Chapter 1
Dark Fermentation and Bioelectrochemical Systems for Enhanced Biohydrogen Production from Palm Oil Mill Effluent: Current Progress, Potentials, and Future Perspectives

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Abstract The global rise in demand for fats and oil has made the palm oil industry grown tremendously over the last decades in countries like Indonesia, Malaysia, and Thailand. Malaysian agro-industrial sector alone accounts for about 51% of the world's palm oil production and 62% of the world's export. The sector has generated billions of dollars in revenues, and tonnes of wastes too. Palm oil mill effluent (POME) is the most abundant waste generated during the crude oil extraction process. Efficient and effective POME treatment technologies are still being actively investigated. POME has great potential as a substrate for biohydrogen production due to the high content of degradable organic matter. Dark fermentation, among the various biological processes for biohydrogen production, is highly favored due to the lower cost and low energy requirement. However, achieving a high biohydrogen yield is the main challenge, due to the co-production of organic acids. Additional treatment steps using bioelectrochemical systems (BES), such as microbial

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electrolysis cell (MEC), can provide the much-needed solution. Enhanced biohydrogen production can potentially be achieved when dark fermentation is coupled with MEC, with better POME treatment. Microbial fuel cell (MFC) can provide additional treatment step, with simultaneous electricity generation. This chapter reviews the various dark fermentation technologies that have been employed in producing biohydrogen from POME, the methods employed to improve biohydrogen yield, the advancements in BES, and the potential integration with dark fermentation for enhanced biohydrogen production.

Keywords Biohydrogen · POME · Dark fermentation · Bioelectrochemical systems

1.1 Introduction

The demand for world's energy is on the increase, and this demand is projected to further increase in the next few decades. Based on the International Energy Outlook 2013 and 2018 reports, energy consumption worldwide is estimated to increase by about 56% between 2010 and 2040, from 524 to 820 quadrillion Btu (Singh et al. 2019). This has led to the search for renewable alternative energy sources. Research on energy production from biomass has been on the rise, in the pursuit to lessen the current dependency on fossil fuels, and to tackle the various environmental problems and health impacts associated with the use (Shahlan et al. 2017). Hydrogen at the moment is touted as the energy carrier of the future, with the highest energy content of 142 kJ/g of any known sources (Lu et al. 2012; Cardoso et al. 2014) and does not produce any greenhouse gases upon combustion (Singh et al. 2013a; Krishnan et al. 2016). The energy obtained from hydrogen can be used directly by direct combustion or used to produce electricity via fuel cells to power electric vehicles (Hames et al. 2018). However, current hydrogen production still relies heavily on thermochemical methods, with fossil fuel as the primary energy source (Kapdan and Kargi 2006; Azwar et al. 2014; Dhar et al. 2015). For hydrogen to fully replace conventional fuels, production has to be from environmentally friendly processes, e.g., via the use of various industrial and environmental wastes as raw material, and produced in large scale (Zhang et al. 2005; O-Thong et al. 2011). Production involving the use of microorganisms (fermentative bacteria, photosynthetic bacteria, cyanobacteria, and algae) has attracted a lot of attention over the last decade, as it requires less energy and potentially environmentally friendly too. At the same time, various organic wastes can be used as substrates (Demirbas 2008).

World's palm oil production is currently dominated by the Southeast Asian region, with countries like Malaysia, Indonesia, and Thailand as the major producers (Mukherjee and Sovacool 2014). Malaysia and Indonesia accounted for about 86% of the world's production in 2011. This industry serves as the key economic driver and the main element of their GDP (Iskandar et al. 2018). In Malaysia alone, the number of hectares of cultivated land increased from 3.79 million in 2003 to 5 million in 2011, resulting in a tremendous increase in the number of operating

mills to about 426 mills (Mohmmed and Chong 2014). The milling process involves the use of a large amount of water that generates a huge amount of wastewater discharge, known as palm oil mill effluent (POME). Malaysian palm oil industry produces tonnes of POME, which accounts for about 60% of the total waste produced. Most of the currently employed treatment systems are the anaerobic–aerobic ponding system and digestion systems for biogas production (mainly methane) (Nasution et al. 2014). Anaerobic digestion are highly favored among other technologies due to the numerous advantages associated with the process, such as less land requirement, reduced sludge production, and easier operation and maintenance (Rizvi et al. 2015). Biogas generated from the treatment process is used to run the daily operations of the mills, which can help decrease the dependency on petroleum fuels, and decrease the impact the palm oil processing has on the environment (Chotwattanasak and Puetpaiboon 2011). It is estimated that from 1 m^3 of POME, around 28 m^3 of biogas can be generated and a net income of MYR3.8 million can be obtained yearly from the electricity generation (Chin et al. 2013). However, the utilization of methane results in the emission of greenhouse gases. Thus, the search for a cleaner, more sustainable, and renewable energy carrier is still ongoing.

The use of POME as a substrate for biohydrogen production is currently under intense investigation, in order to maximize production, from various operational aspects, e.g., different fermentation methods (dark and photo-fermentation), inoculum source, operational temperature, pH, hydraulic retention time, loading rates, and bioreactor types. Commercialization of the process for large-scale production is still farfetched, due to the constraints of low production rate and yield, making it not yet feasible. The low biohydrogen rate is attributed to a number of factors, such as inoculum source, operational mode, bioreactor design, inhibitory substance, hydrogen concentration, soluble metabolites, and concentration of substrates (Dong et al. 2009; Bundhoo and Mohee 2016). This chapter provides a summary of biohydrogen production via dark fermentation using POME, the setbacks associated, and the approaches investigated toward improving biohydrogen production yield. The feasibility of integrating dark fermentation and other fermentation systems is also discussed, focusing on bioelectrochemical systems (BES), toward achieving higher energy recovery and higher pollutant removal. A brief discussion on the challenges facing hydrogen storage and transport is also included, together with the current advancements to address this problem.

1.2 POME as a Substrate for Biohydrogen Production

Malaysia has been the second largest global palm oil producer after Indonesia, which accounts for 39% production and 44% exports in 2016 (Sawe 2018). POME is categorized as extremely high strength wastewater containing an elevated amount of organic material, oil and greases, and suspended solids. It is a mixture of effluents produced and discharged mainly from sterilizer condensate, clarification wastewater,

and hydrocyclone wastewater during crude palm oil extraction process (Lam and Lee 2011). Considering its high content of biodegradable constituents, POME could potentially be the renewable resources for sustainable biohydrogen production through dark fermentation system (Ji et al. 2013). Raw POME is a nontoxic, thick voluminous brownish colloidal suspension of 95–96% water, 0.6–0.7% oil and grease, and 4–5% total solids containing 2–4% suspended solid (Ahmad et al. 2016; Wu et al. 2007). POME generally has a high chemical oxygen demand (COD) and biochemical oxygen demand (BOD), high oil and grease content, high discharge temperature and is acidic in nature with a distinct offensive odor (Ji et al. 2013; Ahmad et al. 2006a). Most of the suspended solids are from palm fruit mesocarp debris composed of a mixture of carbohydrates, ranging from hemicellulose to simple sugars, amino acids, nitrogenous compounds, free organic acids, cell walls, organelles, short fibers, and inorganic nutrients (K, Ca, Mg, Mn, Fe, Zn, Cu, and Co) (Santosa 2008; Ugoji 1997). POME also contains lignin (4700 ppm), phenolics (5800 ppm), pectin (3400 ppm), and carotene (8 ppm) (Sundram et al. 2003). Table 1.1 shows the compositions of POME and its multielement constituents. POME as a substrate for biohydrogen production is still under intense investigation due to its high organic content and its potential for microbial conversion under both mesophilic and thermophilic conditions, using different inoculum sources, reactor configurations, and operating conditions (Atiff et al. 2005; O-Thong et al. 2007, 2008; Chong et al. 2009a; Choi et al. 2013) as summarized in Table 1.2.

1.3 Challenges Associated with Dark Fermentation Process

Biohydrogen production via dark fermentation process is reported to have higher hydrogen production capacities, substrate versatility, low energy requirement, and easier operational conditions compared to other biological methods (Nath and Das 2004; Moreno et al. 2015). However, the feasibility is hindered by the maximum hydrogen yield which is still limited to 33% of the theoretical stoichiometric conversion of glucose (Gomez et al. 2011). Hydrogen yield differs with the fermentation pathway and end-product metabolites production (Dhar et al. 2015; Elbeshbishy et al. 2017), which include propionate, butyrate, lactate, formate, and solvents (butanol, acetone, and ethanol). Metabolite production decreases the overall hydrogen yield (Zong et al. 2009). The main route for producing hydrogen is the acetate–butyrate fermentation pathway of which theoretically, 4 mol of hydrogen can be produced when acetate is the main fermentation product and 2 mol when butyrate is generated, as shown in Eqs. (1.1) and (1.2).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2 \quad (1.1)$$

Table 1.1 POME compositions and the multielement constituents

Parameter	Unit	Range	References	Multielement constituents					
				Elements	Habib et al. (1997)	Wood et al. (1979)	Ahmad et al. (2006b)	Awotoye et al. (2011)	MPOB (2014)
					μg/g dry weight	mg/L			
Temperature	°C	50–90	O-Thong et al. (2007), Ahmad et al. (2011)	Aluminum, Al	16.60 ± 1.44				
pH	–	3.4–6.9	MPOB (2014), Aziz et al. (2019)	Arsenic, As	9.09 ± 0.65				
Biochemical oxygen demand (BOD)	mg/L	10,250–80,400	Ahmad et al. (2006b), Poh et al. (2010)	Boron, B	7.60 ± 0.60		7.6		
Chemical oxygen demand (COD)	mg/L	15,000–100,000	MPOB (2014)	Calcium, Ca	1650.09 ± 160.45	276–405	439	252.41	
Total solids (TS)	mg/L	11,500–79,000	MPOB (2014)	Chromium, Cr	4.02 ± 0.44	0.05–0.43			
Total suspended solids (TSS)	mg/L	5000–54,000	MPOB (2014)	Cobalt, Co	2.40 ± 0.35	0.04–0.06			
Total volatile solids (TVS)	mg/L	9000–72,000	MPOB (2014	Copper, Cu	10.76 ± 1.04	0.8–1.6	0.89		
Total nitrogen	mg/L	180–1400	MPOB (2014	Iron, Fe	11.08 ± 2.20	75–184	46.5	183.49	
Ammoniacal nitrogen	mg/L	4–290	MPOB (2014), Aziz et al. (2019)	Lithium, Li					
Total Kjeldahl nitrogen	mg/L	60–900	Poh et al. 2010, Loh et al. 2013	Magnesium, Mg	911.95 ± 95.50	254–344	615	283.46	345
Oil and grease	mg/L	130–18,000	MPOB (2014)	Manganese, Mn	38.81 ± 3.65	2.1–4.4	2.0	34.25	

(continued)

Table 1.1 (continued)

Parameter	Unit	Range	References	Multielement constituents					
				Elements	Habib et al. (1997)	Wood et al. (1979)	Ahmad et al. (2006b)	Awotoye et al. (2011)	MPOB (2014)
					µg/g dry weight	mg/L			
Total carbohydrates	mg/L	0.02–28,900	Wu et al. (2007), Norfadilah et al. (2016)	Molybdenum, Mo	6.45 ± 0.40				
Total organic carbon	mg/L	21,000–25,000	Poh et al. (2010), Ahmad et al. (2011)	Nickel, Ni	1.31 ± 0.30				
Reducing sugar	mg/L	0.11–1450	Ahmad et al. (2006b), Norfadilah et al. (2016)	Potassium, K	8951.55 ± 256.45	1281–1928	2270	295.74	1958
Total protein	mg/L	2830–12,900	Ahmad et al. (2006b), Wu et al. (2007)	Selenium, Se	12.32 ± 1.35				
Total lignin	mg/L	130–1740	Poh et al. (2010), Ahmad et al. (2011)	Silica, Si	10.50 ± 1.80				
Volatile fatty acids	mg/L	1900	Ahmad et al. (2011)	Sodium, Na					
Acetic acid	mg/L	471–3540	Poh et al. (2010), Poh and Chong (2014)	Sulfur, S	13.32 ± 1.45				
Color	ADMI	>500	Bello et al. (2013)	Tin, Sn	2.30 ± 0.30				
				Vanadium, V	0.12 ± 0.02				
				Zinc, Zn	17.58 ± 2.10	1.2–1.8	2.3	120.95	

Table 1.2 Summary of biohydrogen production from POME via dark fermentation reported in the literature

Inoculum	Reactor type	Inoculum pre-treatment	Operating conditions (pH, temperature)	Maximum H_2 production rate	Maximum H_2 yield	References
POME sludge	Serum bottles	Heated at 100 °C for 1 h	5.5, 50 °C	NA	28.47 mL H_2/g COD	Zainal et al. (2018)
POME digested sludge	UASB-FFR	Heated at 100 °C for 1 h	5.5, 38 °C	N/A	0.31 L H_2/g COD	Mohammadi et al. (2017)
POME pond sludge	UASB	Heated at 90 °C for 60 min	5.5, 55 °C	1.92 L H_2/L/day	N/A	Krishnan et al. (2016)
POME sludge	Serum bottle	N/A	5.5, 37 °C	N/A	5.988 ± 0.5 L H_2/L-medium	Norfadilah et al. (2016)
POME digested sludge	UASB-FFR	Heated at 100 °C for 1 h	5.5, 38 °C	0.514 L H_2/g VSS/day	N/A	Mohammadi et al. (2014)
ASBR sludge	Serum bottle	N/A	5.5, 60 °C	12.12 mmol H_2/L/h	2.53 mol H_2/mol hexose	Nitipan et al. (2014)
Granular sludge seed	UASB	Heated at 100 °C for 30 min	6.0, 37 °C	43.1 mL H_2/h	182.3 mL H_2/g COD	Pisutpaisal et al. (2014)
Clostridium sp. LS2	UASB-PEG	N/A	5.5, 37 °C	336 ml H_2/L/h	0.35 L H_2/g COD	Singh et al. (2013b)
C. butyricum EB6	UASB-PEG	NA	5.5, 37 °C	510 mL H_2/L-POME/h	NA	Singh et al. (2013a)
POME sludge	UASB-PEG	Heated at 80 °C for 50 min	5.5, 37 °C	0.632 L H_2/(L POME h)	N/A	Singh et al. (2013c)
Anaerobic sludge	Serum bottle	N/A	5.5, 60 °C	N/A	5.2 L H_2/L POME	Seengenyoung et al. (2013)
Anaerobic sludge	Serum bottle	Heated at 90–95 °C for 30 min	6.0, 55 °C	75.99 mL H_2/L/h	27.09 mL H_2/g COD	Fernandez et al. (2011)
C. butyricum	Serum bottle	N/A	7.0, 37 °C	N/A	2.18 mol H_2/mol TC	Fatehizadeh et al. (2018)

(continued)

Table 1.2 (continued)

Inoculum	Reactor type	Inoculum pre-treatment	Operating conditions (pH, temperature)	Maximum H_2 production rate	Maximum H_2 yield	References
Anaerobic sludge	Infusion bottle	NA	6.0, 55 °C	4820 mL H_2/L POME	243 mL H_2/g sugar	O-Thong et al. (2011)
POME digested sludge	CSTR	N/A	5.5, 55 °C	2.64 m^3/m^3/day	N/A	Pachapur et al. (2019)
POME sludge	ASBR	N/A	5.5, 60 °C	9.1 L H_2/L/day	0.27 L H_2/g COD	Prasertsan et al. (2009)
POME sludge	N/A	Heated at 70–100 °C for 10 min	5.5, 37 °C	1034 mL H_2/L/h	N/A	Chong et al. (2009a)
C. butyricum EB6	N/A	N/A	5.5, 37 °C	N/A	2.21 mol H_2/mol glucose	Chong et al. (2009b)
POME anaerobic sludge	ASBR	Heated at 100–105 °C for 2 h	5.5, 60 °C	N/A	6.33 ± 0.142 L H_2/L POME	O-Thong et al. (2008)
POME sludge	ASBR	Heated at 100–105 °C for 2 h	5.5, 60 °C	6.1 ± 0.03 L H_2/L	2.24 ± 0.03 mol H_2/mol hexose	O-Thong et al. (2007)
POME sludge	CSTR	N/A	5.5, 60 °C	N/A	4708 mL H_2/L POME	Atiff et al. (2005)

ASBR Anaerobic sludge bed reactor, *UASB* Upflow anaerobic sludge blanket reactor, *UASB-FFR* Upflow anaerobic sludge blanket-fixed film reactor, *CSTR* Continuously stirred tank reactor, *UFA* Upflow anaerobic, *UASB-PEG* Upflow anaerobic sludge blanket-polyethylene glycol reactor, *N/A* Not available

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2H_2 + 2CH_3CH_2CH_2COOH + 2CO_2 \quad (1.2)$$

The microbial component involved in the biological conversion of substrates to biohydrogen is an integral part of the anaerobic fermentation process, performing various complex series of biochemical reactions. Bacteria with the ability to produce biohydrogen can be obtained from various environments such as soil, compost, wastewater sludge, sediment (Hu and Chen 2007; Mohammadi et al. 2011; Wang and Wan 2009). Biohydrogen production with mixed cultures was found to be easier, faster, more practical to operate and has the ability to degrade a wide range of substrate/mixed substrates compared to processes using pure cultures (Fernandez et al. 2011; Fatehizadeh et al. 2018). Hydrogen evolution rate and yields are higher with mixed cultures, as communities work in synergy using metabolites to ensure rapid degradation of complex substrates (Pachapur et al. 2019). Nonetheless, there are other communities of bacteria (i.e., hydrogen consumers) which naturally coexist with the hydrogen producers, such as the methanogens, nitrate-, and sulfate-reducing bacteria, lactate producers, propionate producers, homoacetogens, all of which adversely affects hydrogen yield (Bundhoo and Mohee 2016; Prasertsan et al. 2009).

Another aspect of concern is the accumulation of fermentation products, both hydrogen gas and volatile fatty acids (VFAs), which is detrimental to hydrogen yield. Continuous operating conditions associated with the removal of gaseous products and influent dilutions coupled with vigorous mixing and gas sparging has the potential to improve process efficiency (Kraemer and Bagley 2006; Gomez et al. 2009). Nguyen et al. (2010), established that the removal of accumulated hydrogen from the reactor headspace during batch fermentation resulted in an increase in hydrogen yield. However, accumulated VFAs were unaffected. Further conversion of these by-products to hydrogen requires the action of hydrogen-consuming microorganisms such as methanogens and sulfate-reducing bacteria to maintain low hydrogen pressure by consuming the hydrogen produced to form hydrogen sulfide or methane (Wang et al. 2011). However, this process also prevents additional hydrogen recovery. Several approaches have been employed to increase hydrogen yield in dark fermentation system, as described below.

1.4 Approaches to Improving Biohydrogen Yield Under Dark Fermentation Process

In order to overcome the drawbacks described above, strategies for improving biohydrogen production yield has focused on various aspects, such as bioreactor configuration and operation, the use of biohydrogen-producing microbial community, substrate preparation and recently, the integration of multiple systems (Krishnan et al. 2016; Chang et al. 2002; Hallenbeck et al. 2012; Kumar et al. 2016) (Fig. 1.1).

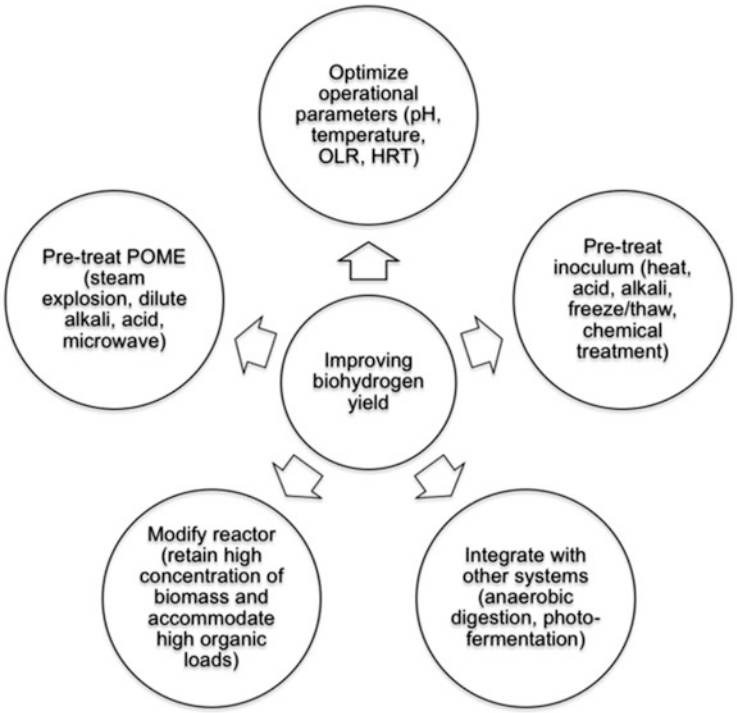

Fig. 1.1 The various strategies employed to improve biohydrogen yield under dark fermentation conditions

1.5 Operational Conditions

1.5.1 Reactor Design

Several anaerobic reactors with different configurations and capacities have been employed to carry out anaerobic digestion/anaerobic fermentation in the treatment of wastewater. Improvements in the reactor designs and configurations have been shown to improve biohydrogen production rate and yield. Continuous stirred tank reactor (CSTR) is the most commonly used reactor system. The reactor has a simple design equipped with an agitator system to enhance the area of contact with biomass (Arriaga et al. 2011). Microbial cells in CSTR are in suspension mode and tend to be sensitive to fluctuation in environmental conditions making it prone to biomass washout and process instability in a continuous operation (Lee et al. 2004; Mu and Yu 2006). Cell immobilization could improve biomass retention and biohydrogen yield (Mu et al. 2006). Cell immobilization matrix is generally made from various supporting materials (both synthetic and natural) and has the ability to create a local anaerobic environment, which is an important requirement for long-term biohydrogen production (Singh et al. 2013c). Higher tolerant to environmental

perturbation, biological activity, and reusability of immobilized cells as a result of higher cell density also makes it attractive (Seol et al. 2011). The use of immobilized cells has led to the development of other reactors such as the upflow anaerobic sludge blanket (UASB) reactor (Abbasi and Abbasi 2012). UASB is able to minimize biomass washout by retaining a high biomass concentration, with short hydraulic retention time (HRT), (Zinatizadeh et al. 2007; Khemkhao et al. 2011) and minimal sludge generation (Abbasi and Abbasi 2012). 69.3% increase in biohydrogen production rate was obtained using cell immobilization of anaerobic sludge using polyethylene glycol (PEG) operated in a UASB reactor, compared to the production using free suspended cells. A further 7% increase in production rate was also observed with the use of heat-treated inoculum (Singh et al. 2013c). Singh and Wahid (2015) investigated the addition of microelements in the immobilization process of *Clostridium* sp. LS2. The maximum biohydrogen production rate of 7.3 L/L POME/day and yield of 0.31 L H_2/g COD was obtained in a continuous operation. Choi et al. (2013) used settleable biomass aggregated and immobilized into granules of the sludge bed. Khemkhao et al. (2011) improved the sludge aggregation process and biogas production by the addition of chitosan a biopolymer. However, UASB reactors have a long start-up time and granules washout due to hydraulic tensions (Mohammadi et al. 2017). Granulated sludge washout was prevented, and a shortened start-up time of 22 days compared to the conventional start-up duration of about 60 days was obtained, with improved biohydrogen production rate of 0.514 H_2/g VSS/d using a modified UASB reactor equipped with a fixed film (Mohammadi et al. 2014). Further modifications include the incorporation of packed bed/fixed bed and membrane-based systems for effective biomass retention in the form of an attached growth system allowing for operation under high organic loading rate (OLR) (Bharathiraja et al. 2016). Upflow anaerobic contact filters (Vijayaraghavan and Ahmad 2006), two-stage UASB (Tahti et al. 2013), and membrane anaerobic system (Fakhrul-Razi and Noor 1999) have all been used to improve biohydrogen yield but will not be discussed further here.

1.5.2 Fermentation Temperature

The activities of biohydrogen-producing enzymes and substrate degradation rate are highly influenced by operating temperatures (Elbeshbishy et al. 2017). Fermentation temperature also influences the production of by-products. Increase in temperature results in a shift in metabolic pathways, by increasing the acetate/butyrate end by-product while decreasing other end product formation (Mu et al. 2006). One way of obtaining such a condition is the use of higher temperatures for the fermentation process (Nitipan et al. 2014). Biohydrogen production at thermophilic or extremely thermophilic (above 60 °C) has the ability to yield more than 2 mol H_2/mol hexose compared to that obtained under mesophilic condition (Ahn et al. 2005). High-temperature biohydrogen production offers several advantages over mesophilic conditions: (1) deactivation of pathogenic microorganisms from waste

materials (Tahti et al. 2013), (2) improvement of the reactions' kinetics, substrates assimilation, and higher hydrolysis rate due to the enhanced thermodynamics (Nguyen et al. 2010). Hydrogen solubility in water decreases with the increase in fermentation temperature (Ismail et al. 2010; Sonne-Hansen et al. 1999). Operation at higher temperatures also avoid the need for seed sludge pre-treatment, since high temperatures can inhibit the activities of hydrogen consumers (methanogen and homoacetogens) (Nath and Das 2004; Saady 2013). Successful biohydrogen production has been obtained with temperatures in the range of 50–80 °C (Kotsopoulos et al. 2009; Boileau et al. 2016), however, the highest temperature used for POME is 55 °C (Krishnan et al. 2016; Ismail et al. 2010; Yossan et al. 2012) and 60 °C (O-Thong et al. 2008; Prasertsan et al. 2009; Nitipan et al. 2014; Seengenyoung et al. 2013) as highlighted in Table 1.2. To obtain an economically sustainable biohydrogen production, energy productivity and yields should be significantly increased. However, production at higher temperatures is not favorable due to the higher energy input, and may also inactivate vital enzymes for cell protein synthesis and growth (Khemkhao et al. 2012). Therefore, to obtain a balance between energy production and energy used, operating the process at mesophilic temperature range seems more favorable (Mu et al. 2006).

1.5.3 Enrichments of Biohydrogen-Producing Microbial Communities

The use of mixed cultures for biohydrogen production offers robustness, and the ability to utilize a wide substrate range (Nitipan et al. 2014). However, enrichment of hydrogen-producing communities while suppressing hydrogen consumers from a mixed environment for increasing hydrogen yield needs to be done via pre-treatment. The reported pre-treatment methods are heat shock treatment (80–100 °C), load shock, acid and alkali treatment, freeze–thaw cycle, aeration, exposure to chemicals (e.g., chloroform, sodium 2-bromoethanesulfonate, and iodopropane) (Mohammadi et al. 2011; Massanet-Nicolau et al. 2008; Wang and Wan 2008), ultraviolet radiation, and ultra-sonication (Fatehizadeh et al. 2018). Majority of hydrogen producers are resistant to extreme environmental conditions (high temperature, acidity, and alkalinity) by forming protective spores. Hydrogen consumers (homoacetogens and methanogens) are mostly obligate anaerobes in nature and are easily affected by aeration (Zhu and Beland 2006). The use of chemical analogs of coenzyme M reductase enzyme complex required for the reduction of methyl-coenzyme M to methanogenesis has been used to inhibit the methanogenesis phase of the degradation process. Analogs such as sodium 2-bromoethanesulfonate (BESA) have been used as methanogen inhibitor (Cheong and Hansen 2006).

The search for the optimum pre-treatment method is still ongoing, as inconsistencies in the outcomes have been obtained without much room for comparison due

Table 1.3 The various pre-treatment methods for enriching biohydrogen-producing bacteria from mixed cultures

Inoculum(s)	Pre-treatment methods	Substrate	Reactor type and operation conditions	Maximum H_2 yield	Optimal method	References
Wastewater anaerobic sludge	HS (70–100 °C, 30 min), UV (150 W high pressure, 20 min) Son (150 W, 20 kHz, 20 min)	Glucose	Batch, pH 7.5 and 37 °C		HS	Fatehizadeh et al. (2018)
POME sludge	HS (100 °C, 1 h), AS (pH 3.0, 24 h), AkS (pH 12.0, 24 h), FT (−10 °C, 30 °C) 24 h, 0.1% (v/v) Cl, 24 h	POME	Batch, pH 5.5 and 35 °C	0.41 mmol H_2/g COD	HS	Mohammadi et al. (2011)
Waste activated sludge	HS (95 °C, 30 min), AS (pH 3.0, 24 h), AkS (pH 10.0, 24 h), Ar for 24 h, 1% Cl for 24 h, and 10 mmol/L of BES for 24 h	Glucose	Batch, pH 7.0 and 35 °C	1.51 mol H_2/mol glucose	AS	Chang et al. (2011)
Anaerobic digested sludge	HS (100 °C, 30 min), AS (pH 3.0, 6 h), FT (−17 °C, 24 h; 25 °C, 12 h), Ar for 2 h, 2% Cl for 24 h. Son at frequency of 20 kHz for 20 min	Rice and lettuce powder	Batch, pH 7.0 and 37 °C	119.7 mL H_2/g VS	HS	Dong et al. (2010)
Digested sludge	HS (100 °C, 15 min), AS (pH 3.0, 24 h), AkS	Glucose	Batch, pH 7.0 and	221.5 mL/g glucose	HS	Wang and Wan (2008)

(continued)

Table 1.3 (continued)

Inoculum(s)	Pre-treatment methods	Substrate	Reactor type and operation conditions	Maximum H_2 yield	Optimal method	References
	(pH 10.0, 24 h), Ar for 24 h, 2% Cl for 24 h		Temp. 35 °C			
Anaerobic sludge from UASB reactor	HS (102 °C, 90 min), AS (pH 3.0–4.0, 24 h), AkS (pH 12.0, 24 h)	Sucrose	Batch, pH 5.5 and Temp. 35 °C	2.00 mol-H_2/mol glucose	HS	Mu et al. (2007)
Cattle manure sludge	HS (dry heat—105 °C, 2 h, wet heat—95 °C, 20 min, AS (pH 2.0, 48 h), FT (−10 °C, 24 h; 30 °C, 6 h), 0.5–1.0 M BESA for 10 min	Glucose	Batch, pH 7.0 and Temp. 35 °C		AS	Cheong and Hansen (2006)
Digested sludge	HS (100 °C, 20 min), AS (pH 3.0, 30 min), AkS (pH 10.0, 30 min), Ar for 30 min, 10 mmol BESA and Io for 30 min	Sucrose	Batch, Temp. 35 °C	5.64 mol-H_2/mol sucrose	Io	Zhu and Beland (2006)
Aerobic activated sludge, Anaerobic digested sludge, soil, lake sediment, aerobic refuse compost	HS (100 °C, 2 h) and AS (pH 3.0, 18 h)	Glucose	Batch, pH 6.0 and Temp. 35 °C	1.4 mol H_2/mol of glucose	HS	Kawagoshi et al. (2005)

HS Heat shock, *AS* Acid shock, *AkS* Alkaline shock, *FT* Freezing and thawing, *Cl* Chloroform, *Ar* Aeration, *BESA* sodium 2-bromoethanesulfonate, *Io* Iodopropane, *Fl* fluvastatin, *Son* Sonication, *UV* Ultraviolent radiation

to the use of different inoculum and substrates, as summarized in Table 1.3. Most studies have shown that heat shock treatment results in the highest biohydrogen yield (Mohammadi et al. 2011; Wang and Wan 2008; Mu et al. 2007; Kawagoshi et al. 2005). A few studies showed different observations, e.g., Zhu and Beland (2006) observed that iodopropane pre-treatment gave the highest biohydrogen yield of 5.64 mol/mol sucrose while heat shock treatment gave 49.9% lower yield. Cheong and Hansen (2006) and Chang et al. (2011) observed that acid shock treatment was the optimum treatment method when cattle manure and waste activated sludge was used. On the other hand, the pre-treatment method can affect the start-up and overall efficiency of the reactors (Fatehizadeh et al. 2018). Thus, manipulation of the fermentation conditions based on the characteristics of biohydrogen-producing communities has been employed in place of pre-treatment methods. Conditions such as hydraulic retention time, pH, loading rate, dilution rates, and biogas circulation have been used to increase yield and avoid the proliferation of hydrogen consumers (Fang and Liu 2002). Zhu and Beland (2006) observed that using untreated control sludge resulted in higher yield than the pre-treated sludge (acid, base, aeration, and heat shock pre-treatment). They concluded that acidic pH developed as a result of the fermentation represses methanogenic activities. Generally, lower yield and traces of methane production are commonly reported, as methanogens could proliferate with time (Wang and Wan 2008). Among the different pre-treatment methods, heat shock and acid treatment have been reported to completely repress methanogenic activities while sonication, freeze/thaw, and aeration and alkaline treatment do not (Dong et al. 2010). Akutsu et al. (2009) and Chang et al. (2011) reported that diversity of the microbial population, fermentation patterns, and the communities enriched differs in different inoculant. Thus, the best pre-treatment method seems to differ with different inoculum used. Nonetheless, heat shock treatment is the widely adopted method of pre-treatment for enriching hydrogen producers using POME (Table 1.3).

1.5.4 POME Pre-treatment Methods

Cellulose and hemicellulose, which are fermentable sugars with monomers linked by β-1,4-glycosidic bonds, are the main components of POME. These polysaccharides are not easily digested and resist hydrolysis when it occurs in a cross-linked matrix with lignin (Baharuddin et al. 2010; Quemeneur et al. 2012). In order to achieve high biohydrogen conversion efficiency, hydrolysis of the complex molecules to simpler molecules through various pre-treatment methods is crucial. This is to allow simpler sugars to be easily accessible to fermentative microorganisms. Pre-treatment methods such as using acid, dilute alkali (Seengenyoung et al. 2013), steam explosion and heat (Kamal et al. 2012), and enzymatic hydrolysis (Silvamany et al. 2015) have been explored.

Exposure of POME to acid and alkali increases its fermentability to produce biohydrogen by catalyzing an efficient hydrolysis process. Mahmod et al. (2017)

obtained a maximum biohydrogen yield of 1.24 mol H_2/mol glucose using POME pre-treated with phosphoric acid, and 1.04 mol H_2/mol glucose when using POME pre-treated with nitric acid. These are equivalent to biohydrogen yield of 97% and 65% higher than using raw POME. Seengenyoung et al. (2013) recorded a 51% increment in biohydrogen yield when using POME pre-treated with 1.5% NaOH. In general, acids decreases the crystallinity of cellulose and catalyze the depolymerization of hemicellulose into xylose and other sugars, and cellulose into glucose (Balat et al. 2008). While alkalis delignify biomass, remove acetyl group and break the intermolecular ester bonds cross-linking lignin and carbohydrates, enabling further reactivity on the released polysaccharides for biohydrogen production (Sun and Cheng 2002).

Meanwhile, Taifor et al. (2017) and Kamal et al. (2012) showed that a combination of chemical and thermal pre-treatments has successfully improved biohydrogen production by elevating soluble sugar concentration. Acids and alkalis rupture the lignin seal whereas heat causes the cellulosic biomass to be converted into simpler sugars such as glucose, fructose, xylose, and arabinose. The study also revealed that microorganisms have different preferences for carbon sources for biohydrogen production, in the following order: glucose, xylose, fructose, arabinose, while no degradation of POME oligomeric sugars was observed (Taifor et al. 2017). Despite the advantages, both strong acid and alkali present safety hazards, and will subsequently need to be neutralized and recovered before disposal. Besides, in addition to the presence of chloride and sulfate ions, acid hydrolysis form by-products such as furfural, phenolics, and hydroxymethylfurfural (HMF) which are inhibitors for fermentative microorganisms (Rodríguez-Chong et al. 2004). Also, excessive heating during thermal pre-treatment will reduce sugar recovery due to sugar degradation into furans (Ruiz et al. 2008). Therefore, considering POME is rich with organic molecules such as triglycerides, carbohydrates, proteins, nitrogenous compounds and minerals, enzymatic hydrolysis present an excellent alternative to heat and chemicals. Rasdi et al. (2012) employed the use of anaerobically treated POME for biohydrogen production. Garritano et al. (2017) demonstrated that POME hydrolysis by plant enzyme preparation improved biohydrogen productivity by 102%. Nevertheless, the high cost of enzymes and low rate of hydrolysis are the potential drawbacks.

In light of the waste disposal issue associated with chemical pre-treatment methods, several physical methods have also been explored. Leaño et al. (2012) reported the feasibility of ultrasonication in pre-treating POME with 38% improved biohydrogen production and 20% higher COD removal. Theoretically, ultrasonicator propagates ultrasound waves in the range of 15–20 kHz in an aqueous milieu which produce cavitation and acoustic streaming. Cavitation generates strong mechanical shear force whereas acoustic streaming increases convection of aqueous slurries (Neis et al. 2008; Nitayavardhana et al. 2008). The combined effects lead to the disintegration of lignin structure, thereby releasing the substrate and increase its bioavailability for subsequent biohydrogen production. Ozonation has also been reported to improve the biodegradability of POME and enhance biohydrogen production with a maximum yield of 182.3 mL/g COD, which is 49% higher than raw

POME (Pisutpaisal et al. 2014). Ozone is a strong oxidant capable of oxidizing a wide range of organics and inorganics into simpler forms suitable for biological conversion (Chaiprapat and Laklam 2011). Álvarez et al. (2005) found that more than 75% of polyphenols were degraded after ozonation and this decreases the toxicity and inhibitory effects of phenolic compound toward the microorganisms in POME. Even though ozonation has the advantages of effectively removing lignin, producing inhibitor-free residues for downstream processes and pre-treatment under room temperature and pressure, large amount of ozone is required thus making the method expensive (Vidal and Molinier 1988).

Various methods are still being investigated in order to eliminate waste products from POME pre-treatments, while at the same time achieve higher efficiency. The effects of several POME pre-treatments on soluble sugar content and biohydrogen yield are summarized in Table 1.4.

1.6 Integration of Dark Fermentation with Other Processes

1.6.1 Dark Fermentation and Anaerobic Digestion

The production and use of methane are desired as it can be used in existing natural gas infrastructures, easily stored, transported, and converted to syngas (Siegert et al. 2015). Biohydrogen and biomethane production share similar biochemical reactions, carried out by hydrolytic and non-hydrolytic fermentative bacteria. Both anaerobic fermentation and anaerobic digestion are initiated by the hydrolysis step, which involves the solubilization and depolymerization of proteins, carbohydrates, lipids to simple sugars, amino acids, long-chain fatty acids, and alcohols (Abbasi and Abbasi 2012). VFAs are utilized by the acetoclastic or hydrogenotrophic methanogens to produce biomethane (Bundhoo 2017). The production of biohydrogen is generally associated with the production of VFAs and electron sink. Accumulation of these VFAs can indirectly affect the productivity by becoming toxic to the hydrogen producers (Rasdi et al. 2012) or by inhibiting the metabolic activity by activating enzymes for solvent production (Khanal et al. 2004), hindering substrate utilization and finally inhibiting microbial growth (Elbeshbishy et al. 2017). Krishnan et al. (2016) used the effluent from a UASB reactor operated under thermophilic condition for biohydrogen production from POME as a substrate for biogas production in a CSTR. 94% COD removal and total energy recovery of 15.43 MJ/kg COD were obtained. Two-stage UASB reactor system operated under thermophilic (70 °C) condition was used for both biohydrogen and biomethane production to give 22% biohydrogen and 78% biomethane (Tahti et al. 2013).

Table 1.4 The effects of different POME pre-treatments on soluble sugar content and hydrogen yield

Pre-treatment conditions	Reactor operation mode	Incubation conditions	Hydrogen yield	References
Ultrasonicated at dose of 195 J/mL	Batch	44 °C, pH 7, 90 rpm	0.7 mmol H_2/g COD (38% higher than raw POME)	Leaño et al. (2012)
Ozonated with ozone loading rate of 300 mg/h	Batch	37 °C, pH 6	182.3 mL H_2/g COD (49% higher than raw POME)	Pisutpaisal et al. (2014)
Dilute acid treatment (a) 0.8% (w/v) H_3PO_4 (b)1% (w/v) HNO_3	Batch	60 °C, pH 5.8, 150 rpm	(a) 1.24 mol H_2/mol glucose (97% increased than raw POME) (b) 1.04 mol H_2/mol glucose (65% increased than raw POME)	Mahmod et al. (2017)
Alkaline treatment 1.5% (w/v) NaOH	Fed-batch	60 °C, pH 5.5	5.2 L H_2/L POME (51% increased than raw POME)	Seengenyoung et al. (2013)
Alkaline treatment autoclaving 10 M NaOH and autoclaved at 121 °C for 20 min	Batch	37 °C, pH 8.5, 120 rpm	0.68 mol H_2/mol total monomeric sugars	Taifor et al. (2017)
Chemicals-heat treatment (a) Added with 10% 1 M NaOH (b) 10% 1 M H_2SO_4 and heated at 80 °C for 1 h	Batch	37 °C, pH 5.5, 300 rpm	(a) 2.18 mol H_2/mol total carbohydrate (56% increased as compared to raw POME[a]) (b) 1.87 mol H_2/mol total carbohydrate (34% increased as compared to raw POME[a])	Kamal et al. (2012)
Enzymatic hydrolysis treatment 0.75% (w/v) of plant enzyme preparation from *Ricinus communis* L. and incubated at 45 °C, 200 rpm for 2 h	Batch	35 °C, pH 6.5, 150 rpm	2.58 mmol H_2/g COD (102% increased than raw POME)	Garritano et al. (2017)

[a]Calculated according to the data presented

1.6.2 Dark Fermentation and Photo-Fermentation

Photo-fermentation has been shown to be able to convert organic residues to more biohydrogen. However, there are difficulties in operating the system due to its

complexity, higher energy demand in the form of light management, low light conversion efficiencies and limitation in scaling up (Zong et al. 2009; Chookaew et al. 2014). VFAs accumulated in single dark fermentation system could be utilized for additional biohydrogen production via the photo-fermentation system. Photo-heterotrophic purple non-sulfur (PNS) bacteria are able to convert short-chain organic acids to hydrogen and carbon dioxide in the presence of light, producing a maximum yield of 4 mol H_2/mol acetate. Theoretically, integrating dark and photo-fermentation systems could yield a maximum of 12 mol H_2/mol glucose, when acetate is the only VFA present (Eroglu and Melis 2011). The yield obtained from the integrated system is higher than 4 mol H_2/mol glucose produced from acetate in a single dark fermentation system. Dark and photo-fermentation systems can be integrated into a sequential two-stage (consecutive operation) or single-stage (simultaneous operation) process. Sequential fermentation was, however, found to be more advantageous and extensively studied than combined fermentation due to higher productivity (Argun and Kargi 2011).

Even though the maximum theoretical yield of biohydrogen production is yet to be achieved practically, the reported overall yields from integrated systems were considerably higher than the single dark or photo-fermentation system (Table 1.5). Lo et al. (2010) used pure sugar (sucrose) to evaluate the performance of sequential dark and photo-fermentation on biohydrogen production using batch and continuous mode of operations. Sucrose was first fermented by *Clostridium butyricum* CGS5 under dark fermentation and subjected to centrifugation and dilution prior to subsequent photo-fermentation by *Rhodopseudomonas palustris* WP3-5. Total biohydrogen yield of sequential dark and photo-fermentation operated under batch mode was 5.45 mol H_2/mol hexose, and 11.61 mol H_2/mol sucrose (equivalent to 5.85 mol H_2/mol hexose) when operated via continuous mode. Interestingly, they further integrated the systems with an autotrophic microalgae reactor to consume carbon dioxide produced from the fermentation process.

Sequential dark and photo-fermentation for biohydrogen production from real wastewater have been reported by Özgür et al. (2010) using sugar beet molasses as feedstock. The dark fermentation was operated under extreme thermophilic condition using *Caldicellulosiruptor saccharolyticus* yielding 4.2 mol H_2/mol sucrose accompanied by acetate and lactate as the main VFAs. The dark fermentation effluent was centrifuged, sterilized, and diluted prior to inoculation of a mutant strain of *Rhodopseudomonas capsulatus* lacking hydrogenase uptake gene (hup-) for photo-fermentation. Cumulative biohydrogen yield of the integrated system was reported to be 13.7 mol H_2/mol sucrose (equivalent to 6.85 mol H_2/mol hexose). Cheng et al. (2011) investigated biohydrogen production from cassava starch in a sequential dark and photo-fermentation operated under batch mode. Heat treated mixed anaerobic bacteria dominated by *C. butyricum* were used as the inoculum in dark fermentation and immobilized mixed photosynthetic bacteria dominated by *R. palustris* in photo-fermentation. Dark fermentation yielded 2.53 mol H_2/mol hexose and VFAs produced were mainly acetate and butyrate. Further conversion of VFAs into biohydrogen in a photo-fermentation system yielded 3.54 mol H_2/mol hexose, resulting in total production of 6.07 mol H_2/mol hexose.

Table 1.5 Integration of dark and photo-fermentation for biohydrogen production

First stage: Dark fermentation						Second stage: Photo-fermentation				Total H_2 yield from sequential dark-photo-fermentation	References
Substrate type	Microbial inoculum	Fermentation conditions	Maximum H_2 production rate	Maximum H_2 yield	Dominant end products	Microbial inoculum	Fermentation condition	Maximum H_2 production rate	Max. H_2 yield		
Sucrose	*Clostridium butyricum* CGS5	37 °C, pH 7.5, Batch	266 mL H_2/L/h	0.98 mol H_2/mol hexose	Lactate, butyrate, formate, acetate and ethanol	*Rhodopseudomonas palustris* WP3-5	N/D, pH 7.1, Batch	12.61 mL H_2/L/h	4.48 mol H_2/mol hexose	5.45 mol H_2/mol hexose	Lo et al. (2010)
	C. butyricum CGS5	37 °C, pH 6.5, CSTR	667.37 mL H_2/L/h	6.56 mol H_2/mol sucrose	Lactate, formate, acetate butyrate and ethanol	*R. palustris* WP3-5	32 °C, pH 7.1, CSTR	6.56 mL H_2/L/h	5.06 mol H_2/mol sucrose	11.61 mol H_2/mol sucrose (5.85 mol H_2/mol hexose)	
Sugar beet molasses	*Caldicellulosiruptor saccharolyticus* DSM 8903	72 °C, pH 6.9, Batch	200 mL H_2/L/h	4.2 mol H_2/mol sucrose	Acetate and lactate	*hup*$^-$ mutant of *Rhodobacter capsulatus*	30–33 °C, pH 6.7, Batch	34.04 mL H_2/L/h	9.5 mol H_2/mol sucrose[a]	13.7 mol H_2/mol sucrose	Özgür et al. (2010)
Cassava starch	Mixed anaerobic bacteria (dominated by *Clostridium* sp.)	31 °C, pH 6.3, Batch	334.8 mL H_2/L/h	2.53 mol H_2/mol hexose	Acetate and butyrate	Mixed photosynthetic bacteria (dominated by *R. palustris* sp.)	31 °C, pH 6.3, Batch	21.1 mL H_2/L/h	3.54 mol H_2/mol hexose	6.07 mol H_2/mol hexose	Cheng et al. (2011)
Cheese whey	Immobilized *Enterobacter aerogenes* MTCC 2822	30 °C, pH 6.8, Batch	1.91 mmol/L/h	3.5 mol H_2/mol lactose	Acetate and butyrate	Immobilized *Rhodopseudomonas* BHU 01	34 °C, pH 6.8, Batch	1.87 mmol/L/h	2.69 mol H_2/mol acetic acid	5.88 mol H_2/mol lactose	Rai et al. (2012)
Sugarcane bagasse	*E. aerogenes* MTCC 2822	30 °C, pH 6.8, Batch	11.90 mL H_2/L/h	1000 mL/L	Acetate and butyrate	*Rhodopseudomonas* BHU 01	34 °C, pH 6.8, Batch	7.86 mLH_2/L/h[a]	755 mL/L	–	Rai et al. (2014)
POME	*C. butyricum* LS2	37 °C, pH 5.5, CSTR	21 mL H_2/h	0.784 mL H_2/mL POME	Butyrate, acetate, ethanol and propionate	*R. palustris*	30 °C, pH 7.0, Batch	26 mL H_2/h	–	3.064 mL H_2/mL POME	Mishra et al. (2016)

CSTR Continuous stirred tank reactor, *N/D* not determined

[a]Calculated according to the data presented

1.6.3 Dark Fermentation and Bioelectrochemical Systems

Bioelectrochemical systems (BES) is an emerging and expanding field of research, with the ability to revolutionize the process of capturing renewable resources by combining biological catalytic redox activity with the typical abiotic electrochemical reactions (Santoro et al. 2017). Energy stored in biodegradable organic substrates can be converted into electricity via the catalytic actions of electrochemically active bacteria, also referred to as electricigens or exoelectrogens (Chookaew et al. 2014; Chae et al. 2009). Microbial fuel cell (MFC) and microbial electrolysis cell (MEC) are the most commonly used BES for bioenergy generation from organic wastes in the form of electricity and biohydrogen, respectively. MEC is similar to MFC in configuration but differs in function with the addition of an electrical power supply and an anaerobic cathode chamber to capture the hydrogen produced, a process referred to as "electrohydrogenesis" (Liu et al. 2005). During the process, bacteria degrade organic substrates, and produce extra electrons, which need to be removed to maintain ionic neutrality as a result of the redox reactions of ferredoxins, and protons. Protons passed through an ion exchange membrane, before hydrogen is formed in the cathode chamber. Biohydrogen and bioelectricity production via MEC and MFC has been demonstrated using a wide range of substrates, such as glucose, fermentation effluents (Logan et al. 2008), bovine serum albumin (BSA), peptone (Lu et al. 2010), hemicellulose, cellulose, and other organic matter (Cheng and Logan 2007; Kadier et al. 2014), industrial wastewater, refinery wastewater, winery wastewater, and dairy manure wastewater (Lu et al. 2012; Logan et al. 2008; Ren et al. 2013) making these systems a highly promising clean energy production technology (Kadier et al. 2016).

In MEC, biohydrogen conversion efficiency using complex fermentable substrates are observed to be lower than that obtained from acetate, a model substrate, which has been extensively used in the investigation of electroactive microorganisms. The highest biohydrogen production yield is close to the theoretical value of 4 mol H_2/mol acetate (Bond et al. 2002). The low conversion efficiency using complex fermentable substrates can be attributed to the slow hydrolysis steps required for the breakdown of the complex polymers to produce electron donors for the exoelectrogenic microbial community (Cheng and Logan 2007).

The use of POME as a substrate in MFC and MEC has been investigated by a few researchers (Jong et al. 2011; Baranitharan et al. 2013; Nor et al. 2015). Jong et al. (2011) evaluated and compared the bioenergy generation from POME and acetate using enriched inoculum in MFC. The maximum power density of 622 mW/m^2 was obtained from the MFC fed with diluted POME having the COD concentration of 200 mg/L. Comparing the efficiency with pure acetate, it showed a 79% decrease as to when the system was operated solely with acetate. Likewise in the anode, a 23% maximum COD removal and 32% coulombic efficiency was obtained when POME was used. As a comparison, 47 and 75% COD removal and coulombic efficiency was achieved when acetate was used. Baranitharan and coworkers observed that different COD concentrations influenced the coulombic efficiency, power density

and COD removal efficiency (Baranitharan et al. 2013). A maximum power density of 45 mW/m^2 was obtained from the raw undiluted POME, resulting in 0.8% coulombic efficiency and 45% COD removal efficiency. Similarly, power density decreased upon dilution at 1:50, while coulombic efficiencies and COD removal increased with maximum values of 24 and 70%, respectively. The power density obtained was, however, lower to that obtained by Jong et al. (2011), using diluted POME. They further concluded that the low coulombic efficiency observed (despite a reasonable percentage of COD removed) was attributed to the complexity of the substrate, and the presence of non-electron transferring bacteria in the community, such as hydrolytic and fermentative bacteria.

To combat the lower electricity production, controlled inoculum was used, which is composed of both fermentative and electrogenic microorganisms isolated from anaerobic sludge and biofilm, respectively (Baranitharan et al. 2015). With the use of the controlled inoculum, a maximum power density of 107.35 mW/m^2 was obtained, which is twice of that obtained from another study (Baranitharan et al. 2013). Likewise, a much higher coulombic efficiency of 50% was also obtained with the controlled inoculum, but COD removal efficiency was found to be lower. The low COD removal was attributed to the possible absence or low abundance of the required fermentative bacterial community in the controlled inoculum. The potential of using pure cultures in the generation of electricity from POME has been attempted (Nor et al. 2015; Islam et al. 2016). Nor et al. (2015) successfully isolated *Pseudomonas aeruginosa* ZH1 from anaerobic POME sludge and compared power productivity between the pure culture and sludge inoculum. The maximum power and current density achieved from the use of POME sludge were 85.12 mW/m^2 and 91.12 mA/m^2, respectively, while the use of *P. aeruginosa* ZH1 yielded 451.21 mW/m^2 and 654.90 mA/m^2, respectively. The power and current density increased by 81 and 86% with the use of pure culture compared to the mixed culture, but showed lower COD removal efficiency. Islam et al. (2016) improved COD removal efficiency to 74.28% by using ultrasonicated POME as the substrate in a single air cathode MFC with *Klebsiella variicola* as the inoculum. Higher power density of 1648.70 mW/m^3 was obtained from the use of the pre-treated POME, compared to untreated POME (1280.56 mW/m^3). Nonetheless, the current density obtained was lower compared to that obtained by others (Nor et al. 2015). These studies have shown that pure electrogenic cultures have the ability to generate much higher power and current density in an MFC, compared to mixed cultures, but are less effective in degrading complex substrates. Thus, utilization of complex substrates such as POME in an MFC, for both electron generation and waste treatment, would require a diverse microbial community of both exoelectrogens and efficient degraders (Lu et al. 2010).

A few researchers have used MEC/MFC to utilize residual organic materials in effluents generated from dark fermentative biohydrogen production as a result of the abundant VFAs content to further improve substrate utilization and energy recovery. Chookaew et al. (2014) investigated the integration of dark fermentation and MFC/MEC using glycerol substrate. The dark fermentation process gave a maximum biohydrogen production rate of 332 mL H_2/L and yield of 0.55 mol/mol

glycerol. Undiluted fermentation effluent gave the maximum current density and COD removal of 50.2% when fed into MFC. However, a 50% dilution effluent showed higher performance than undiluted samples in the MEC reactor with the highest biohydrogen yield of 106.14 mL H_2/g COD consumed, 40.58% COD removal efficiency and 34.8 A/m^3 current density at 1.0 V applied voltage. The studies showed that fermentation effluent resulted in higher power output compared to when raw glycerol was used as a substrate (Nimje et al. 2011).

The possibility of obtaining a self-sufficient system involving the integration of dark fermentation-MFC-MEC was investigated by Wang et al. (2011). Effluent from a continuous dark fermentation system with a maximum biohydrogen yield of 10.1 mmol H_2/g cellulose was used as a substrate in both MFC and MEC. Two MFCs yielding voltage of 0.435 V was used as the source for external power for the MEC setup. The biohydrogen production rate of 0.28 m^3 H_2/m^3 was obtained from the MFC-MEC setup, which is 18 times higher than the rate obtained when acetate was used as a substrate. However, electrohydrogenesis was observed only in the first 28 h of operation resulting in a biohydrogen yield of 33.2 mmol H_2/g COD, no hydrogen recovery was observed upon further reaction time despite substrate availability and current production. This was attributed to COD regeneration as a result of acetogenesis. Moreno and co-workers (2015) explored the use of acidified and enriched cheese whey fermented effluent in a membrane-less MEC as a mean to combat the proliferation of methanogenic or acetogenic activity. They obtained 178 mL biohydrogen production from the fermentation and 1.5 L from the electrohydrogenesis phase, amounting to 2.2 L H_2 which corresponds to 94.2 L H_2/kg versus biohydrogen yield. Similar to the findings of Wang et al. (2011), the total biohydrogen yield of cheese whey through the fermentation-MEC integration was higher than the yield obtained from the use of only fermentation (Islam et al. 2016; Nimje et al. 2011). Babu et al. (2013) studied the effects of different voltage on additional hydrogen recovery via MEC process using acidogenic effluent of dark fermentation. It was observed that the increase in voltage poised resulted in an increase in biohydrogen productivity. However, a further increase beyond 0.6 V resulted in a drop in productivity. Maximum biohydrogen production rate of 0.53 mmol/h and VFA utilization (49.8%) was obtained at 0.6 V. Rivera et al. (2015) supported similar results to those obtained by Babu and co-workers (2013). In addition to the use of high voltage to obtain higher biohydrogen productivity, it was also observed that low COD concentration of UASB effluent used as substrate in a double-chambered MEC setup also influenced biohydrogen production rate. No significant difference between the use of synthetic and dark fermentation effluent was observed. Maximum biohydrogen production rate obtained was 81 mL/L/day with COD consumption of 85%. Table 1.6 summarizes the integration of dark fermentation and bioelectrochemical systems reported in the literatures so far.

Unlike photo-fermentation and anaerobic digestion process for biohydrogen production, MECs are less energy demanding. Nonetheless, for the successful adoption of dark fermentation effluent as feed substrates, feed neutralization, and dilution is critical for obtaining higher productivity. From the economic and environmental point of view, the adoption of MEC as a posttreatment stage of dark

Table 1.6 Research on integrated dark fermentation and bioelectrochemical system (microbial electrolysis cell) reported in the literatures

	Dark fermentation					Bioelectrochemical systems						
Substrate	Seed inoculum	Operational conditions	$BioH_2$ yield	$BioH_2$ rate	% COD removal	Operational conditions	Voltage applied (V)	$BioH_2$ rate	$BioH_2$ yield	% COD removal	% overall energy efficiency	References
Municipal wastewater	N/A	Continuous UASB, 35 °C, pH 4.5	N/A	N/A	N/A	Double chamber		81 mL H_2/L/day		85		Rivera et al. (2015)
Cheese whey	Digested sludge	Batch, 35 °C	N/A	0.7 L H_2/$L_{reactor}$	N/A	Membrane-less chamber, 7.2, 25 °C	1.0	0.5 L H_2/L_a/day	N/A	82	N/A	Moreno et al. (2015)
Sugar beet juice	Anaerobic digested sludge	Batch, 37 °C, 5.5	3.2 mol H_2/mol hexose	N/A	N/A	Double chamber, 7.2, 25 °C	0.4	N.A	12% TCOD	N/A	57	Dhar et al. (2015)
Crude glycerol	Park sediments	Batch, 35 °C, 7.0	0.55 mol H_2/mol glycerol	332 mL/L	20	Double chamber, 7.0		0.050 m^3H_2/m^3/day	106.14 mL H_2/g COD	40.58		Chookaew et al. (2014)
Corn stalk	Cow dung compost	Batch, 36 °C, 7.0	129.8 mL H_2/g-corn stalk	1.73 m^3/m^3 day		Single chamber	0.8	2.43 ± 0.12 m^3/m^3day	257.3 mL H_2/g-corn stalk	44 ± 2	166 ± 10	Li et al. (2014)
Domestic wastewater	USAB anaer-obic consortium	Batch	[a]11.21 mol/kg $COD_{removed}$	N/A	53		0.6	0.53 mmol/h	[a]2.025 mol/kg $VFA_{removed}$	49.8[b]	N/A	Babu et al. (2013)
Cellulose	Rotted wood crumbs	Continuous, 60 °C	10.1 mmol H_2/g cellulose	0.24 m^3 H_2/m^3/day	71[c]	Single cham-ber, 7.0	0.4	0.48 m^3H_2/m^3/day	33.2 mmol H_2/g COD	19	23	Wang et al. (2011)
Corn stover	*Clostridium thermocellum* 27,405	Batch, 55 °C, 6.8	N/A	1.65 L H_2/L/day	N/A					65		Lalaurette et al. (2009)

TCOD total chemical oxygen demand, *N/A* not available, *L* Liter of the anode

[a]Specific hydrogen yield

[b]VFA utilization

[c]Cellulose utilization

fermentation might not be practical. The use of other wastewater streams produced in the same facility or other industrial effluents with a less organic load having similar organic matter composition could be used for the dilution in order to obtain an optimized performance. There has been no report to date of the use of POME dark fermentation effluent as the substrate in an MEC system. However, the potential of adopting the integrated method for improved wastewater degradation and biohydrogen production using POME as the substrate is very attractive.

1.7 Challenges in Using Hydrogen as an Energy Carrier

Despite the environmental benefits using hydrogen to produce electricity or to power vehicles, the issues related to its storage and distribution are still being investigated. Several types of fuel cells can be used for electricity production from hydrogen, e.g., polymer electrolyte membrane fuel cell (PEMFC), alkaline fuel cell (AFC), phosphoric acid fuel cell (PAFC), molten carbonate fuel cell (MCFC), and solid oxide fuel cell (SOFC). If storage and transport of hydrogen are required, there are four main hydrogen storage methods reported: namely (1) as compressed gas, (2) as a cryogenic liquid, (3) physical storage in hydrides and (4) chemical storage in hydrides (Mah et al. 2019). Owing to its physical and chemical properties, the logistics costs for hydrogen are higher than those for other energy sources. Shell in its 2017 Hydrogen Study (Adolf et al. 2017) outlines three different modes of transportation: (1) using compressed gas containers, (2) via liquid transport, and (3) pipeline. Gaseous hydrogen can be transported in small to medium quantities in compressed gas containers by lorry. Hydrogen can also be transported in liquid form, which allows more hydrogen to be carried compared to pressure gas vessels. It is also more cost effective to transport hydrogen in liquid form over longer distances. For comprehensive and longer term use of hydrogen, a pipeline network would be the best option despite being costly. The cost can be offset when larger volumes of hydrogen are used in the future (Adolf et al. 2017).

1.8 Conclusion

Dark fermentation is considered the most feasible among all the methods for biohydrogen production. At the moment, anaerobic digestion is adopted in countries burdened with the treatment of POME for the production of biogas for use in local power generation. At the same time, the utilization of POME for biohydrogen generation has been receiving increasing attention, due to the cleaner nature of hydrogen combustion. Although various technical, operational, and biological improvements have been attempted to increase biohydrogen production yield via dark fermentation, the process still faces several drawbacks. We have described here the potential of integrating bioelectrochemical systems with dark fermentation for

treatment of different waste and wastewater types with simultaneous energy generation, particularly for the treatment of POME. Although the large-scale biohydrogen production might still require further research and development, there is a huge potential in utilizing POME for cleaner energy generation.

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