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Review

Integrated System Technology of POME Treatment for Biohydrogen and Biomethane Production in Malaysia

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Abstract: In recent years, production of biohydrogen and biomethane (or a mixture of these; biohythane) from organic wastes using two-stage bioreactor have been implemented by developing countries such as Germany, USA and the United Kingdom using the anaerobic digestion (AD) process. In Thailand, biohythane production in a two-stage process has been widely studied. However, in Malaysia, treating organic and agricultural wastes using an integrated system of dark fermentation (DF) coupled with anaerobic digestion (AD) is scarce. For instance, in most oil palm mills, palm oil mill effluent (POME) is treated using a conventional open-ponding system or closed-digester tank for biogas capture. This paper reviewed relevant literature studies on treating POME and other organic wastes using integrated bioreactor implementing DF and/or AD process for biohydrogen and/or biomethane production. Although the number of papers that have been published in this area is increasing, a further review is needed to reveal current technology used and its benefits, especially in Malaysia, since Malaysia is the second-largest oil palm producer in the world.

Keywords: dark fermentation; anaerobic digestion; integrated system; biohydrogen; biomethane; POME

1. Introduction

Strategies to produce renewable energy from organic waste have become a high priority topic in any energy, bioconversion, bioresource and sustainability conferences in the world. Conversion of organic and inorganic wastes into useful and valuable end products like biohydrogen, biomethane and bio alcohols are increasingly studied each year as many nations progressively working towards sustainable world development. This is because biohydrogen gas is a clean energy alternative and it acts as a good source of fuel to apply in fuel cells for electricity generation. Meanwhile, biomethane, another clean energy alternative for electricity and transportation, is produced from the anaerobic digestion process. Bio alcohols that include biomethanol and bioethanol that were produced by the action of enzymes and microorganism through fermentation would also be used as fuels for internal combustion engines.

Renewable energy is an energy that can be replaced, sustainable and does not harm the environment as it is derived from non-nuclear and non-fossil sources [1]. Due to its high energy efficiency, hydrogen (H_2) is considered one of the preferable biofuels among various renewable energy sources [2]. It is considered the best and most effective fuels for transportation. This is because, when H_2 is combusted (only water vapor is produced with the absence of carbon monoxide (CO), the energy yields are 2.75 times higher (122 kJ/g) than hydrocarbon fuels [3,4]. This can minimize environmental problems and makes H_2 a future fuel, which has drawn significant attention to the world.

Various biotechnologies such as dark fermentation (DF) can be used to generate H_2 in a green and environmental-friendly way using renewable resources [5,6]. Theoretically, DF is a bioprocess whereby H_2 is produces by microorganisms (i.e., anaerobic bacteria) from organic wastes or wastewaters. Through the activities of fermentative hydrogen producing-bacteria (HPB; obligate anaerobes and facultative anaerobes), the DF process could utilize various types of wastewaters and organic wastes as a feedstock to produce H_2. The fermentative conversion of organic wastes in the DF process involves similar biochemical pathways as in anaerobic digestion (AD) for methane production. As compared to photo-fermentation, the DF process is independent on weather conditions and produce relatively higher H_2 production rate. The other type of fermentation that can use organic wastes is lactic acid fermentation. Compared to the DF process, one mole of glucose will produce two moles of lactic acid under a simple redox reaction with no production of gas as a byproduct.

On the other hand, in the AD process, organic materials were converted into biogas, nutrients and some refractory organic matter under anaerobic condition by a mixture of symbiotic microorganisms [7]. AD consists of four steps, *viz*. hydrolysis, acidogenesis, acetogenesis and methanogenesis. Lactic acid fermentation only involves the first two steps [8]. The AD process could reduce pollution and odor as well as produce renewable energy in an effective waste treatment due to the microbiological conversion. Compared to fossil fuels, renewable methane does not contribute to carbon dioxide (CO_2) emissions in the atmosphere [9].

The palm oil industry in Malaysia is still progressing after developing over the years [10]. It contributes largely to the country's foreign exchange earnings and increased Malaysian lifestyle [11]. In 1917, the oil palm cultivation begins at very slow growth. The plantation developed rapidly only after the last 50 years through cultivation large-scale investment in order to diversify the country's agricultural development [12]. Back then, Malaysia is known as the main producer of cocoa, rubber and coconuts. An inclination for oil palm has prompted a quick extension of its planted regions to the detriment of rubber and different products in the course of the most recent four decades. Oil palm land area has increased within 45 years from 54,000 hectares (1960) to 4.05 million hectares (2005) [12]. This shows the successfulness of the oil palm industry in Malaysia as well as the contribution to global food sources.

Palm oil mill effluent (POME) is the main pollutant produced in palm oil mills in Malaysia. For one ton of crude palm oil processing, it is estimated that 3.05 m^3 of POME is produced [13]. If there is no proper effluent management, POME will be the main source of air and water pollution in the future. POME contains a high nutrient, organic and carbon contents despite having high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) content (Table 1). It also possesses huge potential for the production of biogas [14]. During POME decomposition of organic matters, there are 60%-70% of methane and 30%–40% of CO_2 produced, with the rest consists of a trace amount of H_2S [15].

Table 1. Characteristics of palm oil mill effluent (POME).

Parameter	Unit	Raw POME	Digested POME	References
		Mean ± S.D. *	Mean ± S.D. *	
pH	–	4.3 ± 0.28	7.4 ± 0.05	[15–18]
Volatile fatty acids (VFAs)	mg L^{-1}	470 ± 240	678.83 ± 166.47	[15,19,20]
Chemical oxygen demand (COD)	mg L^{-1}	53,450 ± 10,350	83,800 ± 11,000	[16,21–23]
Total suspended solids (TSS)	mg L^{-1}	29,000 ± 6000	10,200 ± 2500	[22–24]
Suspended solids (SS)	mg L^{-1}	23,600 ± 4400	4126	[22,25]

Table 1. *Cont.*

Parameter	Unit	Raw POME	Digested POME	References
Oil and grease	mg L^{-1}	7000 ± 550	183 ± 10.1	[21,22,26]
Ammonium nitrogen (NH_3–N)	mg L^{-1}	63 ± 24	25 ± 5	[22,23,25]
Biochemical oxygen demand (BOD)	mg L^{-1}	28,000 ± 6750	19,000 ± 5500	[21,22,26,27]

* S.D. = standard deviation

In 1978, Environmental Quality Regulations enactment was proposed for POME discharge standards with the focus on BOD. From 25,000 mg L^{-1} of untreated POME, the discharge standard limit was reduced to 5000 mg L^{-1} in the first generation, down to the current BOD of 100 mg L^{-1} [14]. Initiatives are in the progress to decrease the BOD level to 50 mg L^{-1}, and in places where release into conduits is required. Research and Development (R&D) is effectively sought after to decrease the BOD load to 100 mg L^{-1}. Table 2 represents POME discharge standards starting from 1978 until 2015 [28].

The palm oil and rubber mills effluent discharge standard were first introduced by Malaysia. In 1977, the Department of Environment (DoE) announced the discharge standard for POME. Before the regulation was implemented by all palm oil mills, crude palm oil seemed to be the worst main source of pollution. The daily discharge was more than 300% increased from 1965 until 1977. Hence, the regulation was made in order to reduce pollution without hindering the growth of oil palm industries.

Table 2. POME discharge limit from 1978 to 2015 and thereafter [29].

Parameter	Limits Required Based on the Period of Discharge						
	1 July 1978–30 June 1979	1 July 1979–30 June 1980	1 July 1980–30 June 1981	1 July 1981–30 June 1982	1 July 1982–31 December 1983	1 January 1984–2015	Future Standard Discharge Limit (2015 Onwards)
pH	5–9	5–9	5–9	5–9	5–9	5–9	5–9
Temperature (°C)	45	45	45	45	45	45	45
Oil and Grease (mg L^{-1})	150	100	75	50	50	50	5
Total Solids (mg L^{-1})	4000	2500	2000	1500	-	-	-
Suspended Solids (mg L^{-1})	1200	800	600	400	400	400	200
Total Nitrogen (mg L^{-1})	200	100	75	50	-	-	150
Ammonium Nitrogen (mg L^{-1})	25	15	15	10	150	100	-
COD (mg L^{-1})	10,000	4000	2000	1000	100	100	-
BOD (mg L^{-1})	5000	2000	1000	500	250	100	20

POME Treatment Systems in Malaysia

The anaerobic process has become the most suitable method in treating POME due to its high organic properties. The high concentration of lipid, nitrogenous compounds, carbohydrates, protein and minerals in POME can be converted to valuable products by using microbial process [30]. Due to that, treating POME using the ponding system has been used in an earlier stage for the palm oil industry.

Despite the fact that POME is non-lethal, there is a concern that economic expansion, environmental protection and sustainable development need to be balanced due to the fact that POME is a potential cause of pollution [31]. To ensure that this industry remains sustainable and environmentally friendly, POME needs to be managed properly and cannot directly be discharged into a water body as it can contaminate the water and endanger the aquatic ecosystem [32].

Therefore, a lot of studies have been done by researchers to treat POME using alternative methods. This is because conventional methods such as the aerobic/anaerobic system, open decomposing tank, anaerobic system, closed anaerobic decomposition tank and advanced ventilation system requires extensive land area and producing a foul odor, which results in environmental pollution [33,34].

Due to the presence of untreated palm oil residue, raw POME consists of a high value of degradable organic matter [35]. Biological treatment with the aerobic, anaerobic or facultative process is the most suitable method to degrade/treat POME. This is because biological treatment requires less energy demand, does not liberate foul odor, can minimize sludge accumulation and can produce hydrogen and methane gas by anaerobes under fermentation and digestion processes. Moreover, methane gas produced can further be used for electricity generation.

However, the open ponding system could cause methane gas being released into the atmosphere. This contributes to the thinning of the ozone layer that resulted in the greenhouse gas (GHG) effect. Even though less operational energy and small capital are required, an open ponding system involves a longer retention time (20–60 days) and large area [13]. The implementation of a closed anaerobic system has drawn many changes towards the regulatory standard. It was reported that covered lagoon or closed-tank anaerobic digester has been widely used to treat POME [36]. Table 3 shows different methods studied to treat POME, especially in Malaysia, in order to get the highest removal of BOD, COD and suspended solids (SS) and/or total suspended solids (TSS) as possible.

On the other hand, a hybrid system that combines the conventional and alternative methods such as the anaerobic filter [37], up-flow anaerobic sludge blanket (UASB) [38], sequencing batch reactor (SBR) [39], up-flow anaerobic sludge fixed-film reactor (UASFF) [40] and anaerobic fluidized bed reactor (AFBR) [41] were studied and used to obtain higher efficiency and ensure lesser treatment time. These hybrid reactors were proven to reduce hydraulic retention time (HRT) when studied on a laboratory scale.

Table 3. Different methods studied for POME treatment in Malaysia.

Treatment Method Used	Parameters			References
	BOD removal (%)	COD removal (%)	Total suspended solids/suspended solids removal (%)	
Lab scale				
Using biosorbent	97.41	100	100	[42]
Ultrasonic-assisted membrane anaerobic system	74	95	91–99.5	[43]
Attached growth on rotating biological contactor	91	88	89	[44]
Large scale				
Anaerobic expanded granular sludge bed (EGSB)	88.24	94.89	64.65	[45]
Combined high-rate anaerobic reactors	-	93.50	>90	[46]
Ultrafiltration membrane	86.33	85	99.86	[47]
Activated carbon as bioadsorbent	83	68	90	[48]
Green synthesis	-	94.70	51.50	[49]

Above all, all palm oil millers must meet the standard requirement provided by the Malaysian DoE. The transition of the treatment method makes conventional POME treatment system becomes outdated and the new requirement for BOD discharge limit of 20 mg L^{-1} seems hard to be fulfilled by the respective mills. However, a lot of POME treatment technologies have been studied as an alternative to the above-mentioned problem.

Despite the importance of DF and AD process for biohydrogen and biomethane production, there has been extensive research on two-stage DF or AD for biohydrogen and biomethane production using different bioreactor configurations. However, combining the DF and AD process for biohydrogen and biomethane production utilizing POME is uncommon. Therefore, the major aim of this paper is to highlight current integrated systems treating POME. The sole purpose of this review is to highlight the importance of POME and how an integrated system could turn POME into valuable end products. This review will not focus on various aspects of POME polishing, processing and purification and its treatment methods using different bioreactor configurations as they have been reviewed previously [50,51].

2. Biogas Production from POME

About 53 million tons of palm oil production and 13 million tons of empty fruit bunches were recorded annually in Malaysia [52]. This phenomenon has pulled in researchers and investigators to deal with energy production from POME [53]. To date, most oil palm processes in Malaysia have applied POME as a feedstock for biogas generation [54]. Production of biohydrogen using digested POME as inoculum was examined by Mamimin et al. using the anaerobic sequencing batch reactor (ASBR) [55]. The impacts of hydraulic retention time (HRT), temperature and organic loading rate (OLR) were explored for process stability in ASBR in a continuous process. In their study, they used a thermophilic condition (60 °C) to enhance biohydrogen production. At the end of the experiment, they found out that there was a significant increase in biohydrogen production under thermophilic condition, as compared to mesophilic temperature (37 °C). This is because thermophilic bacteria were present in POME sludge (inoculum) due to a long adaptation time, thus making it more favorable for biohydrogen production.

Different studies on the effects of volatile fatty acids (VFAs) [56], pH [57] and organic loading rate (OLR) [58] using POME were also done, either using single stage or integrated reactors. Studies using a single-stage reactor revealed that the biogas production rate could be accomplished at a HRT of 1.5 days and the system was capable to effectively treat POME [40]. On the other hand, several researchers also reported higher efficiency in energy recovery using an integrated system as compared to a single-stage process, as well as increased process stability [59]. These findings showed that an integrated system using a two-stage bioreactor is better in terms of COD removal efficiency, stability and gives a significant impact on biogas production and yield, in comparison to a single-stage reactor.

Challenges Using POME Wastewater

Raw POME is composed of lignocellulosic material types that make it hard to degrade. A biological pre-treatment, either using specific bacteria or mixed, will take a longer time compared to using the chemical pre-treatment. A study on the pre-treatment of brewery seed sludge for biohydrogen production using raw POME as a substrate was done with the end goal to determine the best pre-treatment strategy for biohydrogen efficiency [60]. Among all the studied strategies, heat-shock pre-treatment was found to produce the highest cumulative hydrogen with highest COD removal efficiency. This is because homoacetogens in the seed sludge (inoculum) had been suppressed during the heat-shock, thus enabling hydrogen-producing bacteria (HPB) to grow.

Mohammadi et al. uncovered that the obtained results were higher than the study done by Mohan et al. using dairy wastewater as a substrate, regardless of the pre-treatment method used [61]. It shows that even though the hydrogen production using raw POME is not as high as another study [62], but this carbohydrate-rich material contains a large amount of starch, simple sugars and cellulose, therefore makes it a suitable substrate for biogas production, especially in Malaysia. Considering the above matter, dark fermentation is clearly the key innovation for producing hydrogen from agricultural wastes. Such wastes, which are complex substrates, can be biologically degraded by complex microbial ecosystems. Furthermore, biological pre-treatment is preferable as it is much cheaper compared to chemical pre-treatments.

Meanwhile, Khemkhao et al. were reported on having a long start-up period using POME [63]. They needed 123 operating days for microbial adaptations and to evaluate the performance of a single-stage up-flow anaerobic sludge batch (UASB) reactor during a temperature shift. This is on account that the UASB reactor can treat high-strength wastewater that contains high levels of suspended solids and deliver a high measure of biomethane. However, a study done by Zainal et al. demonstrated that the two-stage anaerobic high-rate bioreactor could abbreviate the start-up period to just about two months for biohydrogen and biomethane generation [18]. Using a two-stage up-flow anaerobic sludge fixed-film (UASFF) bioreactor, they found out that the start-up period could be shortened by initially acclimatizing the digested POME and using fresh raw POME as a substrate. However, the up-flow velocity in the bioreactor, influent and effluent flow rate, as well as the internal packing material play important roles for the reactor stability and efficiency.

In Malaysia, the current situation does not prepare for the implementation of biohydrogen production technology from POME. The main problems lead to the constraints of up-scale biohydrogen production, which are the HRT, storage and safety problems, and the reactor engineering [64]. However, the conventional POME treatment does require wide land area, longer HRT, mass sludge production and low treatment effectiveness. Therefore, the inexpensive high-rate anaerobic treatment, together with the steady and well-organized bioreactor (in terms of biogas capture), raises an important consideration for oil palm industries.

3. Biohydrogen Production via Dark Fermentation (DF)

Hydrogen is naturally produced by varieties of organisms under anaerobic conditions. Dark fermentation is known to be involved in hydrogen production while dark fermentative microorganisms are those associated with the process. These microorganisms can be distinguished based on their sensitivity to temperature and oxygen. Obligate anaerobes are those that favor anaerobic conditions while facultative anaerobes are those that can survive in both aerobic and anaerobic environments.

Pure microbial species or mixed cultures can both produce hydrogen. In that community, some of the microorganisms can act as hydrogen-producing bacteria (HPB) while some may act as hydrogen-consuming bacteria (HCB) for their energy. In most biohydrogen studies, researchers were using either mixed cultures or pure culture in a laboratory or scale-up bioreactor [65,66].

3.1. Dark Fermentative Bacteria

3.1.1. Obligate Anaerobic Bacteria

Obligate anaerobic bacteria are used in most biohydrogen studies because of their ability to utilize the various types of wastewaters and carbohydrates. In addition, they are also able to produce a higher rate of hydrogen production, compared to facultative anaerobes. Hydrogen production mainly occurs during the exponential growth phase. During the stationary phase, microorganism metabolism is shifted from hydrogen/acid production to solvent production [67].

3.1.2. Mixed Cultures

Mixed cultures are normally applied when the complex substrate is used, for example, raw POME. Mixed cultures can boost substrate consumption compared to using pure cultures. It is also reported that pure cultures are easily contaminated with hydrogen consuming bacteria (HCB) [68]. Compared to mixed cultures, the operation in industries is normally under nonsterile conditions as they have been designated for growth and dominance. Therefore, this makes them robust to environmental changes such as temperature and pH.

The choice of mixed cultures for hydrogen production as inocula can be obtained from anaerobic digester of municipal sewage, sludge from digested POME of an anaerobic pond or fermented soybean meal. However, the presence of methanogens or HCB becomes a major bottleneck in selecting these mixed cultures. Therefore, in some cases, several researchers will pretreat these mixed cultures in order to suppress the activity of methanogens and remove HCB [69,70]. At high temperature, mixed cultures would be favourable to reaction kinetics, thus, contamination by HCB could be avoided [71].

3.1.3. Thermophiles

Most thermophiles are obligate anaerobes. Thermophiles can utilize various types of substrates such as lignin, hemicellulose and cellulose, as well as pectin-containing biomass [72]. According to O-Thong et al. in their study treating POME under thermophilic conditions, nutrient addition helped in promoting the growth of HPB, i.e., *Thermoanaerobacterium thermosaccharolyticum* [73]. Other studies include thermophiles for hydrogen production, which are *Thermoanaerobacterium* sp. [74], *Caldicellulosiruptor saccharolyticus* [75] and *Thermotoga* sp. [76].

3.2. Biochemistry of Dark Fermentation

Under anaerobic condition, the fermentation (metabolic) process occurs to regenerate the cell's energy currency (ATP). The tricarboxylic acid cycle is also blocked under this condition. When reduced metabolic end products (e.g., alcohol and acids) formed, fermentation will dispose of the excess cellular reductant. Similarly, the cellular redox potential is maintained by the production of hydrogen that acts as a reduced metabolic product as well.

For the fermentation process, carbohydrates are the preferred carbon source that contains mainly glucose, which can predominantly increase acetic and butyric acids along with hydrogen gas. Hydrolysis will convert complex organic polymers to glucose. Glucose will then produce pyruvate to generate ATP via the glycolytic pathway. Subsequently, pyruvate may be involved in the formation of hydrogen in two different biochemical reactions [77].

Pyruvate will be oxidized to acetyl coenzyme A (acetyl-CoA) in *Clostridia* [78] as obligate anaerobes and thermophilic bacteria [79] by pyruvate-ferredoxin oxidoreductase [80]. Next, acetyl-CoA will be converted to acetyl phosphate, along with the production of ATP and acetate. Reduction of ferredoxin (Fd) is required for the oxidation of pyruvate to acetyl-CoA. [Fe-Fe]-hydrogenase will oxidize the reduced Fd and catalyzes H_2 formation. The overall reaction is shown in the equations below.

$$\text{Pyruvte} + \text{CoA} + 2\text{Fd(ox)} \rightarrow \text{Acetyl} - \text{coA} + 2\text{Fd(red)}\text{CO}_2 \tag{1}$$

$$2\text{H}^+ + \text{Fd(red)} \rightarrow \text{H}_2 + \text{Fd(ox)} \tag{2}$$

When pyruvate is oxidized to acetate as the sole metabolic end product, four moles of hydrogen per mole of glucose is formed [81]. However, when pyruvate is oxidized to butyrate, only two moles of hydrogen produced per mole of glucose. Therefore, in the mixed acid pathway, a higher acetate to butyrate ratio is critical for higher hydrogen production [82]. Overall biochemical reaction with acetic and butyric acid as metabolic end products is shown in the next equations, respectively.

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

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \tag{4}$$

4. Biomethane Production via Anaerobic Digestion (AD)

Biochemistry of Anaerobic Digestion

In the absence of oxygen, a process by which microorganisms will breakdown biodegradable material is called anaerobic digestion. Since it can provide a significant reduction in the mass of the input material (substrate), therefore, anaerobic digestion is mostly used for wastewater treatment or any organic wastes.

In the anaerobic digestion process of organic polymeric materials, there are seven sub-processes involved [83]. Complex organic materials will be hydrolyzed at first, followed by fermentation of amino acids and sugars in the second phase. The oxidation process will occur next in long-chain fatty acids and alcohols. In the fourth phase, short-chain fatty acids take place in anaerobic oxidation (except acetate), followed by the production of acetate from carbon dioxide and hydrogen in the fifth phase. Then, acetate will be converted to methane. Finally, methane will be produced by carbon dioxide and the hydrogen reduction process [83].

However, even though there are seven sub-processes involved, the principle of bacteria classes is only divided into three categories [83];

I—Bacteria that is responsible for hydrolysis. These bacteria hydrolyzed the substrate and breakdown the insoluble organic polymers (e.g., carbohydrates) and make them accessible for other bacteria.

II—Acid-producing bacteria. There are two acid-producing bacteria involved in this pathway. The first one is acidogenic bacteria while the other is acetogenic bacteria. The former will convert sugars and amino acids into CO_2, H_2, ammonia and organic acids while the latter will then convert the produced organic acid into acetic acid (along with ammonia, H_2 and CO_2).

III—Methane-producing bacteria. In the end, these bacteria convert the products into CH_4 and CO_2. Methane formation is strictly under anaerobic condition in this phase and the reaction is exergonic. It is also reported that not all methanogens will degrade the substrates [84].

Meanwhile, substrates that are acceptable for the methanogenesis process are divided into three groups as mentioned below:

$$\text{(i) Acetoclastic methanogenesis will convert acetate to } CH_4 + CO_2 \tag{5}$$

$$\text{(ii) Hydrogenotrophic methanogenesis will convert } H_2 + CO_2 \text{ to } CH_4 \tag{6}$$

$$\text{(iii) Methylotrophic methanogenesis will convert methanol to } CH_4 + H_2O \tag{7}$$

There are two biochemical components for methanogens that makes them unique; the mechanism of H_2 oxidation and CO_2 reduction. Methanogens will utilize H_2 with acetate, formate, CO_2 and methanol as substrates under the methanogenesis process [79]. Next, they will use CO_2 as the thermal electron acceptor before producing CH_4 [84].

5. An Integrated System as an Innovative Approach for Biohydrogen, Biomethane Production and Wastewater Treatment

A large amount of water was consumed during palm oil mill processing. This contributed to the mass production of POME wastewater that leads to water contamination because of its high BOD and COD content. However, through anaerobic digestion, POME has become one of the potential and valuable sources of bioenergy, viz. biohydrogen and biomethane. Every oil palm industry in Malaysia should consider having a renewable and sustainable bioenergy strategy, as well as the in-house wastewater treatment system [85]. The production of methane and CO_2 by the action of active microorganism requires multi-stage processes for organic matter degradation, i.e., hydrolysis, acidogenesis, acetogenesis and methanogenesis [41].

During the early stage of hydrolysis and acidogenesis, acid-forming bacteria will convert fresh raw POME to volatile fatty acids (VFAs), before being converted to CH_4 and CO_2 in methanogenesis under the anaerobic digestion process [86]. This will lead to the formation of biohydrogen and biomethane from POME, which helps in stabilizing the system through sludge diminishing. Currently, anaerobic digestion systems are springing up like a mushroom. For POME, the most recommended digestion process includes UASB, UASFF, an anaerobic sequencing batch reactor (ASBR) and a continuous stirred tank reactor (CSTR) [87]. Tables 4 and 5 show some comparison studies using a single-stage bioreactor and integrated bioreactor for POME treatment, respectively, while Table 6 summarized the preferences and drawbacks of each bioreactor.

Table 4. Different studies on POME treatment using a single-stage bioreactor for biohydrogen/biomethane production.

Inoculum	Bioreactor	Organic Loading Rate (OLR; g L^{-1} d^{-1})	Temperature (°C)	HPR (L H_2 L^{-1} d^{-1})	MPR (L CH_4 L^{-1} d^{-1})	COD Removal (%)	References
Digested POME	500 mL serum bottle	4.96	37	5.99 ± 0.5	-	42	[65]
Digested POME	UASFF	9.43	50	-	4.40	94	[17]
Digested POME	CSTR	25	55	1.16	-	<30	[88]
Digested POME	UASFF	51.8	38	4.61	-	40–54	[89]
Digested POME	50-L UASB	500–1000	30–35	-	992	>90	[54]

Table 5. Comparison studies of dark fermentation coupled with anaerobic digestion for biogas production from POME using integrated systems.

Inoculum	Integrated System Used	Organic Loading Rate (OLR; g L^{-1} d^{-1}) H_2 CH_4	Temperature (°C) H_2 CH_4	HPR (L H_2 L^{-1} d^{-1})	MPR (L CH_4 L^{-1} d^{-1})	COD Removal (%) H_2 CH_4	References
Anaerobic seed sludge	DF–AD (UASB–CSTR)	75 30	55 37	1.92	3.20	42 94	[90]
Decanter cake	DF–AD (two-stage batch fermentation system)	60 g VS $L^{-1}d^{-1}$ 60 g VS $L^{-1}d^{-1}$	60 60	1.46 [a]	51.59 [a]	-	[91]
POME sludge	DF–AD (UASFF–UASFF)	20 varies	43 43	5.29	9.60	26 79	[18]
POME sludge	DF–AD (ASBR–UASB)	60 6	55 35	1.804	2.60	38 95	[92]
POME sludge	DF–AD (CSTR–UASB)	14.3 g VS $L^{-1}d^{-1}$ 1.58 g VS $L^{-1}d^{-1}$	55 35	3.80	14.00	93	[93]

UASFF—up-flow anaerobic sludge blanket (UASB)-fixed film (FF); AD—anaerobic digester; ASBR—anaerobic sequencing batch reactor; POME—palm oil mill effluent; [a] m^3 $tonne^{-1}$ waste d^{-1}.

Table 6. Advantages and disadvantages of the anaerobic treatment system commonly used for POME treatment using different bioreactor configurations.

Anaerobic Treatment System	Advantages	Disadvantages	References
UASB	High COD removal efficiency and CH_4 production rate	High dependable on sludge settling property	[41]
UASFF	Higher biomass retention, a shorter start-up for sludge granulation	Reactor stability and efficiency depend on the feed flow rate, internal packing, up-flow velocity and effluent recycle ratio	[94]
ASBR	Simple operation, flexible and no separate clarifiers needed.	Low treatment capability under higher OLR	[73]
CSTR	Inexpensive and easy to handle	Poor gas production under high OLR and short HRT	[95]

Theoretically, the same mechanism of fermentation and anaerobic digestion applied in treating different organic wastes, regardless of the types of bioreactor used. However, the characteristics of the microbes, bioreactor configurations, conditions used and process parameters might affect the growth of hydrogen-producing bacteria (HPB), reactor stability, pH and temperature in the bioreactor. Extensive research has been done using an integrated system for biohydrogen and/or biomethane production using various lignocellulosic wastes. The studies conclude that in comparison to a single-stage, the two-stage system could increase energy yield [92], stabilize hydrolysate and improve energy recovery [96,97], stabilize using a high organic loading rate [41] and achieve operational stability [98]. The economic benefits of the waste treatment also could be improved by having a phase separation for H_2 and CH_4 in the respective systems [99].

Studies on biohydrogen/biomethane using an integrated system in Malaysia are also increasing every year [100,101]. This review is important for researchers and industries despite studies treating POME using the integrated system that is new in Malaysia. This is because anaerobic digestion is the cheapest technology for biohydrogen/biomethane production compared to photo-fermentation or electro-hydrogenases. The integrated process of DF–AD can also be applied for different organic wastes such as food wastes for hydrogen/methane production in Malaysia.

Meanwhile, Table 7 shows studies done in different countries treating organic wastes for biohydrogen and biomethane production implementing a two-stage system. The big finding of using the integrated system is that either effluent concentration could be reduced (high COD removal efficiency), or two valuable gases could be produced (H_2 and CH_4) or both. Different ways also have been explored by researchers to achieve the highest output as possible, such as re-used DF effluent to increase methane production [102] and hydrogen production [103], recirculation of digested sludge to shorten HRT and reactor stability [104], finding optimum process parameters [18] and studied the role of microorganisms during the digestion process [105].

Table 7. Different organic wastes used for biohydrogen and biomethane production using integrated systems.

Types of Waste	Inoculum	Integrated System Applied	Organic Loading Rate (OLR; g L^{-1} d^{-1}) H_2 CH_4	Temperature (°C) H_2 CH_4	HPR (L H_2 L^{-1} d^{-1})	MPR (L CH_4 L^{-1} d^{-1})	COD Removal (%) H_2 CH_4	References
Organic Fraction of Municipal Solid Waste	Waste activated sludge	DF–AD (CSTR–CSTR)	16 kg TVS m^3 $^{-1}$ d^{-1} 3 kg TVS m^3 $^{-1}$ d^{-1}	55 55	0.43 ± 0.04	0.60 ± 0.09	43 52)	[105]
Garbage slurry and shredded office papers	Seed microflora	DF–AD (CSTR–Packed Bed Reactor)	97,000 15,700	60 55	5400	6100	- 79	[106]
Food waste from organic fraction municipal solid wastes (OFMSW)	Anaerobic digester sludge	DF–AD (Semi-continuous mode)	39 4.16	55 55	11.1	47.4	90 85	[107]
Sugarcane syrup	Brewery UASB granules	DF–AD (CSTR–ABR*)	2167 10,773	35 35	7.53	75.6	69 94	[108]
Coffee drink manufacturing wastewater (CDMW)	Anaerobic seed sludge	DF–AD (UASB–UASB)	80 3.5	55 35	101.76	2.06	50 93	[109]

* ABR—Anaerobic baffle reactor

6. Importance of Biohydrogen and Biomethane

Application of biohydrogen and biomethane, or the mixture of these, biohythane, has become an increasing interest for the industries as alternative renewable energy. Currently, an increment in energy demand and continuous usage of fossil fuels is vulnerable by the concerns of global warming due to the increase of carbon dioxide (CO_2) released in the climate [110]. Hydrogen is a high presence in nature, contrasted with fossil fuel [111]. When burning the biohydrogen, water is produced as a by-product, which left the hydrogen, which has higher calorific value due to its higher energy value [112]. This high energy (heating) value (142 kJ g^{-1}) makes biohydrogen applicable for combustion engines. Pure biohydrogen can produce electricity in fuel cells. These criteria make hydrogen the most environmentally friendly and an ideal alternative to fossil fuels [113]. For the future energy economy, hydrogen has become a key energy trajectory [114].

Attentions have been focused on the fuel cell efficiency and technology for hydrogen storage for transport applications to meet commercial viability, by having a clean environment and reducing the pollution [115]. In general, hydrogen is applied in ammonia production [116,117], petroleum refining [118,119] and metal refining (tungsten, copper and lead) [120,121]. Hydrogen is highly used for ammonia synthetization, hazardous waste hydrogenization, desulphurization (e.g., hydrodesulfurization and hydrogenation reactions) and refining, food preparation, chemical plants, rocket fuel and high-temperature industrial furnace fuel [122]. In ammonia production, with 500 billion cubic meters (Bm^3) of hydrogen, 250 Bm^3 of hydrogen is consumed for ammonia production, 65 Bm^3 of other chemical products production and 185 Bm^3 of petrochemistry production [122,123]. Jain reported a significant hydrogen application on cooking food, hydrogen-powered industries, electricity generation, jet planes, fuel for automobiles, hydrogen village and not to forget the domestic requirements [124].

Production of biohydrogen from organic waste is followed by the production of organic acids, which become the source of substrate for methane production [125]. Biomethane has the potential to reduce fossil fuels demanding, for example, coal, oil and natural gas that provided power. In order to improve energy yields from other biofuel production processes (e.g., biohydrogen, bioethanol and biodiesel), biomethane production can be applied together. Digestion technology implementation at municipal, industrial as well as agricultural industries will allow effective distribution and decentralized energy generation [7]. Biomethane also can be produced from bioethanol production industries for electricity or fuel usage. Production of biomethane via anaerobic digestion will produce clean fuel, especially from renewable feedstocks. Instead of producing energy from fossil fuels, biomethane can also act as a source of energy that can reduce the environmental impacts (i.e., global warming and acid rain) [126]. Applications of pure methane in appliances, industries, vehicles and power generation are increasing every year. However, different states of purity can also be applied especially in energy conversion and transportation compared to electricity.

7. Conclusions

The dark fermentation system is cheap and utilizes simple technology for biogas production. It is also applicable to a variety of waste streams. However, with a single-stage dark fermentation for biohydrogen production, it produced large amounts of byproducts with a low COD removal. An integrated system would give higher biogas production rate with a good percent of COD removal efficiency, as compared to a single stage. The two-stage fermentation process is more stable in terms of its processes and resulted in higher energy recovery. A biological method in an integrated system for biohydrogen and biomethane production would pose high capacity, clean and inexpensive methods, is sustainable and is a long-term technology. Various organic wastes could be treated using an integrated system for biohydrogen and biomethane production. Therefore, for a future prospect, a large-scale integrated system should be considered, especially from the agricultural and food and beverages industries in Malaysia. While reducing CH_4 emissions from an open-ponding system used, a clean H_2 and CH_4 could also be simultaneously generated using a biological treatment in an integrated bioreactor.

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