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To cite this version:

Yeo-Myeong Yun, Mo-Kwon Lee, Seong-Won Im, Antonella Marone, Eric Trably, et al.. Biohydrogen production from Food Waste: Current Status, Limitations, and Future Perspectives. Bioresource Technology, 2018, 248, pp.79-87. 10.1016/j.biortech.2017.06.107 . hal-02628592

HAL Id: hal-02628592
https://hal.inrae.fr/hal-02628592v1

Submitted on 26 May 2020

Review

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PII: S0960-8524(17)31011-8
DOI: http://dx.doi.org/10.1016/j.biortech.2017.06.107
Reference: BITE 18344

To appear in: *Bioresource Technology*

Received Date: 30 April 2017
Revised Date: 19 June 2017
Accepted Date: 20 June 2017

Please cite this article as: Yun, Y-M., Lee, M-K., Im, S-W., Marone, A., Trably, E., Shin, S-R., Kim, M-G., Cho, S-K., Kim, D-H., Biohydrogen production from Food Waste: Current Status, Limitations, and Future Perspectives, *Bioresource Technology* (2017), doi: http://dx.doi.org/10.1016/j.biortech.2017.06.107

Biohydrogen production from Food Waste: Current Status, Limitations, and Future Perspectives

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ABSTRACT

Among the various biological routes for H_2 production, dark fermentation is considered the most practically applicable owing to its capability to degrade organic wastes and high H_2 production rate. Food waste (FW) has high carbohydrate content and easily hydrolysable in nature, exhibiting higher H_2 production potential than that of other organic wastes. In this review article, first, the current status of H_2 production from FW by dark fermentation and the strategies

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applied for enhanced performance are briefly summarized. Then, the technical and economic limitations of dark fermentation of FW are thoroughly discussed. Economic assessment revealed that the economic feasibility of H_2 production from FW by dark fermentation is questionable. Current efforts to further increase H_2 yield and waste removal efficiency are also introduced. Finally, future perspectives along with possible routes converting dark fermentation effluent to valuable fuels and chemicals are discussed.

Keywords: Food waste; Dark fermentation; Hydrogen; Economic assessment; Integrated system

1. INTRODUCTION

Food waste (FW) is one of the most abundant and problematic organic solid wastes, accounting for 15-63% of total municipal solid wastes worldwide (AIT, 2010; Jang et al., 2015). Unless properly managed, it releases odor and leachate during collection and transportation due to its high volatile solids (VS: 85-95%) and moisture content (75-85%). However, as FW has high energy content, the generation of fuels and chemicals while reducing waste seems ideal (Breunig et al., 2017). In particular, it could be more valuable if clean fuel (also it could be a raw material for chemical processing), hydrogen (H_2), is recovered during the treatment process.

Currently, H_2 is almost exclusively made by physico-chemical methods that split fossil fuels. However, it is an environmental contradiction that a clean fuel is generated from polluting and limited sources under high temperature pressure condition, emitting significant greenhouse gases (Ewan and Allen, 2005). Therefore, it is important to use other sources and methods to obtain H_2 in a renewable, sustainable, and environmentally friendly way. Biological H_2 production processes are more environmentally friendly and less energy consumptive than physico-chemical

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ones. They include a wide range of approaches to generate H_2, including direct biophotolysis, indirect biophotolysis, photo-fermentation, and dark fermentation (Kim and Kim, 2011). Among them, dark fermentation is considered the most practically applicable method since it does not require external energy, and its H_2 production rate is much faster than other processes. In addition, when combined with the treatment of waste such as FW, it can solve two problems simultaneously: the reduction of environmental burden and production of clean energy.

Since FW has higher carbohydrate content and biodegradability than other organic wastes, high H_2 production potential and rate are generally achievable. Dark fermentation performance has been maximized through various pretreatment techniques, the optimization of operation parameters, and the employment of various reactor types. However, from engineering and economical point of views, there are still doubts as to whether this process is ready to be practically applied. In this review article, first, the current status of H_2 production from FW by dark fermentation and the strategies applied for enhanced performance are briefly summarized. Then, the technical limitation of low H_2 yield from FW by dark fermentation is mentioned, and the economic feasibility is discussed. Finally, current efforts to further increase H_2 yield and waste removal efficiency along with future perspectives on dark fermentation of FW are covered.

2. CURRENT STATUS

2.1 Batch operation

H_2 production performances under batch operation and the strategies applied are arranged in Table 1. The main purpose of batch studies was to increase H_2 yield, which have been expressed in three units: mol H_2/mol hexose, mL H_2/g volatile solids (VS), and mL H_2/g chemical oxygen demand (COD). Since the H_2 production potential of carbohydrates is much higher than lipids

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and proteins, the H_2 yield on a hexose basis is an important factor in the evaluation of the performance from a scientific point of view (Dong et al., 2009). Under anaerobic condition, carbohydrate degradation proceeds through several metabolic pathways, as shown in Table 2. Some liquid metabolites such as acetate and butyrate are related with H_2 production, but others are not. Theoretically, the maximum H_2 yield from hexose is 4 mol H_2/mol hexose, if all carbohydrates are degraded into acetate. However, acetate cannot be the only metabolite due to thermodynamic reason. The H_2 yield, in general, does not exceed 3 mol H_2/mol hexose (Lalman et al., 2013).

The expression of H_2 yield on a hexose basis is not sufficient to determine whether a feedstock is suitable for H_2 production. The H_2 yields on VS and COD basis are more directly related with H_2 production potential of organic solid wastes. Similar H_2 yields could be obtained from FW, sewage sludge, and livestock waste on a hexose basis, but FW is considered a much more feasible feedstock for H_2 production, owing to its higher carbohydrate content. The carbohydrate content of FW ranges 30-70%, while those of sewage sludge and livestock waste are lower than than 10% (Kim et al., 2011^b). Considering that 1 kg COD is equivalent to 1.4 m^3 H_2, the H_2 yield of 133 mL H_2/g COD_{added} achieved by Jang et al. (2015) corresponds to the conversion of almost 10% of the energy content of FW into H_2. Authors may choose the unit of H_2 yield on the purpose of their study and results, but it is highly recommended that substrate characteristics including carbohydrate content, total COD, and VS concentrations be presented.

Various pretreatments such as heat-, alkali-, and acid-treatments have been applied to FW, and have increased the H_2 yield by 5-20 times compared to the control (Im et al., 2012; Kim et al., 2009; Kim et al., 2014, Jang et al., 2015). It seemed that the role of pretreatment was not to increase the hydrolysis but to select microbes favorable for H_2 production. In the previous

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studies shown in Table 1, there were no differences in the solubilization (soluble COD/total COD) of untreated and pretreated FW after fermentation. Microbial analysis conducted by next generation sequencing clearly showed that lactic acid bacteria (LAB) were the most abundant species in untreated FW while H_2-producers were dominant in the pretreated one (Kim et al., 2014; Jang et al., 2015). Owing to their unique metabolic characteristics and antibiotic function, LAB are enriched in many fermentation processes of milk, meats, cereals, and vegetables, and their all known metabolic reactions degrading carbohydrates are irrelevant to H_2 production (Stiles and Holzapfel, 1997). LAB are also known to produce bacteriocins, which suppress the activity of H_2-producers (Noike et al., 2002). The strength of pretreatment was also found to be important for increasing H_2 production. As the heating temperature increased from 60°C to 90°C, a H_2 yield gradually increased (Kim et al., 2009). Acid pretreatment at pH 4 did not show any improved effect on H_2 production (Kim et al., 2014), and the amount of H_2 production from alkali-treated FW at pH 13 was half of the amount achieved at pH 11 and 12 (Jang et al., 2015).

The effects of operating parameters including pH, substrate concentration, temperature, and F/M (food to microorganism ratio) on batch H_2 production from FW have been studied. According to Kim et al. (2011[a]) and Xiao et al. (2013), the optimal initial pH was found to be 8.0. Although the period for pH drop from the initial values of 5.0-9.0 to 5.0 was less than one-tenth of the entire fermentation, this short period significantly affected the H_2 production performance. The H_2 yield ranged 1.6-1.7 mol H_2/mol $hexose_{added}$ at 5-60 g COD/L (on a carbohydrate basis), but decreased to 1.39 mol H_2/mol $hexose_{added}$ at 80 g COD/L, indicating substrate inhibition (Kim et al., 2014). Compared to mesophilic condition, thermophilic condition showed higher H_2 yield, which was attributed to the fact that the activity of indigenous LAB was suppressed at high temperature condition (Kim et al., 2011[c]). A high F/M ratio (on a VS basis) ranging 7-10 was

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preferable for H_2 production, while the highest yield of 57 mL H_2/g VS_{added} was attained at an F/M ratio of 7 (Pan et al., 2008).

Even though FW is a suitable substrate for producing H_2 owing to its high carbohydrate content and easily hydrolysable nature, it may be short of nitrogen which is a vital nutrient for the growth of H_2-producers. Sewage sludge is a good candidate as a co-substrate to meet the nitrogen source requirement. Kim et al. (2004) and Sreela-or et al. (2011) found that the addition of sludge to FW led to a more balanced carbon to nitrogen ratio, increasing H_2 production. Unlike previous studies, the increased performance by sewage sludge addition was ascribed to the existence of Fe and Ca at much higher concentrations in the sewage sludge compared to those in the FW (Kim et al., 2011[b]). Zhou et al. (2013) achieved a high H_2 yield from FW by adding primary sludge and waste activated sludge separately.

2.2 Continuous operation

The main goal of a continuous process in organic waste treatment is to retain an active microbial consortium at a high concentration for stable and fast treatment. Therefore, instead of H_2 yield, volumetric H_2 production rate (VHPR) is often considered the main important indicator to tell the performance of continuous operation. To achieve high VHPR, the input of a large amount of FW, often expressed as high organic loading rate (OLR), has been attempted by controlling the substrate concentration and hydraulic retention time (HRT). As shown in Table 3, the highest VHPR achieved from FW was 10.7 L H_2/L/d, which was far lower than the value obtained from liquid-type organics. Wu et al. (2006) designed a reactor containing silicone-immobilized and self-flocculated sludge, and obtained the documented highest H_2 production rate of 15 L H_2/L/h from sucrose containing wastewater. This huge difference resulted from the

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low reaction rate of the hydrolysis step involved in solid waste degradation.

A continuous stirred tank reactor (CSTR) was the most frequently used reactor type for the continuous operation, but other reactor configurations such as anaerobic baffled reactor, membrane bioreactor, and sequencing batch reactor, whose purpose were to separate solid retention from hydraulic retention, have been employed. Kim et al. (2008) controlled solid retention time (SRT) and HRT separately at the same OLR, and achieved the highest VHPR of 2.73 L H_2/L/d at SRT 120 h and HRT 36 h. A high SRT can be attained by providing enough settling time, but there could be a limitation due to the rise of biogas and lipid in the acidified broth during settling time (Kim et al., 2010). It seemed that membrane bioreactors were successful to achieve high VHPR (Lee et al., 2014). However, these configurations may face technical difficulties, such as fouling, in long-term operation.

It was difficult to draw general conclusion on the effect of HRT and substrate concentration on continuous H_2 production from FW. For example, at a fixed HRT of 1.6 d, the highest VHPR was observed at the lowest substrate concentration of 46.4 g COD/L in an anaerobic baffled reactor, while the highest VHPR was observed at the highest substrate concentration of 112 g COD/L at a fixed HRT of 4 d in an intermittent CSTR (Lee et al., 2010[b]). We cannot tell which is right or wrong, but it is reasonable to conclude that the optimal conditions vary depending on the feedstock used, inoculum, and other operating parameters, such as temperature and pH. Compared to the batch studies, thermophilic regime was more frequently used than mesophilic regime in continuous studies. According to Shin et al. (2004), thermophilic operation showed a H_2 yield of 1.8 mol H_2/mol hexose$_{added}$, while it was limited to 0.1 mol H_2/mol hexose$_{added}$ under mesophilic operation. This might be related to the vigorous activity of the indigenous LAB in the mesophilic temperature range. To inhibit the LAB activity, Kim et al. (2008) and Kim et al.

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(2010) fed FW fed after alkali-pretreatment at pH 12 under mesophilic operation. Co-digestion with brown water, which acted as diluting water to decrease substrate concentration of FW, has been attempted (Paudel et al., 2017).

3. LIMITATIONS

3.1 Low H_2 yield

As mentioned '2.1 Batch operation', 4 moles of H_2 can be generated from 1 mole of glucose in dark fermentation. However, the actual H_2 yield is lower than 50% of the theoretical maximum, and this is attributed to the (1) thermodynamic limitation, (2) existence of non-H_2 producers in the broth, and (3) acetogenic H_2-consuming reaction. These reasons can be applied to all types of feedstock used in dark fermentation, and were well explained by Lalman et al. (2013).

FW consists of not only carbohydrates but also other nutrients such as proteins and lipids. Numerous studies have shown that carbohydrates are preferred for H_2 production in dark fermentation. H_2 yields of 19.3-96.0 mL/g VS have been achieved from cabbage, carrots, and rice, while those from the protein-(egg and lean meat) and lipid-(fat and chicken skin) rich FW were negligible (Dong et al., 2009; Okamoto et al., 2000). Experimental results indicated that the H_2 production potential of carbohydrate-rich solid waste was approximately 20 times higher than that of fat-and protein-rich ones (Lay et al., 2003). Glycerol, the main component of lipids, could be a source of H_2 production but small amount. Heyndrickx et al. (1991) reported that glycerol is not a suitable substrate for H_2 production, but is an excellent substrate for solvent production. Proteins are hydrolyzed to various amino acids, and there are two types of anaerobic amino acid degradation reactions by *Clostridium* sp.: sole amino acid degradation and the Stickland reaction. A small amount of H_2 can be generated via sole amino acid degradation, but it would be

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consumed in the Stickland reaction. The carbohydrate content of FW depends on the diet and storage condition (time and temperature), and ranges from 30% to 70% on a COD basis (Li et al., 2008). Achieving a H_2 yield of 2 mol H_2/mol hexose is generally considered as a success, but this corresponds to only 5% of the energy content of FW with a carbohydrate content of 30%.

The other problem associated with obtaining low H_2 yield from FW is the continued supply of indigenous non-H_2 producers, in particular, LAB. When FW was cultivated at 35°C without any pretreatment and addition of inoculum, it was decomposed mainly to lactate (Kim et al., 2009). Jo et al. (2007) observed unstable H_2 fermentation performance in the treatment of FW under continuous operation, and attributed this to the supply of indigenous LAB. By applying various pretreatments such as heat-, alkali-, and acid-shock, indigenous LAB were selectively killed while spore-forming bacteria including *Clostridium* sp. were cultivated (Kim et al., 2009). Also, it was reported that alkali-pretreatment of FW at pH 12.5 for 1 d was an essential step for the stable H_2 production (Kim et al., 2008). Even though FW was pretreated at pH 11 or 12, stable H_2 production was not sustained with the reactivation of LAB (Jang et al., 2015; Kim et al., 2009). To recover the performance, alkali-treatment of the entire broth in the fermenter has been attempted, which increased the H_2 yield from 0.4 to 0.8 mol H_2/mol $\text{hexose}_{\text{added}}$ (Kim et al., 2010). However, this method imposes a high economic burden and cannot warrant stable production since it frequently requires this kind of chemical-shock.

3.2. Economic feasibility

Although H_2 would be derived from waste materials via an environmentally friendly route, there is still a concern as to whether dark fermentation of FW is economically feasible or not. To address this issue, we conducted an economic assessment, assuming a high H_2 yield of 2.26 mol

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H_2/mol hexose at an OLR of 100 kg COD/m^3/d (obtained from the highest values in Tables 1 and 3), and a lifespan of 20 years while operating 360 d per year. The COD concentration of FW and carbohydrate content were assumed to be 200 kg/m^3 and 50% on a COD basis, respectively. The fermenter size was set to 200 m^3, treating 100 tons of FW per day.

Table 4 summarizes the costs and benefits for the economic assessment, including the capital, annual operating cost, and profits. The capital cost includes the construction costs of all facilities, land use, and installation costs. The construction cost was estimated according to the study of Vrije and Claassen. (2013), and the cost of land use and set up was estimated to be 50% of the total construction cost (Van Haandel and Lettinga, 1994). Operating costs consisted of maintenance costs such as those for electricity, water use, and annual expenses like labor, and the use of chemicals. Annual expenses and maintenance costs were estimated to be 25% of the capital cost (Benemann, 1998), and the labor cost was estimated to be 50% of the total operating cost (Luccio et al., 2002). The profit can be gained from the treatment of FW, which is 100 USD/ton waste (NABO, 2012). However, since COD removal is limited to 10% in dark fermentation, the profit was calculated using a rate of 10 USD/ton FW. The cost for purification cost, removing CO_2 from the produced biogas, was calculated that is carried out through pressure swing adsorption (PSA).

Based on the estimated costs above, the total capital cost and annual operating costs are 1,636,560 USD and 548,568 USD/y, respectively. Meanwhile, 360,000 USD of profit could be gained through the treatment of FW. Considering the amount of 949,200 m^3 H_2 (10 ton Carbo. COD/d × 2.26/12 × 1,400 m^3 × 360 d) production per year, the H_2 production cost is estimated to be 3.2 USD/kg H_2 (= [(1,636,560/20 + 548,568) – 360,000]/(949,200/11.2)). This is lower than the previously cost ranging 10-30 USD/kg H_2 (Han et al., 2016[a]; Han et al., 2016[b]; Li et al.,

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2012), which might resulted from the high performance (H_2 yield and OLR) values applied in this economic assessment. However, this production cost is still higher than the current selling price of H_2 (0.5-3.2 USD/kg H_2) (Bartels et al., 2010), indicating that the economic feasibility of H_2 production from FW by dark fermentation is questionable.

4. STRATEGIES TO INCREASE H_2 YIELD AND OBTAIN MORE ENERGY

4.1. Microbial electrolysis cells

Increasing the H_2 yield from dark fermentation remains one of the main challenges for large-scale H_2 production. Dark fermentation only results from partial oxidation of organic substrates, and more than two thirds of the carbon and H_2 contents is converted to microbial metabolic by-products. Among the biotechnologies that could utilize the metabolic byproducts generated by dark fermentation processes, microbial electrolysis cells (MECs), an emerging technology related to microbial fuel cells (MFC), is a very promising candidate for the augmentation of classical, single-stage dark fermentation to generate H_2 with high efficiency (Kumar et al., 2016).

In MEC applications, however, food-processing wastewater rather than FW were more often used. During dark fermentation of FW, 30-60% of VS reduction is generally achieved, but the remained VS concentration (5-50 g/L depending on the dilution rate) is still high to directly apply to MECs. A wide variety of wastes and wastewaters from the food industry has been investigated for electricity generation using MFCs (ElMekawy et al., 2015), but, to date, only few studies dealt with H_2 production by MECs both as a single-stage process or coupled with dark fermentation in a two-stage system (Dhar et al., 2015; Lu et al., 2009; Marone et al., 2017; Moreno et al., 2015).

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By combining an MECs with a fermentation system for treating molasses wastewaters, an overall H_2 recovery of 96% has been obtained. The corresponding electrical energy efficiency, based on voltage input, reached up to 287%, and the overall energy efficiency (relative to the electrical input and substrate) reached 70% (Lu et al., 2009). Similarly, the overall energy recovery achieved from sugar beet juice using an integrated biohydrogen process of dark fermentation and MEC was 57% (6 mol H_2/mol $\text{hexose}_{\text{added}}$), while the individual efficiencies of energy recovery in dark fermentation and MECs were only 35% and 34%, respectively (Dhar et al., 2015). Recently, Marone et al. (2017) evaluated and compared six different industrial wastewaters and by-products coming from cheese, fruit juice, paper, sugar, fruit processing and spirits factories for their potential to generate H_2 by coupling dark fermentation with an MEC in a two-step process. Regardless of the substrate, the amount of energy produced was at least three times higher than the amount of supplied energy, where the electrical energy efficiency ranged from 336 to 725%. The H_2 yield increased by up to 13 times when both processes were used in comparison to dark fermentation alone. Among the tested wastewaters, fruit juice wastewater was the most suitable substrate since it provided the best total hydrogen yield of 1.6 ± 0.3 L H_2/g $\text{COD}_{\text{consumed}}$ (corresponding to around 9.82 mol H_2/mol hexose) with 72% of COD removal, without the need of dilution. Meanwhile, taking into account the need of dilution, the most interesting substrates to be exploited for H_2 production in such a two-stage system were vinasse residues generated from spirit production followed by cheese whey. Indeed, up to 28 ± 5 L of H_2 could be produced per liter of raw vinasse and 8.1 ± 1.4 L of H_2 could be produced per liter of raw cheese whey (Marone et al., 2017).

One of the main current challenges for MECs is scaling up. In this domain, further research is required, since most of the studies carried out until now, which succeeded in H_2 production, dealt

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with bench-scale reactors that provided useful information about the functioning of MEC technology but do not allow any prediction on its success at industrial scale. Indeed, up to now, only Cusick et al. (2011) have attempted to scale-up MECs from bench experiments to a significant pilot-scale system for H_2 production from food industry residues. The authors developed an MEC system to treat winery wastewaters with a maximum capacity of 1 m^3. Although a consistent continuous soluble COD removal of 62 ± 20% was achieved, most of the produced biogas was CH_4, comprising 86 ± 6% of the biogas, and no H_2 was recovered. CH_4 contamination has often been reported as the main cause of process failure in MEC treating complex substrates (Lalaurette et al., 2009). When treating FW or food-processing wastewater and by-products by MEC, excluding the use of chemical inhibitors (Marone et al., 2017; Montpart et al., 2014; Rago et al., 2017) which is not applicable at industrial scale, several strategies have been proposed to prevent CH_4 evolution. These include (i) the use of low operational temperature (i.e. 9°C) (Wang et al., 2014), (ii) a low acetate concentration and hydraulic retention time (Sosa-hernández et al., 2016), (iii) air exposure of the cathode and reduction of the time for a fed-batch cycle (Lu et al., 2009), and (iv) the use of a particular substrate (Montpart et al., 2014; Rago et al., 2017).

4.2. CH_4 production

As shown in Table 5, another approach for both increasing bioenergy recovery and enhancing pollutant (often referred to as COD) removal is to further utilize remaining organic acids by methanogens to produce CH_4. The fundamental difference between the conventional two-stage process and the two-stage process with H_2 and CH_4 co-production is that the latter has a specific environment favorable for H_2 production while the former does not. Co-combustion of H_2 and

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CH_4 mixture (often referred to as 'hythane') could reduce less nitrogen oxides emissions compared to the combustion of CH_4 alone (Cooney et al., 2007).

Han and Shin (2004) found that the cogeneration of H_2 and CH_4 from FW markedly increased the bioenergy conversion efficiency from 8% in only H_2 production to 78%. Antonopoulou et al (2008) also operated the two-stage fermentation system and maximum H_2 and CH_4 yields were found to be 41 mL H_2/g COD and 310 mL CH_4/g COD, equivalent to 71% [H_2 (3%) + CH_4 (68%)] of energy content in the FW with 94% of COD removal efficiency. The H_2 and CH_4 yield in the pilot-scale of two-stage fermentation system were 290 mL H_2/g VS and 240 mL CH_4/g VS, respectively, while COD removal of the process efficiency was 95% (Han et al., 2005). Chu et al (2008) demonstrated that a temperature-phased two stage process for H_2 and CH_4 production was more efficient. The yields in thermophilic H_2 production and mesophilic CH_4 production were 205 mL H_2/g VS and 464 mL CH_4/g VS, respectively, while COD removal efficiency reached 93%. The reason of obtaining high H_2 yield over 200 mL H_2/g VS in above studies was that carbohydrate-rich FW was synthetically collected and used, which is not realistic. Two types of CH_4 fermenter were applied for the treatment of dark fermentation effluent (DFE), where anaerobic sequencing batch reactor (ASBR) showed higher bioenergy recovery performance than up-flow anaerobic sludge blanket reactor (UASB) (Jung et al., 2013). However, the maximum OLR and CH_4 production rate were approximately three times higher, and the HRT was 7.5 times shorter in the UASB as compared to the ASBR. More energy gain and pollutant removal can significantly increase energy selling and treatment income. However, the additional costs for the construction and operation of the CH_4 fermenter should be considered.

An additional advantage of a sequential CH_4 fermentation system is that the methanogenic effluent can be utilized as diluting water in H_2 fermentation. Jung et al. (2013) and Kraemer and

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Bagley (2005) reported that recycling of CH_4 fermented effluent reduced the required amount of alkaline addition for pH control by approximately 40-50%. Lee et al. (2010[a]) successfully adjusted the pH at 5.0-5.5 by returning sludge as an alkali buffer without the addition of any external chemical buffer.

5. FUTURE PERSPECTIVES

Reports on pilot- and full-scale studies on dark fermentation of FW are quite limited. Fermenter size ranged 0.15-0.5 m^3, which was far smaller than the practical size (Cavinato et al., 2012; Jayalakshmi et al., 2009; Lee and Chung, 2010). Homogeneity can be guaranteed with sufficient agitation in a lab-scale fermenter, enabling precise pH control. However, when scaling-up and in practical implementation, it is uncertain whether the pH would be uniform throughout the fermenter (Amanullah et al., 2001). A dead zone may exist with insufficient agitation, and this phenomenon may lower the accuracy of pH control, which, in turn, causes a decrease in H_2 production (Moon et al., 2015). For the design of an agitator and efficient mixing, it is important to be well informed of rheological properties such as viscosity, storage, and loss moduli. These are not constant values, which varies depending on the solid concentration, temperature, shear rate, and the type of feedstock (Dai et al., 2014; Eshtiaghi et al., 2016). Numerous works have been done on anaerobic digestion sludge, sewage sludge, and digested slurry, but little information is available on dark fermentation.

As previously mentioned, one of the biggest obstacles for the practical application of dark fermentation of FW is the low H_2 yield. A few attempts were made to produce H_2 from lipid wastes, but the yields were still low, approximately one third of those from carbohydrates (Liu et al., 2013; Trchounian et al., 2015). Genetic modification such as by knocking out genes related to

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non H_2-producing pathways in the degradation of proteins and lipids can increase the H_2 yield. However, the use of pure culture in treating actual wastes such as FW is not economically promising (Kleerebezem and van Loosdrecht, 2007).

Although the H_2 yield from FW is limited, DFE can be further utilized for valuable fuel and chemical processing (Fig. 1). After successful dark fermentation of FW, short-chain fatty acids (SCFAs) including acetate and butyrate remain, representing 40-60% of the energy content of FW. These SCFAs can be further processed to H_2 by MEC and photo-fermentation. Extensive studies have been carried out on the combined process of "dark- and photo-fermentation" to achieve high H_2 yield, but the research on using FW as a feedstock is limited (Zong et al., 2009). Kim and Kim (2013) fermented FW to lactate using the indigenous LAB, and then the supernatant obtained through centrifugation of latctate-fermented residue was converted to H_2 by a photo-fermenting organism. A high H_2 yield of 8.35 mol H_2/mol hexose was attained by photo-fermentation, which was equivalent to 41% of the energy content in FW. This was the highest H_2 yield ever obtained from FW. However, the low reaction rate of photo-fermentation, which requires a large reactor and footprint can lower the economic feasibility of the integrated system. The possible route to get more H_2 by MEC was mentioned in section '4.1' in detail.

The produced SCFAs can be further converted to liquid biofuels including medium-chain fatty acids (MCFAs) and biodiesel by microalgae growth. Acetate, lactate, and propionate, which are the possible soluble metabolites of dark fermentation, have been biologically elongated to MCFAs such as caprylate and heptanoate (Grootscholten et al., 2013; Kucek et al., 2016; Steinbusch et al., 2011). These have higher energy densities and lower solubilities in water due to their longer hydrocarbon tails, which enable selective separation from the broth. MCFAs can be utilized directly as animal feed, green antimicrobials, or corrosion inhibitors, or indirectly by

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conversion with organic chemistry into biofuels such as biodiesel or jet fuels (Spirito et al., 2014). DFE has been tested for the growth of lipid-rich microalgae, and there was a consensus that microalgae growth is favored on effluents containing high acetate concentration rather than butyrate (Turon et al., 2016). Coupling of dark fermentation and heterotrophic microalgae cultivation seems to be a promising sustainable approach for producing both gaseous and liquid biofuels. However, finding an economically feasible means for harvesting and extracting lipid from microalgae still remains a technical challenge (Kim et al., 2013).

On the other hand, the SCFAs in DFE themselves have own values; they can be applied in the food processing, chemical, and pharmaceutical industries. The price of acetic acid and butyric acid ranges 0.4-0.8 \$/kg and 2.0-2.5 \$/kg, respectively, depending on the purity and grade (Zacharof and Lovitt, 2013). Since SCFAs are highly soluble, their concentration should be high in order to achieve high extraction yield from the broth (Kim et al., 2016). However, the concentrations of acetate and butyrate in DFE were generally below 50 g COD/L due to production inhibition (Kim et al., 2014; Zhu and Yang, 2003). Moreover, in batch operation, the inhibitory effect becomes larger as fermentation proceeds with pH decrease. At low pH, the proportion of undissociated form of SCFAs increases, which has higher toxicity compared to the dissociated form. The other possible candidate of chemical that can be derived from DFE is polyhydroxyalkanoates (PHAs). It is biodegradable and biocompatible, and is currently widely applied in polymers, pharmaceuticals, and fermentation industries. There have been several attempts to produce both H_2 and PHAs from carbohydrate substances, but not from FW (Luongo et al., 2017; Yan et al., 2010).

As described above, dark fermentation can be integrated with various technologies to maximize the value of by-products from FW. It is also essential to generate energy from the solid

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part of DFE by conventional anaerobic digestion. The solid part generally accounts for 30-50% of energy content of DFE, and the derived CH_4 can be utilized for heat and electricity generation, or directly supplied to households and vehicles after upgrading (Kim et al., 2016). This additional energy production can also supplement the energy requirement in the processing of fuels and chemicals from DFE.

6. SUMMARY

Compared to other organic solid wastes, higher H_2 production potential and rate are generally achievable from FW by dark fermentation. Numerous studies have been conducted to maximize H_2 yield and VHPR, but its economic feasibility is still found to be questionable, due to the limited carbohydrate content, thermodynamic limitations, and unwanted fermentation led by indigenous LAB. Attempts have been made to further increase H_2 yield and energy recovery by coupling with MECs and methane fermentation. There are several possible routes to utilize DFE for valuable fuel and chemical processing, which can maximize the value of by-products from FW.

ACKNOWLEDGEMENT

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean Government (MSIP: Ministry ofScience, ICT and Future Planning) (NRF-2015R1C1A1A02037289).

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Fig. 1 Integrated system of dark fermentation of food waste with effluent conversion process (SCFAs = Short-chain fatty acids, MEC = Microbial electrolysis cells, MCFAs = Medium-chain fatty acids, PHAs = Polyhydroxyalkanoates)

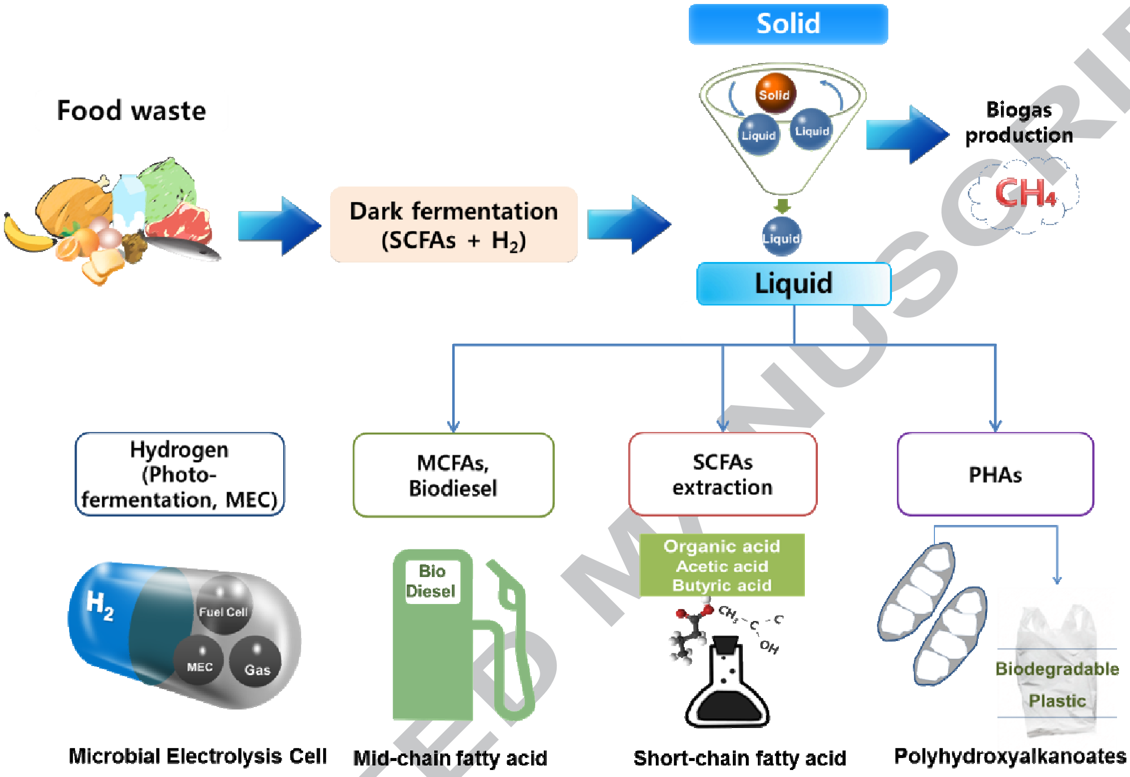

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Table 1 Batch H_2 production performance from food waste by dark fermentation (The H_2 yield value indicated here was obtained under optimal condition.)

Substrate concentration	Temp.	H_2 yield per added substrate	Strategy to enhance performance	Reference
30 g Carbo. COD/L	35°C	2.26 mol H_2/mol hexose	Heat-treatment (90°C for 20 m)	Im et al. (2012)
30 g Carbo. COD/L	35°C	153.5 mL H_2/g VS	Heat-(90°C for 20 m), acid-(pH 1 for 1 d), and alkali-treatment (pH 13 for 1 d)	Kim et al. (2009)
30 g Carbo. COD/L	35°C	1.74 mol H_2/mol hexose	Acid-treatment (pH 1.0-4.0)	Kim et al. (2014)
30 g Carbo. COD/L	37°C	162 mL H_2/g VS 1.71 mol H_2/mol hexose 133 mL/g COD	Alkali-treatment (pH 9-13, 6 h)	Jang et al. (2015)
30 g Carbo. COD/L	35°C	1.92 mol H_2/mol hexose	Initial pH change (5.0-9.0)	Kim et al. (2011[a])
N.A.[a]	37°C	77.0-79.1 mL H_2/g VS	Initial pH change (5, 6, 8)	Xiao et al. (2013)
5-80 g Carbo. COD/L	35°C	1.71 mol H_2/mol hexose	Substrate concentration change (5-80 g Carbo. COD/L)	Kim et al. (2014)
30 g Carbo. COD/L	35-60°C	1.79 mol H_2/mol hexose	Temperature change (35-60°C)	Kim et al. (2011[c])
30 g carbo. COD/L	35°C	2.11 mol H_2/mol hexose	Co-digestion (FW:SWS[a] = 10:0-10:4, 0:10),	Kim et al. (2011[b])
N.A.[a]	37°C	102.63 mL H_2/g VS	Co-digestion (FW + SWS[b]) at different C/N ratios (10:1, 20:1, 30:1, 40:1, and 50:1)	Sreela-or et al. (2011)
5-50 g VS/L	35°C	1.05 mol H_2/mol hexose	Co-digestion at various substrate concentration (FW:SWS[b] = 0:100-100:0),	Kim et al. (2004)

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N.A.[a]	37°C	76 mL H_2/g COD 165 mL H_2/g VS 1.84 mol H_2/mol hexose	Co-digestion (FW + PS[c] +WAS[d])	Zhou et al. (2013)

[a] N.A. = Not available; [b] SWS = Sewage sludge; [c] PS = Primary sludge; [d] WAS = Waste activated sludge

Table 2 Typical glucose degradation reactions in dark fermentation

	Reactions
H_2 production	Glucose + $2H_2O$ → 2Acetate + $2CO_2$ + $4H_2$
	Glucose + H_2O → Acetone + $3CO_2$ + $4H_2$
	Glucose → Butyrate + $2CO_2$ + $2H_2$
No relation	Glucose → 2Lactate
	Glucose → Butanol + $2CO_2$ + H_2O
	Glucose → 2Ethanol + $2CO_2$
H_2 consumption	Glucose + $2H_2$ → 2Propionate + $2H_2O$
	Glucose + $2CO_2$ + $2H_2$ → 2Succinate + $2H_2O$
	H_2 + CO_2 → Formate

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Table 3 Continuous H_2 production performance from food waste by dark fermentation (The VHPR value indicated here was obtained under optimal condition.)

Reactor type	OLR[a]	HRT[b]	Temp.	H_2 yield per added substrate	VHPR[c] (optimal condition)	Strategy to enhance performance	Reference
ABR[d]	29.0-47.0 g COD/L/d	1.6 d	35°C	12.9 mL H_2 /g COD	0.4 L H_2/L/d (29 g COD/L/d)	OLR change (29, 36, 47 g COD/L/d)	Tawfik et al. (2012)
i-CSTR[e]	19, 28 g COD/L/d	4 d	55°C	38.1 mL H_2/g COD	1.0 L H_2/L/d (28 g COD/L/d)	OLR change (19, 28 g COD/L/d)	Lee et al. (2010[b])
Membrane bioreactor	70.2-125.4 g COD/L/d	18.7, 14.0, 10.5 h	55°C	111.1 mL H_2/g VS	10.7 L H_2/L/d (125.4 g COD/L/d)	OLR change (70.2, 89.4, 125.4 g COD/L/d)	Lee et al. (2014)
CSTR[f]	19.0-57.0 g VS/L/d	24-8 h	35°C	11.2 mL H_2/g VS	0.4 L H_2/L/d (38 g VS/L/d)	OLR change (19-57 g VS/L/d)	Castillo-Hernandez et al. (2015)
ASBR[g]	15.4-27.0 g COD/L/d	42-24 h	35°C	61.7 mL H_2/ g VS	2.7 L H_2/L/d (HRT 24 h, SRT 100 h)	HRT change (42-24 h), SRT change (160-24 h)	Kim et al. (2008)
CSTR[f]	1.2 g VS/L/d	5 d	35, 55°C	1.8 mol H_2/ mol hexose	0.1 L H_2/L/d (6 g VS/L, 55oC)	VS concentration change (3- 10 g VS/L), Temp. comparison	Shin et al. (2004)
ASBR[g]	20 g	36 h	35°C	0.9 mol	2.1 L H_2/L/d	C/N ratio change	Kim et al.

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	Carbo. COD/L/d			H_2/ mol hexose	(C/N ratio = 20)	(10-30)	(2010)
CSTR[f]	17.7-106 g VS/L/d	48-4 h	35°C	-	3.5 L H_2/L/d (HRT 8 h)	Co-digestion with brown water, HRT 48-8 h	Paudel et al. (2017)

[a]OLR = Organic loading rate; [b]HRT = Hydraulic retention time; [c]VHPR = Volumetric H_2 production rate; [d]ABR = Anaerobic baffled reactor; [e]i-CSTR = intermittent-continuously stirred tank reactor; [f]CSTR = continuously stirred tank reactor; [g]ASBR = anaerobic sequencing batch reactor

Table 4 Economic assessment for H_2 production from food waste by dark fermentation

	Item	Cost (USD, USD/y)	Reference
Capital cost	Total	1,636,560	
Construction	Storage tank (25% of fermenter cost)	89,000	Han et al., 2016[a]
	H_2 fermenter	356,000	Vrije and Claassen., 2013
	Food waste grinding, Heat exchanger	258,000	Han et al., 2016[a]
	Purification (PSA/Fermenter ratio: 1.09)	388,040	Gim et al., 2008
Additional	Land use, set up (50% of construction cost)	545,520	Van and Lettinga, 1994
Operation cost	Total	548,568	
Materials	Chemicals	48,000	Oh et al., 2006
Maintenance	6% of capital cost	98,194	Benemann et al., 1998
Annual capital	19% of capital cost	310,946	Benemann et al., 1998

cost			
Others	Labor cost (20% of total operating cost)	91,428	Luccio et al., 2002
Profit		360,000	
Waste treatment	10% of treatment cost (100 USD/ton waste)	360,000	NABO, 2012
Production cost	Annual cost (Capital cost/20y + operating cost)/Annual H_2 production	**3.2 USD/kg H_2**	

Table 5 Performance of two-stage fermentation system converting food waste to H_2 and CH_4

1st stage		2nd stage			
H_2 yield per added substrate	Bioenergy recovery (% of FW input)	CH_4 yield (per added substrate)	Bioenergy recovery (% of FW input)	COD removal (%)	Reference
65 mL H_2/g VS	6	546 mL CH_4/g VS	82	N.A.[a]	Wang and Zhao, 2009

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310 mL H_2/g VS	28	210 mL CH_4/g VS	70	73% (on a VS basis)	Han and Shin, 2004
290 mL H_2/g VS	19	240 mL CH_4/g VS	52	95	Han et al., 2005
205 mL H_2/g VS	11	464 mL CH_4/g VS	86	93	Chu et al., 2008
161 mL H_2/g VS	9	250 mL CH_4/g COD	70	89	Jung et al., 2013
41 mL H_2/g COD	3	310 mL CH_4/g COD	68	94	Antonopoulou et al., 2008

[a] N.A. = Not available

- Critical reviews on dark fermentation of food waste (FW)
- Current status of dark fermentation with strategies applied for enhancement
- Technical and economical limitation of dark fermentation performance of FW
- Strategies to increase H_2 yield and gain more energy
- Integrated system converting fermentation effluent to various fuels and chemicals

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