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REVIEW ARTICLE

Anaerobic Digestion of Agri-Food Wastes for Generating Biofuels

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Abstract Presently, fossil fuels are extensively employed as major sources of energy, and their uses are considered unsustainable due to emissions of obnoxious gases on the burning of fossil fuels, which can lead to severe environmental complications, including human health. To tackle these issues, various processes are developing to waste as a feed to generate eco-friendly fuels. The biological production of fuels is considered to be more beneficial than physicochemical methods due to their environmentally friendly nature, high rate of conversion at ambient physiological conditions, and less energy-intensive. Among various biofuels, hydrogen (H_2) is considered as a wonderful due to high calorific value and generate water molecule as end product on the burning. The H_2 production from biowaste is demonstrated, and agri-food waste can be potentially used as a feedstock due to their high biodegradability over lignocellulosic-based biomass. Still, the H_2 production is uneconomical from biowaste in fuel competing market because of low yields and increased capital and operational expenses. Anaerobic digestion is widely used for waste management and the generation of value-added products. This article is highlighting the valorization of agri-food waste to biofuels in single (H_2) and two-stage bioprocesses of H_2 and CH_4 production.

Keywords Anaerobic digestion · Agri-food waste · Biohydrogen · Biomethane · Integrative bioprocesses · Value-added products

Introduction

Nature is progressing via sustainable mechanisms. Therefore living organisms are strongly harmonized through environmental changes. Energy utilization is significantly increasing in developed countries as compared to developing countries, and nearly 15% of the World's population is consuming over half of the total energy consumption [1, 2]. The exponential increase of world populations ($\sim$ 7.9 billion) in the past few decades is pressuring too much burden for sustainable development. Primarily, we rely on fossil-based sources to fulfill its increasing energy demands in societal and industrial areas [3–5]. The economic development hinders due to deteriorating stocks of non-renewable energy-based assets. An alternative to these energy sources, biofuels-based energy sources such as hydrogen (H_2) [6–8], biogas mainly methane (CH_4) [9, 10], ethanol [11], methanol [12–15], and biodiesel [16, 17], are more helpful to minimize the emission of harmful gases via the burning of fossil fuels and also their eco-friendly nature. A large quantum biowaste(s) is generated through our daily lives and various human activities [18–20]. Thus, the utilization of biowaste(s) for generating useful for various kinds of biomolecules such as biofuels [21–25], biopolymers such polyhydroxyalkanoates (PHAs) [26–30], and bioelectricity [31, 32]. Biological processes have been proved more beneficial for biotransformation applications

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than physical or chemical methods, which are primarily considered high energy-intensive processes [33–38]. Further, the biocatalyst's properties can be significantly improved through genetic and protein engineering or related synthetic approaches for their potential applications [39–44]. Also, biological-derived products, materials or microbes themselves can be potentially applied in the area of microbial pathogenesis to improve microbes, human, and plants health [45–53]. Recent pandemic arising due to viral infection is a significant influence of human thinking for better management of population sustainability and environmental issues research over other non-related areas [54–56].

The energy resources-based predictions suggested that coal deposits will be utilized over the next Century. In contrast, petroleum-based deposits will be used up within few decades [10, 57]. Also, the environmental worsening is a significant concern, which is significantly associated with the extensive uses of these non-renewable energies. Renewable energy resources are vital for sustainable development [10, 58–61]. However, from the past few decades, alternative energy sources to fossil fuels are recognized as a significant area of research. The production of biofuels, especially H_2 can be more beneficial using biowaste as a low-cost feed over costly pure sugar [1, 19, 62–64]. The various approaches have been used for the utilization of biowaste(s)-based feedstocks to produce biofuels such as H_2 and CH_4 [10]. The production of these biofuels is extensively studied using various biowastes from agricultural, municipal, industrial, and synthetic origins. The production of H_2 is largely demonstrated using mixed cultures (MCs) over pure cultures as an inoculum due to their better metabolism [10, 65, 66]. The use of integrative processes such as H_2 production followed by CH_4 can be adopted at a large scale to improve the bioprocess economy. This article presents the status of the production of biofuels from agri-food waste in single- and two-stage. Further, the bioprocess improvement strategies for sustainable development have been discussed.

Biowastes

Globally, a significant advancement in life-routine and industrialization has generated a severe problem by accumulating various kinds of waste (including biowastes) and their negative environmental impact [3, 67, 68]. The challenges of their management have earned considerable public and political recognition in current times. Therefore, minimization of wastes generation through their management is highly recommended for sustainable development. In addition, we are highly relying on unsustainable fossil fuels-based energy sources that can lead to environmental pollution via the emission of harmful gases, and they (fossil fuels) may be depleted in the following centuries. However, in the past few decades, the generation of biofuels such as H_2, CH_4, methanol, ethanol, and biodiesel is demonstrated as an alternative to fossil fuels [9, 11, 17, 69]. The production of biofuels from biowastes can be carried out to solve these waste management issues and biofuels and the environmental benefits. The primary sources of wastes can distribute in various groups based on their origin, such as agricultural, industrial, municipal, and biomedical [1, 19]. The quantum of wastes can be varied at regional and cultural levels. Despite the numerous environmental regulations and rules, a small level has been accomplished primarily in developing countries to minimize the generation of wastes [10]. In recent times, the generation of wastes in Indian major cities is escalating high rate ($\sim$ 1.5%) of total wastes quantum [2]. However, the handling of a large quantum of waste is needed through practical methods in an economical manner. Various wastes management technologies have been used, includes—(1) AD, (2) composting, (3) incineration, (4) landfilling, (5) recycling, and (6) dumping (especially in the open) [10]. These methods can be used individually or in combination for effective waste management and showed some benefits over each other. The brief benefits of different waste management methods such as landfilling and dumping (open) are widely adopted globally, contributing up to 80% of the total waste management methods presented in Table 1 [10]. In contrast, AD and composting are equally used with very low combined contributions of 10–12% that is equal to the recycling method. The agri-food waste such as cereals (no-edible parts), fruits, and vegetables are generated in considerable amounts in markets. These kinds of biowaste are highly biodegradable that can be easily managed via their valorization to value-added products or other envirometal applications [66, 70–72]. However, biowastes-based generation of biofuels is considered to be potentially applicable technologies for sustainable development.

Biofuels Production from Biowastes

The major biofuels such as H_2 [8, 10], CH_4 [10, 73], methanol [74, 75], ethanol [11], biodiesel [17], production is expected to reduce global warming. These are probable to take fundamental developments in biofuels production [10]. The biofuels production are broadly classified into four generations: (1) 1st generation—this type of biofuels (biodiesel, bioethanol, biogas) is produced largely from agricultural-based crops, sugarcane, sugar beet, wheat, rice, corn, and sunflower through hydrolysis and fermentation, (2) 2nd generation—this generation of fuels are produced

Table 1 The management procedures for valorization or disposal of wastes

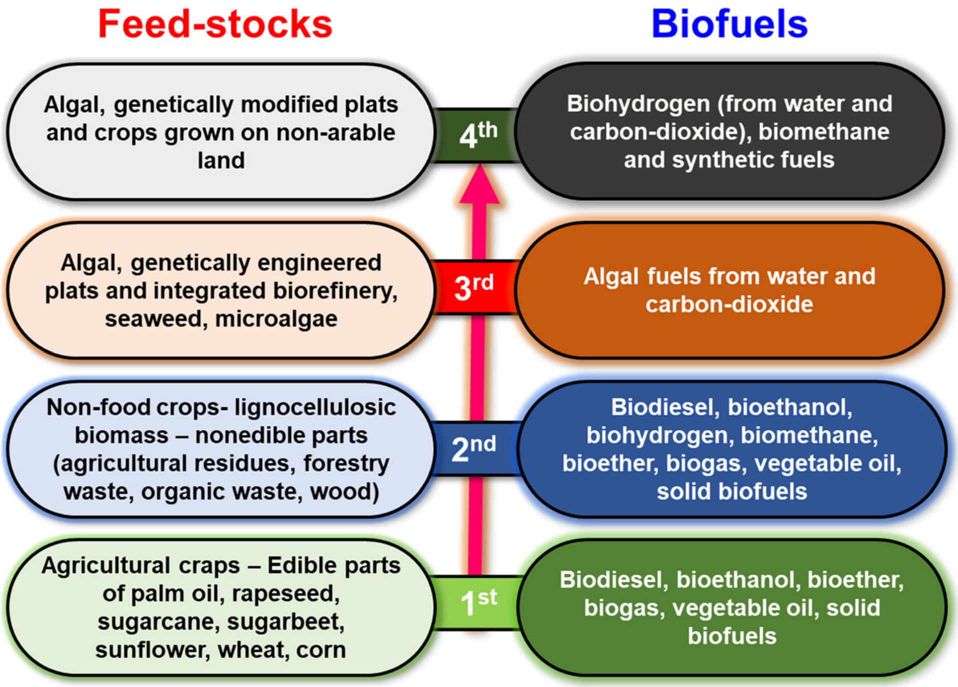
Process	Contribution (%)	Benefits
Anaerobic digestion	6.30	Provide renewable energy (biogas) and/to generate electricity Reduce pollution, smell, pathogens, and weed seeds Conservation of agricultural land Generate fertilizer
Composting	5.05	Embolden microorganisms to produce humus (nutrient-filled materials) Soil enrichments and conquer plant infections and pests Decrease methane emissions Reduce chemical fertilizers requirement
Incineration	6.45	Reduce waste quantity, and efficient waste management Generation of energy and pollution reduction It prevents methane generation and operated in any weather Reduce harmful microbes and chemicals
Landfilling	37.4	Advanced landfills are eco-friendly, and an excellent energy source An easy method to keep clean city and town Helpful to manage all kinds of wastes Economical
Dumping (in open)	32.2	The simplest method and requires a small area Very economical Convenient Source for shelter and nutrients
Recycling	12.6	Provide a livable environment for a sustainable future Reduce quantity for waste management by other methods Conserve natural resources Improve economy and save energy

using non-edible plant parts, (3) 3rd generation—biofuels such as ethanol and biodiesel were produced via photosynthetic algae and genetically engineered plants through biochemical and thermochemical bioprocesses, and (4) 4th generation—this type of biofuels are produced through advanced photobiological solar or electric fuels (Fig. 1). The main drawback of this generation of fuels is a conflict of “food vs. fuel” [76].

The selection of suitable fuel for future uses can meet different criteria such as (1) convenient in transportation, (2) safe to use, (3) easily transform to another form of energy, (4) environmentally friendly nature, (5) high utilization efficiency, and (6) inexpensive to use [1, 10]. Among various available biofuels based on the above criteria, H_2 can be considered as a wonder fuel for sustainable development. Biologically H_2 has been produced from numerous microbes by using cheap raw materials such as biowastes. Lignocellulose-based biowastes are abundantly accessible [77]. Due to their complex nature, the pretreatment of biowastes is considered a satiable approach to produce soluble sugars for easy utilization towards biofuels (H_2) through fermentation. Primarily, lignocellulosic biowastes are consists of cellulose, hemicellulose and lignin. However, the hydrolysis of biomass largely depends on the type of pretreatment approaches due to significant variations in their compositions. The different pretreatments of biomass approaches have been used to generate fermentable sugars, includes physical (microwave and pyrolysis), chemical (acidic and alkaline), (3) physical–chemical-based (ultra-sonication and steam explosion), and (4) biological (microbial and enzymatic) [11, 76]. In the case of enzymatic pretreatment of biowastes, the following cellulase, xylanase, β-glucosidase, and laccase can be used for direct hydrolysis or to decrease the toxicity of hydrolysate [8, 78–81].

The biological pretreatment methods can be considered as eco-friendly as compared to physical, chemical, or their combinations [10, 76]. Still, the economic H_2 production from biowaste is challenging due to partial utilization of feed and bioprocess scaling-up. Also, the present production cost of H_2 through biological routes is higher than available energy sources. In general, the integrative approaches are proved more beneficial for value-added bioproducts that can improve the process economy.

Fig. 1 The generations of biofuels production from various feed-stocks



Various integrative approaches such as H_2 followed photo fermentative H_2, CH_4 or PHAs have been reported [10, 21, 66]. The utilization of PHAs for the biotechnological applications can be more useful because of their novel therapeutic uses such as antimicrobial, tissue engineering, and drugs carrier [82–86]. Also, the techno-economics analysis suggested that these integrative processes will be more desirable over single-stage H_2 production from sugars or biowastes [10, 21].

Anaerobic Digestion

AD is considered one of the oldest bioprocesses for wastes utilization. Biowastes are very complex; thus, different strategies such as AD have been employed for their valorization to useful bioproducts such as H_2 and CH_4 [10, 57]. AD is a multi-step process and primarily carried to utilize complex materials such as biowastes using indigenous microbial populations or externally added cultures. The AD is carried out in four steps that are classified as (a) hydrolysis, (b) acidogenesis, (c) acetogenesis and (d) methanogenesis [10]. In the AD 1st step, the biowaste (complex organics) are hydrolyzed to simple sugars, fatty and amino acids by hydrolytic enzymes such as amylase, cellulase, protease, and lipase activity of microbial cultures. This group of cultures is known as hydrolytic fermentation bacteria, and they provide hydrolyzed substrates to the next step of the bacterial population (Acidogenesis). At the 2nd step of acidogenesis (fastest step in the AD), the partially hydrolyzed substrate was further broken down by enzymatic reaction of cultures. Acidogenic bacteria are very fast growing with lower than an hour of doubling time and especially generates volatile fatty acids (VFAs), and gases, includes H_2, carbon dioxide (CO_2), and ammonia. The 3rd stage of AD is known as acetogenesis, and during this stage, largely acetic acid is produced by acetogens along with H_2 and $CO_{2.}$ This stage microbial population is slow-growing with a more significant doubling time about 50-fold higher to acidogens (2nd stage). Thus, this stage's success primarily depends on cooperation between their microbial populations to achieve better efficiency. The 4th stage of AD is known as methanogenesis and is considered the terminal stage of AD (Fig. 2). At this step, methanogens are producing CH_4 directly from acetate or H_2 and CO_2 mixture as a biogas [10, 18]. Methanogens are phylogenetically diverse groups of unique bacteria that are called archaebacteria. Through the AD of biowastes, the biological oxygen demand, as well as chemical oxygen demand (COD), can be significantly reduced, and this process can all offer various environmental, and socio-economic benefits via the generation of renewable fuels. In addition to numerous benefits, AD can exhibit limitations such as strict anaerobic conditions requirement susceptible towards even low presence of oxygen amount) concentrations, and slow metabolic activities of methanogens [1, 10]. Apart from H_2 and CH_4, the VFAs generated during the acidogenesis stage in AD can be potentially used to produce PHAs.

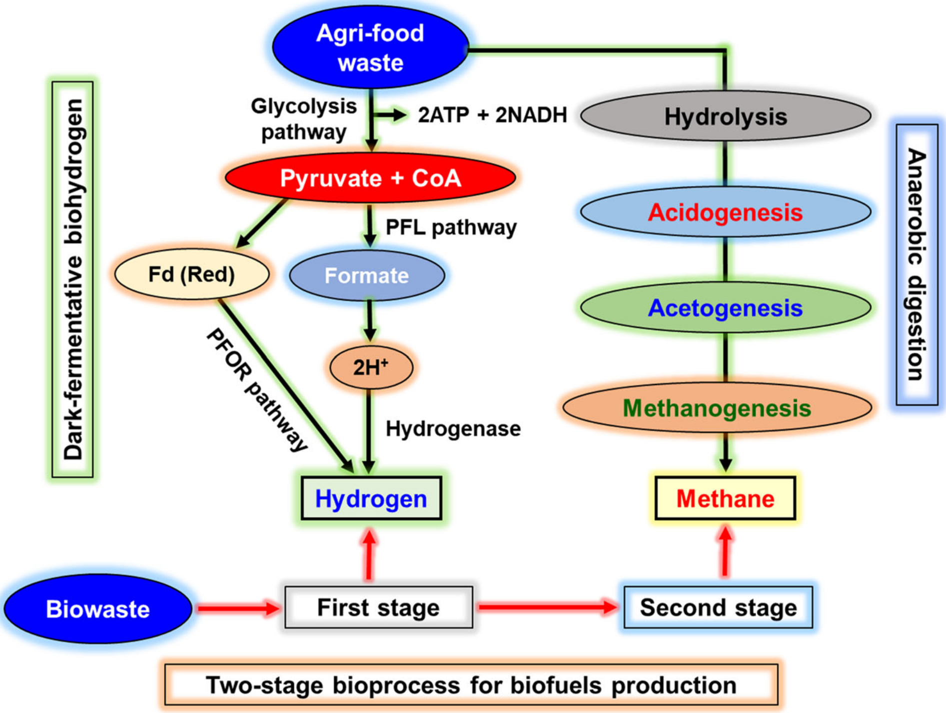


Fig. 2 Bioprocess illustrations for the first-stage (hydrogen) and second-stage (hydrogen and methane) biofuels production from agri-food waste

Biohydrogen Producers and Their Biodiversity

Among various candidates, H_2 is recognized as a promising future fuel due to its high caloric energy (141.9 MJ/kg) and non-polluting potential [10]. The H_2 can be produced using natural gases, biomass, coal, and fossil fuels. In the present scenario, ~ 90% of H_2 is produced through fossil-fuels [1, 10]. Biologically produced H_2 showed benefits like moderate production conditions, and an environmenal-friendly bioprocess over various physicochemical processes [65]. The biological methods to produce H_2, include—dark-fermentation (DF), photo-fermentation, photolysis, and electrochemical processes. The fermentative H_2 generation is a novel aspect, and it is considered suitable when biowaste is used as feed. H_2 production is occurred by hydrogenases through excess protons release via reversible reaction of $H_2 \leftrightarrow 2H^+ + 2e^-$ [1–3]. Based on the type of metal contents, hydrogenases are categorized into [Fe–Fe]- (naturally involves for H_2 generation), [NiFe]- (such as uptake-hydrogenases, bidirectional cytoplasmic-hydrogenases, cytoplasmic H_2 sensors and cyanobacterial uptake-hydrogenases, and H_2-evolving hydrogenases), and [Fe]-containing enzymes. The metabolic pathway of H_2 involves the generation of pyruvate from glucose via Embden–Meyerhof–Parnas cycle or glycolytic pathway [3]. Further, formate is produced from pyruvate through pyruvate formate lyase. The generation of H_2 involved different pathways into facultative (such as *Escherichia* via hydrogenase and formate-dehydrogenase) and strict anaerobic (like *Clostridium* through pyruvate ferredoxin oxidoreductase (POR) and H_2-POR) organisms (Fig. 2). In photo-fermentation H_2 evolution occurs in bacterial by nitrogenase via capturing solar energy [19]. The biotransformation of hexose to H_2 by dark- and photo-fermentative organisms are demonstrated as following from Eqs. 1, 2, 3, 4, 5, 6 [1, 10]:

$$\text{Hexose} + 2H_2O \rightarrow 2\ \text{Acetate} + 4H_2 + 2CO_2 \tag{1}$$

$$\text{Hexose} \rightarrow \text{Butyrate} + 2H_2 + 2CO_2 \tag{2}$$

$$\text{Hexose} + 6H_2O + \text{light (Sun)} \rightarrow 12\ H_2 + 6\ CO_2 \tag{3}$$

$$\text{Hexose} \rightarrow \text{lactate or ethanol} \tag{4}$$

$$\text{Acetate} + 2H_2O + \text{light} \rightarrow 4H_2 + 2CO_2 \tag{5}$$

$$\text{Butyrate} + 6H_2O + \text{light} \rightarrow 10H_2 + 4CO_2 \tag{6}$$

The taxonomically diverse microbes have been used to generate H_2—(1) Archaea such as *Methanobacterium*, *Pyrococcus* and *Methylotrophs*; (2) Actinobacteria such as *Mycobacterium*; (3) Cyanobacteria like *Anabaena*, *Calothrix*, *Nostoc*, and *Spirulina*; (4) Firmicutes such as *Bacillus*, *Clostridium*, *Caldicellulosiruptor*, and *Frankia*; (5) Bacteroidetes or Chlorobi like *Acetomicrobium*, *Chlorobium*, and *Bacteroides*; (6) Thermotogae such as *Thermotoga*; (7) Fusobacteria like *Fusobacteriai*; (8) Alpha-proteobacteria such as *Rhizobium*, *Rhodobacter*, and *Rhodopseudomonas*; (9) Beta-proteobacteria like *Alcaligenes* and *Rubrivivax*; (10) Delta-proteobacteria such as

Desulfovibrio; (11) Epsilon-proteobacteria like *Campylobacter*; and (12) Gamma-proteobacteria like *Azotobacter*, *Enterobacter*, *Escherichia*, *Pseudomonas*, *Citrobacter* and *Klebsiella* [1]. Overall, along with a few unique H_2-producers, a lower H_2 production to stoichiometric yield has been described. In DF production, H_2-producers like *Bacillus*, *Clostridium*, *Caldicellulosiruptor*, and *Enterobacter* have shown yield ~ 3.8 mol of H_2/mol of gelucose [19]. Whereas photo-fermentative H_2-producers like *Rhodobacter* and *Rhodopseudomonas* have reported yield ~ 9.0 mol of H_2/mol of hexose [10, 87]. The key benefits are associated with DF over photo-fermentative include—lower energy input, and high production efficiency. The fermentative H_2 yield can be improved by various approaches such as (1) pretreatment of biowaste as feed, (2) uses of nanoparticles and metal ions, (3) use of selective defined MCs (DMCs) over pure culture, (4) co-digestion of feed, (5) use of metabolically engineered H_2-producers. The H_2-produces can be engineered to eliminate lactate dehydrogenase, uptake hydrogenase, or fumarate reductase encoding genes [1, 10]. These genetically modified H_2-producers are limited by the fact that H_2 production is associated with undesirable influences such as lower yield and poor utilization of feed [1, 10, 88]. Overall, H_2 production by engineered microbes can be boosted through inhibition of H_2 production competitive pathways, designing unique pathways, or over-expressing genes related to H_2-production [10]. Alternatively, the uses of immobilization of biocatalysts (either cell-free or cell-based systems especially enzymes) are well stabilized to improve various biotransformations [89–97]. Numerous kinds of support such as solids and polymeric materials have been used to developed efficient biocatalysts especially whole cells [14, 36, 67, 77]. Additionally, the uses of low-cost supports such as lignocellulosic-derived biowastes can be more beneficial for economical biotransformation over costly polymers. However, immobilized H_2-producers can be potentially enhanced H_2 yield over free cells, especially under continuous culture conditions [2, 77]. Nanomaterials play a crucial role in biohydrogen production and improved yield up to sixfold as compared to control [10, 64]. Also, nanomaterials exhibit selective antimicrobial properties towards specific organisms that potentially can be effectively employed for the enrichment of H_2-producers in mixed populations containing non-producers [64, 98, 99].

Biofuels Production from Agri-Food Wastes

Single-Stage Biohydrogen

The maximum 2 and 4 mol/mol of glucose can be produced through the generation of acetate and butyrate as soul metabolite intermediates, respectively [1, 19]. In contrast, H_2 generation is inhibited in the fermentative conversion of hexose to lactate or ethanol. From the past few decades, primarily various initiatives carried out to identify efficient H_2-producers with desirable features to use diverse kinds of feed. Broadly, undefined MCs (UMCs) have been adopted to produce H_2 from biowaste over pure cultures due to their higher substrate specificity and stability towards undesirable changes during fermentation like pH and feed. Still, lower H_2 yields are achieved to 4 mol/mol of hexose because of the generation of undesirable metabolite intermediates such as butyrate, propionate, lactate, and ethanol instead of acetate [1, 10]. The production of H_2 is highly varied by the composition of feed. The agricultural-based food wastes composition for cellulose, hemicellulose, and lignin are presented in Table 2. The cellulosic (cellulose and hemicellulose) and lignin contents are highly varied among wastes. However, the production of H_2 is mainly dependent on the cellulosic content of wastes and the potential of H_2-producers to metabolize them directly or after pretreatment [100].

The H_2 production under batch and continuous culture conditions from various agricultural-based food waste has been shown in Table 3. Under batch conditions, the H_2 production of ranges from 8.3 L/kg of COD to 239 L/kg of feed [101, 102]. Whereas H_2 yields from 54.0 L/kg of total solids (TS) to 635 L/kg of volatile solids (VS) under continuous mode [21, 103]. Overall, these studies suggested that the continuous mode of H_2 production is more beneficial to achieve nearly 6.5-folds better H_2 yield than the batch culture conditions. Agri-food wastes such as *Agave tequilana* bagasse, cheese whey, rice husk, sugar beet, and sugarcane molasses reported H_2 yield of 0.92–2.10 mol/mol of glucose [104–108]. Among these feeds and cultures, the association of molasses and *Caldicellulosiruptor saccharolyticus* DSM 8903 founded more beneficial to achieve a maximum yield of 2.10 mol/mol of glucose over other cultures either in pure form (*Bacillus cereus* and *Clostridium thermocellum* DSMZ 1313) or anaerobic sludge as UMCs and different biowastes combinations. Under batch mode, the combination of potato peals with *Parageobacillus thermoglucosidasius* KCTC 33,548 and DMCs resulted in yields up to 0.83 L/L of feed and 92 L/kg of TS, respectively [5, 59, 109]. In contrast, potato starch founded more beneficial to achieve higher production of 151 L/kg of feed

Table 2 Cellulose, hemicellulose, and lignin composition of few agricultural origin wastes

Agricultural waste	Cellulose	Hemicellulose	Lignin	Others
Rice husk	35.1–41.1	17.6–38.3	18.8–26.6	11.8–22.5
Banana peels	11.5–44.0	18.4–25.5	8.05–9.80	29.5–53.3
Barley bran	37.1–44.1	30.4–34.9	19.8–25.5	8.20–19.4
Sugarcane bagasse	39.2–58.2	9.20–25.8	13.4–18.4	16.6–19.2
Apple pomace	36.0–42.5	11.0–18.8	19.0–23.7	15.0–34.0
Cassava	38.8–56.5	7.2–12.6	11.8–12.2	18.7–42.8
Olive husk	31.9–36.4	21.9–26.8	26.0–26.5	10.8–19.7

[110]. The supplementation of glucose to pea-shells hydrolysate recorded high production up to 75.0 L/kg of TS over pea-shells (microbially hydrolyzed) as compared to pea-shells with yields of 65.0 L/kg of TS [20, 21]. Also, the organic fraction of municipal solid waste (OFMSW) showed quite similar H_2 production of 62.5 L/kg of VS under batch-mode by UMCs [111]. These findings suggested that a suitable combination of feed and H_2-producing cultures can be desirable to achieve a high yield.

The vegetables, fruit, and cheese whey mixture exhibited $\sim$ 44-folds lower H_2 yields to those recorded of 553 L/kg of VS from mixed fruit wastes (Table 3) [101, 103]. The combinations of the various agri-biowastes mixture (two to six different combinations) along with corresponding controls proved beneficial to produce H_2 by DMCs and the high H_2 production varied between 54.0 and 102 L/kg of TS [5, 59, 62]. Similarly, higher H_2 yield of 166, and 199 L/kg of feed from cassava and sweet potato-based starch, respectively, were also reported [110]. In contrast, an association of banana, grape, melon, orange to MCs noted maximum H_2 yields up to 403 L/kg of VS under continuous mode [103]. In batch mode, the H_2 production from food waste recorded a higher production of 220 L/kg of feed over 35.1, 93.4, and 119 L/kg of feed from cassava pulp, cheese whey, and cassava waste, respectively [101, 112, 113]. Based on yield among the various agri-food wastes, apple waste can be potentially utilized as a suitable feed for commercial biohydrogen production in the near future.

Two-Stage Process of Biohydrogen and Methane

In general, under single-stage DF H_2 production the partial valorization of biowaste has occurred and bioprocess seems less economical due to the maximum H_2 yield achievable of only 33% to total theoretical production of 12 mol/mol of hexose [1, 19]. Therefore, to improve DF process efficiency, various over integrating approaches have been demonstrated to produce value-added biofules biomolecules (H_2, CH_4, butanol, and biodiesel) and eco-friendly biodegradable polymers (PHAs) a substitute to manmade plastics [10, 70]. Thus, such integrative bioprocesses as the biorefinery approach can endorse better management of wastes and environmental pollution along with the generation of various renewable products. The combination of DF H_2 generation followed with AD to produce CH_4 can achieve almost complete utilization of feed (Fig. 2). [1, 10]. The two-stage integrative generation of H_2 and CH_4 from agri-food wastes is presented in Table 4. Generally, the MCs inoculum employed at the H_2 production stage requires pretreatment like heat to enrich H_2-produces and minimize methanogens (H_2 consumers). In contrast, the CH_4 stage inoculum can be directly used as an inoculum instead of any initial pretreatment (naturally selected). Giordano et al. demonstrated integrative production of 177 L of H_2/kg of COD, and 243 L of CH_4/kg of COD from wheat (Common and durum), mashed and steamed peels of potato, respectively. These findings suggested that feed can significantly altered the production of H_2 and CH_4 by granular sludge [114]. In contrast, a quite similar production of H_2 and CH_4 was recorded from potato peels and rice by anaerobic sludge as inoculum [115].

Agri-food pure wastes such as banana peels, beans, cassava residues, potato, cheese whey, and sugarcane bagasse reported H_2 and CH_4 up to 253 and 507 L/kg of total VS (TVS), respectively (Table 4) [116–120]. In contrast, a lower H_2 up to 87.5 L/L of feed and higher CH_4 yields up to 570 L/kg of COD observed from OFMSW as a mixture of agri-food to other type wastes [121–123]. A quite comparable production of 223 L of H_2/kg of VS and 277 L of CH_4/kg of VS was noted from rice residue and *Chlorella pyrenoidosa* [124]. The association of food wastes resulted in yields of H_2 and CH_4—(1) up to 218 and 432 L/kg of VS by anaerobic sludge, and (2) 135 and 510 L/kg of VS by seed sludge as inoculum, respectively [125–127]. Similarly, food waste in different combinations to olive husk, garden, and activated sludge produced up to 87.0 NL of H_2/kg of VS and 682 L of CH_4/kg of TS [128–130]. Significant variations are observed to integrative generation of H_2 and CH_4 from agri-food wastes that can be associated with compositions of sugars in feed, fermentation conditions, mode of production, and types of inoculums (Table 2). Overall, among the other agri-food wastes, potato, and a mixture of vegetables to other wastes

Table 3 Biohydrogen generation by dark-fermentation of various agri-food wastes

Agri-biowaste	Culture	Biohydrogen		Reference
		Mode	Yield	
Agave tequilana bagasse	Anaerobic sludge	Continuous	1.53 mol/mol of hexose	[105]
Agri-biowaste mixtures	Defined mixed cultures (DCMs)	Batch	54.0–102 L/kg of TS	[5, 62]
Apple	Mixed culture	Continuous	635 L/kg of VS	[103]
Apple pomace	DCMs	Batch	60.0–83.0 L/kg of TS	[5, 59]
Banana	Mixed culture	Continuous	403 L/kg of VS	[103]
Cassava pulp	Soil-based mixed culture	Batch	35.1 L/kg of feed	[113]
Cassava starch	Anaerobic sludge	Batch	166 L/kg of starch	[110]
Cassava waste	Cattle dung	Batch	119 L/kg of feed	[112]
Cheese whey	Anaerobic sludge	Continuous	1.97 mol/mol of hexose	[106]
	Mixed culture	Batch	93.4 L/kg of COD	[101]
Corn starch	Anaerobic sludge	Batch	177 L/kg of starch	[110]
Grape	Mixed culture	Continuous	384 L/kg of VS	[103]
Date fruit waste	*Enterobacter aerogenes* ATCC 13,408	Batch	144–239 L/kg of feed	[102]
Food waste	Cattle dung	Batch	220 L/kg of feed	[112]
Melon	Mixed culture	Continuous	352 L/kg of VS	[103]
Mixed fruit wastes	Mixed culture	Continuous	553 L/kg of VS	
Onion-peels	DCMs	Batch	56.0–86.0 L/kg of TS	[5, 59]
Orange	Mixed culture	Continuous	403 L/kg of VS	[103]
The organic fraction of municipal solid waste	Anaerobic digestion sludge	Batch	62.5 L/ kg of VS	[111]
Pea-shells	DMCs	Batch	65.0 L/kg of TS	[20]
Pea-shells hydrolysate	DMCs	Batch	75.0 L/kg of TS	[21]
	DMCs	Continuous	54.0 L/kg of TS	
Potato peels	*Parageobacillus thermoglucosidasius* KCTC 33,548	Batch	0.83 L/L	[109]
	DCMs	Batch	64.0–92.0 L/kg of TS	[5, 59]
Potato starch	Anaerobic sludge	Batch	151 L/kg of starch	[110]
Rice husk	*Bacillus cereus*		1.37 mol/mol of hexose	[107]
Sugar beet molasses	*Caldicellulosiruptor saccharolyticus* DSM 8903	Batch	2.10 mol/mol of hexose	[104]
Sugarcane bagasse	*Clostridium thermocellum* DSMZ 1313	Batch	0.92 mol/mol of hexose	[108]
Sweet potato starch	Anaerobic sludge	Batch	199 L/kg of starch	[110]
Vegetable and fruits	Mixed culture	Batch	8.3 L/kg of COD	[101]
Vegetable, fruit, and cheese whey	Mixed culture	Batch	12.5 L/kg of COD	

recorded maximum productions of 253 L of H_2/kg of TVS and 730 L/kg of VS at first and second stages of integrative bioprocess, respectively [120, 130]. Thus, these wastes combinations can be more beneficial to produced higher H_2 and CH_4 in the future. Additionally, the lower H_2 generation at the first-stage of the integrative bioprocess can be improved via the uses of DMCs, novel culture or genetically engineered culture over UMCs [10, 132, 133].

Conclusions and Prospects

The key challenges of H_2 production are associated with the costly sugars as primary feed and lower H_2 yield to 4 mol/mol of hexose under DF conditions. Biowastes, including agri-food wastes, are desirable alternative low-cost feed to produce biohydrogen. However, the available quantum of these wastes is highly variable, especially in

Table 4 Two-stage bioprocesses for hydrogen and methane production from various agri-food wastes

Agri-waste	Stage I—Biohydrogen		Stage II—Biomethane		Reference
	Culture	Yield	Culture	Yield	
Banana peels	Anaerobic sludge	210 L/kg of VS	Anaerobic sludge	284 L/kg of VS	[117]
Bean waste	Seed sludge	152 L/kg of TVS	Seed sludge	463 L/kg of TVS	[120]
Cassava residues	Mixed culture	118 L/kg of TS	Mixed culture	308 L/kg of TS	[119]
Cheese whey	Anaerobic sludge	137 L/kg of COD	Anaerobic sludge	250 L/kg of COD	[116]
Common wheat	Granular sludge	47.0 L/kg of COD	Granular sludge	202 L/kg of COD	[114]
Durum wheat	Granular sludge	76.0 L/kg of COD	Granular sludge	243 L/kg of COD	
Food waste	Anaerobic sludge	215 L/kg of COD	Anaerobic sludge	311 L/kg of COD	[125]
		218 L/kg of VS	Anaerobic sludge	432 L/kg of VS	[127]
	Seed sludge	135 L/kg of VS	Seed sludge	510 L/kg of VS	[126]
Food and olive husk	Anaerobic sludge	87.0 NL/kg of VS	Anaerobic sludge	505 NL/kg of VS	[128]
Food waste and activated sludge	Seed sludge	76.8 L/kg of VS	Seed sludge	148 L/kg of VS	[130]
Garden and food	*C. saccharolyticus* DSM 8903	46.0 L/kg of TS	Anaerobic sludge	682 L/kg of TS	[129]
Mashed potato	Granular sludge	177 L/kg of COD	Granular sludge	207 L/kg of COD	[114]
Organic fraction of municipal solid waste	Anaerobic sludge	24.0 L/kg of VS	Anaerobic sludge	570 L/kg of VS	[122]
		87.5 L/L	Mixed culture	241 L/L	[123]
	Mixed culture	41.7 L/kg of VS	Anaerobic sludge	300 L/kg of VS	[121]
Potato	Seed sludge	253 L/kg of TVS	Seed sludge	507 L/kg of TVS	[120]
Potato peels	Anaerobic sludge	103 L/kg of VS	Anaerobic sludge	237 L/kg of VS	[115]
Rice	Anaerobic sludge	125 L/kg of VS	Anaerobic sludge	232 L/kg of VS	
Rice residue and *Chlorella pyrenoidosa*	Anaerobic sludge	223 L/kg of VS	Anaerobic sludge	277 L/kg of VS	[124]
Steam potato peeling	Granular sludge	134 L/kg of COD	Granular sludge	183 L/kg of COD	[114]
Sugarcane bagasse	Cow dung	93.4 L/kg of VS	Anaerobic sludge	222 L/kg of VS	[118]
Vegetables and other wastes mixture	Seed sludge	79.4 L/kg of VS	Seed sludge	730 L/kg of VS	[131]

cases of seasonal waste that can be a limiting factor for sustainable H_2 production via environmentally friendly technologies. Also, feedstocks (biowastes) mobilization is a vital concern to produce from biomass. Due to the complex nature of biowastes and variations in their cellulosic contents can also influence biohydrogen production. Thus, the utilization of biowastes (type) can impact overall prospects of their use such as the production of H_2 through DF and CH_4 via AD. These obstacles may be undertaken through the development of cost-effective biowastes pretreatment techniques via focusing on the improvement of bioprocess efficiency by the valorization of waste to increase H_2 yield. The bioprocess-based technologies to produce H_2 are in different levels of developmental stages. In typical, various studies have been focused on H_2 production bioprocess through—(1) reduce capital investments, different operational expenses, including maintenance), revenue (profits either directly or indirectly) and cost of the product (H_2), and (2) improvement of technical efficiency of H_2 production such as (1) use of inexpensive-pretreatment methods for hydrolysis of biowaste to fermentable sugars, (2) screening efficient and novel H_2-producers, (3) use of metal and nanoparticles to influence biocatalytic activity especially hydrogenases, (4) co-digestion of biowastes to improve nutrition balance as suitable feed, (5) use of selective consortia of DMCs instead of pure or UMCs (it requires additional pretreatment to the elimination of CH_4-producers and enrichment of H_2-producers) to improve metabolization of feed towards H_2, (6) selection of desirable reactor type and (7) metabolic engineering of biocatalysts. In the current scenario, still, the H_2 production cost is substantially high in addition to the uses of biowaste as low-cost feed due to higher capital and operation costs. The integration of pure or MCs-based bioprocesses from agri-food wastes can be more economically desirable to produce H_2 followed by value-added products at the second stage such as (1) H_2 via photo-fermentative, (2) CH_4 through AD, (3) PHAs, or (4) electricity production.

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Declarations

Conflicts of interest The authors declare no conflict of interest.

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