are very versatile anaerobes, which convert a variety of different substrates to acetate as the major end product [108]. This implies, therefore, that in experimental studies the biohydrogen production measured might be lower than the expected

572 value calculated from the accumulation of acetate [83]. Thomas *et al.* [25] used pig slurry as
573 substrate in a CSTR and observed that the actual production of hydrogen was substantially
574 lower than the value expected from VFA accumulation. As no methane was detected in the
575 biogas and the propionate mass balance did not explain hydrogen losses, hydrogen was
576 assumed to be consumed by acetogenic bacteria [25]. Siriwongrungson *et al.* [109] reported
577 that considerable homoacetogenesis occurred in CSTR reactors using digested dairy manure
578 as inoculum and operated under thermophilic temperatures [109]. It was shown that the
579 biohydrogen produced from butyrate oxidation reacted rapidly with CO_2 to form acetate by
580 homoacetogenesis [109]. Unfortunately, the pretreatment of the inoculum by heating to select
581 spore-forming bacteria is not suitable for inhibiting of homoacetogenic bacteria since some of
582 them belong to the same genus *Clostridium* [110]. Thus, only operating parameters could
583 favor biohydrogen production, *e.g.* by removing CO_2 from the headspace [111].
584
585 **4.2.2 Sulfate-Reducing Bacteria**
586
587 According to theoretical thermodynamics, the most efficient biochemical reaction using
588 hydrogen involves the sulfate/nitrate-reducing microorganisms ($\Delta G^0 = -165$ kJ.mol^{-1}), even at
589 a low hydrogen concentration of only 0.02 ppm in the presence of sulfate or nitrate [112]. It
590 has been shown that SRB have a thermodynamic advantage over MPB and HAB [82]. Some
591 waste especially from pulp/paper industry, sea-food processing, distilleries, edible oil and wet
592 corn milling, contains high sulfate concentrations which perturb hydrogen anaerobic digestion
593 as well as produce sulfide gas which is hazardous for fuel cells [113] [114]. Short HRTs are
594 not sufficient to inhibit these microorganisms. Even at a HRT of 2h, the interspecies transfer
595 metabolites such as hydrogen, carbon dioxide and VFA, are immediately consumed by SRB
596 under sulfate-rich conditions [82]. At longer HRT, hydrogen is converted either to methane
597 with carbon dioxide by MPB under sulfate-limited conditions, or to sulfidic acid by SRB if
598 sulfate is abundant in the substrate [115]. Along with the concentration of sulfate and HRT,
599 pH is a key factor in sulfate reduction. pH values lower than 6 significantly inhibit the activity
600 of SRB [115] [113].
601
602 4.2.3 **Methanogens**
603
604 Methanogens are considered as the main hydrogen-consuming microorganisms in
605 anaerobic environments [116] [117] [118]. Many options exist for inhibiting methanogenesis:
606 chemical inhibition, low pH control, heat treatment of the inoculum, short hydraulic retention
607 times.
608 The most commonly used chemical inhibitors are Bromoethanesulfonate (BES), acetylene
609 and chloroform [57]. BES is specific against methanogens and acts as an analog of the
610 coenzyme M in the respiratory chain. However, treating with effective concentrations of BES

is not environmentally friendly and too costly for large-scale operations [57]. pH is also a factor in preventing methanogenic activity since most methanogens can only grow at a narrow pH range from 6 to 8 [119]. In absence of pH control during a batch process, an acidic initial pH is strongly recommended [120] [121]. The most common treatment of inoculum is heating the medium to around 100 degrees for approximately ten minutes to select spore-forming, hydrogen-producing bacteria. Methanogens do not sporulate and do not survive such conditions [122] [123]. Because methanogens present low growth rates (approx. 0.2 h^{-1}), the application of short HRT (< 8 h) quickly leads to a washout of methanogens from the reactor, when no biofilm is formed. To obtain stable hydrogen production in a methane-free biogas, the optimal HRT observed were 3-6 h, 9h, 18h up to 48h for respectively, molasses, bean curd waste, brewery waste and food waste [44] [95] [56] [75]. In a kinetic study of hydrogen production in an anaerobic system, Chen *et al.* [124] calculated a maximum specific growth rate for methanogenic microflora of 0.172 h^{-1}. They concluded that HRT of less than 6h are recommended to selectively wash out the methanogens in continuous reactors [124] [82].

4.2.4 Lactic Acid Bacteria

Noike *et al.* [125] studied the inhibition of hydrogen production by lactic acid bacteria (LAB). They observed the replacement of hydrogen fermentation by lactic acid fermentation when two lactic acid bacteria (LAB) strains, *i.e. Lactobacillus paracasei* and *Enterococcus durans* , were cultivated with two hydrogen-producing strains, *Clostridium acetobutylicum* and *Clostridium butyricum*. Secretion of bacteriocins was recognized as the inhibitory effect and temperatures above 50°C were proposed to prevent LAB influence [125]. In mesophilic systems, LAB growth could not be limited by temperature, and the accumulation of lactic acid led to the instability of the mixed culture processes. Indeed, Wang *et al.* [42] showed that lactic acid inhibited hydrogen fermentation in a two-stage continuous system using food waste as substrate [42]. The hydrogen yield dropped from 71 to 49 $mL_{H2}.g_{VS}^{-1}$ when the lactic acid increased from 2.3 to 4.4 $g.L^{-1}$. Increasing the organic loading rate resulted in an increase in lactic acid concentration and in the microflora indigenous in food waste, *i.e.* lactic acid bacteria, and then led to the perturbation of the system if no pretreatment had been previously carried out [42]

5. Conclusion

The present review reports recent findings on biohydrogen production from agricultural waste by dark fermentation. Three categories of agricultural residue have been considered in the present review: (i) the waste directly generated from agricultural production (ii) animal manure and (iii) food waste. It is shown that all three possess great potential as a substrate for

650 hydrogen production by dark fermentation, in decreasing order: food waste, crop residues and
651 livestock waste. But further research is necessary to better understand the impact of the
652 composition of the substrate on biohydrogen performances. Moreover, the biological
653 processes involved are not only restricted by the composition of the organic waste, but also
654 they are highly dependent of the operating conditions. Key operational parameters such as
655 low pH, low partial pressure, high temperature and acclimated microbial communities are
656 recommended. These operating parameters affect not only the yields of biohydrogen in mixed
657 culture, but also redirect by-product spectrum and impact the structure of the microbial
658 communities. Since a pattern of metabolites are concomitantly produced, the association of a
659 hydrogen fermentor with a methanogenic reactor is strongly recommended to achieve the
660 conversion of biodegradable organic matter to bioenergy. Finally, we suggest it is important to
661 distinguish three classes of microorganisms that require further characterization in mixed
662 cultures: hydrogen producers, hydrogen consumers and metabolic competitors. The presence
663 of various hydrogen consumers and the control of the occurrence of H_2 consuming pathways
664 in mixed cultures constitute the main challenge to improving the stability of bioreactors
665 treating agricultural waste.
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List of Figures and Tables

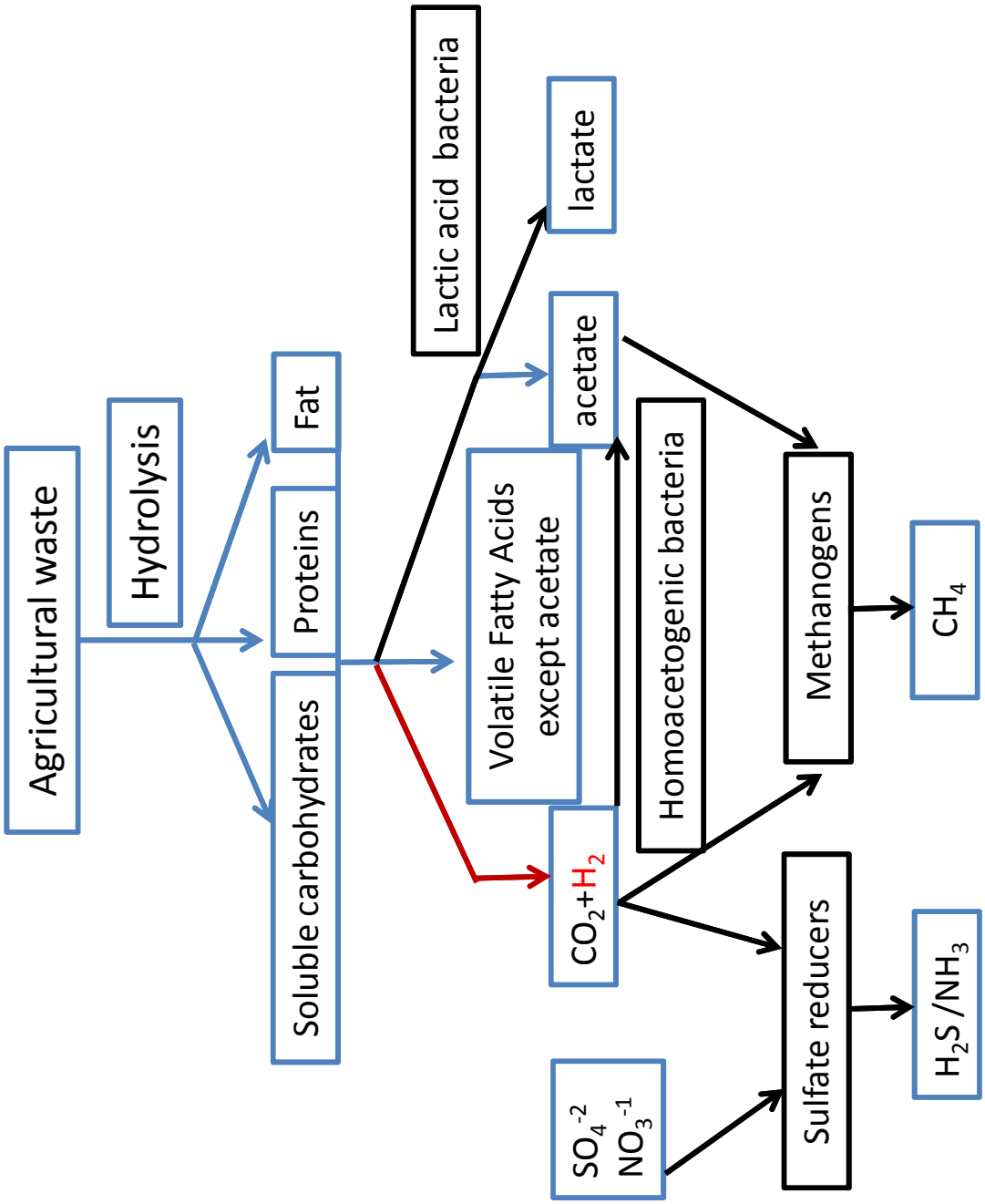
Figure 1: Microbial pathways in an ecosystem degrading agricultural waste, in which red arrows indicate hydrogen producers and black arrows hydrogen consumers.

Figure 2: Different types of anaerobic digestion plant, adapted from Weiland 2006 [73]. a/b/c: Vertical, completely-stirred tank reactor (a/b: mechanical stirring; c: biogas mixing), d/e: Horizontal plug-flow reactor (mechanical stirring)

Table 1: Estimated H_2 production yields of anaerobic reactors treating agricultural waste (*calculated from literature data, - no pretreatment of feedstock, n.d. not determined)

Table 2: Optimal pH for biohydrogen production according to the organic substrate.

Figure


Agricultural waste
Hydrolysis
Fat
Proteins
Soluble carbohydrates
Lactic acid bacteria
lactate
acetate
Volatile Fatty Acids
except acetate
CO_2+H_2
Homoacetogenic bacteria
Methanogens
CH_4
SO_4^{-2}
NO_3^{-1}
Sulfate reducers
H_2S /NH_3

Figure

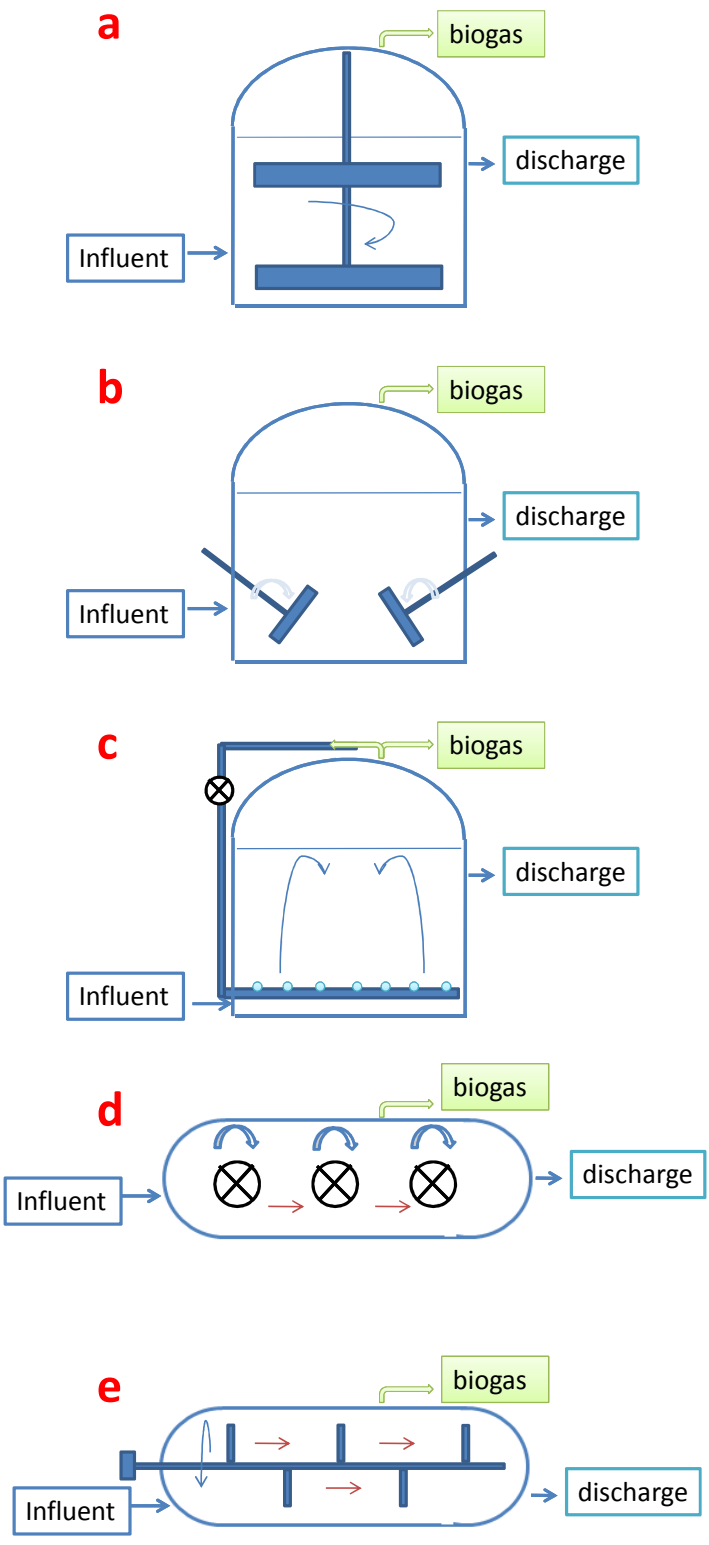

a
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Table 1: Estimated H_2 production yields of anaerobic reactors treating agricultural waste

(*calculated from literature data, - no pretreatment of feedstock, n.d. not determined)

Substrate	Maximum assessed production yield ($ml_{H_2}.g_{VS}^{-1}$)	Pretreatment*	Temperature (˚C)	Reactor operation mode	Reference
Corn straw	9	-	35	Batch	[12]
Corn straw	68*	1.5MPa10min	35	Batch	[12]
Corn stover	49*	220˚C 3min	35	Batch	[132]
Corn stover	66*	1.2% HCl+200˚C 1min	35	Batch	[132]
Cornstalk	3	-	36	Batch	[14]
Cornstalk	57	0.5% NaOH	36	Batch	[14]
Cornstalk	150	0.2%HCl boiled 30min	36	Batch	[14]
Grass silage	6	-	35	Batch	[13]
Grass silage	16	-	70	Batch	[13]
Maize leaves	18	-	70	Batch	[98]
Maize leaves	42	130˚C 30min	70	Batch	[98]
Rice bran	61	n.d.	35	Batch	[93]
Sweet sorghum plant	32.4*	130˚C 30min	70	Batch	[98]
Sugarcane bagasse	19.6*	130˚C 30min	70	Batch	[98]
Silphium trifoliatum leaves	10.3*	130˚C 30min	70	Batch	[98]
Wheat straw	1	-	36	Batch	[11]
Wheat straw	68	HCl 2%+microwave heating	36	Batch	[11]
Wheat straw	49*	130˚C 30min	70	Batch	[98]
Wheat bran	43	n.d.	35	Batch	[93]
Cow feces and urine	18*	-	75	Batch	[23]
Cow feces and urine	29*	-	60	Batch	[23]
Cow feces and urine	0.7*	-	37	Batch	[23]
Cattle manure	65	90˚C 3h	52	Batch	
Cattle wastewater	53*	-	45	Batch	[89]
Dairy manure	18	0.2%HCl boiled 30min	36	Batch	[133]
Dairy manure	14	0.2%NaOHboiled30min	36	Batch	[133]
Dairy manure	14	infrared radiation 2h	36	Batch	[133]
Pig slurry	4	-	70	CSTR	[25]
Swine liquid manure	209*	-	35	Semi-continously -fed fermeter	[30]
Rice	96	-	35	Batch	[40]
Carrot	71	-	35	Batch	[40]
Cabbage	62	-	35	Batch	[40]
Chicken skin	10	-	35	Batch	[40]
Egg	7	-	35	Batch	[40]
Lean meat	8	-	35	Batch	[40]
Foodwaste	196	160˚C 2h	36	Batch	[32]
Foodwaste	60*	n.d.	35	Batch	[41]
Foodwaste	77	-	35	Batch	[122]
Foodwaste	125*	-	35	CSTR	[75]
Foodwaste	63	pH12.5 1day	35	ASBR	[45]
Foodwaste	65	-	40	Demi-continuous rotating drum	[42]
Foodwaste	13	-	20	CSTR	[13]
Foodwaste	3	-	37	CSTR	[13]
Foodwaste	16.5	-	55	CSTR	[13]
Kitchen waste	72	-	n.d.	Inclined plug flow reactor	[34]
Molasses	2.5 $molH_2/molsucrose$	-	37	CSTR	[44]
Molasses	2.1mol_{H2}/mol_{hexose}	-	35	CSTR	[95]
Sweet lime peelings extracts	76.4ml/g CODr*	121˚C pH=7 40min	32	Batch	[43]
Bean curd manufacturing waste	21	n.d.	35	CSTR	[93]
Cheese whey	290*	$NaHCO_3$ 20g/L	35	CSTR	[38]
Palm oil mil effluent	84.4*	-	60	Batch	[37]

Table 2: Optimal pH for biohydrogen production according to the organic substrate.

Substrate	Reactor	pH range	pH optimum	Reference
Corn straw	Batch	4-8 each0.5unit	7.0-7.5	[12]
Grass silage	Batch	4; 5; 6	6	[13]
Rice bran	Batch	7initial	-	[93]
Wheat bran	Batch	7.0initial	-	[93]
Wheat straw	Batch	4-9	7	[11]
Cow waste slurry	Batch	6-7.5	7.0	[23]
Cattle wastewater	Batch	4.5-7.5	5.5	[89]
Foodwaste	Batch	6initial		[41]
Foodwaste	CSTR	5.0-6.0	5.5	[75]
Foodwaste	ASBR	5.3 constant	-	[45]
Foodwaste	CSTR	5.5-6.0 constant	-	[13]
Foodwaste	CSTR	5.5 constant	-	[47]
Vegetable kitchen waste	Batch	5.5-7 constant test	6.0-7.0	[54]