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Dark Fermentative Biohydrogen Production from Palm oil Mill Effluent: Operation Factors and Future Progress of Biohydrogen Energy

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ABSTRACT

Malaysia is one of the largest producers and exporters of palm oil, thus, a large amount of palm oil mill effluent (POME) is generated through this process. POME contributes to environmental pollution if it is not properly treated. This complex effluent consists of colloidal matters and mainly organic components with more than 90% water. Thus, it is useful to be used as a substrate for fermentative processes, including biohydrogen production. Biohydrogen from POME is a renewable source that can potentially serve as an alternative to substitute fossil fuels. The abundance of POME and the rising price of fossil fuels in the global market create a demand for this source of energy. However, the complexity of the substituents in POME makes the optimisation of this effluent as a substrate in dark fermentation a challenge. This review article explores the important parameters that need to be considered for optimal biohydrogen production, such as the bioreactor operational parameters and the microbial consortium. Besides, the potential of metabolic engineering as a tool to overcome the limitations of the microbial strains to metabolise POME for increased

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biohydrogen production was also reviewed. However, further research and development are needed to increase the biohydrogen yield on par with commercial demand.

Keywords: Biohydrogen, dark fermentation, hydrogen-producing microorganisms, palm oil mill effluent

INTRODUCTION

As the availability of fossil fuels is steadily decreasing over the years, a renewable alternative source of energy needs to be considered. Biohydrogen is one of the potential replacements of fossil fuels. Biohydrogen refers to hydrogen that has been generated through biological processes (Kapdan & Kargi, 2006). Biohydrogen is a clean energy source and considered renewable since it can be produced continuously from various renewable sources such as oil palm biomass and food waste (Mohd Yasin et al., 2013). This gas is odourless, colourless, combustible, and nontoxic. Unlike hydrocarbon fuels, hydrogen gas burns cleanly without emitting any environmental pollutants. This is because hydrogen combustion only produces water vapour (H_2O) (Mokhtar et al., 2019).

Table 1 shows the comparison between gasoline, ethanol, methane, and hydrogen, respectively as energy carriers. Biohydrogen is also an interesting biofuel since it is scale-independent and has a high conversion rate to electricity via fuel cells compared to other gaseous fuels (Groot, 2003). According to the International Energy Agency (IEA), in 2018, the cost of hydrogen gas production from renewable sources is approximately USD 3.0-7.5/kg, while the cost of using coal as a feedstock is about USD 1.2-2.2/kg of hydrogen (IEA, 2020). In 2020, it can be seen that the prices of alternative fuels (biodiesel B20 USD 2.36/gallon, biodiesel B99-B100 USD 3.51/gallon) are comparative to hydrocarbon fuels (diesel USD 2.61/gallon and gasoline USD 1.91/gallon) (Energy, 2020).

In order to make the production of biohydrogen more sustainable and cost-effective, the use of renewable resources as the feedstock is more ideal compared to the conventional simple sugars. Thus, in conjunction with a sustainable and environmentally friendly strategy, many researchers have preferred to utilise organic biomass as alternative substrates for biohydrogen production.

The POME generated will be utilised by anaerobic microorganisms in a bioreactor as a substrate to generate biohydrogen through a process called dark fermentation. Thus, to take full advantage of this inexhaustible resource, the efficiency of the dark fermentation process by the microbes needs to be optimised. Therefore, this review also covers the crucial factors that may contribute to the efficiency of biohydrogen production through dark fermentation. This includes microbial limitation, environmental limitation, operational condition, and the application of metabolic engineered microorganisms. The current development and future projection of biohydrogen production from the renewable substrate were discussed thoroughly.

Table 1
Properties of energy carriers; H_2, CH_4, ethanol, and gasoline

Energy Carrier Properties	Hydrogen	Methane	Ethanol	Gasoline
Density, gas (NTP) (kg m^{3-1})	0.0899	0.651	N.A.	N.A.
Density, liquid (kg m^{3-1})	70.8	422.6	789.3	720 -780
Melting point (°C)	-259.1	-182.3	-114.15	-40
Boiling point (°C)	-252.76	-161.15	78.29	N.A.
Lower heating value (MJ kg^{-1})	119.9	50.0	N.A.	44.6
Energy per unit mass (MJ kg^{-1})*	141.9	55.5	29.9	47.4
Energy per unit volume (GJ m^{3-1})	0.013	0.651	23.6	34.85
Flame temperature (°C)	2045	1875	N.A.	2200
Self-ignition temperature (°C)	585	540	423	228-501
Minimal ignition energy (mJ)	0.2	0.29	N.A.	0.24
Ignition limits in air (vol %)	4 – 75	5.3 – 15	4.3 -19	1.0 – 7.6
Flame propagation in air (m s^{-1})	2.65	0.4	N.A.	0.4
Diffusion coefficient in air (cm^2 s^{-1})	0.61	0.16	N.A.	0.05
Toxicity	No	No	No	Yes
N.A. = not available				

Sources: (Najafpour et al., 2015: Xu et al., 2009)

Substrate for Biohydrogen Production via Dark Fermentation

The increasing trend in palm oil production gains the concern of environmental activist groups. This is because palm oil plantation expansion leads to deforestation, causing loss of biodiversity. An increase in palm oil production also reflected the increase in crude palm oil (CPO) production. CPO production becomes an environmental issue due to the massive generation of POME from the process of CPO extraction where a tonne of CPO produces approximately 3.05 tonnes of POME (Singh et al., 2010). POME is the largest wastewater produced and the most problematic environmental pollutant in the palm oil industry (Singh et al., 2010). This complex effluent is viscous, brownish in colour, and consists of colloidal matters, with more than 90% of water. The solids content of POME comprises more than 5% total solids and around 4% suspended solids (Taifor et al., 2017). The POME also has a discharge temperature of 80–90°C.

The pre-treatment of POME is vital before its utilisation, not only as a substrate for biohydrogen production through microbial fermentation but also for the production of various products such as biosolvents (Hipolito et al., 2008), bioacids (Mumtaz et al., 2008) and polyhydroxyalkanoates (PHA) (Hassan et al., 1997). The alkaline-heat supernatant pre-treatment was shown to produce the highest biohydrogen production (2.18 mol H_2/mol total carbohydrate) by POME compared to other pre-treatment like acid (Kamal et al., 2012).

MATERIALS AND METHODS

General Factors that Influence Biohydrogen Productivity: Nutrients

Macronutrients and micronutrients are essential in dark fermentation which includes: carbon and nitrogen sources (Lin & Lay, 2004a), ammonium, phosphate (Lin & Lay, 2004b), sulphur, sulphate (Cheng et al., 2011), iron (Yang & Shen, 2006), and elemental traces (Lin & Lay, 2005). The concentration of these nutrients also influences the growth of microbes and hydrogen production. The ranges of nitrogen concentration around 0.1-2.0 g N/L with a C/N ratio of 3.3 to 130 were found to result in optimal growth.

Besides microbial growth, the efficiency of biohydrogen production also relies on the microbial hydrogenases that are involved in hydrogen metabolism. The most important element that influences the action of hydrogenases is ferredoxin (Chou et al., 2007). This is because iron is important for hydrogenase activity and may deviate the fermentation pathways away from biohydrogen production (Yang & Shen, 2006). Reported that magnesium, sodium, and zinc were reported as the most significant elements for biohydrogen production. The optimum concentrations of elements were (mg/L) 0.25 Zn^{2+}, 4.8 Mg^{2+}, 1 Fe^{2+} and 393 Na^{+}. The maximum biohydrogen yield was 233 mL H_2/g/hexose from sucrose-containing wastewater (Lin & Lay, 2005).

Buffer. Organic acids are by-products produced from the dark fermentation of biohydrogen production. The accumulation of these acids will reduce the pH of the growth medium of the microbes, resulting in a decrease in biohydrogen production or stunting the microbial growth. Therefore, a strong buffer in the medium is required to oppose the pH change caused by organic acids produced. Carbonate buffers ($NaHCO_3$ and NH_4CO_3) are widely used in biohydrogen dark fermentation studies. However, the use of these buffers may result in the formation of additional CO_2 due to the interaction of HCO_3^- with acidic metabolites (Lin & Lay, 2005). This situation should be avoided as the gas build-up will induce toxicity of the microbial environment. Hence, the use of phosphate buffer is preferable to alleviate this concern. This is because some studies have found that the use of phosphate buffers like K_2HPO_4 and Na_2HPO_4 could maintain the pH values of the medium and promote hydrogen production (Lin et al., 2011).

Hydrogen Partial Pressure. The theory predicts that by reducing the partial pressure of hydrogen may increase the biohydrogen yields from glycerol. High dissolved H_2 concentration in the culture medium inhibits H_2 production and favour the hydrogen consumption pathway instead (Mandal et al., 2006). Immediate removal of H_2 from the culture medium is recommended to facilitate maximum H_2 yields that showed hydrogen yield was doubled to 3.9 mol H_2/mol glucose (Chong et al., 2009). Another method that can be employed is by adding chemicals like KOH and NaOH, to absorb carbon dioxide

from the headspace and by removing the dissolved gases (Saady, 2013). The addition of the chemicals will create a vacuum environment. However, the addition of the chemicals will increase the pH of the medium, thus, affecting the optimal pH needed to maintain bacterial growth. Agitation served to remove dissolved gases such as CO_2 and H_2 from the fermentation medium. Ferchichi et al. (2005) revealed the agitation up to 100 rev/min yielded 1.66 mol-H_2/mol.

Limitation of Dark Fermentation for Biohydrogen Production

Physicochemical Conditions. Table 2 depicts the advantages, disadvantages, and mechanisms of biohydrogen production through dark fermentation. The metabolism of bacteria for biohydrogen production through dark fermentation is highly dependent on the physicochemical factors. Among the crucial factors are the pH, hydraulic retention time, partial pressure of hydrogen, temperature, fermentation products, by-products inhabitation and growth media.

Table 2
Microbial biohydrogen production mechanisms by dark fermentation: advantages and disadvantages

Mechanism	$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2$
Advantages	• H_2 production from various carbohydrates and organic wastes • High H_2 production rates • No light required • Simpler process for engineering than the others • H_2 can be produced along with the high-value compounds (e.g.: glucogenic acid and 1,3-propanediol)
Disadvantages	• CO_2 present in the product gas • Incomplete oxidisation of organic materials to H_2, low H_2 yields • Effluent treatment required • Impurity of product gas, traces of H_2S, methane and carbon dioxide.

Sources: Mohd Yasin et al. 2011; Vignais & Billoud 2007

Substrate Inhibition. The mechanisms of biohydrogen production involving microbes are catalysed by mainly hydrogenase and nitrogenase enzymes (Vignais et al., 2006). Both mechanisms utilise the presence of protons as the electron sink during the metabolism of organic substrates that act as electron donors. The nitrogenase enzyme catalyses the reduction of nitrogen gas to ammonia (Tamagnini et al., 2002). The absence of N_2 will, therefore, shift the total electron flux to biohydrogen production instead. This reaction is irreversible and can produce biohydrogen even at saturated biohydrogen concentration in the medium and this reaction is energy-intensive (Vignais et al., 2006).

The hydrogenases can be distinguished based on the types of electron donors and acceptors used in hydrogen metabolisms such as NAD, cytochrome, coenzyme, and ferredoxins. The enzymes can also be classed based on the metallic cofactors and

sequence similarity of the hydrogenases. There are currently three known classes: [NiFe]-hydrogenases (Forzi & Sawers, 2007), [FeFe]-hydrogenases and [Fe]-hydrogenases (Fang et al., 2017). Interestingly, it was found that the functionality of the different Hyd enzymes largely depending on the pH (Sanchez-Torres et al., 2013). The majority of studies on biohydrogen production involves the metabolism of simple sugars. POME is a complex substance which could not be readily available for microbes to metabolise. It leads to long adaptive phases and low conversion rates into the product. However, the components of the complex substrates will be transformed into simple compounds through the degradation process. The stoichiometric reactions involved in the dark fermentation were explicated in Table 3.

Table 3
Stoichiometries reaction of dark fermentation of glucose for biohydrogen production

Reaction	Stoichiometry	ΔG0' (kJ reaction)	Reference
Oxidation of glucose	$C_6H_{12}O_6 + 12H_2O \rightarrow 12H_2 + 6HCO_3^- + 6H^+$	+ 3.2	(Chou et al., 2008)
Acetate production	$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 4H_2 + 2HCO_3^- + 4H^+$	- 206.3	
Butyrate production	$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COO^- + 2H_2 + 2HCO_3^- + 3H^+$	- 254.8	
Ethanol production	$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3CH_2OH + 2HCO_3^- + 2H^+$	- 235.0	
Acetate and ethanol Production	$C_6H_{12}O_6 + 3H_2O \rightarrow CH_3CH_2OH + CH_3COO^- + 2H_2 + 2HCO_3^- + 3H^+$	- 215.716	(Hwang et al., 2004)
Lactate production	$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOO^- + 2H^+$	- 198.1	(Kim et al., 2009)
Butanol production	$C_6H_{12}O_6 + H_2O \rightarrow CH_3CH_2CH_2OH + 2HCO_3^- + 2H^+$	- 280.5	(Chin et al., 2003)
Propionate production	$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COO^- + 2H_2O + 2H^+$	- 359.0	(Morimoto et al., 2005)
Valerate production	$C_6H_{12}O_6 + H_2 \rightarrow CH_3CH_2CH_2CH_2COO^- + HCO_3^- + H_2O + 2H^+$	- 330.9	(Chou et al., 2008)
Acetogenesis	$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 4H_2O$	- 104.6	
Acidogenesis	$C_6H_{12}O_6 \rightarrow 3CH_3COO^- + 3H^+$	- 310.6	(Kim et al., 2009)

Temperature. The temperature influences the microbial growth and consequently increases enzymatic reactions and the rate of chemical synthesis (Dasgupta et al., 2010). Dark fermentation metabolism can occur within a wide range of temperatures 15-45°C (mesophilic), hyper-thermophilic (more than 80°C). Previous studies on the production of biohydrogen under thermophilic conditions were compared in Table 4.

The effects of temperature from mesophilic to thermophilic (25 - 55°C) during the fermentation were investigated by Yossan et al. (2012) and the results showed that the

Table 4
Advantages and disadvantages of biohydrogen dark fermentation in thermophilic condition

Advantages	Disadvantages
• Increase in the rates of chemical and enzymatic reactions • Increase in thermodynamic favourability of H_2-production. H_2 production becomes less affected by the partial pressure of H_2. • The solubility of H_2 and CO_2 to water decreases • Reactors are less prone to contamination by H_2-consuming organisms • Decreased diversity of side products • Some thermophiles excrete exoenzymes, which can hydrolyze biopolymers • Suitable for direct processing high-temperature wastewaters • Destruction of pathogens in the reactor effluent	• Low cell densities achieved • Energy need for heating

Source: (Hallenbeck, 2005; Hawkes et al., 2002)

biohydrogen yield was optimum at 37°C. The highest yield of biohydrogen was obtained using 55°C reactor temperature with 985.3 mL/L POME. O-thong et al. (2011) optimised three different temperatures between 35-75°C, which produced the biohydrogen production at 1104 mL H_2/L POME (35°C), and maximum at 4750 mL H_2/L POME (55°C), respectively. Based on the statistical analysis, the optimal condition for biohydrogen production was at 60°C, with a maximum production of biohydrogen at 4820 mL H_2/L POME. These studies have clearly shown that high temperature is the most ideal fermentation temperature to achieve the highest biohydrogen yield using POME.

pH. The optimal growth pH is microbial dependent and an important factor in suppressing the behaviour of the hydrogen-consuming methanogens. Studies showed different initial pH yielded different biohydrogen value, 2584 mL H_2/ L POME (pH 4.5), 4750 mL H_2/L POME (pH 5.5) and 4300 mL H_2/ L POME (pH 6.5), respectively (O-thong et al., 2011). The optimal initial pH for biohydrogen production was found at 5.5, where the reaction achieved the maximum production of biohydrogen at 4820 mL H_2 L/POME. RSM analyses from different studies showed the optimum production of biohydrogen was found at 272 mL H_2/g substrate with an initial pH around 5.70. Another experiment using microflora in POME sludge also showed that the maximum biohydrogen production rate was 98 mL H_2/h with initial pH at 5.98 (Rasdi et al., 2009).

Under slightly acidic conditions, the bacteria growth of methanogens will be suppressed. The ability of biohydrogen-producing bacteria to develop will be increased. Moreover, controlling the pH in dark fermentation is important because organic acids generated as by-products tend to reduce the pH of the culture medium (Li & Chen, 2007).

Products Inhibition. Biohydrogen is typically produced from the metabolism of glucose or sucrose that also produces secondary products such as acetate and butyrate. The organic

acids can reduce the rate of cell growth at lower concentrations and cause changes in cell metabolic process (Kyazze et al., 2006). The organic acids (undissociated) may pass through the cell membrane and dissociate within the cell of bacteria. This occurs when the pH inside the cell is higher than its surroundings. Therefore, high organic acid concentrations can disrupt the proton motive force (pH gradient) across the cell membrane, resulting in metabolic inhibition (Van Ginkel & Logan, 2005). Thus, biohydrogen production is typically more influenced by the disassociated butyric acid than by acetic acid, due to butyric acid having lower pK_a value than acetic acid at 4.7 (Hawkes et al., 2007).

Another end-product which could suppress the biohydrogen production is ethanol. Lack of bacteria tolerance against ethanol. Thermophilic bacteria are less ethanol-tolerant than mesophilic bacteria (Burdette et al., 2002). The most ethanol-tolerant strains are the *Thermoanaerobacter* sp. strain A10 (Georgieva et al., 2007) and *Clostridium thermocellum* sp. strain SS22 (5% (v/v) (Rani & Seenayya, 1999).

Inoculum. Most of the microbes that are studied for biohydrogen production are obligate anaerobes (i.e *Clostridia*). However, the combination of facultative anaerobes with obligate anaerobes in the biohydrogen production may create more advantageous (Chong et al., 2009). Several bacteria can metabolise the complex material such as POME into simple sugars or organic acids, while others utilise these intermediate products to produce biohydrogen. The highest yield of biohydrogen was reported to be at 2.15 mol H_2/mol-hexose from 3.2 L anaerobic batch sequencing reactor (ASBR) (O-Thong et al., 2007). In another study, the highest biohydrogen yield was 1773 N mL H_2/L POME using continuous batch. The studies evidenced that mixed cultures are more advantageous compared to pure cultures.

Single culture also plays a significant role particularly its metabolism and the optimal growth conditions during biohydrogen production. O-Thong et al. (2009) showed that the *Thermoanaerobacterium* had produced 25.9 mmol H_2/d from POME. Chong and colleagues found that the *Clostridium butyricum* EB6 generated 948 mL H_2/mL glucose from POME (Chong et al., 2009). Besides, it is also important to reduce the presence of bacteria that may inhibit the production of biohydrogen like methanogens and sulphate reducers. Methanogens may be depleted using shorter hydraulic retention time (HRT), provided that the HRT is not exceeding the crucial value where biohydrogen producing bacteria may be washed out (Ismail et al., 2010).

Metabolic Engineering Approaches

Metabolic engineering is one of the available strategies to address the limitations presented by dark fermentation in the production of biohydrogen. The theoretical biohydrogen yield from dark fermentation using glucose as a carbon source is 12 mol H_2 and 6 mol CO_2 per

mole glucose, but there are no reported natural bacteria that possess the metabolism that is capable to generate this value (Chaudhary et al., 2012). However, based on several known fermentation reactions, the theoretical maximum H_2 yield is only 4 mol H_2/ mol glucose produced by strictly anaerobic bacteria. Meanwhile, facultative bacteria can only produce biohydrogen yield of 2 mol H_2/mol glucose (Mohd Yasin et al., 2013). Therefore, the theoretical maximum yield represents only 25% of substrate conversion into biohydrogen, signalling the inefficiency of the system. This is because other metabolic by-products of dark fermentation like butyrate, propionate, ethanol, lactate, including biomass, are also generated in significant amounts (Table 3).

E.coli has been employed as a robust model strain for metabolic engineering and protein engineering to improve the productivity of hydrogen-producing bacteria (Sanchez-Torres et al., 2013). Even though obligate anaerobes like *Clostridia* spp. showed higher hydrogen production compared to *E. coli* (Table 5), they require more sophisticated cultivation set-up because they are obligate anaerobes.

Table 5
Types of microorganism, bioreactor types and scales and biohydrogen production from POME through the dark fermentation process

Microorganism	Hydrogen Yield	Hydrogen Production Rate	Reactor Type	References
C. butyricum EB6	298 mL H_2/g carbohydrate	849.5 mL H_2/h	3L Reactor	(Chong, Sabaratnam, et al., 2009)
Thermoanaerobacterium-	6.5 L H_2/L-POME	25.9 mmol H_2/L/d	150 mL bottle	(O-Thong et al., 2007)
Thermosaccharolyticum	4.6 L H_2/L-POME	-	1L ASBR	
Mixed culture	199 mmol H_2/L-POME	-	1L Reactor	(O-Thong et al., 2007)
Mixed culture	0.27 L H_2/g-COD	9.1 L H_2 /L/ POME/d	3.2L ASBR	(Prasertsan et al., 2009)
Mixed culture	840 NmL H_2/L-POME	35 N mL/H_2/L/ POME/h	50L CSTR	(Yusoff et al., 2009)
Suspended Mixed culture	145.9cm^3H_2/g-COD	240.5 cm^3 H_2/g-VSS/d	122 cm^3 vials	(Ismail et al., 2010)
Mixed culture	1054 NmL H_2/L-POME	44 N mL H_2/L/ POME/h	50L CSTR	(Yusoff et al., 2009)

Maeda and his colleagues designed the robust engineered *E. coli* strains for enhanced biohydrogen production (Maeda et al., 2008). To date, the framework for metabolic engineering is restricted to the well-described microorganism, limiting the window of opportunity to discover novel genes that may increase the production of biohydrogen.

Future Progress of Dark Fermentation for Biohydrogen Production

The largest obstacle to biohydrogen production using POME was low biohydrogen molar yield, which only reached 10-20% of the total energy the substrate can provide (Angenent et al., 2004). In addition, estimation of the use of POME to produce biohydrogen is determined using simple sugars such as glucose, since it is impossible to measure the moles of complex substrates like POME. Many studies have shown that the lower yield of biohydrogen production through dark fermentation is due to the bioconversion of POME into multiple by-products (Hipolito et al., 2008). The presence of multiple by-products will not only increase the pH of the culture medium but also cause the purification of products more difficult.

Technologies and systems for biohydrogen production are well known, but currently imperfect for complex substrates. Most of the technical issues are related to the use of stand-alone technology, such as exclusive use of dark fermentation (Levin, 2004). The integrated biohydrogen and methane production system is currently the best solution to these issues. The advantages of the two-stage system include the efficiency of the process, higher yield of biogas and high total energy recovery (Hawkes et al., 2002). Figure 1 shows the emerging POME biohydrogen manufacturing approach using hybrid systems. In the first stage, POME will be transformed into organic acids and biohydrogen using dark fermentation, followed by the conversion of the organic acids into biohydrogen via photo fermentation. By implementing this hybrid system, biohydrogen production efficiency increased from the first stage at 50% to 70% in the second stage (Cheng et al., 2011). However, the main problem in the hybrid system is the implementation of the second stage

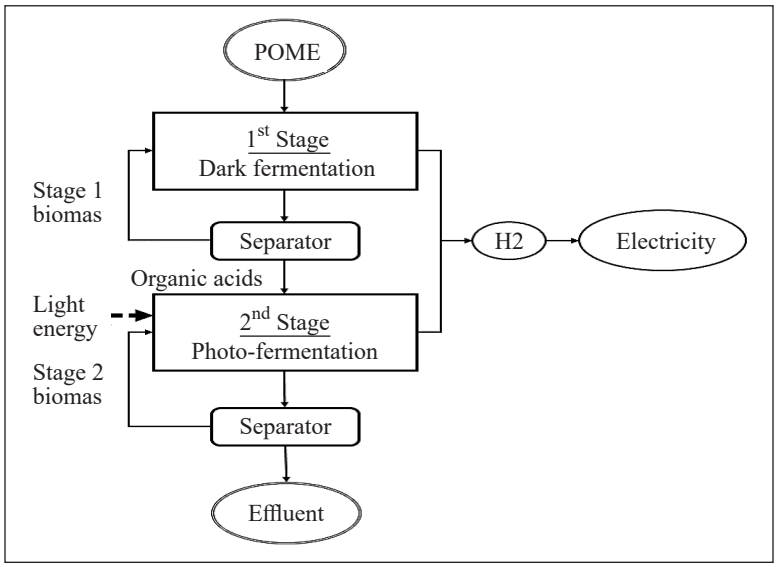

Figure 1. The proposed strategy of an integrated system by combining dark and photo fermentation to produce biohydrogen from POME as a carbon source

(photo fermentation), where it requires high cost and complex infrastructure to set up the photobioreactor (Cheng et al., 2011).

Another method to improve the fermentation flux is through the integration of metabolic engineering of the microbial strains with optimal fermentation parameters. For example, integrating the metabolic engineering and low partial pressure during fermentation process was found to significantly enhance the hydrogen production (Mandal et al., 2006). However, not many kinds of research have been conducted in pairing metabolic engineering and bioprocessing technology. Most efforts in influencing the metabolic pathway of POME fermentation for biohydrogen production are centred on editing the Fe and Ni hydrogenases pathways as mentioned in another section.

The modification of existing pathways using metabolic engineering approaches will also lead to the creation of a new robust strain with higher hydrogen yield and better productivity. In addition, through metabolic engineering, genetic modification can be made to fully exploit the abundant substrate availability of POME and its derivatives (Taifor et al., 2017). These strategies can also be applied for other applications to construct recombinant strains to produce a wide range of chemicals and bioproducts. Therefore, the application of the metabolic engineering methods in industrial-scale bioprocessing is a promising study to improve the production of biohydrogen.

CONCLUSION

Dark fermentation is one of the anaerobic fermentation processes applied for biohydrogen production. The performance can be recovered by manipulating the factors that have tremendous influences on biohydrogen production, including pH, temperature, medium formulation, and the application of genetic engineering. Bench studies provide basic essential information to know and understand the microbial limitation, environmental limitation, and operational condition for biohydrogen production. The production strains can be manipulated by genetic engineering to obtain the strains that can utilise POME for biohydrogen production with minimal intermediate products, such as organic acids, solvents, or amino acids. Another important step is to shift the biohydrogen production from bench to pilot scale-mostly operated by continuous systems, without compromising the ability of microorganisms to convert complex substrate into biohydrogen. Nevertheless, further investigation from the bench studies and the industry are needed to enhance the efficient utilisation of wastewater, like POME, towards maximal biohydrogen production through dark fermentation.

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