Prospects for the Improvement of Bioethanol and Biohydrogen Production from Mixed Starch-Based Agricultural Wastes

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Abstract: The need for fossil fuel alternatives keeps increasing. Bioethanol and biohydrogen have emerged as significant renewable options. However, these bioprocess routes have presented various challenges, which constantly impede commercialization. Most of these bottlenecks are hinged on feedstock logistics, low biofuel yield and enormous process costs. Meanwhile, a large output of renewable energy can be generated from mixed starch-based agricultural wastes due to their intrinsic bioenergy characteristics. This study, therefore, focuses on the production of bioethanol and biohydrogen from mixed starch-based agricultural wastes. The content further highlights the current challenges of their individual processes and elucidates the prospects for improvement, through an integrated biofuel approach. The use of mixed starch-based agricultural wastes as substrates for integrated bioethanol and biohydrogen production was proposed. Furthermore, the use of mixture-based experimental design for the determination of optimal values of critical factors influencing biofuel production emerges as a viable prospect for profitable bioethanol production from the starch-based biomass. Additionally, biohydrogen production from effluents of the mixed starch-based waste bioethanol looked promising. Thus, the study proposed valuable insights towards achieving a cost-effective biofuel technology.

Keywords: bioethanol; biohydrogen; integrated biofuel; agricultural wastes

1. Introduction

The escalating demand for energy in recent years, food security concerns and increasing environmental pollution has positively driven the growth and focus on bioenergy and subsequently, biofuel production [1]. Fossil fuels, in the form of petroleum, have been the most prominent, especially for transportation, for many decades. However, it is anticipated that in the next fifty years, fossil fuel sources will be completely depleted [2]. Hence the need for the development of sustainable fuels. Biofuels such as bioethanol, biodiesel and biogas are produced from renewable and biological raw materials. These are sustainable natural sources due to their global abundance [3]. Thus, biofuels represent a sustainable alternative to fossil fuels. Currently, bioethanol is considered as one of the most promising and environmentally friendly biofuels for transportation and other energy applications. Global bioethanol supply is majorly from food crops such as corn and sugarcane [4].

As of 2019, the world's largest producer of bioethanol is the United States, with the national bioethanol output of 15.78 billion gallons, followed by Brazil (8.57 billion gallons) [5].

However, these two largest contributors to the global bioethanol stream utilize corn and sugarcane as their major crops for bioethanol production. Bioethanol from these crops is regarded as first generation, and there are concerns over food scarcity, drought and other agricultural side effects usually associated with the production of first-generation biofuels [3]. These have led to a consistent shift towards a full exploration of non-food alternatives in recent years [6]. Bioethanol and other liquid biofuels from non-food sources are referred to as second-generation biofuels [3].

On the other hand, hydrogen is a versatile fuel that has found usage as automobile fuel, rocket fuel and raw materials for chemical industries. As of 2019, annual global hydrogen production was 70 million tons [7]. The fuel is commonly produced through the partial oxidation of fossil fuels, coal gasification and other energy-intensive thermochemical methods. Although the combustion of hydrogen does not result in environmental pollution, the route to production greatly does [8]. This has led to the emergence of biohydrogen as a rather sustainable form of hydrogen due to its production from organic sources such as wastes by the biological process of dark fermentation. Despite the environmentally benign advantages of this biofuel, the challenge of low yield coupled with decreased substrate conversion efficiency still limits the sufficient satisfaction of the high demand for the fuel [9].

A critical observation of the production process of bioethanol and biogas reveals the cost of production as a crucial challenge influencing their economic viability. The general cost of biofuel production is largely determined by the issues around feedstock logistics and substrate intrinsic characteristics [10]. These issues are described as the cost of feedstock, the energy-intensive process of biomass harvesting, cost of pretreatment, enzyme costs and the unavoidable cost of high-throughput fermentation technologies [11]. Essential strategies that have been reported for tackling the challenges as mentioned above to produce cost-competitive biofuels include the development of economically viable pretreatment methods and combined fermentation models [12–16]. Other strategies are the development of cheap but effective machinery, generation of other important by-products from the biofuel stream (coproduct formation) and the utilization of integrated biofuel production techniques [17–22].

Firstly, in addressing the challenge of feedstock logistics for biofuel production, the choice of the feedstock is to be considered as a critical factor. Potential non-food sources such as agricultural waste can greatly drive profitable biofuel production. Many agricultural wastes which include by-products of forestry, industrial and municipal activities are considered wealthy and renewable sources of biofuel due to the presence of high-energy compounds in the form of polysaccharides and various carbohydrate derivatives. Large output of renewable energy can be generated from agricultural wastes due to their lignocellulosic characteristics [23]. Agricultural wastes containing starch, e.g., cassava peels and potato peels, are usually generated in huge amounts annually across the globe. They are an arsenal of crucial polysaccharide compounds that can be harnessed for biofuels and bioenergy. Starch-based agricultural wastes can be explored for bioethanol production through the application of relevant strategies directed towards improving product yield and process feasibility. Lignocellulosic biomass and waste-based integrated biofuel production have been reported for bioethanol, biohydrogen, biodiesel and/or biogas production. These fuels have been reportedly generated from the combination of pure lignocellulosic substrates and non-starch-based agricultural wastes [17,24–26], or a combination of food crops and agricultural wastes [27]. Thus, the feedstock logistic challenge in biofuel processes could be addressed by the use of random mixtures of starch-based agricultural wastes [28].

Additional strategies to address the current challenges of biofuel production is an investigation into the possibilities of multiple biofuels streams from mixed starch-based wastes in an integrated process. Integrated biofuel production allows for the generation of two or more biofuels from energy-rich biomass, thereby increasing the profitability of the production pathway. Agricultural waste-biofuel processes generate effluents, which constitutes a nuisance to environmental bodies and soil microorganisms. These waste streams contain organic and inorganic compounds that can be utilized for additional biofuel products, thereby increasing the profitability of the biorefinery.

This review, therefore, focuses on the integrated production of bioethanol and biohydrogen from mixed starch-based agricultural wastes. The content elucidates the separate conventional

bioethanol and biohydrogen processes from agricultural wastes and highlights the current challenges of both processes. A full pathway of integrated bioethanol and biohydrogen production from mixed starch-based agricultural waste was then proposed as a panacea to the challenges of the individual processes.

2. Conventional Bioethanol Production

Bioethanol is ethyl alcohol with a chemical formula of C_2H_5OH. It is an eco-friendly oxygenated fuel with various properties. These properties include a high octane number, which helps enhance gasoline performance when blended [29]. Other properties are broader flammability limits and higher evaporation enthalpy. Bioethanol flammability limits of between 3.3 and 19 and evaporation enthalpy of 50.43 KJ/mol have been reported [30,31]. These properties make bioethanol one of the most promising alternatives to fossil fuel [4]. Bioethanol is a product of the fermentation process. The most common substrates for bioethanol fermentation are sugar and starch crops, and these include sugarcane, sweet sorghum, dates, corn, watermelon and sugar beet. In Brazil, 79% of the national bioethanol stream is produced from sugarcane [4], whereas the primary substrate for bioethanol production in the United States is corn (a starch crop). These crops are referred to as first-generation biofuel sources, and predominantly, the starch present in starchy first-generation feedstocks is highly desirable for bioethanol production. This is because plant starch is heavily loaded with glucose monomers usually stored as bioenergy. However, the constant feud between these sources and food supply, coupled with the challenges associated with feedstock availability, has rendered this bioethanol route non-preferrable. Nevertheless, the huge amounts of wastes usually generated from starchy crops are not consumable and are usually discarded. These starch-based agricultural wastes can thus be explored as a second-generation alternative for bioethanol production using the established routes for the production of bioethanol from the starchy first-generation feedstocks [3]. The established route of bioethanol production from first-generation feedstocks are shown in Figure 1. Sugar crops are subjected to an extraction process that releases the sugar content, which is subjected to fermentation to bioethanol by yeasts. Similarly, starchy crops are subjected to preliminary processes that extract the starch, which is subsequently subjected to the enzyme-inclusive stages of liquefaction and saccharification.
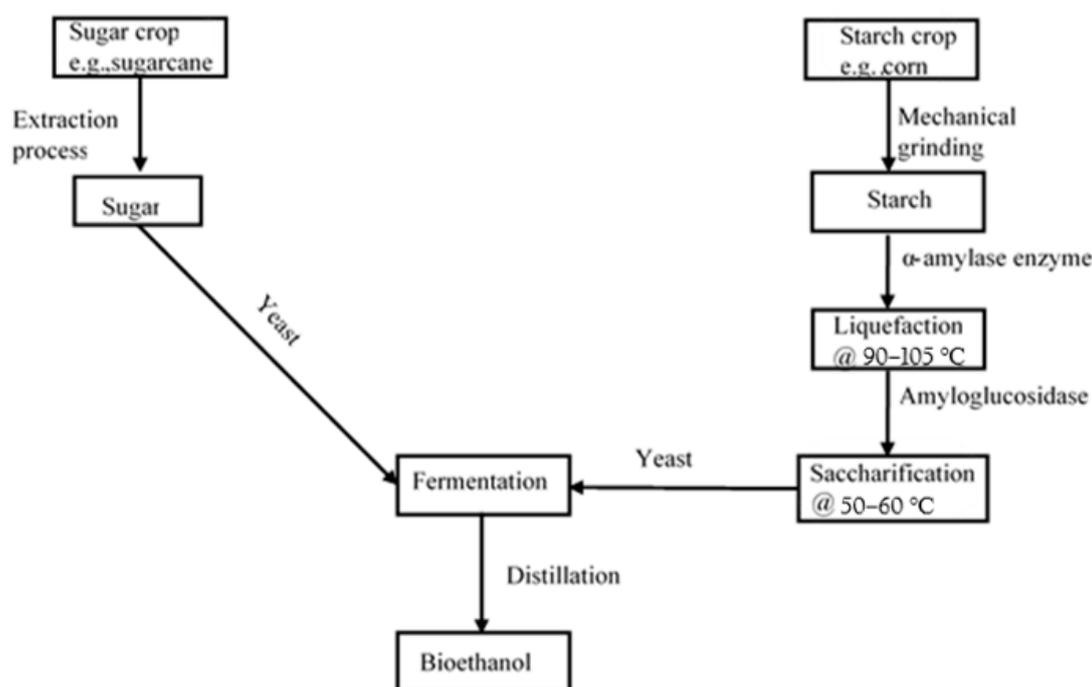
Figure 1. General schematic diagram of bioethanol production from first-generation feedstocks.

The most common microbe used in bioethanol fermentation is *Saccharomyces cerevisiae*. It converts glucose obtained from starch and sugar crops into bioethanol in an anaerobic process that takes place in the cytoplasm. This microbial step by step breakdown of sugars is referred to as glycolysis. Biochemically, the presence of glucose in the cell's environment stimulates the synthesis of an enzyme system involved in the glycolytic pathway. This pathway proceeds through the induction of genes encoding these enzymes. One mole of glucose successfully transported via the glucose transporters into the yeast's cytoplasm will be catabolized into two moles of pyruvate. Each mole of pyruvate, in turn, undergoes a reduction reaction generating one mole of ethanol and one mole of carbon dioxide. Energy generated during the process, i.e., Adenosine Triphosphate (ATP), is utilized by the organism for the biosynthesis of essential macromolecules needed for the growth of its cells [4,32]. Another common microorganism capable of bioethanol fermentation is the Gram-negative bacterium, *Zymomonas mobilis*. However, it is not commonly used like *S. cerevisiae* due to its narrow substrate range [33]. Many efforts have been directed towards the modification of yeast strains as a strategy towards the improvement of bioethanol processes. Bioethanol fermentation exposes yeasts to various types of stress that lowers their performance and productivity. These stressors include increased bioethanol concentration, toxic compounds inhibition, high process temperature and osmotic pressure from a high concentration of sugar [34]. Increased bioethanol concentration followed by high process temperature is considered as the major stressor for yeasts, and thus, most efforts have been directed towards the development of yeast strains with ethanol-tolerant and thermo-tolerant capabilities. Strategies towards the creation of such modified strains for improved bioethanol production include random chemical mutagenesis, genome shuffling and ultraviolet exposure [35].

Similarly, focusing on the substrates for bioethanol production, genetic modifications and genotypic variations have also been applied on bioethanol substrates with the aim of improving their bioethanol yield. However, in other instances, they are supplemented with additional nutrients with the aim of increasing the extractable sugar, ethanol yield and fermentation efficiency.

Studies on the improvement of bioethanol production through yeast genotypic variations and first-generation feedstock are shown in Table 1. A study which evaluated the potential of genotypically varied sweet sorghum for improved bioethanol production observed that out of five sweet sorghum genotypes evaluated, maximum bioethanol production was achieved with a Keller variety [36]. In another study, juice from sugarcane was subjected to bioethanol fermentation with the use of a thermo-tolerant yeast strain, *Pichia kudriavzevii,* under adaption to galactose medium conditions. The adapted cells produced higher bioethanol than the non-adapted ones [37]. Other studies involving the improvement of bioethanol production include the use of sugar beet with ultrafiltration techniques on the sugar concentrates before fermentation [38]. The fermentation process was carried out under various media conditions where the ultra-filtered concentrate was feasible for bioethanol production with the $Mg_3(PO_4)$-supplemented media, giving the highest bioethanol yield [38]. In another study, cassava starch was fermented to bioethanol using partially purified enzymes produced from several filamentous fungi. A bioethanol yield of up to 84% was achieved after the fermentation, subsequent to a dual enzymatic process involving the produced α-amylase and amyloglucosidase [39].

In a similar study, the possibility of minimizing enzyme requirement prior to fermentation was evaluated using high sugary corn genotypes (HSCG) against the parent field corn lines (PFCs). Higher bioethanol concentrations were achieved with the HSCG [4]. Duvernay et al. [40] also demonstrated the potential of industrial sweet potatoes (ISP) for bioethanol production using fermentation media with or without salt nutrients. A bioethanol concentration of up to 62.6 g/L was achieved with the flour form of ISP. Adaptation of the aforementioned basic principles and strategies which were applicable to the improvement of the conventional and first-generation bioethanol can foster the product profitability from the more abundant second-generation substrates.

Table 1. Studies towards the improvement of first-generation bioethanol.

Substrate(s)	Study Focus	Organism(S) Used	Study Outcome	References
Sweet sorghum (*Sorghum bicolor*)	The genotypes: Keller, SSV84, Wray, NSSH 104 and BJ 248 were examined for bioethanol production.	*S. cerevisiae* CFTR 01 and *S. cerevisiae* SG	The Keller variety gave the highest ethanol concentration of 9.0% w/v.	Ratnavathi et al. [36]
Sugar beet	Investigation of the feasibility of membrane ultra-filtered sugar concentrate and nutrient enrichment for bioethanol production.	*S. cerevisiae* strain (Ethanol Red) and *S. cerevisiae* strain (Safdistill C-70)	Ultra-filtered concentrate was feasible for bioethanol production and media supplemented with $Mg_3(PO_4)$ nutrient gave the highest bioethanol yield of 91.16–92.06 g/L.	Kawa-Rygielska et al. [38]
Sugarcane	Evaluation of the effect of galactose-adapted medium containing *Pichia kudriavzevii* on bioethanol production potential.	Thermo-tolerant yeast strain: *Pichia kudriavzevii* (*Issatchenkia orientalis*)	Over 30% increase in ethanol production than from non-adapted cells.	Dhaliwal et al. [37]
Cassava	An investigation into the effect of dual enzymatic starch degradation method using partially purified α-amylase and amyloglucosidase enzyme on bioethanol production.	*S. cerevisiae*	High fermentable glucose of 40 g/L was obtained resulting in 84% ethanol yield.	Pervez et al. [39]
Corn	Evaluation of the effects of grain sugar on the fermentable sugar and ethanol yields from four high sugary genotypes (HSGs) and their parent field lines (PFCs).	*Saccharomyces cerevisae* (ATCC 96581)	Higher ethanol concentrations (15.25–17.5% (v/v)) were obtained from HSGs compared to the PFCs (11–13.65% (v/v))	Zabed et al. [4]
Industrial sweet potato (ISP)	Production of ethanol from industrial sweet potatoes with or without the addition of salt nutrients	Ethanol Red yeast (*S. cerevisiae*)	Ethanol concentration of 62.6 and 33.6 g/L was achieved for flour and fresh ISP, respectively (without salt nutrients)	Duvernay et al. [40]

3. Conventional Biohydrogen Production

Hydrogen has gained significant relevance as an alternative to fossil fuel owing to several advantages of high energy density (143 KJ/g), zero carbon emissions and multiple storage forms [9,41]. In addition to fuel relevance, the hydrogen commodity has huge industrial applications in ammonia synthesis and methanol production [41]. Hydrogen (H_2) can be produced through thermochemical and biological routes (Figure 2). The thermochemical methods include gasification of hydrocarbons, steam reforming, oil reforming, coal gasification and water electrolysis [9,42]. Additionally, hydrogen can be obtained indirectly through some heterogeneous catalytic approaches using lignocellulosic biomass as a substrate, where the cellulose and the hemicellulose portions are first hydrogenated into polyols [43]. These polyols, mainly xylitol and sorbitol, are subjected to aqueous phase reforming processes generating molecular hydrogen and syngas (CO). The syngas is further converted to

hydrogen and carbon dioxide through water gas shift (WGS) reactions [44]. However, these are highly energy-intensive processes with enormous environmental emissions of carbon compounds. On the other hand, the biological route is a much cleaner and sustainable alternative leading to the formation of "biohydrogen". Biohydrogen can be produced through bio-photolysis, photo-fermentation and dark fermentation methods [8,45]. In the dark fermentation method, biohydrogen is produced through the fermentation of organic materials such as lignocellulosic wastes and industrial organic wastewaters using mesophilic and thermophilic microorganisms [9]. This method utilizes sustainable raw materials and is less energy-intensive compared to the thermochemical route and has thus gained much attention as the most promising route of biohydrogen production. However, dark fermentative biohydrogen production is still largely challenged by low substrate conversion efficiencies, low production rate and low yield [46,47].
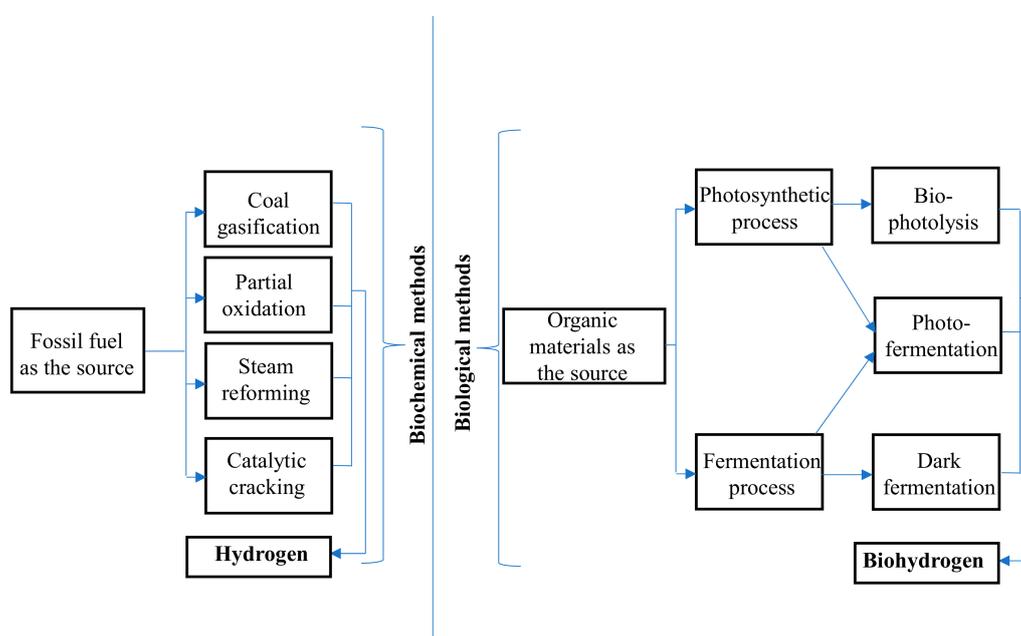

Figure 2. Schematic diagram of the hydrogen production process.

Dark Fermentative Biohydrogen Production

Dark fermentative biohydrogen production is a biologically friendly process for the use of sugar/carbohydrate-rich organic materials as substrates for biohydrogen production. The major drivers of this route of hydrogen production are mesophilic and thermophilic microorganisms, preferably the facultative and obligate anaerobes. Anaerobic bacteria produce biohydrogen from the carbohydrate (glucose) composition of organic substrates via two major catabolic pathways in the absence of oxygen. In the first pathway, which describes that of facultative anaerobes such as *Klebisiella* sp., pyruvate formed from glucose is ultimately decarboxylated into 4 moles of hydrogen-producing acetic acid as an intermediate compound. On the other hand, the pathway of strict anaerobes (obligate) such as *Clostridia* sp. generates 2 moles of hydrogen from pyruvate, while producing butyric acid as an intermediate in the process [48]. Thus, the complex process of dark fermentative biohydrogen production can be summarized as hydrolysis (breaking down of the organic matter into glucose), acidogenesis (formation of acetic acid or butyric acids) and methanogenesis [9].

Methanogenesis occurs in the presence of methanogens which compete with hydrogen production by converting acetic acid, hydrogen and carbon dioxide formed into methane. This process must be completely inhibited in order to maximize hydrogen production. Generally, the factors affecting dark fermentative biohydrogen production include seed inoculum, substrate, temperature, alkalinity, hydraulic retention time, hydrogen (and CO_2) partial pressure and pH [7,44]. Utilization of substrates

such as agricultural wastes for biohydrogen production has the potential to significantly enhance the sustainability of this biofuel.

4. Agricultural Waste for Biofuel Production

Agricultural wastes are generated due to operations such as farming and related activities [49,50]. These include animal wastes, product packaging materials such as plastics and papers, hazardous wastes (insecticides and pesticides) and crop wastes [51]. Huge amounts of agricultural wastes are usually produced annually across the globe, an example is China and Egypt, which both generate an approximate fifty-six and twenty-seven million tons of agricultural wastes per annum, respectively [50–52].

Crop-based agricultural wastes contribute significantly to the annual global production of agricultural wastes. Examples of crop wastes include corn stover, cornstalk, sugarcane bagasse, cassava peels, potato peels, sugarcane leaves and rice straw. Agricultural wastes originating from crops (crop wastes) can be classified into two categories, based on their general structural composition. These include starch-based and non-starch-based agricultural wastes. In the bio-industrial sector, much attention is centered on crop-originated agricultural wastes. This is because they contain high-energy polysaccharides, which can be harnessed for the production of high-value products. These polysaccharides include the polymeric structures of cellulose, hemicellulose and lignin. Some others contain starch as an additional polymer. These can be classified as starch-based wastes (SBW), while those without starch are non-starch-based wastes (NSBW). Agricultural wastes naturally take on part or full biochemical composition of the crops from which they originate.

4.1. Biochemical Composition of Agricultural Wastes

Generally, most NSBWs reportedly contain an approximate composition of 6–42% lignin, 4–50% cellulose and 7–30% hemicellulose, while SBWs have a similar composition with an additional starch component that ranges from 2% to 70% (Table 2) [4,13,52–55]. Starch is a large polymer of monomeric sugars that are linked together in the form of amylose and amylopectin chains. Amylose is the unbranched polysaccharide type of starch linked by α-1-4 glycosidic bonds, while amylopectin is the branched type that contains α-1-6 bonds in addition to α-1-4. Cellulose is a linear polymer of glucose molecules [55], while hemicellulose is a short-chain polymer of hexoses and pentoses linked by α-1,4 and β-1,4-glucosidic bonds, respectively. Lignin is an aromatic polymer of coumaryl, conipheryl and sinapyl alcohols [4]. Cellulose, hemicellulose and lignin are usually intertwined in the cell wall of plants from which SBW and NSBW originate, giving them their rigid, robust structure.

4.2. Starch-Based Agricultural Wastes for Bioethanol and Biohydrogen Production

Agricultural wastes such as starch-based wastes contain starch in addition to their holocellulose (hemicellulose and cellulose) composition and are thus suitable as lignocellulosic substrates for bioethanol fermentation. Similarly, agricultural wastes can be utilized as alternative organic substrates to crop-based organic sources for biohydrogen production.

4.2.1. Bioethanol Production from Starch-Based Wastes

Sugars are present in starch-based agricultural wastes and all lignocellulosic substrates, which need to be released from the carbohydrate polymers for the yeast to ferment them to bioethanol. Yeast cannot utilize the carbohydrate polymers directly as a substrate for bioethanol fermentation [56]. Thus, pretreatment is essential for the disruption of the holocellulose structure in order to facilitate enzymatic hydrolysis and microbial fermentation. First, the pretreatment process removes lignin and hemicellulose from the lignocellulosic matrix, decreases cellulose crystallinity and increases the porosity and the surface area of the substrate (Figure 3). The released cellulose and hemicellulose molecules are thereafter exposed to enzymatic hydrolysis. Although the depolymerization of the hemicellulose fraction which takes place at the pretreatment stage releases some fermentable sugars, the enzymatic hydrolysis stage is crucial for optimum sugar recovery.

Pretreatment methods include physical, chemical and biological pretreatments. Physical pretreatment involves milling and grinding while chemical pretreatment can be achieved with the use of acids, bases and various salts, which reduces the crystallinity of the substrate. Biological pretreatment involves the use of enzymes. Currently, the pretreatment of agricultural wastes for bioethanol production still requires research efforts in order to achieve process consistency, efficiency and economy [57].

Table 2. Biochemical composition of starch-based wastes and non-starch-based wastes.

Agricultural Wastes	Lignin (%)	Starch (%)	Cellulose (%)	Hemicellulose (%)	References
Starch-based wastes	–	–	–	–	–
Cassava stem	22.1	15.0	22.8	28.8	Pooja and Padmaja [53]
Cassava leaves	20.1	2.4	17.3	27.7	Pooja and Padmaja [53]
Cassava peels	1.5	81.4	NR	NR	Moshi et al. [52]
	–	44.53	9.05	7.5	Aruwajoye et al. [13]
Potato peels	6.07	20	4.03	10	Chohan et al. [54]
Pumpkin waste	NR	65.3	NR	NR	Chouaibi et al. [58]
Non-starch-based wastes	–	–	–	–	–
Corn cobs	6.32	–	34.21	39.08	Sewsynker-Sukai and Kana [12]
Corn stover	7–19	–	38–40	24–26	Zhu et al. [59], Saini et al. [60]
Sugarcane bagasse	20–42	–	42–48	19–25	Saini et al. [60], Kim and Day [61]
Sugarcane leaves	9.39	–	44.78	27.38	Moodley and Kana [62]
Rice straw	12–14	–	28–36	23–28	Saini et al. [60]
Wheat straw	17–19	–	33–38	26–32	Saini et al. [60]

NR—Not reported.

Inefficient chemical pretreatment can generate toxic compounds as by-products, which eventually inhibits microbial metabolism during ethanol fermentation [63]. Furthermore, this strategy is challenged by partial degradation of inherent sugars, high severity and corrosion in process equipment during large-scale pretreatment [13,64]. Other methods which are physicochemical are challenged by the incomplete disruption of the holocellulose-lignin framework, causing low fermentable sugar yields. Biological pretreatment also requires innovative modifications in order to improve the rate of hydrolysis and sugar yield [57]. Addressing these challenges will prevent severe drawbacks on the process cost and economics.
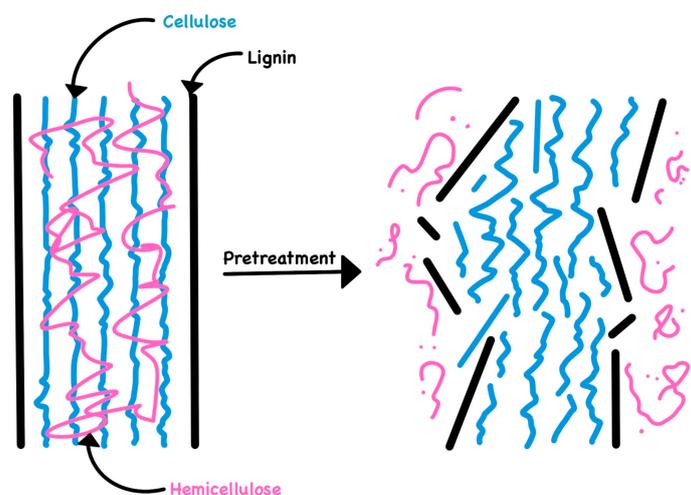
Figure 3. Schematic representation of pretreatment of lignocellulosic substrates (Adapted from Ji et al. [65] and Mosier et al. [66]).

In the enzymatic hydrolysis stage, cellulose and hemicellulose molecules released from the pretreatment stage are exposed to hydrolysis through cellulase enzyme activities. The cellulase enzyme deconstructs cellulose molecules into actual monosaccharide sugars [65]. The enzyme system includes exoglucanase, endoglucanase and β-glucosidase. Endoglucanase randomly breaks down the β-1,4 glycosidic bonds in the amorphous region of the cellulose molecule, producing short D-glucan chains. On the other hand, the exoglucanase cleaves the glucan chains into cellobiose from the non-reducing end. The cellobiose units are, thereafter, converted to glucose by the action of β-glucosidase [4]. In the case of SBWs, additional enzymatic stages of liquefaction by α-amylase, and saccharification by amyloglucosidase, is needed for the breakdown of the starch component of the biomass. Starch polymers are converted into dextrins and maltose in the liquefaction stage. The action of α-amylase and amyloglucosidase enzymes releases glucose units by cleaving the α-1,4-D-glucosidic linkages and 1,4-linked maltooligosaccharides of the starch from the non-reducing end.

Subsequent to maximum fermentable sugar release from the pretreatment and enzymatic hydrolysis stages, the processed slurry is subjected to bioethanol fermentation. The microbial process involved in crop-based bioethanol fermentation also applies to agro-based lignocellulosic wastes. Bioethanol production from agro-based lignocellulosic wastes using various fermentation modes is shown in Table 3. The traditional fermentation process is usually executed separately from the enzymatic hydrolysis stage (separate hydrolysis and fermentation—SHF). However, a major limitation of this process is the possibility of enzyme inhibition by sugars released from the hydrolysis stage. Alternatively, the simultaneous saccharification and fermentation (SSF) process allows for enzymatic hydrolysis/saccharification and fermentation to be executed in a single vessel under the same conditions [14,15]. This reduces time and leads to higher product yield. However, despite the advantages of SSF over SHF, finding an optimum temperature for both fermentation and hydrolysis could be a difficult task [67]. Additionally, the cost of the enzyme to be used for saccharification processes could amount to a significant drawback during large-scale processes [11].

Table 3. Bioethanol production from agro-based lignocellulosic wastes.

Substrate	Fermentation Mode	Fermentation Conditions	Microorganism	Bioethanol Concentration (g/L), Yield	References
Cassava peels	SSF	[a] 5.31, [b] 125.30 U/g, [c] 14.61 U/g, [d] 15.32 U, [f] 33.73 °C, [h] 10.16%	*S. cerevisiae* BY4743	16.42, 143.31 g/Kg	Aruwajoye et al. [68]
Waste potato mash	SHF	[a] 5.5, [b] 18 mg/mL, [c] 300 U/mL, [f] 60 °C, [g] 3%, [h] 4.04% w/v	*S. cerevisiae*	30.99, 0.864 g/g	Izmirlioglu and Demirci [69]
Potato peels waste	SHF	[a] 5, [b] 90 U, [c] 9 U, [f] 30 °C, [h] 15%	*S. cerevisiae*	21, 0.46 g/g	Khawla et al., [70]
Cassava peels	PTSSF	[a] 4.02, [b] 125.30 U/g, [c] 18.80 U/g, [d] 19.12 U, [e] 6.36 h, [f] 33.73 °C, [h] 10.16%	*S. cerevisiae* BY4743	14.56, 161.61 g/Kg	Aruwajoye et al. [15]
Raw cassava waste	SSF	[a] 6, [b] 11.25 mg/g, [c] 5 mg/g, [d] 0.15 g/g, [f] 30 °C, [h] 10% w/v	*Z. mobilis*	13.6, 64.5%	Pothiraj et al. [71]
Cassava starch waste	SHF	[a] 4.5, [b] 8.4 U/µL, [d] 62 U/g, [f] 30 °C, [g] 1.96 × 10⁷, [h] 6% w/v	*S. cerevisiae*	11.9, 0.44 g/g	Akaracharanya et al. [72]
Wheat straw	PTSSF	[a] 5, [c] 15 IU/g, [d] 15 FPU/g, [e] 6–8 h, [h] 10% w/v	*S. cerevisiae* F12	23.7, 0.43 g/g	Tomás-Pejó et al. [73]
Furfural	PTSSF	[a] 4.5, [c] 4 h, [d] 15 FPU/g, [f] 30 °C, [h] 10%	*S. cerevisiae*	19.3, 76.5%	He et al. [74]
Sugarcane bagasse	SSF	[a] 4.5, [d] 100 U/g, [f] 39 °C, [g] 9.10 × 10⁶, [h] 10% w/v	*S. cerevisiae* BY4743	4.88, 0.49 g/g	Jugwanth et al. [75]

Footnote: [a] = fermentation pH, [b] = Amylase enzyme, [c] = Amyloglucosidase enzyme, [d] = cellulase enzyme, [e] = prehydrolysis time, [f] = fermentation temperature, [g] = yeast cell concentration, [h] = solid loading. SSF: Simultaneous Saccharification and Fermentation, SHF: Separate Hydrolysis and Fermentation, PTSSF: Simultaneous Saccharification and Fermentation with Prehydrolysis time, IU: international unit for enzyme, FPU: Filter-paper unit

4.2.2. Biohydrogen Production from Starch-Based Wastes

Organic materials are suitable substrates for biohydrogen production. These include starch and sugar crops such as cassava, sorghum, wheat, molasses, sago and potato [8]. However, the use of edible crops for biofuel production greatly competes with food availability and threatens global food security. Thus, the use of agricultural wastes for dark fermentative biohydrogen production affords a globally friendly, clean and sustainable alternative. The agricultural wastes suitable for biohydrogen production include starch and cellulose-containing food wastes, pure lignocellulosic wastes and starch-containing lignocellulosic wastes [7,8,76,77]. Biohydrogen production from pure lignocellulosic wastes such as corn stalk [78], rice straw [79], wheat straw [80] and sugarcane bagasse [81] have been reported. Similarly, starch-containing/starch-based lignocellulosic wastes have been utilized for biohydrogen production. These include starch-rich kitchen waste [82], potato waste [83], potato peels [77,84] and steam potato peels [85]. Production of biohydrogen from various agricultural wastes is shown in Table 4.

In the preparation of agricultural wastes for biohydrogen production, pretreatment activities are sometimes necessary for the deconstruction and hydrolysis of their complex carbohydrate-lignin structure. Pretreatment processes prior to dark fermentation produce glucose, which is then converted to organic acids [8]. Exploring the potential of high-energy biomass as substrates for biohydrogen production could address the challenges of dark fermentation such as low yield and high production cost [47,86]. The use of cheap, abundant and renewable waste alternatives will further enhance the feasibility of the process. Another potential strategy that could reduce the cost of biohydrogen production is the integration of the production process with the generation of other biofuels.

Table 4. Biohydrogen production from agricultural wastes.

Substrate	Fermentation Mode	Process Conditions	Microorganism	Biohydrogen Concentration/Yield	References
Sugarcane bagasse	Dark and photo-fermentation	[a] 6.8, [b] 30 °C, [c] Serum bottle	*Enterobacter aerogens* and *Rhodopseudomonas*	755 mL/L	Rai et al. [87]
Platanus orientalis leaves	Photo-fermentation	[a] 6.18, [b] 35.59 °C, [c] Batch reactor, [d] 26.29%	*R. rubrum, R. capsulatus,* and *R. palustris*	64.10 mL H_2 g^{-1} TS	Li et al. [88]
Sugarcane bagasse	Dark fermentation	[a] 7, [b] 55 °C, [c] Serum bottle, [d] 10%	*T. thermosaccharolyticumMJ1*	6.2 L-H_2/L	Hu et al. [89]
Apple waste	Photo-fermentation	[a] 7.14, [b] 30.46°C, [c] 20% v/v, [d] Batch reactor	*Rhodospirillum rubrum, Rhodobacter capsulatus,* and *Rhodopseudomonas palustris*	111.85 ± 1 mL H_2/g	Lu et al. [90]
Agricultural Residue Powders (ARPs)	Photo-fermentation	[a] 7, [c] 20% v/v, [d] Batch reactor	*Rhodospirillum rubrum, Rhodobacter capsulatus,* and *Rhodopseudomonas palustris*	228.94 mmol L^{-1}	Zhang et al. [91]

Footnote: [a] = fermentation pH, [b] = fermentation temperature, [c] = microbial load, [d] = process vessel. TS = total solids

5. Prospects of Integrated Bioethanol and Biohydrogen Production

Integrated biofuel production is a hybrid process that allows for the generation of two or more biofuels from energy-rich biomass, thereby increasing the profitability of the production pathway. This process has the potential to mitigate some of the aforementioned challenges and drawbacks often encountered during the single production of biofuel. Several researchers have reported integrated bioprocess routes for bioethanol and biohydrogen.

Integrated bioethanol production was reported by Boonchuay et al. [19]. The study combined the production of bioethanol with xylooligosaccharides (XOs) using corncobs. Bioethanol fermentation was conducted on cellulose-rich corncobs (CRC) obtained from XO production stage, which was conducted subsequent to KOH pretreatment. SHF and SSF mode of fermentation using thermo-tolerant Candida glabrata was employed, yielding 21.92 g/L (0.28 g/g) and 31.32 g/L (0.27 g/g) of bioethanol, respectively. In another process, Nair et al. [17] integrated the production of bioethanol and biogas together with the generation of high-protein fungal biomass. The fungal biomass was produced subsequent to

the bioethanol fermentation of phosphoric acid-pretreated wheat straw. Furthermore, the pretreated straw slurry and the post-ethanol fermentation slurry were both utilized for methane production, which resulted in a combined total energy output of 15.8 MJ/kg wheat straw. Moreover, the addition of the waste stream from the ethanol process as a co-substrate for the biogas production resulted in an additional increase of 27% total energy output compared to the only wheat straw-based biogas process. In a non-traditional integrated biofuel study, combined production of bioethanol and arabinoxylan extraction was conducted using an integration-based methodology of pinch analysis [20]. The goal of the bioethanol pinch strategy was to reduce the amount of the bioethanol product stream required for the traditional precipitation of the second product, arabinoxylan. A significant 94% reduction in the utilization of the bioethanol stream was achieved. Another study conducted by Nguyen et al. [92] evaluated a novel process of combined production of bioethanol and mannose. The bioethanol-producing yeast employed for the fermentation process was manipulated to only utilize glucose and galactose for ethanol production while retaining D-mannose sugar in the fermentation broth. This process gave an approximate 15.7 g of D-mannose and 11 g of ethanol from coffee residue waste used as substrate.

Integrated biofuel production has also been reported for biohydrogen. The most common strategy is the combination of dark fermentation and photo-fermentation alone or with biophotolysis [48,93]. The studies reveal the production of biomethane through a photo-fermentation (or dark fermentation) stage or as a second product following biohydrogen production. For instance, Intanoo et al. [94] investigated the production of hydrogen and methane from cassava wastewater using an up-flow anaerobic sludge blanket reactor (UASB). The study gave a yield of 54.22 mL H_2/g hydrogen in the first stage (dark fermentation) while 164.87 mL CH_4/g methane was obtained in the second stage (photo-fermentation). The effluent from the hydrogen reactor contributed to the increase in methane yield from the second stage. In a similar study, Kumar et al. [95] also achieved a 2.5- to 3.5-fold increase in biomethane production from food waste and vegetable wastes when effluents from the biohydrogen process were utilized. The study finally gave a hydrogen yield of 17 and 85 L/kg TS, and 61.7 and 63.3 L/kg TS (methane) for the vegetable waste and food waste, respectively. Channeling of product effluent is an inherent and workable method of obtaining a higher yield of a second product during integrated biofuel production.

6. Mixed Starch-Based Agricultural Wastes for Integrated Biofuel Production

Starch-based agricultural wastes are potential substrates for biofuel production because they are cheap, affordable and do not compete with food availability. Additionally, they contain an added high-energy starch polymer together with the general holocellulose backbone generally present in all lignocellulosic biomass. Furthermore, the utilization of these high-energy substrates relieves the environment of the potential health hazards associated with their disposal and decay. Implementing biofuel generation from these substrates on a commercial scale is also a possibility if the general concerns of biomass-based biofuel production are properly addressed in light of the starch-based agricultural waste pathway.

The limitations of commercial biomass-based biofuel have been attributed primarily to the high cost of feedstock supply and associated technological complexities of the downstream and upstream process [96,97]. The feedstock supply limitations originate from the costs associated with the collection and transportation of the substrates to the biorefinery. Furthermore, the seasonal availability of the single biomass from which the waste-substrates are generated disrupts their prompt supply to the biorefinery. However, the use of mixed starch-based agricultural wastes (MSBAW) in varying or equal proportions is a potential solution to the aforementioned challenges. Although there are limited studies and literature on bioenergy generation from mixed lignocellulosic biomass (MLB), they offer several advantages compared to the use of single feedstock [10]. For instance, the use of MSBAW specifically has the potential of generating a high throughput during pre-processing. Additionally, seasonality of feedstock which usually necessitates extensive storage of the single biomass is avoided due to the

availability of various starch-based wastes from different seasons. This reduces the timeframe for biomass collection and the use of equipment, personnel and resources needed under strict use of singular feedstock [98]. Moreover, the use of MSBAW for integrated bioethanol and biohydrogen production meets the fundamental criteria for the use of multiple lignocellulosic biomass for biofuel generation. These include similar characteristics [99], the concession for a higher yield of fermentable sugar (presence of starch), and thus bioethanol or biohydrogen [8], and cheapness and abundance [100]. However, after designing a workable scheme for the production pathway from MSBAW, it is necessary to select the processing methods that will be optimal for all the mixture components during key stages of the bioprocess. This is essential because of the presence of varying characteristics of the MSBAW such as moisture, ash and percentage chemical composition. A proposed scheme of integrated bioethanol and biohydrogen production from MSBAW is shown in Figure 4. Modifications associated with bioethanol from MSBAW followed by biohydrogen must align with the major stages of lignocellulosic biofuel generation. The three critical stages of bioethanol production from lignocellulosic biomass are pretreatment, hydrolysis and fermentation [68].
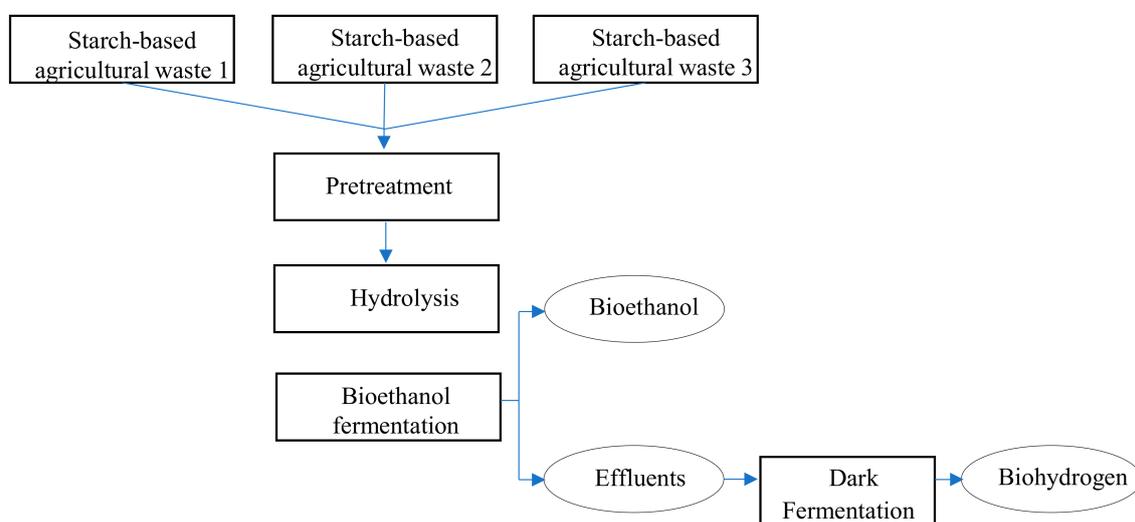
Figure 4. A proposed scheme of integrated bioethanol and biohydrogen production.

The proposed scheme of integrated bioethanol and biohydrogen production from MSBAW (Figure 4) suggests pretreatment as the first stage. Pretreatment of MSBAW is necessary for the reduction of cellulose crystallinity, altering of the holocellulose-lignin framework and the enhancement of biomass porosity and surface area [12]. As proposed, two or more starch-based agricultural wastes are collected and pre-processed through pretreatment activities.

6.1. Pre-Processing, Mixture Design and Pretreatment

Effective pretreatment (biological or chemical) may only be achieved subsequent to a pre-processing by drying of the individual biomass and reduction of their particle size through grinding or milling. The mixture of milled starch-based wastes in adequate or optimal proportions is key to successful biofuel production from multiple lignocellulosic biomass [10]. Although few reports have investigated the production of bioethanol from random mixtures of starch-based agricultural wastes [28,101], there is a dearth of knowledge on the optimized proportions or best values. The use of optimized substrate concentrations or appropriate ratios of lignocellulosic biomass can significantly enhance bioethanol yield [11,102].

Response Surface Methodology and Mixture Design

Optimal substrate proportions of starch-based wastes can be attained through the mixture design of the response surface methodology (RSM). Response surface methodology (RSM) is a modeling technique that elucidates both individual and interactive effects of parameters on their responses [68]. In mixture design, the total amount of two or more components is held constant while the response of their mixture varies depending on the change in proportions of the components of the mixture [25,103]. The most frequently used types of mixture designs are the simplex-lattice design, simplex centroid design and D-optimal [104]. They are employed in solving problems related to simple mixtures. However, more complex approaches have been reported. These complex mixture-dependent processes include categorized components [105], multifactor mixtures [106], mixture of mixtures and cross-mixture experiments [107]. Categorized components, multifactor mixtures and mixture of mixtures are used for solving problems associated with major components that are mixtures of some other minor components [105]. On the other hand, cross-mixture designs can solve problems related to combined minor component mixtures and major component mixtures that are related in a more complicated manner. Thus, cross-mixture designs have the potential to generate models for the determination of the optimal proportions of starch-based wastes with the understanding of the interactions between their mixtures while subjected to various pretreatment conditions.

Following the pre-processing or mechanical pretreatment of MSBAW substrates, chemical pretreatment can be executed. Chemical pretreatment methods include alkali-, acid-, organosolvent-, microwave- and inorganic salt-based pretreatments. Although literature on the most appropriate chemical treatment on MSBAW are few, low acid or alkali concentrations are preferable because higher concentrations corrode pretreatment vessels [108]. Specifically, the potential of inorganic salt-based treatments (alkali and metal salts) on sugar release from MSBAW could be explored due to their laudability for cheapness, less invasiveness and recyclability [12].

6.2. Hydrolysis and Bioethanol Fermentation

The hydrolysis process follows pretreatment during the valorization of all SBWs to bioethanol. This is a two-stage traditional route of liquefaction and saccharification using industrial α-amylase and amyloglucosidase (with or without cellulase) enzymes, respectively [68]. α-amylase enzymes used in the valorization of SBW are usually of bacterial and fungal origin. The common strains of bacteria for amylase production are *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus subtilis* and *Bacillus stearothermophilus*, while that of fungi are *Aspergillus oryzae* and *Aspergillus niger* [109]. Industrial amyloglucosidase or glucoamylase are commonly produced from Aspergillus and Rhizopus species of fungi [110], while large-scale production of cellulases is mostly from Trichoderma, Aspergillus and Penicillum species of Fungi [111]. Although the enzymatic hydrolysis stage is significant, the cost of enzymes accrued at this stage remains a bottleneck to the industrial feasibility of bioethanol production from SBW [68]. Thus, more economical strategies need to be employed for the enzymatic hydrolysis of MSBAW in order to overcome this challenge. The potential of using the organisms that produce the enzymes instead of the commercially packaged enzymes should be explored. The presence of the carbohydrate polymers present in the MSBAW biomass can induce the extracellular production of the hydrolytic/saccharifying enzymes needed for the process if conducted under well-monitored and optimized conditions. The most influencing process parameters of this stage that can be investigated include substrate (solid) loading/concentration, pH, mixing rate and temperature [55].

Further improvements to MSBAW bioethanol can be achieved through the combination of the enzymatic hydrolysis and the fermentation stages in an SSF process. The limitation of the SHF process is the feedback inhibition of the enzymes by the sugar produced during the hydrolysis process. This occurrence has the potential to negatively impact on the overall fermentable sugar yield and thus, the bioethanol produced. Additionally, the effect of a short prehydrolysis/presaccharification process on the SSF could be investigated for possible improvement

in bioethanol yield. The prehydrolysis/presaccharification stage allows for a short time where the saccharifying enzymes involved in the SSF process are subjected to their optimal temperatures. This results in reduced viscosity of the medium and thus, improved sugar yield and bioethanol productivity [73]. Additionally, comparative assessments on SHF, SSF and SSF (with prehydrolysis) of MSBAW can further provide needed knowledge on the appropriate mode of fermentation for high bioethanol yield and productivity.

Another strategy to improve MSBAW bioethanol is to explore the possibility of reusability of the fermenting organism through Simultaneous Saccharification Filtration and Fermentation (SSFF). Unlike the simple SSF process where the fermenting organism is mixed with the biomass, the biomass hydrolysate is rather transferred to the fermenting organism via a cross-flow membrane [112]. This strategy allows the retaining of the fermenting organism separately from the fermented slurry. Additionally, the introduction of a short prehydrolysis step has the potential to increase the sugar concentration and thus, the productivity of the SSFF process. However, the number of batches of the organism reusability must be minimal in other to circumvent the risk of infecting the fermenting organism.

Another approach to improving MSBAW bioethanol is to take advantage of the available hemicellulose component of the mixed substrate. Hemicelluloses are made up of pentoses in addition to their glucose units. The provision for the fermentation of the pentose sugars in addition to the glucose fermentation can positively impact on the bioethanol yield and productivity. Thus, recombinant xylose-fermenting yeast strains can be employed for the fermentation process. On the other hand, pentose fermenting organisms such as *Pichia stipitis* [113,114] can be added to the fermentation process in a Simultaneous Saccharification and Co-fermentation strategy (SSCF) [115].

Further improvements in MSBAW bioethanol could be achieved by exploring the possibility of consolidating the different stages of the process into a single step. The consolidation of the stages of pretreatment of biomass, enzymatic hydrolysis and fermentation into one step is referred to as Consolidated Bioprocessing (CBP). Production of bioethanol from MSBAW through the CBP process has the potential to significantly reduce process costs and the risk of contamination by achieving the different stages of production in a single vessel [57]. Additionally, the environmental impact of multiple energy-consuming stages of the bioprocess is minimized [73,116].

6.3. Bioreactor Design for MSBAW Bioethanol

Bioreactors are very central to bioprocesses, especially at scale-up, and their design should be based on the characteristics of the biological process involved. The design of a bioreactor must be such that it can effectively control basic factors such as the temperature, pH and agitation. These factors are essential for all the stages of MSBAW bioethanol, and thus the bioreactor must be configured to guarantee the aforementioned conditions. The operational mode of fermentation to be adopted can also significantly influence the bioreactor design. Generally, the existing operational modes are batch (discontinuous), fed-batch (semi-continuous) and continuous. Although the batch mode is currently widely used, when the substrate is fed into the reactor at a high solid load, it could ultimately result in lower bioethanol yields [117]. This occurrence could be influenced by inadequate mixing. On the other hand, the semi-continuous mode affords the addition of the MSBAW substrate to the reaction vessel in an intermittent manner while accumulating the ethanol product [35]. Additionally, more nutrients can be introduced to the mixture should the initial starters be spent. Moreover, considering the highly starchy nature of the MSBAW substrate, the fed-batch mode will prevent a possibly viscous slurry that may be formed either during pretreatment or the enzymatic saccharification stages [117]. In continuous processes, the volume of the reaction slurry is kept constant due to continuous feeding of substrate and removal of product. Although this strategy paves the way for better process control and higher productivity, there is a major drawback of diminished activity of the fermenting yeast due to anaerobic cultivation over a long period of time. Furthermore, the substrate is not completely consumed, thus, reducing the yield [35,118].

Bioreactor Configurations

Focusing on general bioreactor configurations, the most common bioreactors are the stirred tank reactors (STR) and the membrane bioreactors (MBR). The STRs are cylindrical vessels with one or more impellers coupled with an external motor, while the membrane bioreactors employ specialized membranes that aid the selective movement of available species in the reaction slurry (e.g., enzymes and the substrate) [117]. The MBR configuration is preferable when recovering and reusing the enzymes (through immobilization) or fermenting yeasts is of paramount importance. However, a more advanced configuration that supports lower energy consumption can be adopted for MSBAW bioethanol. Unlike the STRs and MBRs where the use of motors drives the mixing efficiency of the bioreactors, some other bioreactors have been developed that induce mixing into the vessel through the use of air. In a simple process, the air could be introduced at the bottom of the vessel through nozzles. These are referred to as pneumatically agitated bioreactors [117,119].

Other bioreactor designs with great potential to significantly improve MSBAW bioethanol are microfluidic bioreactors. Microfluidic bioreactors are designed using vessel dimensions of micrometers, thus gaining the advantage of portability and the ease of regulation of important process factors [120,121]. Additionally, an ethanol bioprocess that involves the use of genetically modified strains could be challenged with the difficulty of screening of the strains that have been transformed. Microfluidic techniques through the use of microdroplets have the potential to facilitate the effective screening of such strains for higher bioethanol productivity by separating them from their wild-types [121].

6.4. Conversion of Bioethanol Effluents to Biohydrogen

Efficient management and disposal of bioethanol effluents are essential for the viability of the process, especially at a large scale. Although there is scant literature available on the production of biohydrogen from the bioethanol effluents of SBW, studies using the effluents from MSBAW bioethanol are worth investigating. The generation of the second product biohydrogen from MSBAW bioethanol effluent is an integrative phenomenon with possible potentials. Analysis of bioethanol effluent from lignocellulosic biomass [62] and SBW biowaste effluent [41] reveal the presence of valuable nutrients which could facilitate dark fermentative biohydrogen production. Thus, there is a high possibility of significant biohydrogen yield from MSBAW bioethanol effluent. Furthermore, the influence of important operational parameters of biohydrogen production such as temperature, pH and nutrient supplementation could be assessed and optimized for improved yield [9].

Process of Biohydrogen Production from MSBAW Bioethanol Effluent

The commencement of the biohydrogen process from MSBAW bioethanol effluent must be consequent upon effective separation of the bioethanol product from the effluents of the fermentation process. This can be achieved through distillation or evaporation processes which may be inbuilt to the fermentation vessel or executed separately. Separated effluents of the ethanol fermentation process are composed of organic and inorganic compounds which are essential for biohydrogen production. Although glucose compounds may be exhausted from the slurry after fermentation, a small percentage of other organic components remains. These include some unfermented sugars from the hydrolyzed hemicellulose portions, and some oligomers. Recently, Tobin et al. [122] observed that subsequent to bioethanol fermentation of poplar trees, only 76% of the total carbohydrates were utilized and 98% of the remaining carbohydrates were oligomers inaccessible to the yeast during fermentation. Thus, remnant organic compounds from MSBAW bioethanol effluents can be employed as the carbon source for biohydrogen fermentation.

The fermentation of MSBAW bioethanol effluents can be achieved through a conventional dark fermentation process. Pure or mixed cultures may be employed; however, the use of pure cultures may be expensive due to strict process control and sterile conditions [123]. On the other

hand, in order to avoid low hydrogen yields from the use of mixed cultures (e.g., anaerobic sludge), inoculum pretreatment must be carried out. Inoculum pretreatment inhibits methanogens and other non-hydrogen-producing populations. Inoculum pretreatment methods include acid or base treatment, freezing and thawing, heat shock, the use of methanogen inhibitors and hybrid pretreatment [124].

Further stages of the dark fermentation process include nutrient supplementation, and the setting of bioreactor control setpoints of pH, temperature, agitation and process time. The use of STR and sludge bed bioreactors have been reported for dark fermentative biohydrogen production [9,124].

7. Conclusions

There is an urgent need for fossil fuel alternatives. Although various renewable options have been investigated for important biofuels such as bioethanol and biohydrogen, the search for more viable solutions to the persistent challenges is inevitable. Most of the challenges are entrenched in feedstock logistics, low biofuel yield and extreme process costs. The use of mixed starch-based agricultural wastes for integrated bioethanol and biohydrogen production is promising to overcome these challenges. Experimental mixture designs focused on the basic interactions between the substrate mixture and specific parameters at the initial stages of bioethanol production could significantly contribute to improving the integrated process. Furthermore, selection of the appropriate lignocellulosic-fermentation mode coupled with energy-saving bioreactor configurations could further enhance the productivity of the bioprocess. Additionally, efficient conversion of the effluents of mixed starch-based wastes bioethanol to biohydrogen is potentially viable for maximum profitability from the agricultural waste substrate.

Abbreviations

HK	Hexokinase (HK)
ATP	Adenosine Triphosphate (ATP)
CBP	Consolidated Bioprocessing (CBP)
HSCGs	High Sugary Corn Types (HSCGs)
PFCs	Parent Field Corn Lines (PFCs)
ISP	Industrial sweet potato (ISP)
SBW	Starch-based waste (SBW)
NSBW	Non-starch-based waste (NSBW)
SSF	Simultaneous Saccharification and Fermentation (SSF)
SHF	Separate Hydrolysis and Fermentation (SHF)
SSFF	Simultaneous Saccharification, Filtration and Fermentation (SSFF)
SSCF	Simultaneous Saccharification and Co-fermentation (SSCF)
STR	Stirred Tank Reactor (STR)
WGS	Water Gas Shift (WGS)
XOs	Xylooligosaccharides (XOs)
CRC	Cellulose-rich corn cobs (CRC)
MLB	Mixed lignocellulosic biomass (MLB)
MSBAW	Mixed starch-based agricultural wastes (MSBAW)
RSM	Response Surface Methodology (RSM)
UASB	Up-flow Anerobic Sludge Blanket (UASB)

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