

Review

Advances and Perspectives in Biohydrogen Production from Palm Oil Mill Effluent

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Abstract: Palm oil, the main vegetable oil produced globally, serves diverse purposes, ranging from cooking to the production of processed foods, cosmetics, and biodiesel. Despite contributing significantly to the economies of major producing nations, the escalating production of palm oil raises serious environmental concerns, including deforestation, biodiversity loss, and various forms of pollution. Palm oil mill effluent (POME), a byproduct of palm oil extraction, poses a severe environmental threat when left untreated. As an eco-friendly alternative, anaerobic digestion in controlled bioreactors has emerged, offering simultaneous POME treatment and biofuel generation, particularly hydrogen, with high energy efficiency. This review explores the challenges and opportunities associated with biohydrogen production from POME. Key considerations involve optimizing parameters through pretreatments, nanoparticle incorporation, defining optimal bioreactor conditions, determining hydraulic retention times, and integrating multi-stage processes like dark fermentation followed by photofermentation. This review also emphasizes the significance of sustainable practices and economic analyses in shaping the future of hydrogen production from POME, positioning it as a pivotal player in the palm oil industry's circular economy and the global energy transition.

Keywords: palm oil mill effluent; dark fermentation; bioprocess; bioreactor



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1. Introduction

Palm oil is currently the most consumed edible oil in the world, used as an ingredient in cooking, in the formulation of processed foods, and in chemical, cosmetic, and biodiesel industries [1]. It is a source of solid fat at room temperature, rich in carotenoids, antioxidants, polyphenols, and fatty acids such as palmitic, oleic, and the omega-6 linoleic acid [2], having a higher yield per hectare and less need for fertilizers and pesticides compared to other oilseed crops [3].

In 2023, palm oil reached a gross production of about 77.2 million tons [4], with Malaysia and Indonesia responsible for 85% of its total production. It is estimated that palm oil production should increase by 25 million tons by the year 2035 [5], generating jobs in these regions and a significant increase in the gross domestic product (GDP) [6].

Despite its important socioeconomic impact on the main exporting countries, palm oil production and its growing demand are configured as a scenario of great environmental concern [7], as it leads to deforestation, the loss of biodiversity, greenhouse gas emissions, water and air pollution, and the generation of large amounts of palm oil mill effluent (POME) during the extraction process [8,9].

POME is often discarded in nature without any treatment, threatening the soil, groundwater, bodies of water, and consequently, human and other animal life. Due to the low capital and operating cost, the open pond treatment system is the most common method of treating POME in palm oil mill industries [10]. However, this treatment system has a series

of disadvantages, such as the need for a large land area, a long hydraulic retention time, from 20 to 200 days depending on the POME characteristics, and the non-capture of biogas, which causes foul smell and the emission of greenhouse gases such as CH₄ and CO₂ into the atmosphere [11,12].

Thus, alternatives such as the anaerobic digestion of POME in controlled bioreactors have become attractive as they enable the simultaneous treatment of POME and generation of biofuels. Hydrogen is particularly interesting as a biofuel due to its high energy efficiency, 122 kJ g^{−1}, which is 2.5 times higher than hydrocarbon-derived fuels [13] and the emission of H₂O as the only by-product of its combustion [14,15], and also it can be later utilized in the plant for thermal and electrical energy [12].

In addition, the production of hydrogen from this effluent is an alternative to the current production method, based mainly on fossil fuels, playing an important role in the circular economy of the palm oil industry and the transition of the world energy matrix [5].

However, hydrogen production from POME still faces numerous challenges for its large-scale production, requiring the adjustment of several parameters to obtain high yields [5]. This can be achieved by employing pretreatments to increase the availability of organic POME components [11], nanoparticles addition [16], optimal bioreactor definition, optimal hydraulic retention time (HRT) [17], and integrating processes such as two-stage hydrogen production with dark fermentation followed by photofermentation [18].

Therefore, this review assesses the different forms and parameters of hydrogen production from palm oil mill effluent (POME) as well as the methodologies available to increase its production, such as pretreatments, the addition of nanoparticles, and types of bioreactors, highlighting their sustainability and economic analysis.

2. Physicochemical Properties of POME

POME is generated in large proportions; it is generated in three stages of the oil production process: sterilization, threshing and digestion, and extraction and clarification. The amounts of POME generated in each step are 0.9 ton, 1.5 ton, and 0.1 ton per ton of oil, respectively.

For each ton of crude palm oil produced, 2.5 to 3.8 tons of POME are estimated to be generated [4]. POME is a complex and potentially polluting effluent with a viscous appearance and brownish color, acidic pH, and high concentrations of COD (chemical oxygen demand), BOD (biological oxygen demand), and TSS (total suspended solids), with its composition presenting organic compound fractions such as proteins, lipids, and carbohydrates and some of these are difficult to biodegrade, such as cellulose, hemicellulose, and lignin (Table 1) [12,19–21]. The energy content of POME is approximately 28 kWh/m³ [22]. Besides the macronutrient fractions [23,24], the residue contains minerals such as manganese, zinc, copper, iron, potassium, calcium, molybdenum, sulfur, and selenium [25,26].

Table 1. Physicochemical characteristics of POME.

Parameter	Raw POME				
	[19]	[12]	[27]	[20]	[21]
Reference	[19]	[12]	[27]	[20]	[21]
pH	4.3 ± 0.3	4.40 ± 0.07	4.63	4.5 ± 0.5	4.67
Color	-	-	-	-	brown
Turbidity (NTU)	67,500 ± 1910	-	-	690 ± 5	7560
Chemical oxygen demand (COD) (mg/L)	84,450 ± 19,500	50,000 ± 1081	89,591	52,000 ± 2000	42,482
Biochemical oxygen demand (BOD) (mg/L)	-	-	34,771	26,000 ± 1000	20,838
Total solids (TS) (mg/L)	-	38,793 ± 395	47,050	41,000 ± 1000	32,157
Dissolved solids (DS) (mg/L)	-	-	9310	-	17,436

Table 1. Cont.

Parameter	Raw POME				
Total suspended solids (TSS) (mg/L)	19,610 ± 7.900	5000 ± 438	36,560	19,500 ± 500	14,721
Total nitrogen (TN) (mg/L)	650 ± 300	750 ± 0.00	-	720 ± 5	340
Ammonical nitrogen (NH ₃ -N) (mg/L)	-	-	-	32 ± 1	133
Phosphorus (PO ₄ -P) (mg/L)	-	-	-	-	210
Total reducing sugars (mg/L)	-	-	228	-	-
Oils and greases (mg/L)		4000 ± 0.13	37,883	4050 ± 20	1927
Fe (mg/L)	70.7 ± 1.65	-	-	-	-
Zn (mg/L)	7.53 ± 1.07	-	-	-	-
Mn (mg/L)	6.47 ± 1.43	-	-	-	-
Mg (mg/L)	1144 ± 7.00	-	326.89	-	-
Al (mg/L)	334 ± 22.65	-	-	-	-
Cellulose (% dry basis)	-	14.34 ± 0.07	-	-	-
Hemicellulose (% dry basis)	-	10.76 ± 0.28	-	-	-
Lignin (% dry basis)	-	13.58 ± 0.10	-	-	-
Glucose (mg/L)	-	2070 ± 14	-	-	-
Xylose (mg/L)	-	640 ± 0.00	-	-	-

(-) Not specified in the study.

In addition, POME is a source of several amino acids (aspartic acid, glutamic acid, serine, glycine, arginine, alanine, proline, methionine, isoleucine, and leucine) [26]. The physicochemical characteristics of POME make it a rich culture medium for the growth of bacteria, including those that produce hydrogen. In addition, the minerals and amino acids contained in the effluent can also improve biohydrogen production, as they are precursors of hydrogenases (enzymes responsible for hydrogen production) [28,29].

Hydrogen is a product of low commercial value but is widely demanded in the energy, food, and industrial sectors, so its production requires cheap raw materials, mainly waste. Hydrogen production costs vary depending on the source. The cost of producing 1 kg of hydrogen ranges from US\$ 0.9 to 3.2 when conventional raw materials such as coal or natural gas are used. When renewable sources are used to produce this energy vector, production costs rise to the range of US\$ 3.0 to 7.5. However, this renewable hydrogen can be considered green. Hydrogen can be produced from POME by thermochemical methods (POME steam reforming) [30,31], electrochemical (microbial electrolysis cells) [32], or biological (dark fermentation, photofermentation, and two-stage process) [18]. The production through dark fermentation is the most explored and constantly reported in the literature as the most promising due to numerous benefits, such as higher hydrogen yield and removal of COD, BOD, and TSS, not needing to use light and additional nutrients, in addition to being operated under conditions of low temperature and pressure [33].

POME is a byproduct of low or no commercial value, generated in large quantities and with interesting physicochemical properties due to its composition; it becomes a promising residue for hydrogen production via biological means. In addition, using POME as a substrate for hydrogen production via dark fermentation would serve as the first pretreatment of the effluent, as up to 90% of the organic matter can be removed in this process [34]. The main bottleneck in hydrogen production via dark fermentation is in the yields obtained as theoretically the maximum yield of hydrogen is 4 mol H₂/mol glucose [35], in addition to logistical problems.

3. POME Pretreatments

Palm oil mill effluent (POME) is commonly treated in anaerobic stabilization ponds due to the lower cost of this technique. However, it has several disadvantages, such as long residence times, low efficiency, and emission of gases such as CH₄ and CO₂, which are responsible for enhancing the greenhouse effect [36]. To solve this problem, several studies have demonstrated the potential of POME degradation to produce biogas and biohydrogen, combining the treatment of this effluent with the generation of energy [37].

However, POME has a high load of sugars that are difficult to biodegrade (Table 1), such as cellulose and hemicellulose, derived from palm fiber (10–14% *w/w* on a dry basis), which limits the amount of carbon available to fermentation microorganisms [12]. Thus, several POME pretreatments have been proposed to increase the bioavailability of these carbohydrates, improve solubility, and adjust the size and porosity of the substrate particles, thus reducing hydraulic retention time (HRT) and increasing biohydrogen production in anaerobic digestion [38].

The ideal pretreatment must obligatorily meet some criteria, such as improving the availability of sugars, preventing the degradation or loss of carbohydrates, limiting the formation of degradation compounds that inhibit microbial growth, minimizing energy demand, and having a low cost [39].

Compared to the literature on the pretreatment of lignocellulosic waste and solid urban waste, studies that specifically highlight the pretreatment of POME to increase biohydrogen production are still scarce. These pretreatments can be primarily classified as physical, chemical, or biological. According to Table 2, pretreatment efficiency depends on the physicochemical characteristics of POME [40] and should be considered when selecting the pretreatment method. Among the most efficient pretreatments for POME are chemical and enzymatic ones; the first can improve biohydrogen production by up to 75%, while enzymatic ones achieve improvements of up to 2.5 times.

3.1. Physical Pretreatment

3.1.1. Ultrasonication Pretreatment

Considered a simple pretreatment technology, ultrasonication consists of the application of ultrasound waves to liquid media (>20 kHz) [19], leading to cavitation bubbles that, on collapse, generate shear forces, an increase in temperature, and even the formation of free radicals. Together, these physical and mechanical effects lead to the disruption of cell walls, increasing the bioavailability of organic matter due to the disruption of chains of lignocellulosic materials, reducing the size of particles, and increasing their porosity, which later affects mass transfer rate [4], thereby culminating in increased biohydrogen production [40].

Leaño [41] employed ultrasonication as a pretreatment of POME employing four pretreatments in terms of ultrasound dose: (i) 0 J/mL, (ii) 91 J/mL, (iii) 143 J/mL, and (iv) 195 J/mL. Results showed that increasing the ultrasound dose from 91 to 195 J/mL led to an increase in H₂ yield, reaching 0.68 mmol H₂/g COD, and a COD removal of 62.24%. These results were 38% and 20% higher, respectively, when compared to the production using POME without ultrasonication pretreatment. These results were also corroborated by the study of Budiman and Wu [19], which combined POME and pulp and paper mill effluents, varying the energy input from 0 (control) to 1245 J/mL with defined durations of 5, 15, 30, 45, and 60 min. An increase in the solubilization of organic matter and consequently in the increase of soluble carbohydrates were observed in all treatments, reaching the hydrogen production yield with pretreatment of 775 J/mL for 45 min at 30 °C of 8.72 mL H₂/mL medium with a total COD removal of 36.9%. In contrast, the lowest biohydrogen yield (4.67 mL H₂/mL medium) and total COD removal of 28.8% were obtained in the control without pretreatment. As with the results obtained by Leaño [41], the solubilization of compounds increases with the ultrasound dose; however, Budiman and Wu [19] verified that this increase occurs only up to the use of ultrasound doses of 775 J/mL, with a slight improvement in biohydrogen production with higher ultrasound doses.

Furthermore, increases in ultrasound duration from 45 to 60 min for higher amplitude values did not significantly affect organic matter solubilization.

Isa [42] performed the ultrasonic pretreatment of POME at a density of 0.88 W/mL for 16.2 min. Fermentation carried out with pretreated POME operated at 30 °C produced the highest amount of biogas (3465 mL), yielding 21.5% higher yield than that of raw POME. Although the method was effective, increasing the ultrasound dose and pretreatment time could have further improved COD removal and biogas production since the ultrasound dose and experiment time were relatively lower than some of the studies previously described [19,41].

3.1.2. Microwave Pretreatment

Microwaves have also been used as an efficient methodology for the pretreatment of effluents [43–45] but are still rarely used in POME pretreatment. Microwaves are a part of the electromagnetic spectrum, with radiation ranging from 300 MHz to 300 GHz, corresponding to a wavelength range from 1 m to 1 mm [46]. The microwave field generates energy by realigning dipoles with oscillating electric fields to generate heat internally and at the material's surface. The water present in the effluent absorbs the microwaves, generating heat, and the thermal effect causes the breakdown of polymers, releasing fermentable carbohydrates into the environment [47].

Mishra [40] investigated the effects of three pretreatments on the POME effluent: ultrasonication, microwave, and combined ultrasonication–microwave. The pretreatment combining ultrasonication and microwave was more efficient in hydrogen production and COD removal, achieving the highest cumulative hydrogen production of 4080 mL H₂/L-POME with a COD removal efficiency of 75.56%. Raw POME achieved a maximum cumulative hydrogen production of 3360 mL H₂/L-POME.

Compared with the study of [19,41], the study of Mishra [40] showed the highest COD removal, indicating that combining different pretreatment methods is more effective for the solubilization of compounds.

3.1.3. Heat Pretreatment

Heating causes diverse effects depending on the temperature and times reached, from increasing solubilization and reducing foaming to breaking the long polymer chains and releasing the monomers [11].

The thermal pretreatment of POME presents a series of positive points, such as high solubilization of volatile solids, smaller digester volume, well-dehydrated cake, high yield of biogas and biohydrogen, and quality of the treated effluent [11,48]. In addition, heat pretreatment is the most suitable both due to its efficiency and technical–economic feasibility since the POME discharge temperature is already extremely high, requiring the additional use of a small amount of energy to reach the desired temperature, value easily offset by the amount of biogas produced [11,48]. The production of biogas, including hydrogen, can be favored by substrates containing monomers.

Khadaroo [49] carried out the thermal pretreatment of POME by heating it at 120 °C for one hour. Using thermally treated POME, the biogas production was 1886.11 ± 21.63 mL, with a methane percentage of $83.40 \pm 0.31\%$ and a yield of 328.73 mL CH₄/g COD removed and with a COD removal efficiency of $80.63 \pm 0.46\%$. When POME was not thermally pretreated, the biogas production dropped by 25%, with a methane yield of 58.40 mL CH₄/g COD removed and a COD removal percentage of 48.89%.

Khadaroo [11] also tested the effect of heat treatment and the change in solid loading on biogas production in a 5 L continuous thermophilic bioreactor. POME pretreatment was carried out at 120 °C for 1 h. The initial COD of raw POME was 20.4 ± 0.578 g/L, dropping to 10.7 ± 0.651 g/L after anaerobic digestion, showing a COD removal efficiency of 53% with a daily methane yield of 294 ± 25 mL CH₄/g COD removed. The thermally pretreated POME presented an initial COD of 23.1 ± 0.18 g/L and a final one of 0.6 ± 0.092 g/L, a COD removal efficiency of 97.4%, and a yield in the production of 414 ± 17 mL CH₄/g

COD removed. Considering that biogas production was improved with the application of a thermal pretreatment, it is expected that the same pretreatment will also provide high yields in hydrogen production. After all, biohydrogen is also a component of biogas. Both studies showed a significant improvement in COD removal and biogas production, confirming the feasibility of the pretreatment technique.

3.2. Chemical Pretreatment

3.2.1. Acid Hydrolysis Pretreatment

Acid hydrolysis using chemical substances such as hydrochloric, sulfuric, phosphoric, and nitric acids and other chemicals is one of the most used pretreatment methodologies [5] due to its low cost and effectiveness in breaking down compounds such as hemicellulose [50], by cleaving the glycosidic bonds that hold the monomeric sugar units together [51].

However, their dosages must be strictly controlled, and it is recommended that the acids be used in their diluted form since concentrations above 20% cause operational disadvantages, requiring expensive specialized constructions to withstand the corrosive effects [5]. More concentrated dosages of acid can also lead to the additional hydrolysis of monomeric carbohydrates, leading to the formation of toxic compounds that inhibit the production of biogas and biohydrogen, such as (2-furaldehyde, furfural, and 5-hydroxymethyl-2-furaldehyde (HMF)) [10]. The need to neutralize the effluent after acid hydrolysis makes this process less commercially attractive than other pretreatment methods due to the cost of required chemicals [5].

Mahmod [10] used phosphoric acid (H_3PO_4) and nitric acid (HNO_3) in separate trials to pretreat POME. Pretreatment with phosphoric acid was the most efficient, resulting in the maximum hydrogen yield of 1.24 mol H_2 /mol of substrate and reduced COD. POME pretreated with nitric acid culminated in a hydrogen yield of 1.04 mol of H_2 /mol of substrate, values 97% and 65%, respectively, higher than the yield of biohydrogen from crude POME, which was 0.63 mol of H_2 /mol of substrate.

Arisht [5] performed several experiments with acidic and basic chemical compounds, such as sodium hydroxide (NaOH), and mineral acids, such as hydrochloric (HCl), sulfuric (H_2SO_4), phosphoric (H_3PO_4), and nitric (HNO_3) acids, at concentrations of 1.5% (w/v) to verify the best pretreatment for POME. Corroborating the results achieved by Mahmod [10], the most suitable pretreatment was obtained by acid hydrolysis performed with H_3PO_4 at 1.5% (v/v) due to lower furfural production (1.39 ± 0.02 mg/L). These results led to additional studies varying the concentrations of H_3PO_4 (1%, 2.5%, 5%, 7.5%, and 10%), where the highest concentration of reducing sugars available in the medium of 28.23 ± 0.21 g/L was reached in 160 min and a furfural production of 1.77 mg/L, a considerable increase in the concentration of total reducing sugars when compared to raw POME (15.96 ± 0.86). However, the highest yield of H_2 and percentage of carbohydrate utilization occurred in the treatment with 2.5% H_3PO_4 , which culminated in 21.79 ± 0.37 g/L of total reducing sugars in the medium and 1.37 ± 0.08 mg/L of furfural, obtaining values of 1.69 mol H_2 /mol substrate.

3.2.2. Alkaline Hydrolysis Pretreatment

Alkaline hydrolysis can be performed using different hydrolytic alkaline substances such as NaOH , $\text{Ca}(\text{OH})_2$, CaO , and others [51–53]. The alkaline treatment led to the solvation and saponification of organic compounds, causing an increase in particle surface area and leading to the degradation of the lignin–cellulose bond, thereby releasing carbohydrate monomers [5]. Alkaline hydrolysis can be carried out under ambient conditions, not requiring high temperatures and pressures. However, it can be relatively slow [51].

Seengenyong [54] compared acid and alkaline pretreatments carried out with different concentrations (0–2.5 w/v) of NaOH and HCl , verifying their effects on the cumulative production of H_2 . The highest cumulative hydrogen production occurred from POME pretreated with 1.5% (w/v) NaOH , reaching 4.6 L H_2 /L-POME, a value three times greater

than the amount of H_2 obtained from raw POME, while the amount of H_2 obtained from pretreatment with 1.5% (*w/v*) HCl was 2.56 L H_2 /L-POME. Under the optimal conditions of pH (5.5) and temperature (60 °C), POME pretreated with 1.5% (*w/v*) produced a maximum hydrogen yield of 5.2 L H_2 /L-POME, approximately 50% greater than the amount obtained from raw POME under the same conditions. It was observed that the amount of hydrogen produced increased as the amount of NaOH increased until reaching a concentration of 1.5% (*v/w*). After this value, the amount of hydrogen produced decreased.

Kamal [55] compared the effects of different pretreatments on POME: alkaline (10% NaOH), acidic (10% H_2SO_4), heat (80 °C for 1 h), and a combination of alkaline, heat, and acid. The highest hydrogen production occurred from alkali-pretreated POME. Subsequently, through tests in 2 L reactors with a working volume of 1.8 L, the highest hydrogen yields were obtained from POME pretreated with acid due to the higher initial sugar concentration. However, this treatment also showed the highest production of butyric acid. Although the hydrogen production was higher with this treatment, the yield was lower (1.87 mol H_2 /mol of substrate) compared to the production of POME pretreated with NaOH and heat (2.18 mol H_2 /mol of substrate).

3.2.3. Ozonation Pretreatment

Ozone has an oxidizing action, capable of oxidizing a wide range of inorganic and organic compounds, breaking the long polymeric chains of the organic compounds present in POME, and, consequently, releasing compounds that are easily biodegradable [4]. However, this pretreatment method is expensive and less preferable to other methods [5].

Tanikkul and Pisutpaisal [56] carried out the ozonation of POME as a pretreatment methodology to increase biohydrogen production by dark fermentation. POME was ozonated at a rate of 300 mg h^{-1} , and fermentations were carried out in batches varying the COD/L (5, 10, 15, 20, 25, 30, 25 g/L). The results demonstrated that the maximum hydrogen production was higher from the ozonated POME compared to raw POME. The maximum hydrogen production yield of 77.1 mL H_2 /g COD was achieved from ozonated POME with a concentration of 35,000 mg/L.

The results obtained by Pisutpaisal [57] were similar to the results obtained by Tanikkul and Pisutpaisal [56], achieving a hydrogen yield of 182.3 mL H_2 /g COD, 49% higher than the hydrogen yield from raw POME, which was 122.0 ± 1.4 mL H_2 /g COD, under optimized pH conditions, at 37 °C and with an initial COD of 30,000 mg/L. Tanikkul [58] also obtained a higher hydrogen production, reaching a yield of 182 mL H_2 /g COD when POME was pretreated with ozone.

3.3. Biological Pretreatment

Enzymatic Hydrolysis

Biological pretreatment of POME is commonly considered safe, environmentally friendly, and with a lower energy cost [4], being more commonly performed through hydrolysis using enzymes that are capable of breaking the long polymeric chains of organic compounds such as cellulose and hemicellulose present in POME, making fermentable monomers available [33]. However, despite the promising results presented (Table 2), biological pretreatment also has some disadvantages, such as process delay, careful control, problems with contamination, and technical-economic challenges, with pretreatment being less commercially attractive [33,59].

Leaño and Babel [60] compared the effect of pretreatment of POME with the enzyme OPTIMASH BG[®] and with Tween 80[®] in biohydrogen production. Crude POME produced a maximum hydrogen yield of 1.12 mol H_2 /g COD and a COD reduction of 30%. In contrast, POME pretreated with 0.2% of the enzyme OPTIMASH BG[®] led to a maximum yield of 1.88 mol H_2 /g COD and COD removal of 52%, and pretreatment with Tween 80[®] with the same concentration of 0.2% achieved a yield of 2.76 mol/g COD and a reduction rate of 58% of COD, with a maximum yield of 4.91 mol H_2 /g COD achieved with a concentration

of 0.5% of Tween 80[®]. Although efficient in pretreatment, the cost of these reagents must also be considered before their practical application.

Positive results of enzymatic hydrolysis can also be observed in the study of Al-Shorgani [61], who used a mixture of cellulase enzymes, including Celluclast 1.5 L and Novozyme 188 (Novozymes, Malaysia), resulting in the production of hydrogen of 2075 mL/L of POME, while POME that was not pretreated resulted in the production of 873.6 mL of biohydrogen/L of POME in 72 h.

Prasertsan [12] pretreated POME with a commercial xylanase to increase biogas production, testing various enzyme concentrations ranging from 5 to 20 U/mL, and varying the temperature to find the optimal pretreatment conditions. The maximum CH₄ yield of the four POME hydrolysates was about three times greater than that of the control. COD removal from POME by xylanase hydrolysis followed by biogas production was 78 to 89%, which was also three times higher than the control.

To solve the problem of the value of commercial hydrolase, ref. [33] carried out the pretreatment of POME with a vegetable enzymatic preparation (PEP), consisting of a pool of non-commercial and low-cost hydrolases obtained from dormant seeds of *R. communis* with high hydrolytic activity. Raw POME fermentation, POME hydrolysis, and fermentation simultaneously (one step) and hydrolysis followed by fermentation (two-step process) were performed. Crude POME culminated in a yield of 2.26 ± 0.05 mmol H₂/g COD with a yield of 0.09 mmol H₂/g COD over 24 h.

The one-step production reached a yield of 2.58 ± 0.02 mmol H₂/g COD with a productivity of 0.13 mmol H₂/g COD in the first 16 h, reducing by 50% the fermentation time required to initiate biohydrogen production relative to raw POME. The best treatment consisted of POME fermentation in two stages, where the authors obtained a yield of 2.56 ± 0.05 mmol H₂/g COD with a maximum productivity of 0.174 ± 0.004 mmol H₂/g COD.

Table 2. Different pretreatments of POME applied to produce biohydrogen.

Pretreatment	Process	POME	pH	TSS (mg/L)	COD Removal %	H ₂ Yield	Increase in H ₂ Production	Reference
Ultrasonication	Dark fermentation	Raw	4.63	17.53	-	0.52 mmol H ₂ /g COD	16% to 86%	[41]
		Pretreated	7.00	-	62.24	0.68 mmol H ₂ /g COD		
	Photofermentation	Raw	4.3 ± 0.3	19,610 ± 7900	-	4.67 mL H ₂ /mL POME		[19]
		Pretreated	-	-	36.9	8.72 mL H ₂ /mL POME POMEH ₂ /mL POME		
Ultrasonication and microwaves	Dark fermentation	Raw	5.2 ± 0.2	-	-	3360 mL H ₂ /L-POME		[40]
		Pretreated	3.9 ± 0.4	-	75.56	4080 mL H ₂ /L-POME		
Surfactant	Dark fermentation	Raw	4.63	60.46	-	1.13 mol H ₂ /g COD	334%	[2]
		Pretreated	7.00	-	58	4.91 mol H ₂ /g COD		
Acid hydrolysis	Dark fermentation	Raw	4.5	37,750	-	0.14 mol H ₂ /mol of total carbohydrates	45% to 75%	[55]
		Pretreated	2.00	26,100	-	1.87 mol H ₂ /mol of total carbohydrates		
	Dark fermentation	Raw	4.2	12,000	-	1.5 L H ₂ /L-POME		[54]
		Pretreated	4.1	9200	-	2.56 L H ₂ /L-POME		
	Dark fermentation	Raw	4.5	52,650	-	0.63 mol H ₂ /mol of glucose		[10]
		Pretreated	2.46	39,200	-	1.24 mol H ₂ /mol of glucose		
	Dark fermentation	Raw	4.24 ± 0.6	48,560	-	0.72 mol H ₂ /mol of total carbohydrates		[5]
		Pretreated	5.3	-	-	1.69 mol H ₂ /mol of total carbohydrates		

Table 2. Cont.

Pretreatment	Process	POME	pH	TSS (mg/L)	COD Removal %	H ₂ Yield	Increase in H ₂ Production	Reference
Alkaline hydrolysis	Dark fermentation	Raw	4.5	37,750	-	0.14 mol H ₂ /mol of total carbohydrates	Until 90%	[55]
		Pretreated	7.7	25,050	-	2.18 mol H ₂ /mol of total carbohydrates		
	Dark fermentation	Raw	4.2	12,000	-	1.5 L H ₂ /L-POME		[54]
		Pretreated	5.4	8500	45	4.6 L H ₂ /L-POME		
	Dark fermentation	Raw	4.24 ± 0.6	48,560	-	0.72 mol H ₂ /mol of total carbohydrates		[5]
		Pretreated	5.14 ± 0.43	-	-	1.08 mol H ₂ /mol of total carbohydrates		
Ozonation	Dark fermentation	Raw	4.4–4.7	15,000–20,000	-	51.5 ± 5.0 mL H ₂ /g COD	25 to 49%	[56]
		Pretreated	6	8000–12,000	-	77.1 ± 8.1 mL H ₂ /g COD		
Ozonation	Dark fermentation	Raw	4.4–4.7	-	-	122.0 ± 1.4 mL H ₂ /g COD		[57]
		Pretreated	6	-	-	182.3 mL H ₂ /g COD		
Ozonation	Dark fermentation	Raw	4.3–4.5	15,000–20,000	-	122 mL H ₂ /g COD		[58]
		Pretreated	4.4–4.5	8000–12,000	-	182 mL H ₂ /g COD		
Enzymatic hydrolysis	Dark fermentation	Raw	4.63	60.46	30	1.12 mol H ₂ /g COD	13 to 137%	[60]
		Pretreated	7.00	-	52	1.88 mol H ₂ /g COD		
Enzymatic hydrolysis	Dark fermentation	Raw	-	-	-	873.6 mL H ₂ /L POME		[61]
		Pretreated	6.2	-	-	2075 mL H ₂ /L POME		
Enzymatic hydrolysis	Dark fermentation	Raw	6.5	-	-	2.26 ± 0.05 mmol H ₂ /g COD		[33]
		Pretreated	6.5	-	-	2.56 ± 0.05 mmol H ₂ /g COD		

4. Processes and Parameters for Green Hydrogen Production

Hydrogen can be produced from fossil fuels, water, and biomass through thermochemical (gasification and steam reforming), electrochemical (electrolysis and microbial electrolysis cells), and biological methods (biophotolysis, dark fermentation, photofermentation, and two-stage process) [62]. About 500 billion m³ of hydrogen are produced annually [63], and 95% of which is produced from fossil fuels using the steam reforming of methane, naphtha reforming, and coal gasification [64,65], while another 5% is mainly produced using water electrolysis [66].

However, these methods are problematic from an environmental point of view due to the emission of greenhouse gases when produced with fossil fuels [67], and due to the high amount of energy required, especially in the case of water electrolysis, where the energy cost is equivalent to about 80% of the total cost of the process [68].

To circumvent this problem, research involving the biological production of hydrogen has intensified. Biohydrogen is of global interest because its production takes place mainly from the use of low-value waste as a substrate, as agro-industrial waste [69], industrial processing wastes [70], municipal wastes [71], and livestock waste [72], being a method of production that is more energy efficient and sustainable, which combines obtaining this value-added compounds with the recovery and treatment of these wastes, which are abundant and have continuous and increasing production [73].

The biological production of hydrogen can be carried out using methods that are dependent or not on solar energy, depending on the microorganism used in the process, which is heterotrophic, photoheterotrophic, or photoautotrophic [74].

Biophotolysis and photofermentation are light-dependent methods, and dark fermentation is an independent one. Dark fermentation can also be integrated with photofermentation to produce hydrogen in two stages, ensuring better use of the substrate [18]. Hydrogen can also be produced using microbial electrolysis cells (MEC), combining the metabolism of microorganisms and bioelectrochemical reactions [75].

Specifically, in the case of POME, hydrogen has been produced by thermochemical (POME steam reforming), electrochemical (microbial electrolysis cell), and biological (photofermentation, dark fermentation, and two-stage process) methods (Figure 1). There are no reports in the literature of hydrogen production from POME using gasification, electrolysis, and biophotolysis methods.

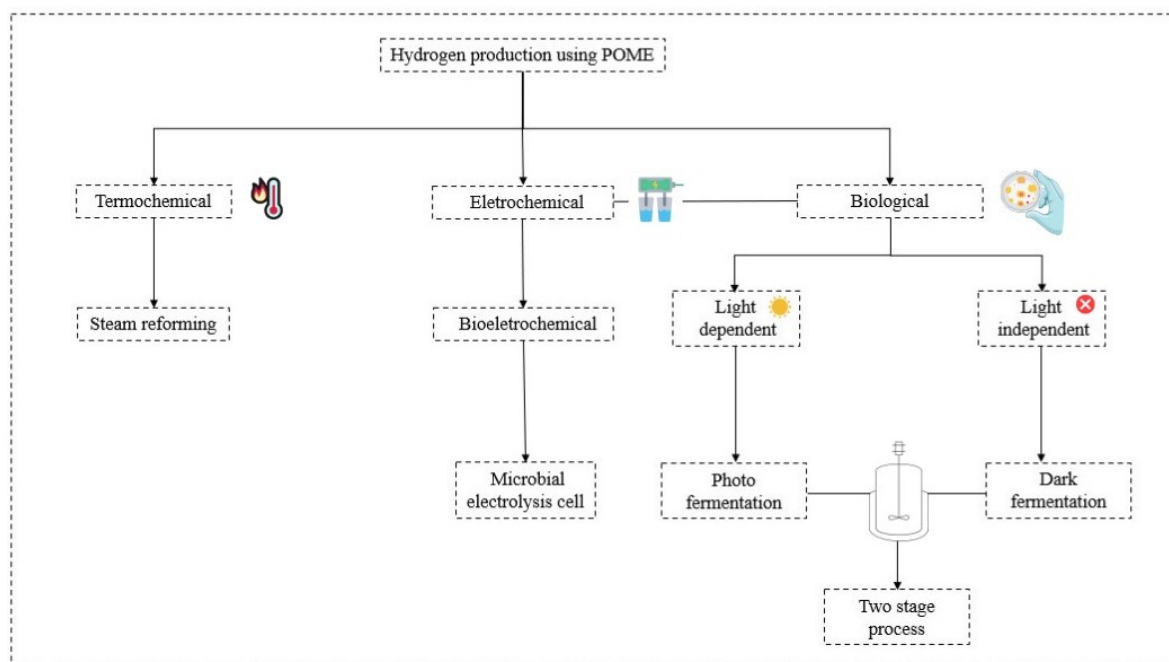


Figure 1. Processes to produce hydrogen using POME.

4.1. Steam Reforming of POME

The steam reforming of fossil fuels is one of the most used methods currently to produce hydrogen, which can be carried out using a wide range of raw materials, such as methane, ethane, ethanol, and others, with methane being the most used. In general, the reaction takes place in a steam reformer under high temperatures that can reach up to 850 °C and a pressure of approximately 20 bar, with the presence of a catalyst that converts the compound used into a gas rich in hydrogen, which is subsequently purified to obtain hydrogen with high purity [76].

The high rates of organic compounds present in POME (Table 1) demonstrate that it can be valued through steam reforming to produce hydrogen [77]. However, the production of hydrogen through this technique is little explored, being first reported in 2018 from the experimental evaluation of the technique using empirical modeling [78], and the later reports of the use of steam reforming of POME for the production of hydrogen belong to the same research group [30,31]. The technical–economic viability of producing hydrogen from the steam reforming of POME was attested by [79].

Due to the few reports in the literature, the processes and parameters of hydrogen production from the steam reforming of POME are not well established. Ng [78] describes the catalyst, process partial pressure, and gas hourly space velocity (GHSV) as the main process parameters.

Nickel-based catalysts have been conventionally used for the steam reforming of fossil fuels; however, their main disadvantage is coke formation [80]. This problem can be mitigated by introducing some metals such as perovskite LaNiO_3 . Cheng [31] added LaNiO_3 as a catalyst to the POME steam reforming, being the main factor in increasing the production of synthesis gas rich in H_2 and ensuring that the reaction occurred at a lower temperature (500 °C), with its optimal operating temperature at 600 °C, generating 73.91 $\mu\text{mol}/\text{min}$ of synthesis gas with a high H_2/CO ratio of 107.88. In addition, the acidity of POME was neutralized, achieving a COD reduction of 98.38% and a TSS reduction of 100%.

Ng [78] used a catalyst consisting of 20% (weight) of nickel and 80% of (weight) of Al_2O_3 , which resulted in a reduction of 95.4% of COD. The highest hydrogen production rate in this study, 62.09 $\mu\text{mol}/\text{min}$, was achieved at a temperature of 900 °C, a partial pressure of 95 kPa, and a GHSV of 50,000 $\text{mL}/\text{h g}_{\text{cat}}$. With the optimization of temperature (900 °C), partial pressure of 95 kPa, and GHSV of 40,000 $\text{mL}/\text{h g}_{\text{cat}}$, the reduction of COD reached 99.7%, also demonstrating the importance of these parameters of hydrogen production from the reform with POME steam, since the high temperature and pressure cause an increase in the collision between the molecules and consequently increases the reaction rate.

Ng [81] focused on evaluating the impact of liquid hourly space velocity (LHSV) and reaction temperature of POME steam reforming on the production of hydrogen-rich synthesis gas. It was found that the reduction in POME COD can be enhanced by the increase in GHSV, achieving a reduction of 93.8% under the flow of 90 $\text{mL}/\text{h g}_{\text{cat}}$ of POME and 20 mL/min of N_2 carrier gas at 600 °C. However, this high GHSV culminates in severe carbon deposition, decreasing H_2 production over time, and this problem can be solved with an increase in temperature, which also culminates in a greater reduction of COD. At the reaction temperature of 900 °C, 99.41% COD reduction was achieved in GHSV of 90 $\text{mL}/\text{h g}^1$ of LHSV and 20 mL/min of N_2 carrier gas, generating product gas with 66.38% H_2 gas using a catalyst consisting of 20% (weight) of nickel and 80% of (weight) of Al_2O_3 .

4.2. Microbial Electrolysis Cell

The generation of bioelectricity and hydrogen using microbial fuel cells (MFCs) and microbial electrolysis cells (MECs), respectively, is already a process widely described in the literature using various industrial effluents [82–85]. In addition, a series of studies have used POME to generate bioelectricity [32,86–88], but hydrogen generation from this effluent using MECs is still scarce [21].

According to studies available in the literature, pH, temperature, organic loading rate, and applied voltage are the main parameters that affect hydrogen production in microbial electrolysis cells from POME [21].

Kadier [21] optimized the incubation temperature, initial pH, and effluent dilution rate to produce hydrogen from POME in a microbial electrolysis cell. The maximum H_2 production rate (HPR) of $1.1747 \text{ m}^3 \text{ H}_2/\text{m}^3 \text{ d}$ was achieved under conditions of a temperature of 30.23°C , pH of 6.64, and effluent dilution rate of 50.71%. As described in the review, pH and temperature jointly affect microbial metabolism, demonstrating the impact of pH in obtaining a higher HPR, which was achieved when the pH was adjusted to 6.4–6.6 since the pH close to the neutral (7.0) is ideal for keeping biofilms on the anodes. In addition, the pH within this range is also ideal for enzymatic action on the anode, affecting proton transfer and cathodic reaction. The increase in temperature ($26\text{--}31^\circ\text{C}$) also influenced the significant increase in HPR due to its influence on the development of microorganisms and the fact that enzymatic reactions are directly proportional to temperature. Higher concentrations of COD ($>60\%$) resulted in a decrease in HPR because the higher rate of COD leads to an increase in cycle duration. Consequently, methanogenic microorganisms consume COD, causing its decrease and leading to a decrease in HPR. Higher COD rates may also inhibit the metabolism of hydrogen-producing microorganisms.

Khongkliang [89] produced hydrogen using POME as a substrate in a microbial electrolysis cell with an initial COD of 66 g/L and an initial pH of 6.5 under different voltages to evaluate its influence on the process. The voltages applied were 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 V, respectively, obtaining yields of 25, 68, 102, 114, 116, 134, 76, and $69 \text{ mL H}_2/\text{g COD}$. It can be observed that the yields of hydrogen increased in the range of applied voltages from 0.2 to 0.7 V, also obtaining greater efficiency in the removal of COD. However, there was a decrease in hydrogen yield at applied voltages above 0.7 V, which is possibly caused by the fact that excessive voltages can restrict the activity of exoelectrogenic bacteria, impairing electron transfer.

To remedy the problem regarding the need for an external source of energy, Kadier [21], in an unprecedented study, proposed the development of a renewable energy source based on Pico-Hydro-Power (PHP) that could produce 176 V to power the microbial electrolysis cell, improving its performance in hydrogen production and COD removal from POME. The PHP-MEC integrated system obtained an average hydrogen yield of $894.42 \pm 0.3 \text{ mmol H}_2/\text{g COD}$ and COD removal of about 73%.

Microbial consortia are typically used in this process and may come from POME itself [60] or anaerobic sludge from reactors running with other effluents [89]. During the electrolysis process, the presence of bacteria such as *Geobacter* sp., *Desulfovibrio* sp., *Thermoanaerobacterium* sp., *Shewanella* sp., and *Geobacillus* sp. are capable of oxidizing organic compounds and transferring protons and electrons to the cathode.

4.3. Dark Fermentation and Photofermentation of POME

As observed in Table 2 and in other studies present in the literature, dark fermentation is currently the most explored biological method to produce biohydrogen from POME and other substrates due to the lower complexity of the process, which requires less maintenance, smaller bioreactors, and is less expensive as it does not use luminescence [90].

This process is carried out by microorganisms containing enzymes called hydrogenases, with FeFe and NiFe being the main hydrogenases described in the literature [91]. This production occurs in anaerobic bioreactors through complex and diverse biochemical steps, where numerous substrates can be used, such as lignocellulosic biomass and other raw materials containing fractions of carbohydrates and starch, such as organic fractions of urban solid waste and wastewater from food industries such as POME [92].

Dark fermentation results in the formation of a mixed gas consisting mainly of H_2 and CO_2 [92], presenting an estimated theoretical hydrogen yield of $4 \text{ mol of H}_2/\text{mol of glucose}$ [93] varying according to the metabolic route used by the microorganism (acetate, butyrate, ethanol, butanol, etc.) [91].

In general, among the parameters that affect hydrogen production by dark fermentation, we can mention the microorganism used, the pretreatment of the raw material, temperature, pH, availability of nutrients, partial pressure of hydrogen, and hydraulic retention time (HRT).

The microorganisms responsible for dark fermentation may come from pure cultures or consortia of facultative or strict acidogenic–acetogenic anaerobic bacteria [91] that can carry out hydrogen production at mesophilic (25–40 °C) or thermophilic temperatures (40 to 65 °C) depending on the characteristics of their metabolism. Several species, mainly of the genera *Clostridium*, *Enterobacter*, *Bacillus*, *Escherichia*, and *Thermoanaerobacterium*, are reported in the literature to produce biohydrogen [27,94].

Pure cultures offer advantages in terms of better process control, the detection of metabolic alterations, and the control of parameters that help obtain higher hydrogen yields. However, using mixed cultures is more desirable because it does not require a sterile process, allowing the synergy of microorganisms and contributing to the degradation of more complex substrates [95].

The microorganisms used in dark fermentation can be a part of the natural microflora of the substrate being used, or they can also be a source of an external inoculum such as sludge from anaerobic digesters, soil, animal manure, and other places [60]. However, when using mixed cultures, there is a risk of also having species of methanogenic bacteria that consume hydrogen, requiring pretreatment of the inoculum through various methodologies, such as acid pretreatment, basic pretreatment, and heat, among others, inhibiting the growth of these bacteria [95].

However, in addition to the production of hydrogen, dark fermentation also leads to the formation of numerous organic compounds of added value, such as ethanol, butanol, acetone, butyric acid, and acetic acid, formed depending on the metabolic pathway of the microorganism and cannot be reused by these microorganisms for the additional formation of hydrogen, limiting the theoretical yield of this compound [96].

As discussed in Section 3 of this review, substrate pretreatment can be carried out by physical, chemical, or biological methods (Figure 1), aiming at the degradation of complex compounds such as lignin, cellulose, and hemicellulose, making fermentable compounds available and subsequently leading to COD reduction and higher yield of hydrogen production [92]. In the case of POME, given the complexity of the compounds present in its composition (Table 1), its pretreatment becomes an indispensable step and culminates in significantly higher hydrogen yields when compared to the yield of hydrogen from raw POME fermentation [60].

The temperature used in dark fermentation is defined by the optimum temperature range of the microorganism used in the process, which can be classified as mesophilic (25–40 °C) or thermophilic (40–65 °C) as described above. Higher temperatures increase the activity of the enzymes responsible for catalyzing the production of hydrogen and culminate in a lower solubility of gases in water, which, when present in high concentrations, can inhibit bacterial growth. It is estimated that the ideal temperature for dark fermentation is 5 to 55 °C [92]. Regarding the dark fermentation of POME, it is described at different temperatures [97], more frequently in the range of 30 °C [27,94].

The ideal pH of the medium for hydrogen production primarily depends on the ideal pH range for the growth of the microorganism used, where the activity of its enzymes is maximal. It is estimated that the ideal pH to produce biohydrogen from dark fermentation is around 5 to 7 [96], also inhibiting the growth of methanogenic bacteria that consume hydrogen, affecting its yield. As noted in Table 2, the pH of dark fermentation using POME as a substrate ranges from four to eight.

The availability of nutrients is another parameter that affects hydrogen production, since they act as enzymatic cofactors and, therefore, are extremely important not only in enzymatic activities but also in microbial growth [98]. Some of the essential nutrients are nitrogen, phosphorus, and micronutrients, such as metal ions. Nutrient supplementation is an alternative to increase biohydrogen production [92].

The partial pressure of hydrogen is also a parameter that must be carefully controlled, since the metabolic pathways responsible for the production of hydrogen are highly vulnerable to an increase in the partial pressure of hydrogen due to the feedback phenomenon, causing the blockage of the conversion of the substrate into hydrogen [98], and favoring the formation of other metabolism products, such as ethanol, lactic acid, butanol, acetone, and others, making it extremely important that hydrogen is removed from the process as it is produced [96].

In hydrogen production processes on larger scales, carried out in semi-continuous and continuous processes, the hydraulic retention time (HRT) is an important parameter of the process, and the ideal HRT value depends on the substrate used and its biodegradability potential, and may vary from hours to days. Zainal [99] investigated the effect of temperature and HRT on biohydrogen production from POME in a rising sludge fixed film anaerobic bioreactor (UASFF) and observed a 28.8-fold increase in biohydrogen production when the temperature increased from 37 °C to 53.5 °C in a 3 h HRT. The maximum hydrogen production of 10.39 L H₂/d and removal of 35.9% was achieved with a 7 h HRT, and a temperature of 57 °C [100] obtained the highest hydrogen yield of 2.99 mol of H₂/mol of sugar at 55 °C and HRT of 12 h.

Photofermentation consists of the oxidation of organic substrates, preferably organic acids such as succinate, malate, butyrate, lactate, and pyruvic acid, by non-sulfur purple bacteria capable of using them in anaerobic conditions, nitrogen deficiency, and in the presence of sunlight, affecting their conversion into H₂ and CO₂ [101].

Among the genera of non-sulfur purple bacteria most used in this process, we can mention *Rhodospseudomonas* [102–104], *Rhodobacter* [105,106], *Rubrivivax* [106], and others.

As in dark fermentation, hydrogenase enzymes also play an important role in hydrogen production. However, in photofermentation, hydrogenases act in conjunction with nitrogenase enzymes that produce hydrogen through a series of biochemical reactions [98]. The oxidation reaction of organic matter generates protons and electrons. These electrons are excited by sunlight and transformed into high-energy electrons that are later used to form ATP, which is used by the enzyme nitrogenase together with protons and electrons to fix nitrogen and transform it into molecular nitrogen and ammonia, producing hydrogen simultaneously, so it is essential that nitrogen is present under stressful conditions [101,106]. About 1.6, 4.0, and 2.8 mol of hydrogen are produced from 1 mol of acetic acid, butyric acid, and propionic acid, respectively [98].

The parameters that affect hydrogen production by photofermentation and dark fermentation are very similar, such as temperature around 30 °C and pH around 7.0, within the optimal range for the growth of microorganisms and the performance of their enzymes [107]. In addition, hydrogen production can be improved through substrate pretreatment, macronutrient and micronutrient supplementation, nanoparticles, and HRT optimization. However, unlike dark fermentation, lighting is the key point for photofermentation, and optimizing the reactor design is also essential to guarantee biohydrogen production by photofermentation [107,108].

However, little attention is given to photofermentation due to the high cost of the process, low productivity, low conversion of sunlight, low conversion of substrate, need for a light source, unstable hydrogen production, and other problems [108]. To solve this problem, many studies have pointed to hydrogen production in two stages: dark fermentation followed by photofermentation [18].

As previously mentioned, the production of hydrogen by dark fermentation also leads to the formation of organic acids that are not reused by the bacteria, limiting the production of hydrogen, which has a theoretical maximum of about 4 mols of H₂/mol of glucose [93]. However, this feature makes the residual substrate of dark fermentation perfect for use in photofermentation, since non-sulfur purple bacteria preferentially use organic acids during this process, significantly increasing the theoretical maximum yield of hydrogen, which is about 12 moles of H₂/mole of hexose [103,109], therefore, being more favorable than dark fermentation and photofermentation carried out separately.

Mishra [18] investigated hydrogen production in two stages using POME, obtaining a yield of 0.78 mL H₂/mL POME in the first stage and 2.86 mL H₂/mL POME in the second stage. Moreover, Mishra [18] carried out the photofermentation of POME from dark fermentation using purple non-sulfur bacteria *Rhodopseudomonas palustris*. The optimization of the culture medium was carried out using the response surface methodology, investigating various light intensities, medium agitation, and dilution rates of the fermented medium. The maximum yield of hydrogen obtained was 3.07 ± 0.66 mol H₂/mol acetate under optimized light intensity conditions of 250 W/m², agitation rate of 200 rpm, and POME dilution of 30%, respectively.

5. Bioreactor Types and Operational Conditions

Different types of bioreactors have been developed for the biological production of hydrogen. These can be divided into photobioreactors and dark fermentation reactors. Photobioreactors are used for photosynthetic microorganisms, such as microalgae, cyanobacteria, and some purple bacteria, such as *Rhodopseudomonas Palustris* [110]. However, they are rarely used with POME because the effluent is dark and contains large amounts of suspended material, reducing light penetration. Therefore, photobioreactors are not addressed in this review. Dark fermentation reactors, conversely, are frequently used in non-photosynthetic biohydrogen production, using cultures of bacteria of the genera *Clostridium*, *Escherichia*, *Pseudomonas*, *Enterobacter*, and *Bacillus*, among others; moreover, these bioreactors have been widely used for the treatment of POME.

5.1. Bioreactors for Dark Fermentation

Dark fermentation is one of the most promising routes for biological hydrogen production. Different types of bioreactors operated in batch and continuous mode have been used in this process. Batch systems are generally preferred due to their simplicity and ease of operation; they generally operate with suspended cells. However, it has a limiting effect on the amounts of effluent that can be treated. Continuous systems can operate with cells in suspension or when immobilized; however, they are more complex [111].

5.1.1. Suspension Cell Bioreactors

Among the bioreactors that operate with cells in suspension and that have been used for the production of biohydrogen are continuous stirred tank reactors (CSTR), anaerobic sequential batch reactors (ASBR), and anaerobic membrane reactors (AnMBR). In these systems, there is high contact between the microorganisms and the substrate, which results in a higher mass transfer rate [112].

Continuous Stirred Tank Reactor (CSTR)

CSTRs are versatile systems widely used in industry and normally used for biohydrogen production due to their simplicity of operation. The system has an impeller that performs the complete agitation of the growth medium or substrate (Figure 2A). In CSTRs, feed and discharge occur simultaneously. According to Han [113], CSTRs are the cheapest and simplest bioreactors operating with suspended cells. The main disadvantage of CSTR is the instability of the process generated by the type of inoculum and substrate. Some important parameters that must be controlled in the CSRT are hydraulic retention time (HRT) and organic loading rate (OLR), because these parameters, in addition to affecting the characteristics of the environment, also affect the microbial population. Reference [114] evaluated the effect of different HRTs (5, 3, 2, 1, and 0.75 days) for hydrogen production. The maximum hydrogen production rate and yield were 1.72 L H₂/LR and 0.54 mol H₂/mol carbohydrate consumed, respectively, and were obtained with an HRT of 0.75 d. Reference [115] reported a maximum removal of 45% of DOC and a maximum hydrogen production rate of 2.16 L H₂/L d in a 4 L CSRT with a working volume of 2.5 L and using POME as a substrate. The operational conditions were inoculum volume of 30% (*v/v*) and HRT of 4 d, corresponding to OLR of 11.3 g COD/L d. According to Carolin [111],

short HRTs can lead to biomass washing, while very high HRTs can favor the formation of volatile fatty acids, so the HRT should not be defined arbitrarily.

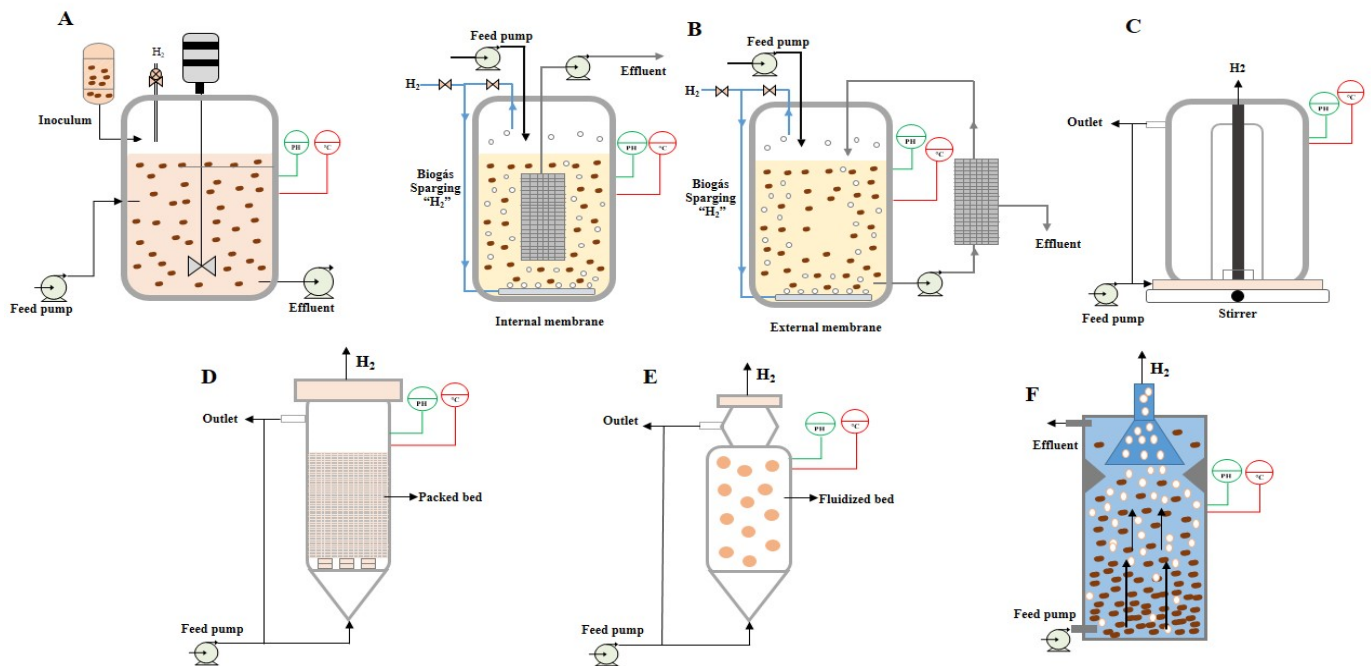


Figure 2. Bioreactors for dark fermentation: (A) CSTR; (B) AnMBR; (C) ASBR; (D) PBR; (E) FBR; and (F) UASB.

Anaerobic Membrane Bioreactor (AnMBR)

AnMBR can be defined as an anaerobic bioreactor coupled with membrane filtration. The membrane filtration component can exist in three configurations: external cross flow, internal submerged, or external submerged [116]. In AnMBRs, the membranes can be placed inside or outside the bioreactor (Figure 2B). This last configuration facilitates the membrane cleaning and replacement processes, allowing the isolation of the membrane unit in an external chamber; however, it requires greater energy input than the configuration with membranes inside the bioreactor [111].

In general, in AnMBRs, long SRT and HRT are used to improve the quality of the inoculum and remove COD. The main drawbacks of this system are membrane fouling and the generation of large amounts of inhibitory products, such as volatile fatty acids, alcohols, and hydrogen-consuming microbes, which ultimately affect the generation rate and hydrogen yields. Conventional AnMBRs employ microfiltration and ultrafiltration processes to reuse cells in converting the substrate into biohydrogen. Oh [117] used an AnMBR to produce microbial hydrogen using glucose as a substrate. A significant improvement was observed when the HRT was increased from 3.3 h to 12 h. With longer HRTs, the glucose utilization increased to 98%, the biogas production rate increased from 500 mL/h to 640 mL/h, and the glucose-to-hydrogen conversion efficiency slightly improved from 22% to 25%.

Anaerobic Sequencing Batch Reactor (ASBR)

The ASBR has been widely studied as an alternative to continuous systems due to its better biological solid retention and process control [118]. The ASBR operation occurs in four stages: feeding, reaction, sedimentation, and liquid removal (Figure 2C). To produce hydrogen in these systems, in addition to controlling the HRT, the solid retention time (SRT) must also be controlled, as these two parameters directly influence the characteristics of the medium and the microbial population [111]. An important characteristic of ASBR systems is that they have a good biomass retention capacity in the case of hydrogen production;

an important aspect because hydrogen is a metabolite associated with microbial growth, and its production is directly linked to the maintenance of the redox balance during the cellular energy production [94]. Santiago [119] evaluated the effect of HRT (4.6 to 27 h) and SRT (17 to 102 h) on biohydrogen production. The results show that the highest rate of hydrogen production (1.86 L H₂/L d) was obtained under the conditions of 60 h and 16 h for HRT and SRT, respectively. Maaroff et al. [100] reported a maximum hydrogen yield of 2.99 mol H₂/mol from POME in an ASBR operated with HRT and SRT of 12 h and 20 h, respectively; in the process, 89% of the effluent sugar was removed, while Mamimin et al. [37] obtained a hydrogen productivity rate of 9 L H₂/L d using POME as a substrate in an ASBR operated with a 24 h HRT.

5.1.2. Immobilized Bioreactors

The immobilized systems may solve the biomass washing problems in the systems that work with the cells in suspension, generated mainly when these bioreactors are operated with short HRT. Simple barriers retain the immobilized microbial biomass, which theoretically could be used continuously as long as the stability of the culture is not altered, and this being the main advantage compared to bioreactors with suspended cells. In immobilized systems, cells and enzymes are physically or chemically trapped into a solid matrix. The most commonly used support materials are ceramic granules, activated carbon, clay, clay granules, chitosan, polyethylene glycol, low-density polyethylene, and alginate granules [120]. Among the systems that operate with immobilized cells are packed/fixed-bed bioreactors (PBR), fluidized bed reactors (FBR), and upflow anaerobic sludge blanket reactors (UASB).

Packed/Fixed-Bed Bioreactor (PBR)

PBR is considered one of the best systems for hydrogen production via dark fermentation. Among its advantages are low energy consumption, easy operation, high cell retention time, and versatility in terms of support materials that can be used [120]. Generally, the support material in this system is fixed inside the tank (Figure 2D). Karapinar [121] evaluated the effect of HRT on the continuous production of biohydrogen in an upflow packed bed reactor containing polyester fiber spheres as material for immobilizing microorganisms. The system was operated under thermophilic conditions at 48 ± 2 °C and an HRT between 2 and 4 h. The reported results showed that the biohydrogen productivity increased from 4331 mL/d to 6624 mL/d when HRT was increased, consequently also improving the volumetric production rate, which increased by 50%, and the yield increased from 0.49 mol/mol glucose to 0.89 mol/mol glucose with the highest HRT. Andreani [122] evaluated biohydrogen production using cassava processing wastewater as a substrate in an upflow anaerobic fixed bed reactor loaded with bamboo stems as a support medium. The highest rate of hydrogen production was 1.1 L/L d. obtained with an organic loading rate (OLR) of 35 g/L d and a 3 h HRT. In the packed/fixed-bed bioreactor, the HRT can vary from 2 to 24 h [111].

Fluidized Bed Reactor (FBR)

The FBR is a column containing inert materials kept in suspension due to upward flotation (Figure 2E). The FBR system is one of the bioreactors with the highest cell production capacity, which reduces contamination. Furthermore, the stirring system is pneumatic, which means that the fermentation medium is highly homogenized, one of its main advantages. Jamali [123] evaluated the performance of cells immobilized on granular activated carbon in an FBR to produce biohydrogen from POME. According to the researchers, the highest hydrogen productivity rate was 5.2 mmol H₂/L h, obtained with a 12 h HRT. In addition, it was found that *Thermoanaerobacterium thermosaccharolyticum* sp. was the dominant species in all HRTs, indicating that this species is resilient to environmental disturbances, which would be considered promising for hydrogen production processes in immobilized systems.

Upflow Anaerobic Sludge Blanket Reactor (UASB)

The UASB basically consists of a tank with a digestion compartment at the bottom. This compartment is initially formed by a bed of sludge, with a high concentration of solids, and above it is a blanket of sludge, which has a lower concentration of solids, and at the top, the system has a three-phase separator (Figure 2F). Essentially, the process consists of an upward flow of effluent through a bed of dense and highly active sludge that is responsible for producing biogas. Initially, the system was developed for anaerobic methane production, and later, it was used to evaluate the biological production of hydrogen. This system's main advantages are simple construction, low operation and maintenance cost, robust treatment efficiency, and broad small-to-large-scale applicability [124]. Krishnan [125] evaluated the effect of OLR (25, 50, 75, 100, and 125 kg-COD/m³·d) and a constant 6 h HRT on hydrogen production in a UASB using POME as a substrate. The highest volumetric production rate and maximum yield were 2.1 LH₂/d, and 49.22 mL H₂/g COD applied, respectively, obtained with an OLR of 75 kg-COD/m³·d. Senan et al. [126] and evaluated the effect of HRT (3 h to 48 h) on biohydrogen production using hydrolyzed POME in a UASB. The maximum hydrogen production and yield rates were 11.75 LH₂/L_{POME}·d and 2.45 mol-H₂/mol-sugar, respectively, which were obtained with a 6 h HRT. In this condition, *Clostridium* spp. predominated in the fermentation, using the metabolic route of butyrate.

6. Nanoparticles and Other Technologies to Increase Biohydrogen Production

Despite the inherent advantages of using dark fermentation process to produce biohydrogen, the low production yield (on average 1–3 mol/mol of glucose) limits its industrial applicability, since around 60–70% of the substrate remains unused in the form of volatile fatty acids and alcohols [127]. One of the ways to try to overcome the dilemmas related to the low productivity of biohydrogen would be by adding nanoparticles, genetic engineering, and cell immobilization [62].

6.1. Nanoparticles (NPs)

The use of nanoparticles is one of the promising strategies to improve the activity of enzymes involved in biohydrogen production [128]. With a size of 1–100 nm, nanoparticles have the essential characteristics for application in biofuels, such as high specific surface area, porosity, chemical stability, and absorption capacity. More precisely, they act directly to improve catalytic activity, reduce inhibitors, and transfer electrons [129]. Regarding classification, NPs can be grouped into several groups based on their morphology, size, and properties, namely, carbon-based NPs, metallic, ceramic, polymeric, and others [130].

Hydrogen production in dark fermentation depends directly on the activity of the hydrogenase enzyme, which catalyzes the redox proton transfer reaction [131]. In bacteria, the expression of [Fe-Fe]-hydrogenase or [Ni-Fe]-hydrogenase can occur. Therefore, nickel and iron are essential to promote the proper functioning of these enzymes [62]. Thus, introducing nanomaterials, mainly metallic, allows the greater production of hydrogen [132]. This arises from the fact that these nanoparticles act as enzymatic cofactors and create an anaerobic environment essential for the activity of hydrogenases [133].

For example, Mishra [18] investigated the impact of metal oxide NPs (NiO and CoO) on hydrogen production using POME as a substrate. As a result, it was found that supplementation with 1.5 mg/L NiO produced a yield of 0.563 L H₂/g COD, and supplementation with 1.0 mg/L CoO produced a yield of 0.487 L H₂/g COD. These results show an increase of 1.51 and 1.61 times in the hydrogen yield by adding NiO and CoO NPs, respectively.

Similarly, Mishra [134] also studied the impact of different concentrations of NiO and CoO NPs on hydrogen production from POME. This study found that the ratio of Ni/Co NPs of 3:1 showed a greater production of H₂ (2844 mL). Furthermore, with the addition of NPs in that proportion, the COD removal efficiency was 74%, representing an increase of 45%. Mishra [135] studied the addition of nickel oxide NPs at a concentration of 80 mg/L, which increased the production of H₂ to 15 mL H₂/h, and the removal of

COD was 59%. However, when the concentration of NPs was 140 mg/L, H₂ production decreased. Therefore, choosing an ideal concentration of NPs is essential for producing H₂ and avoiding the toxicity of these nanomaterials in microbial cells [62].

It is worth highlighting that, in general, unlike other more expensive techniques such as pretreatment processes and the application of enzymatic engineering, the use of nanotechnology, even in its initial phase, has provided a simpler and cheaper option to improve the rate of biohydrogen production [136].

Nanoparticle technologies to improve biohydrogen production are being patented due to the promising results being obtained with these technologies. One of the first patents on nanoparticles for hydrogen production is US9688718B2. In this patent, 20 nm nanolipoprotein particles are developed to catalyze biohydrogen production. The mimetic particle contains a lipid bilayer and a hydrogenase associated with it. The enzyme catalyzes the conversion of hydrogen protons into molecular hydrogen. Furthermore, these proteins are associated with biological membranes. According to Baker [137], proteins and lipids are in a ratio between 1:0.025 and 1:1. Hydrogenases can be obtained from the microbial species of *Allochromatium vinosum*, *Methanosarcina barkeri*, *Escherichia coli*, *Rhodospirillum rubrum*, *Desulfomicrobium baculatum*, and *Ralstonia* sp.

Other patents are focused on synthesizing metallic nanoparticles, which, in addition to being easier and more economical, can also be recovered. For example, Qin [138], in patent CN114524470A, presented a process for synthesizing nanoparticles of nickel ferrite (NiFe), consisting of mixing nickel and iron salts with water hyacinth extraction, which are mixed, dried, calcinated, and finally washed. When used in maximum concentrations of 50 ppm, these nanoparticles can improve biohydrogen yield by up to 88%. Ji [139], in patent CN115215386A, presented the process to produce nanoparticles of nickel cobaltate (NiCo)₂ as these improve the productivity of the energy vector. The nanoparticle synthesis process begins with separating the reagents Ni(NO₃)₂ · H₂O and Co(NO₃)₃, followed by adding metal ions. Subsequently, ammonia is added, and the samples are dried and calcined. Finally, the samples are washed with ethyl alcohol and dried. These (NiCo)₂ nanoparticles improved biohydrogen production via dark fermentation by up to 50% when used at a maximum concentration of 400 ppm.

6.2. Cell Immobilization

Cell immobilization using adsorption, entrapment, encapsulation, and polymer-based techniques has been developed to resolve the limitations arising from the biohydrogen production process and increase its yield [140]. Typical materials used are activated carbon, polyurethane, clay, low-density polyethylene, ceramic spheres, polyethylene pellets, alginate spheres, chitosan, and polyethylene glycol [120]. Table 3 summarizes some applications of materials used in cell immobilization in POME fermentations for biohydrogen production.

Compared to the suspended system, the immobilized system is more tolerant to changes in pH, temperature, organic load, stability, and activity [140,141]. Furthermore, another advantage of the immobilized system is the permanence of microbial cells inside the bioreactor, since biomass washing is low in this configuration [111].

In the study of Singh and Wahid [142], the impact of *Clostridium* sp. immobilization on polyethylene glycol (PEG) gel beads was investigated, as well as the addition of K₂HPO₄, NiCl₂, FeCl₂, and MgSO₄ and the effect of temperature on biohydrogen production with POME. At the end of the continuous process, at 37 °C, a maximum H₂ production rate of 7.3 L/L -POME/day and a yield of 0.31 L H₂/g COD were obtained, using 10% PEG, 2000 mg/L of K₂HPO₄, 1 mg/mL of NiCl₂, 300 mg/mL of FeCl₂, and 100 mg/mL of MgSO₄. According to the authors, PEG is a suitable matrix due to its mechanical and physical properties, besides being a non-toxic material for microbial cells.

Table 3. Applications and materials used in cell immobilization in POME fermentations for biohydrogen production.

Immobilization Technique	Immobilization Media	Inoculum	Operation Mode	Conditions	H ₂ Yield	H ₂ Yield Increase	Reference
Imprisonment	Polyethylene glycol (PEG) gel—10% <i>w/v</i>	<i>Clostridium</i> sp.	Continuous—working volume of 5000 mL	37 °C 100 rpm pH 5.5 36 h (HRT)	0.31 L H ₂ /g COD	-	[142]
Adsorption	Granular activated carbon (GAC)—10%	<i>Caldicellulosiruptor saccharolyticus</i>	Batch—250 mL working volume	Medium enriched with 10% POME Initial pH 7, 100 rpm, 1:1% <i>wv</i> (inoculum: GAC), and thermophilic temperature of 70 °C	2.6 mol H ₂ /mol of substrate	1.94 fold	[143]
Imprisonment	Alginate (1:1)	<i>Bacillus anthracis</i> 15% <i>v/v</i>	Batch—Scott bottle with 350 mL working volume	POME and FW sterilized (1:1 <i>v/v</i> ratio), pH 5.0, 35 °C	Maximum hydrogen production rate of 47 mL/h	-	[144]
Adsorption	Glass beads (3 mm)	POME anaerobic sludge	Fed-batch—100 mL bottles	pH 6, 37 °C, 72 h	479.3 ppm	1.34 fold	[145]
Adsorption	<i>Moringa oleifera</i> Seeds (MOS)—0.7–1.3 cm (addition of 5% <i>w/v</i>)	Anaerobic sludge	Batch—100 mL bottles	37 °C, pH 6	560 ppm	25.5 fold	[146]

Alginate is also used in cell immobilization in dark fermentation processes due to its accessibility, compatibility, and large surface area [147]. This biopolymer was explored in the study of Mishra [144], in which *Bacillus anthracis* cells were immobilized. As a result, using a medium composed of POME and domestic food waste in a 1:1 ratio in a batch process, there was a higher H₂ production rate of 47 mL/h in 60 h and a removal of 72% of the COD after 96 h.

Similarly, granular activated carbon (GAC) has certain properties and characteristics that encourage its use in cell immobilization processes, such as a large surface area and porous structure that facilitate cell adhesion [148]. In the study of Jamali [143], *Caldicellulosiruptor saccharolyticus* cells were immobilized in GAC to produce H₂ from a medium enriched with POME (10, 20, 30, 40, and 50%) in batch. As a result, the best result was obtained with supplementation of 10% POME (20.7 mL H₂/L/h), generating a yield of 2.6 mol H₂/mol sugar. Furthermore, the immobilized bacteria also showed good results when crude POME was used in sterile (20.8 mL H₂/L/h) and non-sterile (29.8 mL H₂/L/h) environments.

Ashah [149] compared the use of different supports, namely, GAC, glass spheres, and MOS, to improve batch H₂ production using POME as a substrate. In their analysis, it was discovered that the best performance was obtained with GAC (1.52 mol H₂/mol glucose), followed by glass beads (1.43 mol H₂/mol glucose) and MOS (1.08 mol H₂/mol glucose). However, Hamid [146] used MOS as an immobilization support for anaerobic sludge cells in a batch process under 37 °C and pH 6, achieving a rate of 39.0 mL/h.

6.3. Genetic Tools and Metabolic Engineering

The production of H₂ by biological means can also be increased through the genetic improvement of microorganisms [150]. This can be done by changes in metabolic pathways and genes to improve the use of the substrate or increase the resistance of microorganisms to inhibitors [151]. For example, improving microorganisms can lead to using different carbon sources, such as cellulose and hemicellulose. At the same time, the overexpression of essential enzymes such as hydrogenases is also a possible strategy to increase H₂ yields [152]. Although promising, studies regarding modified strains must be carried out to allow viable scaling steps [153].

One of the most studied microorganisms to produce H₂ is *Escherichia coli* due to the knowledge of its genomic sequence and metabolic routes [154,155], in addition to presenting rapid growth [154]. Among the strategies employed, we highlight the deletion of competitive metabolic pathways such as the formate pathway, the positive regulation of the operon that regulates the expression of Hyd-3 hydrogenase, the overexpression of enzymes related to glycerol assimilation, and others [156]. Likewise, genetic modification has also been explored in a study by Zhao [157], in which the overexpression of genes, namely, the HycG and HycE gene that codes for the two [NiFe]-hydrogenase 3 subunits of *Enterobacter aerogenes*, promoted an increase in hydrogenase activity: around 124% (*E. aerogenes*/HycE) and 67% (*E. aerogenes*/HycG) when compared to the original strain. Furthermore, hydrogen yields from glucose increased by 86.2% and 69.8%, respectively.

Bacteria of the genus *Clostridium* are extremely important in H₂ production processes. Due to the sensitivity of the hydrogenase enzyme to oxygen, many researchers have studied protein engineering as a tool for improving the oxygen tolerance of [FeFe]-hydrogenases [151]. Furthermore, genetic modifications mediated using the CRISPR-Cas technique, mainly to improve substrate utilization and H₂ yields in several species of the genus *Clostridia*, also emerge as a potential alternative [158].

Taifor [159] compared biohydrogen production yields from POME substrates using unengineered and engineered strains of *E. coli*. The process was carried out in batch at 37 °C for 24 h, and at the end, a maximum hydrogen yield of 0.66 mol H₂/mol monomeric sugars was obtained with the engineered strain. In other words, hydrogen production yield was 3.5-fold higher when using the modified strain over the wild-type strain.

7. Economic Evaluation

Biohydrogen production has emerged as a promising alternative for sustainable and environmentally friendly processes. The evaluation of its economic viability is essential in establishing its potential as a practical and scalable energy source. This involves a comprehensive analysis that combines technical and economic aspects, shedding light on the economic sustainability of biohydrogen production methods. Central to this evaluation are tools such as techno-economic analysis (TEA) and Life Cycle Assessment (LCA), which play a pivotal role in quantifying the sustainability of processes.

Studies focusing on the economic evaluation of biohydrogen production in a broader context have laid the foundation for understanding the economic dynamics of various methods. These analyses often consider factors such as capital investment, operational costs, and overall efficiency. The comparison of different biohydrogen production pathways, including gasification and anaerobic digestion, elucidates the economic scenario of biohydrogen on a larger scale [160].

One key aspect examined in these economic evaluations is the capital investment required for setting up biohydrogen production facilities. For instance, reference [160] explored gasification and anaerobic digestion pathways, quantitatively evaluating their associated capital costs. Gasification, recognized as one of the most economical methods, incurred a cost of US\$ 1.2 per kg of hydrogen produced, showcasing its economic competitiveness.

Operational costs constitute another crucial factor in economic assessments. These costs encompass the day-to-day expenses involved in running biohydrogen production processes. The analysis conducted by [160] demonstrated that anaerobic digestion, with a cost of US\$ 1.25 per kg of hydrogen, proved to be a financially viable method, although slightly less cost-effective compared to gasification.

Efficiency, an important economic factor, has been one of the most explored aspects in the studies conducted by Ganeshan [160], Wijayasekera [161], and Siddiqui and Dincer [162]. Wijayasekera's [161] comprehensive review evaluates waste-to-hydrogen technologies, addressing performance, greenhouse gas emissions, production costs, and societal acceptance. Siddiqui and Dincer's [162] study specifically compares the efficiency of different biohydrogen production pathways, providing valuable insights into the economic implications of these methods. Ganeshan [160] highlighted the efficiency of gasification, with an impressive carbon footprint reduction of $-1.6 \text{ kg CO}_2\text{-eq./kg H}_2$, indicating not only economic advantages but also environmental benefits.

Operational costs, a crucial component in economic assessments, have also been studied by many researchers. Akhbari [163], for instance, quantifies the operational costs associated with biohydrogen production from palm oil mill effluent (POME), providing a nuanced perspective on the economic feasibility of this specific approach. By considering factors such as electricity usage, these studies offer valuable data that aid in understanding the financial implications of biohydrogen production.

Considering POME-based biohydrogen, specific economic factors must be considered due to process characteristics. The economic sustainability of POME-based biohydrogen production is intrinsically linked to the efficiency in harnessing waste resources, simultaneously addressing environmental concerns associated with POME disposal [163]. For example, the efficiency of upflow anaerobic sludge blanket fixed-film reactors when POME is used for biohydrogen production has key technological aspects associated with economic impact. Factors such as organic loading rates, hydrogen production rates, and acclimatization periods play a crucial role in determining the economic viability of the semi-pilot scale UASFF reactor Akhbari [164].

Akhbari [163] provides several insights, emphasizing the vital role of electricity usage, which constitutes 50–98% of total environmental impacts in POME-based biohydrogen production. These data underline the economic significance of optimizing energy consumption, as evidenced by the quantification of a 77% reduction in global warming impact from 818 to 189 kg CO₂-eq per kg biohydrogen through improvement analysis. Such find-

ings spotlight the economic implications of enhancing process efficiency in POME-based biohydrogen production.

The subsequent study by Akhbari [164] examines the economic details of a semi-pilot scale upflow anaerobic sludge blanket fixed-film (UASFF) reactor designed specifically for POME utilization. This study quantifies essential parameters such as organic loading rates, hydrogen production rates, and acclimatization periods, offering a quantitative basis for assessing the economic viability of the UASFF reactor. With achieved hydrogen content ranging from 20% to 68% and a hydrogen production rate varying between 12 L H₂/d and 39 L H₂/d, coupled with a maximum COD removal of 35%, this study provides quantitative data for evaluating the economic efficiency of POME as a raw material for biohydrogen production.

Wee [79] offers a comprehensive exploration of the economics involved in biohydrogen production, particularly emphasizing different pathways. In their analysis, the Total Fixed Capital Investment (TFCI) for the production plant is quantified at US\$ 12,745, with 71.71% attributed to total direct costs and the remaining 28.29% to total indirect costs. Breaking down the direct costs, US\$ 9140 is allocated to total direct costs, while US\$ 3605 represents total indirect costs. Additionally, a working capital investment of US\$ 2250 has been identified, bringing the Capital Expenditure (CAPEX) to approximately US\$ 15,000. The original values originally presented in RM were converted to US\$ here, considering the exchange rate in 2022 which was approximately US\$ 1.00 = RM 9500.00. Moving to operational aspects, the Total Fixed Operating Cost (OPEX) is calculated in detail, amounting to US\$ 1860, complemented by a total variable operating cost of US\$ 3670. The general plant overhead cost is US\$ 650 million, contributing to a total OPEX of US\$ 6180 [79]. These quantifications provide a detailed breakdown of the financial considerations involved in the operational phase of biohydrogen production.

Regarding revenue generation, Wee [79] analyzes the sensitivity of key economic parameters. The selling price of hydrogen is crucial in this context, with values of US\$ 6630, US\$ 8285, and US\$ 9945 per kg identified for worst, base, and best-case scenarios, respectively. In the best-case scenario, the annual revenue of US\$ 9580 is projected, accompanied by a gross profit of US\$ 3695 per year. The plant demonstrates an average cash flow of US\$ 2985 annually, with an estimated payback period of 5 years and a Return on Investment (ROI) of 33.18% in 20 years.

Furthermore, the study reveals the impact of a 20% increase in hydrogen selling prices. In this scenario, the gross profit suffers a 76% increase, reducing the payback period by 43% and enhancing the ROI by 79.5% [79]. These quantitative insights show the great level of correlation between economic parameters, offering a robust basis for evaluating the economic viability and sustainability of biohydrogen production pathways from POME.

In another study, a comprehensive examination of the Total Fixed Capital Investment (TFCI) is undertaken, shedding light on the overall financial commitment involved in producing biohydrogen from POME. The study establishes a foundational understanding of the capital investment required for POME-based biohydrogen production [165]. A subsequent study [166] offers further insights regarding Capital Expenditure (CAPEX) and Operating Expenditure (OPEX), exploring essential aspects related to investments and ongoing expenses, contributing to the overall economic evaluation.

Mahmod [167] performed a sensitivity analysis, which added depth to the economic evaluation. The study quantifies the impact of varying factors, such as hydrogen selling price and production-related costs. For instance, with a 20% increase in hydrogen selling price from its base value, the gross profit experiences a significant rise of 76%. The payback period is shortened by 43%, while the Return on Investment (ROI) is enhanced by 79.5%.

These quantified findings from Mahmod's collective studies [164,166,167] offer a precise and tangible exploration of the economic viability of biohydrogen production from POME. The integration of TFCI, CAPEX, OPEX, and sensitivity analyses provides a solid basis for informed decision making, emphasizing the potential for sustainable and economically viable biohydrogen production pathways.

The economic evaluation of biohydrogen production becomes more robust when coupled with sustainability metrics. Life Cycle Assessment (LCA) studies, such as the one conducted by Akhbari [163], offer quantifiable insights into the environmental implications of biohydrogen production from palm oil mill effluent (POME). In this study, the reduction in global warming potential is a quantifiable indicator of the sustainable aspects integrated into economic analyses. Specifically, the research quantifies a substantial 77% reduction in global warming impact, lowering it from 818 to 189 kg CO₂-eq per kg biohydrogen. Additionally, the potential for further reduction to 54.9 kg CO₂-eq is highlighted when considering the environmental burden avoided due to POME utilization in biohydrogen production. These reductions highlight the synergies between economic and environmental facets, emphasizing the importance of adopting sustainable practices in biohydrogen production [163].

8. Conclusions

Biohydrogen production from wastewaters such as POME is an alternative to the current production method, based mainly on fossil fuels. Furthermore, it plays an important role in the circular economy of the palm oil industry and the transition of the world energy matrix. Although the production of hydrogen from POME is promising, there are some challenges to be addressed. The main aspects highlighted and discussed in detail in the topics of this article involve the types of POME pretreatments, production processes and parameters, bioreactor models, and strategies to improve biohydrogen yield. Due to its composition rich in cellulose and hemicelluloses, POME is difficult to biodegrade and, consequently, to produce biohydrogen.

Many pretreatments have been proposed to increase the availability of organic POME components. With acid and alkaline hydrolysis of POME, higher yields of hydrogen can be achieved compared to untreated POME, and up to a 90% increase in hydrogen production can be obtained. Likewise, the enzymatic pretreatment of POME appears as an option that favors the production of hydrogen from dark fermentation. Increased yields of up to 2.5 times have been reported. Dark fermentation is currently the most explored biological method to produce biohydrogen from POME. In addition to being considered a low-complexity process, the bioreactors used are smaller and cheaper when compared to processes that require illumination. Another strategy to improve hydrogen production is integrating processes such as two-stage hydrogen production with dark fermentation followed by photofermentation. Photofermenting bacteria can use the residual substrate of dark fermentation; since these microorganisms use organic acids during the process, hydrogen yield could be significantly increased. Despite the advantages of dark fermentation, the low yield of hydrogen (1 to 3 mol H₂/mol glucose) can make its industrial production unfeasible. The search for improving biohydrogen productivity is still the subject of much research, which proposes the addition of nanoparticles, using genetic engineering and cell immobilization. As hydrogen production in dark fermentation depends directly on the activity of enzymes such as hydrogenases, the presence of nickel and iron is essential for hydrogen-producing bacteria. Using metallic nanoparticles acting as enzyme cofactors can double hydrogen production. Considering the potential of biohydrogen as a sustainable energy source, the economic assessment of its production is an essential step. The use of POME increases the need for personalized economic analyses considering the unique characteristics of this wastewater. The economic sustainability of POME-based biohydrogen production is intrinsically linked to the efficiency in utilizing waste resources while simultaneously addressing the environmental concerns associated with POME disposal.

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