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Biohythane Production from Organic Wastes by Two-Stage Anaerobic Fermentation Technology

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Abstract

The combination of biohydrogen and biomethane production from organic wastes via two-stage anaerobic fermentation could yield a biohythane gas with a composition of 10-15% H_2 , 50-55% CH_4 and 30-40% CO_2 . Biohythane could be upgraded to biobased hythane by removing of CO_2 . The two-stage anaerobic fermentation process is based on the different function between acidogens and methanogens in physiology, nutrition needs, growth kinetics, and sensitivity to environmental conditions. In the first stage, the substrate is fermented to H_2 , CO_2 , volatile fatty acids (VFA), lactic acid and alcohols by acidogens with optimal pH of 5–6 and hydraulic retention time (HRT) of 1–3 days. In the second stage, the remaining VFA, lactic acid, and alcohols in the H_2 effluent are converted to CH_4 and CO_2 by methanogens under optimal pH range of 7–8 and HRT of 10–15 days. The advantage of biohythane over traditional biogas are more environmentally, flexible of H_2/CH_4 ratio, higher energy recovery, higher degradation efficiency, shorter fermentation time, and high potential to use as vehicle fuel. This chapter outlines the general approach of biohythane production via two-stage anaerobic fermentation, principles, microorganisms, reactor configuration, process parameters, methods for improving productivity as well as technical challenges toward the scale-up process of biohythane process.

Keywords: biohythane, microbiology and biochemistry, physicochemical parameters, reactor configuration, improvement methods, two-stage anaerobic fermentation, organic wastes

1. Introduction

Currently, development of biofuels to replace fossil fuels by the biological process has been attracting attention as an environmentally friendly process. Among the various processes, biohydrogen and biohythane are the promising future energy carriers due to their potentially higher conversion efficiency and low pollutants generation [1]. Dark fermentation shows high H_2 production rate under realistic conditions, which is approaching practical levels [2]. In addition, the major advantages are rapid bacterial growth rates, relatively high H_2 production capacities, operation without light sources, no oxygen limitation problems, and low capital cost of at least at small-scale production facilities [3, 4]. The dark fermentation process can utilize organic materials for H_2 gas production, such as cellulose and starch-containing agricultural and food industry wastes, and some food industry wastewaters, such as cheese whey, olive mill, palm oil mill, and baker's yeast industry wastewaters [5]. H_2 yields from dark fermentation of organic wastes such as food waste, apple processing wastewater, starch wastewater, palm oil mill effluent, and potato processing wastewater were 57, 92, 92, 115, and 128 mL H_2 /gCOD, respectively [6–9]. However, dark fermentation has low substrate conversion efficiency as only 7.5–15% of the energy contained in organic wastes are converted to H_2 and the rest of the energy still remains in the liquid (H_2 effluent) as VFA (mainly butyric acid and acetic acid), lactic acid, and alcohols [1]. The disadvantage of dark fermentation must be overcome before biohydrogen can become economically feasible. The conversion of VFA, lactic acid, and alcohols to CH_4 through anaerobic digestion (AD) [10] is faster and simpler than the conversion of these components to H_2 by photo-fermentation and microbial-electrolysis process [1]. In addition, it has been shown to be an energy efficiency strategy for the production of a mixture of H_2 and CH_4 , known as biohythane, via two-stage anaerobic fermentation [11, 12].

Biohythane has attracted growing attention worldwide due to its potential use as vehicle fuel, high potential to produce from conversion of organic wastes and probably an alternative to the fossil-based hythane [10]. Normally, hythane gas was produced from a thermo-chemical process using natural gas as a starting material. This process is a high-energy consumption and still depends on fossil fuel. Biohydrogen and biomethane production from organic wastes by fermentation process and anaerobic digestion process, respectively, are already established. The combination of these two processes via two-stage anaerobic fermentation processes could yield a H_2 and CH_4 gas with a composition like hythane (10–15% H_2 , 50–55% CH_4 , and 30–40% CO_2) called biohythane [13], which could be upgraded to biobased hythane by removing of CO_2 . The two-stage anaerobic fermentation for biohythane production is involved with the fermentation of organic wastes to H_2 , CO_2 , VFA, lactic acid, and alcohols in the first stage and conversion of these substances in H_2 effluent to CH_4 and CO_2 via anaerobic digestion process in the second stage (**Table 1**). The optimum condition for the first stage is a pH range between 5 and 6 and a hydraulic retention time (HRT) range of 1–3 days that are suitable for acidogens for the conversion of organic wastes to H_2 via the acetate and butyrate pathways. In the second stage, the acetic acid in the H_2 effluent is converted to CH_4 and CO_2 by acetoclastic methanogens under an anaerobic condition with optimal pH range of 7–8 and optimal HRT of 10–15 days [11]. Others VFA, lactic acid, and alcohols in the H_2 effluent are anaerobically converted by acetogens to H_2 and CO_2 , which are consequently converted to CH_4 by hydrogenotrophic methanogens [14].

Technology	Processes	Substrates	Products
Hythane	Thermo-chemical	Natural gas	5–7% H ₂ , 90% CH ₄ and 5% CO ₂
Biomethane	Anaerobic digestion (AD)	Organic wastes	50–60% CH ₄ and 40–50% CO ₂
Biohydrogen	Fermentation	Organic wastes	40–60% H ₂ and 40–60% CO ₂
Biohythane	Two-stage fermentation/AD	Organic wastes	5–10% H ₂ , 60% CH ₄ and 30% CO ₂

Table 1. Biohythane technology development from two-stage anaerobic fermentation technology.

The two-stage anaerobic fermentation process could increase energy recovery, degradation efficiency, reactor stability, CH₄ production rates, and purity of gas products when compared to one-stage H₂ or CH₄ fermentation [15]. In addition, the two-stage process has advantages of improving negative impacts of inhibitive compounds in feedstocks (such as wheat hydrolysate, molasses, and skim latex serum), operated at high organic loading rates and reduced fermentation time with total HRT of 10–18 days for overall processes. Advantages of biohythane over traditional biogas are improved energy recovery, shortened fermentation time, flexible H₂/CH₄ ratio, and more environmentally benign and process robustness for handling the organic wastes [10, 16]. Integrated biohydrogen with biomethane process worth for commercialization could get the biogas in the form of biohythane. Typically, the suggested H₂ content in biohythane is 10–15% by volume. Biohythane is considered to be a clean fuel for vehicles compared to gasoline or diesel due to low greenhouse gas emission from the combustion process [17].

Biohythane via two-stage anaerobic fermentation using organic wastes could be a promising technology for higher energy recovery and cleaner transport biofuel than biogas. Various types of organic wastes can be used as substrate for biohythane production such as starch wastewater, wheat straw hydrolysate, palm oil mill effluent, food waste, and organic solid waste [13, 18–20]. Wheat straw hydrolysate was used for biohythane production by *Caldicellulosiruptor saccharolyticus* with maximum H₂ production rate of 5.2 L H₂/L·d and maximum CH₄ production rate of 2.6 L CH₄/L·d. The maximum energy output of the process was 10.9 kJ/g of straw with energy recovery of 57% of energy contained in the wheat straw [20]. Biohythane production of starch wastewater achieved H₂ and CH₄ yields of 130 mL H₂/gCOD and 230 mL CH₄/gCOD, respectively [18]. Biohythane production of food waste achieved H₂ and CH₄ yields of 205 mL H₂/gVS and 464 mL CH₄/gVS, respectively [21]. Biohythane production of palm oil mill effluent (POME) was achieved with H₂ and CH₄ yields of 201 mL H₂/gCOD and 315 mL CH₄/gCOD, respectively [13]. Nathao et al. [22] obtained two-stage process for biohythane production from food waste with H₂ and CH₄ yields of 55 and 94 mL/gVS at F/M of 7.5. Kongjan et al. [11] used UASB reactors for extreme thermophilic H₂ and thermophilic CH₄ production from wheat straw hydrolysate via a two-stage anaerobic fermentation process. Specific H₂ and CH₄ yields of 89 mL H₂/gVS and 307 mL CH₄/gVS, respectively, were achieved. Successful continuous biohythane production from POME by two-stage thermophilic fermentation and mesophilic anaerobic digestion was reported by Mamimin et al. [13]. The continuous biohythane production rate of 4.4 L/L·d was achieved with biogas containing 51% CH₄, 14% H₂, and 35% CO₂. Energy analysis suggested that the two-stage fermentation process for biohythane production had greater net energy recovery than the single H₂ fermentation

and CH_4 fermentation process. This chapter provides the information on general approach of biohythane via two-stage anaerobic fermentation, principles of biohythane process, microorganisms involved in H_2 and CH_4 production, reactor configuration for biohythane production, methods for improve biohythane production, process parameters affecting biohythane production and technical challenges toward the scale-up process.

2. Principles of biohythane process

Most of wastewater and organic wastes were usually treated in an anaerobic process for CH_4 recovery as energy. Regarding clean energy of H_2 , anaerobic process was modified for H_2 production by suppression of methanogenic activity. To harvest H_2 from the first stage, the H_2 -consuming pathway has to be inhibited [23]. Most H_2 -producing bacteria can form endospores in stress environment. Various selection methods can be used to enrich H_2 -producing bacteria [24]. The most common selection methods are heat treatment and pH control. However, some researchers reported the invalidity of such selection methods [25], because not all H_2 -producing bacteria are associated with the ability to form endospores. In addition, there are many H_2 -consuming bacteria that can form endospores, such as acetogens and sulfate-reducing bacteria [26]. The pH control is an important method for maintaining H_2 -producing bacteria in continuous systems of first stage. The pH varies depending on the microbial species, microbial activities, reactor configuration, feedstock characteristics, organic loading rate, buffer capacity, and temperature. The change of pH is due to acetic acid and butyric acid production accompanies with H_2 production, whereas the low pH influences on the shift of metabolic products from acidogenesis to solventogenesis [27]. Low pH is also critical strategies to inhibit the activity of methanogenesis. The suggestion for optimal pH of H_2 production could range from 5.0 to 6.5. From the perspective of thermodynamics, changes of Gibbs free energy during H_2 production were much larger than those of methanogenesis. This means faster rates for microbial growth in biohydrogen fermentation. On the basis of this characteristic, the manipulation of hydraulic retention time (HRT), temperature, and oxidation-reduction potential (ORP) can achieve microbial H_2 process feasible in continuous operation.

Continuous biohythane production by integrating biohydrogen with biomethane process worth for commercialization could get the biogas that has composition like hythane gas. In the first stage, substrate is fermented to H_2 , CO_2 , VFA, lactic acid, and alcohols whereby the non-gas metabolites are converted to CH_4 and CO_2 in the second stage [10]. The fermentation products from H_2 production process are very important for the whole biohythane system performance because they can affect the loading, degradation efficiency, and operating stability of the methanogenesis stage [28]. The conversion rate from VFA to acetic acid will affect the methanogenic archaea quantity, and subsequently affect the degradation rate of acetic acid and CH_4 yield. The basic principle of a two-stage process is shown in **Figure 1**. The first stage includes hydrolysis and acidogenesis where hydrolytic and fermentative bacteria excrete enzymes to break down complex organic compounds of carbohydrate, protein, and lipid into single molecules of mono sugar, amino acid, and long chain fatty acids and/or glycerol respectively. The acidogenesis, fermentative, and acidogenic bacteria convert the hydrolysis products into CO_2 ,

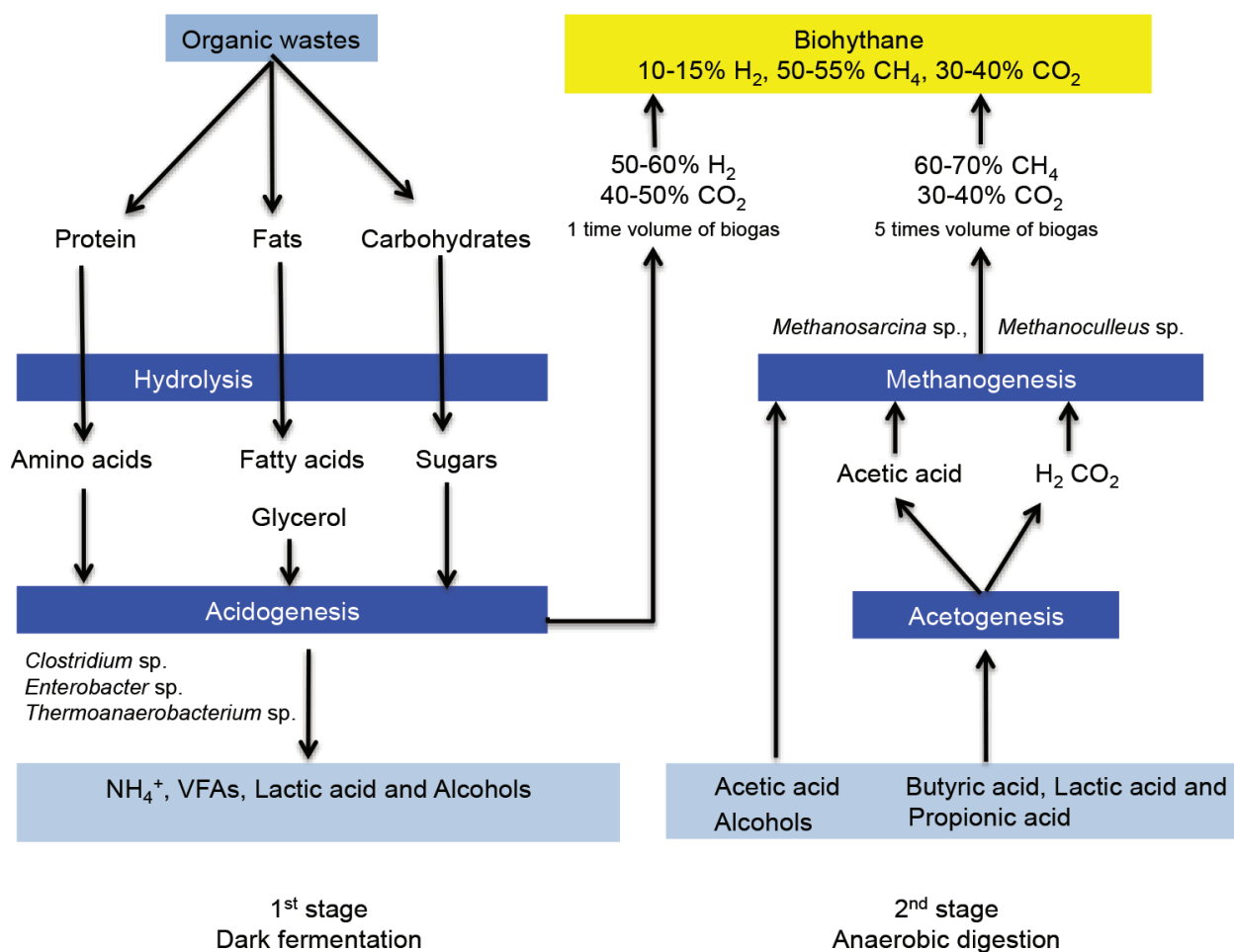


Figure 1. Modification of anaerobic digestion for biohythane production from organic wastes via two-stage anaerobic fermentation process.

H₂, VFA, lactic acid, and alcohols. High H₂ production was achieved by fermentative bacteria via acidogenesis process under pH range of 5-6 and operating at short HRT of 1-3 days. Under the optimum condition, acidogenic bacteria could convert carbohydrate to H₂ and CO₂ via the acetate and butyrate pathways and competition to other microorganisms. In the second stage, the acetic acid in the H₂ effluent is anaerobically converted to CH₄ and CO₂ by acetoclastic methanogens. The acetogenic bacteria could produce acetic acid along with additional H₂ and CO₂ from butyric acid, propionic acid, and lactic acid. H₂ and CO₂ are consequently converted to CH₄ by hydrogenotrophic methanogens [29]. These reactions occur under an optimal pH range of 7-8 and HRT of 10-15 days [30]. The two-stage anaerobic fermentation process is also characterized by a significantly reduced fermentation time with overall fermentation time of 13-18 days [10].

The two-stage anaerobic fermentation process is based on two physiologically different groups of microorganisms. One group of acidogenic bacteria that converts organic matter into H₂, CO₂, soluble VFA, lactic acid, and alcohols, is fast growing, prefers a slightly acidic environment of pH 5-6, and is less sensitive to environmental changes. A large number of microbial species, including strict and facultative anaerobic bacteria such as *Clostridium* sp.,

Enterobacter sp., *Caldicellulosiruptor* sp., *Thermotoga* sp., and *Thermoanaerobacterium* sp., are efficient H_2 producers, while degrading various types of carbohydrates [31]. The other group in second stage is methanogenic archaea, which converts VFA, lactic acid, and alcohols into CH_4 and CO_2 , is slow growing, prefers neutral to slightly alkaline environments, and is very sensitive to environmental changes. *Methanosarcina* sp. and *Methanoculleus* sp. were dominant and played an important role in second stage [14, 15]. *Methanosarcina* species were reported to be dominant at high acetate concentration (>1.2 mM), and the results were consistent with the high acetate concentrations in H_2 effluent that feed to CH_4 reactors. *Methanoculleus* species were responsible for hydrogenotrophic methanogenesis that convert H_2 and CO_2 to CH_4 [11]. Obtaining the optimum environmental conditions for each group of organisms by the two-stage anaerobic fermentation process provides several advantages over the conventional single stage [32–34], e.g., high net energy efficiencies, more stable operation, allowing higher organic loading rate operation, smaller-size reactor (40–60% smaller), thus better economics for construction cost and higher CH_4 content in the biogas (65–75%) [15, 35]. High CH_4 content and production was found in the second stage due to CO_2 in the second stage is mainly generated by acetoclastic methanogen and then consumed partly by hydrogenotrophic methanogen also existed in the second stage. The higher CH_4 content is definitely a better fuel value for on-site use and higher digestion efficiency, thus more CH_4 is recovered [36].

3. Microorganisms in biohythane process

The two-stage anaerobic fermentation process is based on the differences between acidogens and methanogens in physiology, nutrition needs, growth kinetics, and sensitivity to environmental conditions. The acidogens and methanogens are enriched separately in two tanks enabling optimized growth by maintaining proper environmental conditions in each reactor [37]. Microorganisms involved in the first stage H_2 production and in the second stage CH_4 production via two-stage anaerobic fermentation process are shown in **Table 2**. First stage (H_2 reactor) involved with the several bacterial strains is capable to produce H_2 through dark fermentation of various carbohydrates. Obligate anaerobic *Clostridia* are potential H_2 producers and are well known for high H_2 yield [38]. *C. butyricum*, *C. welchii*, *C. pasteurianum*, and *C. beijerinckii* were used for H_2 production [39]. *Clostridium* sp. is capable of utilizing a wide range of carbohydrates such as xylose, arabinose, galactose, glucose, cellobiose, sucrose and fructose with a H_2 yield of 2.1–2.2 mol H_2 /mol sugars [40]. Facultative anaerobes *Enterobacteriaceae* are H_2 producers that are resistant to trace amount of dissolved oxygen. *Enterobacter* sp. has lower yield (1.0 mol H_2 /mol sugars) when compared to *Clostridium* sp. [41]. *Citrobacter* sp. also belongs to family *Enterobacteriaceae* known to produce H_2 from CO and H_2O by water-gas shift reaction under anaerobic condition [42]. *Escherichia coli* is capable of producing H_2 and CO_2 from formate in the absence of oxygen. The H_2 yields of *E. coli* were 0.6–1.3 mol H_2 /mol glucose [43]. *Bacillus* sp. also has been identified as H_2 producers such as *B. licheniformis* [44] and *B. coagulans* [45]. Its H_2 yield was 0.5 mol H_2 /mol glucose with lactic acid as main soluble metabolites. Dark fermentation at thermophilic temperatures (55–60°C) showed favorable kinetics and stoichiometry of H_2 production compared to the mesophilic systems. Metabolism at higher temperatures becomes thermodynamically more favorable

Stages	Mesophilic condition (30–35°C)	Thermophilic condition (55–60°C)	Extreme thermophilic condition (70–90°C)
1st hydrogen production (Bacteria)	<i>Clostridium</i> sp.	<i>Thermoanaerobacterium</i> sp.	<i>Caldanaerobacter</i> sp.
	<i>Enterobacter</i> sp.	<i>Clostridium</i> sp.	<i>Caloramator</i> sp.
	<i>Citrobacter</i> sp.	<i>Thermoanaerobacter</i> sp.	<i>Thermotoga</i> sp.
	<i>Bacillus</i> sp.		
2nd methane production (Bacteria)	<i>Clostridium</i> sp.	<i>Clostridium</i> sp.	<i>Caloramator</i> sp.
	<i>Bacillus</i> sp.	<i>Thermoanaerobacterium</i> sp.	
	<i>Desulfobacterium</i> sp.	<i>Desulfomicrobium</i> sp.	
2nd methane production (Archaea)	<i>Methanobacterium</i> sp.	<i>Methanothermobacter</i> sp.	<i>Methanothermus</i> sp.
	<i>Methanoculleus</i> sp.	<i>Methanosarcina</i> sp.	<i>Methanothermococcus</i> sp.
	<i>Methanospirillum</i> sp.		
	<i>Methanococcus</i> sp.		
	<i>Methanobacter</i> sp.		

Table 2. Microorganisms involved in the first stage H₂ production, and the second stage CH₄ production via two-stage anaerobic fermentation process.

and less affected by the partial pressure of H₂ in the liquid phase. Dark fermentation under thermophilic condition was involved with *Thermoanaerobacterium* sp., *Thermoanaerobacter* sp., and *Clostridium* sp. [15]. *Thermoanaerobacterium thermosaccharolyticum* has an optimal growth at moderate thermophilic temperature (60°C) and can convert carbohydrate to H₂ via butyrate- and acetate-type fermentation [46]. *Thermoanaerobacterium* species are well known as good H₂-producing bacteria [8, 47]. *Thermoanaerobacterium* sp. represents anaerobic spore forming thermophilic microorganisms previously found in thermophilic H₂-producing reactors [8, 9]. Genus *Thermoanaerobacterium*, especially *Tbm. thermosaccharolyticum*, is capable of H₂ production from various types of substrate under the thermophilic conditions. Various *Tbm. thermosaccharolyticum* strains have been isolated such as strain PSU2 [46], strain GD17 [48], strain W16 [49], strain KKKU19 [50], and strain IIT BT-ST1 [51]. In addition, *Tbm. thermosaccharolyticum* can grow on various organic wastes including hemicellulosic waste and lignocellulosic waste [48, 52]. *Thermoanaerobacter* sp. has optimal growth at moderate thermophilic temperature (60°C) and can convert carbohydrate to H₂ via ethanol- and acetate-type fermentation, but cannot degrade cellulose. These species produce H₂, ethanol, lactate, acetate, and CO₂ as the major products, but no butyrate production. Thermophilic *Clostridium* sp. was found to degrade cellulose using cellulase enzymes and can ferment the lignocellulosic biomass to H₂ with the yield of 1.6 mol H₂/mol hexose [53]. Dark fermentation at extreme thermophilic temperatures (70–90°C) showed more favorable kinetics and stoichiometry of H₂ production compared to the thermophilic and mesophilic systems. Dark fermentation under extreme thermophilic condition was involved with *Thermotoga* sp. and *Caldicellulosiruptor* sp. [54]. The H₂ production ability of *Caldicellulosiruptor* sp. was explored at extreme temperatures. These microbes are known to have various kinds of hydrolytic enzymes that can utilize a wide range of substrate such as cellulose, cellubiose, and xylan. *Caldicellulosiruptor* sp. has high poten-

tial to use lignocellulosic waste for H_2 production with the yield of 3.3 mol H_2 /mol hexose. The predominant metabolites formed by these organisms are acetic acid and lactic acid [55]. *Thermotoga* sp. was isolated from geothermal spring and capable to grow and produce H_2 at temperatures of 90°C. *Thermotoga* sp. can use elemental sulfur as electron source with H_2 yield of 3.5 mol H_2 /mol hexose [56]. The soluble metabolites of these strains are mostly acetic acid, H_2 , CO_2 , and trace amount of ethanol [57].

Microbial consortium or mixed cultures are providing more enzymes for the utilization of complex substrate than pure cultures. Mixed microbial consortium can be developed from various sources such as anaerobic digested sludge, soil samples, and wastewater by heat treatment and load-shock treatment [58]. These two treatments could eliminate unwanted microorganisms such as methanogens and H_2 -consuming bacteria while enriching an H_2 -producing bacterium. Heat treatment inhibits the activity of the methanogens and H_2 consumers, while the spore forming H_2 -producing bacteria was survived. Additionally, continuous operation at a low hydraulic retention time (1–2 days) helps in washing out slow-growing methanogens from H_2 reactor. Industrially, the use of mixed cultures for H_2 production from organic wastes in the first stage could be more advantage than pure cultures. Enriched H_2 -producing bacteria from anaerobic sludge could utilize cellulose as a substrate for H_2 production with the yield of 2.4 mol H_2 /mol hexose [59]. The fermentation of various organic wastes by mixed cultures gave the H_2 yields in the range of 57–128 mL H_2 /gCOD, depending on type of waste [6–9]. This indicates the practical potential to commercialize H_2 production from organic wastes by mixed microbial consortium.

The second stage CH_4 reactor involved with several archaea strains is capable to produce CH_4 through anaerobic fermentation of VFA, lactic acid, and alcohols. The order *Methanobacteriales* comprises of two families (*Methanobacteriaceae* and *Methanothermaceae*) is CO_2 , H_2 , and methanol consuming methanogens. The family *Methanobacteriaceae* including *Methanobacterium* sp., *Methanothermobacter* sp., *Methanobrevibacter* sp., *Methanothermus* sp., and *Methanospaera* sp. are commonly found in CH_4 -producing reactor. *Methanothermobacter* sp. is a thermophilic *Methanobacteriaceae* that is commonly found in thermophilic CH_4 -producing reactor. *Methanothermus* sp. is an extreme thermophilic *Methanobacteriaceae* that is commonly found in extreme thermophilic CH_4 -producing reactor. *Methanothermus* sp. grows at a temperature of 83–85°C and assimilates CO_2 and H_2 [60]. The order *Methanococcales* consists of *Methanocaldococcus* sp., *Methanothermococcus* sp., and *Methanococcus* sp. These archaea produces CH_4 from CO_2 and H_2 or formate as the energy source. [61]. The order *Methanomicrobiales* consists of *Methanomicrobium* sp., *Methanocorpusculum* sp., *Methnanoplanus* sp., *Methanospirillum* sp., and *Methanoculleus* sp. These archaea produce CH_4 from acetic acid and exception of *Methanocorpusculum* sp. and *Methanoculleus* sp. using CO_2 and H_2 for CH_4 production [62]. The order *Methanosarcinales* consists of *Methanosarcina* sp., *Methanohalobium* sp., *Methanohalophilus* sp., *Methanolobus* sp., and *Methanosaeta* sp. *Methanosarcina* sp. are hydrogenotrophic or acetoclastic and thus can reduce CO_2 to CH_4 or can utilize acetic acid to CH_4 and CO_2 . *Methanosarcina* sp. also can convert methyl-group-containing compounds such as methanol, methylamines, and methyl sulfides to CH_4 and CO_2 . *Methanosaeta* sp. utilizes acetic acid as the energy source through acetoclastic reaction.

Acidogenic H_2 producers grow faster than methanogens and eventually produce VFA in effluent. Major genres related to acidogenic H_2 production are *Enterobacter* sp., *Clostridium*

sp., *Citrobacter* sp., *Thermoanaerobacterium* sp., and *Caldicellulosiruptor* sp. After H₂ production, effluents rich in VFA such as acetic acid, butyric acid, lactic acid, and ethanol would be consumed by methanogenic archaea at neutral pH. High acetic acid concentration promotes the growth of *Methanosarcina* sp. On the contrary, lower acetic acid concentration is preferred by *Methanosaeta* sp. For acetoclastic methanogens such as *Methanosarcina* sp., the minimum thresholds for acetate utilization are typically in the range of 0.5 mM and higher. The minimum thresholds for acetic acid utilization of *Methanoseata* sp. are in the micromole range. The presence of *Clostridium*, *Bacillus*, and *Desulfobacterium* in CH₄ production stage is in accordance with the significant removal of lactic acid in the H₂ effluent since *Clostridium* and *Desulfobacterium* spp. are able to degrade lactic acid to acetate and/or H₂ [63]. Meanwhile, some acidogenic bacteria, *Thermoanaerobacterium* sp., *Clostridium roseum*, and *Clostridium isatidis*, which are H₂ producers [64–66] were also detected in CH₄ stage, confirming that some H₂ and CO₂ were also produced. However, the presence of the hydrogenotrophic methanogens of *Methanothermobacter defluvii* and *Methanothermobacter thermautotrophicus* could possibly consume H₂; thus, no H₂ could be detected when the methanogenic stage reached stable conditions [67].

4. Process parameters affecting biohythane production

Biohythane production processes are greatly influenced by complex biochemical and physical parameters. The process parameters such as inoculum properties, complexity of substrate, nutrient, alkalinity, H₂ concentration, hydraulic retention time (HRT), and toxic compounds have influence on biohythane process (Table 3). Inoculums and feedstocks compositions greatly affect first stage H₂ fermentation when using mixed cultures and non-sterile feedstocks [1, 70, 74]. Environmental and physical factors greatly affect the second stage CH₄ production [75, 76]. To stabilize and maximize H₂ production, it is necessary to direct the metabolic pathway toward acetic acid and/or butyric acid and also to maintain the right H₂-producing bacteria during first stage operation. The performance of microorganisms in the conversion of substrate to H₂ is also dependent on the efficiency of its enzymatic machinery. The main factors affecting two-stage anaerobic fermentation are described as follows.

4.1. Feedstocks

Biohythane can be produced from various substrates mainly carbohydrate. In terms of H₂ rate and yields, carbohydrates are the most suitable feedstock followed by protein and peptides, while fat is considered very limited [77]. Most of dark fermentation for H₂ production has been conducted with glucose or sucrose. Glucose is the monomeric unit of cellulose and starch which is a major component in organic wastes [78]. Carbohydrate-rich organic waste is a favorable substrate for H₂ fermentation [79, 80]. The H₂ yield from bean curd manufacturing waste was significantly low compared to carbohydrate-rich substrates [80]. For stable H₂ fermentation, a carbon/nitrogen (C/N) ratio of feedstock greater than 20 is recommended [81]. The H₂ fermentative microorganisms showed improvement in H₂ production when they were grown in a fermentation media having a C/N ratio greater than 20. The C/N ratio of 20–30 also has positive effect on CH₄ production stage. Phosphate concentration in feedstock is also

Factors	Effects on biohythane process	References
Feedstocks	<ul style="list-style-type: none">• Fermentation metabolism, microbial activity, and microbial community	[68]
Inoculum	<ul style="list-style-type: none">• Fermentation metabolism and microbial community	[69]
pH and Alkalinity	<ul style="list-style-type: none">• Fermentation metabolism, microbial activity, and microbial community	[70]
Temperature	<ul style="list-style-type: none">• Cell membrane charge• Metabolic shift to solvent production• Fermentation metabolism, microbial activity, and microbial community	[71]
HRT	<ul style="list-style-type: none">• Fermentation metabolism, microbial activity, and microbial community• Microbial growth rate	[72]
H ₂ Partial Pressure	<ul style="list-style-type: none">• Fermentation metabolism and activity• Activity of acetogens and methanogens	[70]
Trace element	<ul style="list-style-type: none">• Essential for cell growth,• Enzyme activity	[73]

Table 3. Main factors affecting the two-stage anaerobic fermentation for biohythane production from organic wastes.

important in dark fermentation. Phosphate helps in maintaining buffered condition during fermentation and provides the building blocks of nucleic acid and ATPs. In dark fermentation, an increase in phosphate concentration leads to enhancement of the H₂ production [47].

4.2. Inoculums

Developing an enriched inoculum is very important for obtaining H₂ in first stage fermentation. In the enrichment process, selection procedure was applied to selectively promote H₂-producing bacteria and eliminate H₂ consumers. Different selective procedures such as heat, acid, ultrasonic, ultraviolet, organic and alkali treatment were commonly used [58]. Most of H₂-producing bacteria are spore forming, while H₂-consuming bacteria and methanogens are non-spore forming, which get eliminated with selection methods. The selection methods are promoting endospores formation in a certain group of bacteria that also include H₂-producing bacteria. Thus, under favorable conditions, the endospores germinate and the H₂-producing bacteria dominate in the system. The H₂-producing inoculum might consist of sporulating bacteria like *Bacillus* sp. and *Clostridium* sp. Furthermore, the bacteria capable of producing H₂ widely exist in natural environment in the form of mixed cultures such as anaerobic sludge, municipal sewage sludge, hot spring sediment, compost and soil have been widely used as inoculum for fermentative H₂ production [82–84]. Using mixed cultures is more practical than using pure cultures due to the easy operating and control under the non-sterile condition. Mixed cultures also have a broader source of feedstock [85]. The selection of H₂-producing bacteria suitable for introduction into H₂ reactor may be regarded as inoculum preparation. It should consider the revival of bacteria from the stock, successive of subculturing to active bacteria, short lag phase and high active

cells [86]. Inoculum size for dark H_2 fermentation was varied in the range of 10–20% (v/v). This depends on the characteristics of the species and medium used. Obligate anaerobes produce very less amount of biomass; thus, larger inoculum volume and concentration are required. The inoculum age also matters during the fermentation. Cells growing at the exponential phase have the entire enzymatic machinery active which is required for H_2 and CH_4 production.

4.3. Hydrogen partial pressure

The H_2 partial pressure in the liquid phase is the major factor affecting H_2 production, as high H_2 partial pressure causes deactivation of hydrogenase enzyme. Decreasing H_2 partial pressure by intermittent nitrogen sparging of batch reactor headspace could enhance H_2 production during thermophilic fermentation [87]. In addition to a high H_2 partial pressure, the NADH, which is an electron carrier in the cell, will be oxidized mainly to lactate during extreme thermophilic fermentation with *Caldicellulosiruptor saccharolyticus* [88]. The formation of lactate during the overloading or unstable conditions might be caused by a high H_2 partial pressure.

4.4. Hydraulic retention time (HRT)

The total time that cells and soluble nutrients reside in the reactor is called the HRT. H_2 production occurring at low HRT is dependent on the volume of the reactor and the flow rate of feed. It is generally well known that the H_2 -producing bacteria are fast growing [70]. By applying this principle, Liu et al. [48] produced H_2 free of CH_4 in continuously CSTR feeding with household solid waste at acidic pH range of 5.0–5.5 and a short HRT of 3 days without any pretreatment to inhibit methanogens contained in the initial digested manure. HRT is the main optimization parameters of continuous H_2 dark fermentation bioprocesses. In the CSTRs, short HRTs or high dilution (D) rates can be used to eliminate methanogens, which have significant low growth rate [70, 89]. However, HRT is needed to be maintained in a proper level that still gives a D value less than specific growth rate of H_2 -producing bacteria. Generally, short HRT is considered to favor the H_2 fermentation metabolism [3]. On the other hand, too high loading rates may result in substrate inhibition effects, improper food to microorganism (F/M) ratios of H_2 producers or washout of microorganisms [90]. These shock loads could reduce the H_2 production metabolism through decreasing of pH and metabolite inhibition (accumulation of intermediates). The HRT could also help in the enrichment of microbial consortium, since it directly affects the specific growth rate of bacteria. By manipulating the HRT, slow-growing microbes like methanogens and H_2 -consuming microbes can be expelled out of the reactor, thus leading to selective enrichment of H_2 -producing bacteria [91]. This approach of using short HRT for suppressing methanogens led to improvement in H_2 production [92]. In second stage, the HRT is a measure to describe the average time that a certain substrate resides in a digester. If the HRT is shorter, the system will fail due to washout of microorganisms. HRT for anaerobic digestion process are typically in the range of 15–30 days at mesophilic conditions and 10–20 days at thermophilic conditions [13]. Long retention times also benefit hydrolysis of the particulate matter of complex structure such as lignocellulose biomass [93]. On the other hand, organic loading rate (OLR) or amount of organic matter in the system is relative with HRT. The shorter HRT will achieve high OLR that leads to the accumulation of VFA which consequently leads to a pH drop and inhibition of methanogenic

activity. This causes a system failure. During methanogenesis, the HRT should be kept two-fold greater than the generation time of the slow-growing microbes [94]. The HRT should be held for a suitable duration so that the dead zones get eliminated, and it would also help in promoting an efficient syntrophy among the microorganisms present in the mixed culture.

4.5. pH and alkalinity

Among all the chemical factors influencing dark fermentation, pH is considered the most influential. It influences the stability of the acid-producing fermentative bacteria and acetoclastic CH_4 -producing archaea. It plays a major role in the oxidation-reduction potential of the anaerobic process. Thus, it directly impacts the metabolic pathway. In most of literature reports, a pH of 5.5 has been considered to be the optimum pH for H_2 production [3, 47, 70, 95]. The optimal initial pH range for the maximum H_2 yield or specific H_2 production rate is between pH 5.5 and 6.5 [95]. The optimal pH is highly dependent on the microorganism. The control of pH and alkalinity of a substrate is essential for first stage dark fermentation since organic acids produced tend to decrease the pH. The pH lower than 4.5 trends to inhibit the activity of hydrogenases. Low pH also causes a shift of metabolic pathways of dark fermentation microorganisms away from H_2 production. H_2 -producing bacteria like *Clostridium acetobutylicum* can change metabolism from H_2 (acetate and butyrate pathway) to the production of solvents (acetone and butanol pathway) when the pH is decreased to less than 5.0. Alternatively, depending on the organism, low pH can shift the metabolism toward ethanol production [72]. Carbohydrate-based substrates provide good carbon and energy sources for H_2 -producing bacteria. The fermentation process needs buffering of the growth medium, and to be supplemented with nutrients to enhance the growth of microorganisms and resist the pH change caused by organic acids produced [9, 55, 96]. CH_4 production is favored at alkaline pH exhibiting maximum activity at pH of 7.8–8.2 [97]. The rate of CH_4 production may decrease if the pH is lower than this optimal range. The pH is also an important factor for the stability of CH_4 production. The H_2 effluent which is rich in VFA, may cause a drop in pH if fed with high OLR. The pH adjustment can be achieved by an addition of alkali chemical, typically calcium carbonate or sodium hydroxide. A cheap material like ash was used to adjust the pH in an anaerobic reactor [98]. A stable CH_4 production process is characterized by the bicarbonate alkalinity in the range of 1000–5000 mg/L as CaCO_3 . The ratio between VFA and alkalinity should be in the range of 0.1–0.25.

4.6. Temperature

Temperature is one of the most important factors affecting the growth of microorganisms. The operating temperature influences the growth rate of bacteria by influencing the biochemical reactions responsible for the maintenance of homeostasis and their metabolism. H_2 -producing dark fermentation reactors can be operated in various temperature ranges from mesophilic (35–45°C), thermophilic (55–60°C) to extreme thermophilic (70–80°C) conditions. Most of the H_2 dark fermentation studies have been conducted at temperature range of 35–45°C. Many mesophilic bacteria such as *Clostridium* sp. and *Enterobacter* sp. showed optimal H_2 production in the temperature range of 35–45°C [99]. A thermophilic H_2 -producing bacterium gave higher H_2 yield compared to mesophilic bacteria [100]. When temperature rises, microbial growth rates increase due to the increase in the rates of chemical and enzymatic reactions in

their cells. Thermophilic temperature makes the H_2 production process thermodynamically favorable with the H_2 yield of ~ 2.1 mol H_2 /mol glucose, while mesophilic H_2 production gave the yield of ~ 1.7 mol H_2 /mol glucose [101]. Although the H_2 yield from thermophilic temperature was slightly higher than that for mesophilic temperatures, the specific H_2 production rate (mmol H_2 /h·gVSS) for thermophilic temperatures was 5–10 times higher than that from the mesophilic temperatures. Thermophilic H_2 -producing bacteria has certain operation advantages such as low solubility of H_2 and CO_2 , less influenced by the H_2 partial pressure, better solubility of the substrate, improved hydrolysis reaction as well as thermodynamic efficiency. Temperature is also a very important operation factor in the second stage for anaerobic digestion process. It determines the rate of anaerobic digestion process, particularly the rate of hydrolysis and methanogenesis. The thermophilic process could accelerate the biochemical reactions and give higher degradation efficiency as well as higher CH_4 production rates compared to mesophilic condition [102]. As temperature increases, the rate of retention time process is much faster and this results in more efficient operation and lowers the retention time requirement [97]. Thermophilic condition also increases in thermodynamic favorability of CH_4 -producing reactions, decreases solubility of CH_4 and CO_2 , and destruction of pathogens in the reactor effluent. Methanogens are extremely subtle to change in temperature and even a small temperature variation ($2\text{--}3^\circ\text{C}$) can lead to VFA accumulation [103]. This decreases the CH_4 production rate for methanogens, especially at the thermophilic conditions. Maintaining the stable temperature is important for biohythane production.

4.7. Trace elements

Biohydrogen and biomethane production required various types of metal ions as micronutrients. These metal ions play a critical role in the metabolism of microorganisms. Metal ions such as Fe^{2+} , Zn^{2+} , Ni^{2+} , Na^+ , Mg^{2+} , and Co^{2+} play a pivotal role in both biohydrogen and biomethane process. Metals are essential to supplement in media for dark fermentation. These micronutrients might be required in trace amounts but they have an influential role as cofactors, transport processes facilitators, and structural skeletons of many enzymes (Fe-Fe hydrogenase and Ni-Fe hydrogenase) involved in the biochemistry of H_2 formation [104]. Therefore, several researchers have studied the effect of supplementation of Fe ion on biohydrogen production. For example, Lee et al. [105] studied the effect of Fe ion concentration ($0\text{--}4000$ mg/L) on H_2 fermentation and found that the H_2 production increased with iron concentration of 200 mg/L. The addition of Fe ion 200 mg/L influences the system positively with increasing H_2 production from 131 to 196 mL H_2 /g sucrose. Ferchichi et al. [106] suggested that the supplementation with Fe^{2+} ions (12 mg/l) led to a shift in their metabolic profile, for example, supplementation with Fe^{2+} ion concentration of 12 mg/l caused a metabolic shift from lactic acid fermentation to butyric acid fermentation. Magnesium ions function as a cofactor of many enzymes such as kinases and synthetases. In glycolysis, many enzymes require magnesium ions as a cofactor. The activation of hexokinase, phosphofructokinases, glutaraldehyde-3-phosphate dehydrogenases, and enolases helps bacteria to metabolize substrate and produce energy component ATP [107]. Fe ion also plays a critical role in biomethane stage. The Fe ion is required by methanogenic archaea like *Methanosarcina barkeri* to synthesize protocheme via precorrin-2, which is formed from uroporphyrinogen III in two consecutive methylation reaction utilizing S-adenosyl-L-methionine [108]. Nickel is also an

essential metal which plays a critical role in functioning of many enzymes that are responsible for CH_4 production such as monoxide dehydrogenase, hydrogenase, and methyl coenzyme M reductases.

5. Reactors configuration for biohythane production

The bioreactors in which the microorganisms are grown also play a crucial role. The design and the configuration of the fermenter help in the improvement of mixing characteristics and manipulation of overhead gas partial pressure. Parameters such as HRT and recycle ratio are influenced by the bioreactors configuration. The progress on two-stage system was presented based on the type of feeding substrates, classified as sugar-rich biomass, food/municipal waste, cellulose-based biomass, and palm oil mill effluent (POME). Over 20% of the publications reported so far focused on a system using sugar-rich synthetic wastewater. The most commonly used sugars were glucose and sucrose [10]. The maximum biohythane production was 3.21 mol H_2 /mol hexose and 3.63 mol CH_4 /mol hexose from glucose and acetic acid (synthetic wastewater) in CSTR reactor [109]. The summarized H_2 and CH_4 yield from various two-stage reactors configuration used for biohythane production is shown in **Table 4**. The schematic flow diagrams of each two-stage anaerobic fermentation systems for biohythane production are shown in **Figure 2**. The two-stage anaerobic fermentation is suitable for individual optimization of the H_2 and CH_4 production processes. For example, temperature-dependent process will be favored by the two-stage process, where high yield of H_2 could be achieved under thermophilic conditions, and stable maintaining of CH_4 production might be achieved under mesophilic conditions [13, 15, 21, 110]. Solubilization and saccharification of organic wastes with high solid content can be realized simultaneously during the first stage H_2 production [17, 74]. The two-stage anaerobic fermentation systems by integrated continuous stirred-tank reactor (CSTR) with anaerobic baffled reactor (ABR), CSTR with UASB, CSTR with CSTR, UASB with UASB, ASBR with UASB and stepped anaerobic baffled (SAB) were used for biohythane production (**Figure 2**). The system with a CSTR and an upflow biofilter reactor for H_2 and CH_4 production from sucrose was established [89]. This system inoculated with heat-treated sludge as inoculum achieved a maximum H_2 yield of 1.62 mol H_2 /mol hexose. The second stage reactor inoculated with raw anaerobic sludge achieved a maximum CH_4 yield of 323 L CH_4 /kg COD. The analysis of COD balance showed that 13.5% of the influent COD was transformed to H_2 and 70% of the influent COD was transformed to CH_4 . A CSTR H_2 and CSTR CH_4 system fed with synthetic glucose medium using the same anaerobic sludge as inoculums was reported [18]. By optimizing the inoculums-to-substrate ratio (2:1) in this CSTR-CSTR system, the H_2 yield and the methane yield increased to 2.75 and 2.13 mol/mol hexose, respectively, with 10 g/L glucose as a substrate, which corresponded to a total energy recovery of 82%. A similar reactor configuration was also used by Lee et al. [25] and Hafez et al. [109]. A synthesis wastewater containing glucose and acetic acid produced 2.6 mol H_2 /mol hexose and 426 mL CH_4 /kg COD via continuous fermentation in CSTR [109]. The stable H_2 production in the CSTR was possibly due to the introduction of a gravity settler after the H_2 CSTR for H_2 -producer retention. A complete CSTR system for H_2 and CH_4 production from cassava stillage was developed [12]. The gas yields under thermophilic conditions with high

Reactors (H ₂ and CH ₄)	Feedstock and conditions	H ₂ production yield (L-H ₂ /kg VS)	CH ₄ production yield (L-CH ₄ /kg VS)	Biogas composition	References
CSTR and CSTR	Olive pulp, temperature of 35 and 35°C, pH of 5 and 7	190	160	1.6% H ₂ 38.3% CO ₂ 60% CH ₄	[110]
UASB and UASB	Desugared molasses, temperature of 70 and 55°C, pH of 5 and 7	89	307	16.5% H ₂ 38.7% CO ₂ 44.8% CH ₄	[11]
CSTR and UASB	Sugarcane syrup, temperature of 37 and 30 °C, pH of 5.5 and 7.5	88	271	19.6% H ₂ 62.6% CO ₂ 10.9% CH ₄	[111]
ASBR and UASB	POME, temperature of 55 and 35°C, pH of 5.5 and 7.5	210	315	14% H ₂ 32% CO ₂ 51% CH ₄	[13]
CSTR and UASB	POME, temperature of 55 and 35 °C, pH of 5.5 and 7.5	135	414	13.3% H ₂ 32.2% CO ₂ 54.4% CH ₄	[15]
CSTR and CSTR	Biowaste, temperature of 55 and 35 °C, pH of 5.5 and 8	41	102	6.7% H ₂ 40.1% CO ₂ 52.3% CH ₄	[112]
CSTR and UASB with gas upgrade systems	Wheat straw, temperature of 70 and 37°C, pH of 6.9 and 7.5	270	179	46–57% H ₂ 0.4% CO ₂ 43–54% CH ₄	[113]
CSTR and ABR	Food waste, temperature of 55 and 35°C, pH of 5.5 and 7.5	205	464	15% H ₂ 54.5% CO ₂ 30.5% CH ₄	[21]
SAB	Petrochemical wastewater, temperature of 21 and 21°C, pH of 5.5 and 7.5	88	318	16% H ₂ 27% CO ₂ 52% CH ₄	[114]

Table 4. Hydrogen and methane yield from various reactor configurations used for two-stage biohythane production.

organic loading (13 g COD/L·d) were 56.6 L H₂/kg TS, and 249 L CH₄/kg volatile solid (VS), respectively. Chu et al. [21] developed a two-stage thermophilic CSTR reactor and a mesophilic ABR reactor with the heat-treated digested sludge to recirculation to first reactor for H₂ and CH₄ production from organic fraction of municipal solid wastes (OFMSW). The separation of H₂ and CH₄ production was successful by operating the H₂ reactor at a controlled HRT of 1.3 days, and pH of 5.5. Kongjan et al. [11] established a biohythane process from wheat straw hydrolysate by two-stage extreme thermophilic UASB and thermophilic UASB. Specific

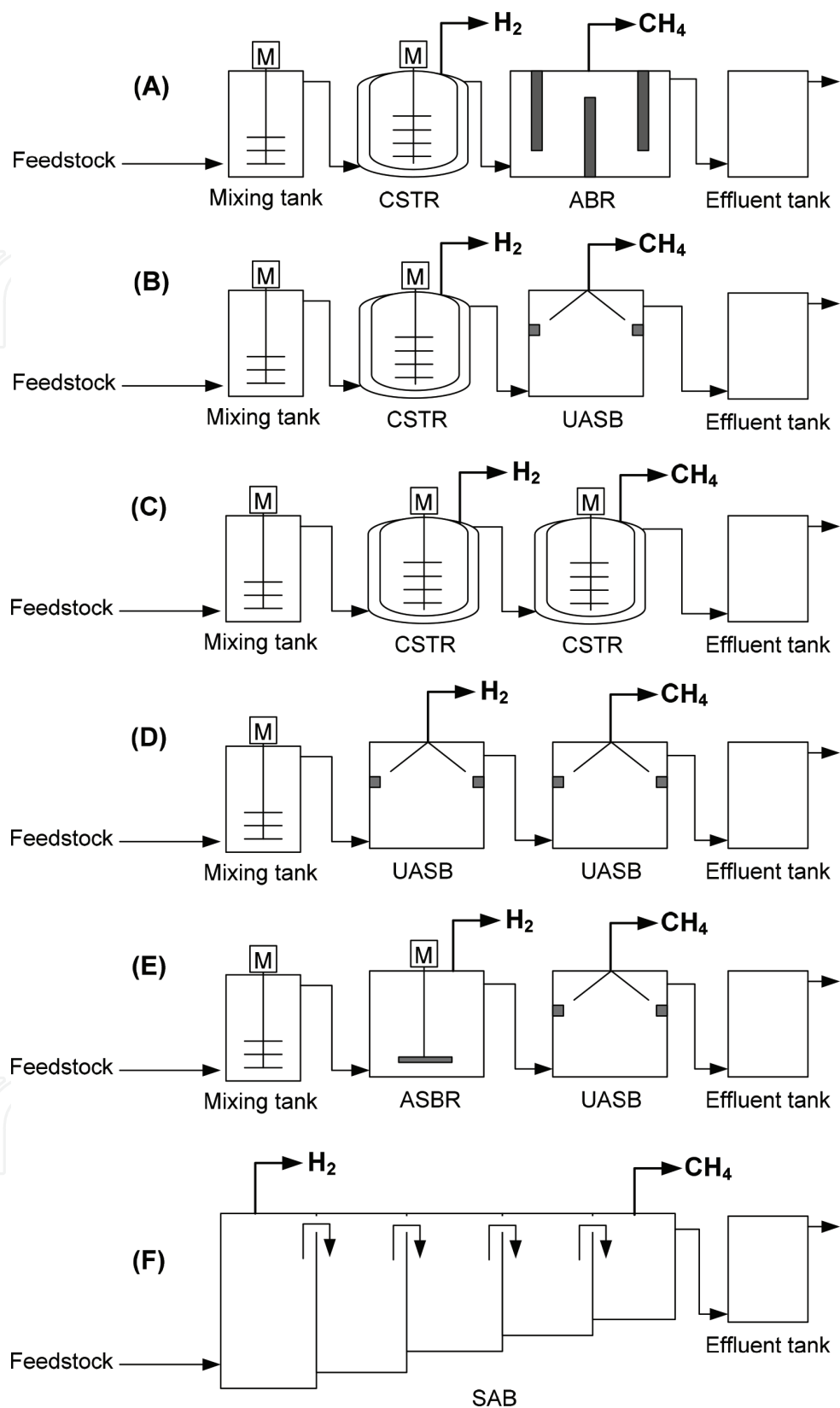


Figure 2. Schematic flow diagrams of two-stage anaerobic fermentation systems for biohythane production by integrated CSTR with ABR (A), CSTR with UASB (B), CSTR with CSTR (C), UASB with UASB (D), ASBR with UASB (E) and SAB (F).

H₂ and CH₄ yields of 89 mL-H₂/g-VS (190 mL H₂/g sugars) and 307 mL CH₄/gVS, respectively were achieved simultaneously with the overall VS removal efficiency of 81% by operating with total HRT of 4 days. A biohythane gas with the composition of 16.5% H₂, 44.8% CH₄, and 38.7% CO₂ could be produced at high production rates (3.5 L/L·d). *Thermoanaerobacter wiegelii*, *Caldanaerobacter subteraneus*, and *Caloramator fervidus* were responsible for H₂ production in the H₂-UASB reactor. Meanwhile, the CH₄-UASB reactor was dominated with methanogens of *Methanosarcina mazei* and *Methanothermobacter defluvii*. Successful biohythane production from palm oil mill effluent (POME) by two-stage thermophilic ASBR followed by mesophilic UASB was achieved by Mamimin et al. [13]. The continuous biohythane production rate of 4.4 L/L·d with biogas composition of 14% H₂, 51% CH₄ and 35% CO₂ was achieved. O-Thong et al. [15] established two-stage thermophilic CSTR and mesophilic UASB with methanogenic effluent recirculation to H₂ reactor for biohythane production from POME. The 30% recirculation rate of methanogenic effluent could keep pH at optimal pH with two times increase in H₂ production when compared with non-recirculation systems. The H₂ and CH₄ yields were 135mL H₂/gVS and 414 mL CH₄/gVS, respectively. Biohythane gas composition was composed with 13.3% H₂, 54.4% CH₄, and 32.2% CO₂. *Thermoanaerobacterium* sp. was dominated during H₂ production from POME, whereas archaea belonging to *Methanosarcina* sp. and *Methanoculleus* sp. were dominated in the CH₄ reactor. A two-stage process with methanogenic effluent recirculation favored *Thermoanaerobacterium* sp. in the H₂ reactor and efficiently for energy recovery from POME. Elreedy et al. [114] established biohythane production from petrochemical wastewater containing mono-ethylene glycol by a novel stepped anaerobic baffled (SAB) reactor. The reactor was continuously operated for 5 months at constant hydraulic retention time (HRT) of 72 h with hydrogen and methane yield of 88 mL H₂/gVS and 318 mL CH₄/gVS, respectively.

Reactors are considered to be practical and economical for industrial H₂ production, particularly via mixed culture fermentation [70, 100]. The two main bioreactor configurations: suspended and attached, or immobilized, growth types have been applied to optimize fermentation process for H₂ production through advancements in active biomass concentration and substrate conversion efficiency [101, 115]. Most studies on H₂ production from carbohydrate rich substrates have been conducted in suspended CSTRs, which are simple to construct, easy to regulate both acidity and temperature, and give complete homogeneous mixing for direct contact between the substrate and active biomass [1, 70, 72]. Furthermore, the CSTR is very suitable for substrates with a high-suspended solid (SS) content, typically with a volatile solid (VS) content of 2–12% [48]. However, in CSTR reactor, HRTs must be greater than the specific growth rate of the microorganisms in order to control the proper concentration of microbial biomass, but faster dilution rates risk active biomass washout [1, 67] leading to process failure. In addition, cell density retained in CSTR is limited, since the active biomass has the same retention time as HRT, resulting in process instability caused by the fluctuation of environmental parameters, including acidity and then having the consequence of limiting substrate degradation and H₂ production. To overcome the above mention problem, a new configuration of a continuous flow reactor is required to decouple the cell mass retention from HRT and subsequently retain higher cell densities in the reactor, such as UASB and ASBR, which can be achieved through granules and biofilm [47, 91, 115, 116]. Cells immobilization can be

employed successfully by using a diluted waste stream with relatively small reactor volumes in ASBR, SAB, and UASB reactors. However, such a reactor configuration has a poor mass transfer system, which is mainly caused by a lack of mixing; this can lead to gases accumulating in the biofilm or granular sludge that risk losing H_2 by H_2 -consuming bacteria [92, 101]. Mass transfer can be improved by mechanical stirring or liquid recirculation, depending on the reactor type and configuration. Also, applying proper bioreactor shapes and optimizing reactor dimensions such as the height to diameter ratio can help to improve mass transfer efficiency [91, 98, 117–119].

The anaerobic conversion of VFA to CH_4 is mainly associated with sequential stages of acetogenesis and methanogenesis. When optimizing a methanogenic process using VFA rich, soluble organic matters, the goal is to maximize both CH_4 production and VFA degradation, while keeping the reactor stable [37]. The acetogenesis is limited mainly by VFA degradation, especially propionate that is the rate-limiting factor in the second stage anaerobic process. The investigation into optimizing the methanogenic reactor is mostly carried out by varying OLRs via increasing the substrate concentration or decreasing the HRTs to obtain satisfactory performance [25, 120]. The main signs of methanogenic reactor instability or overloading are decrease in pH [121]. As a drop of pH actually corresponds to VFA accumulation, pH below 6.3 has an impact on enzyme activity in the microorganisms involved in the second stage anaerobic digestion. Methanogenic archaea can function properly in a pH range between 6.5 and 7.8 [122]. Thus, a buffering solution is needed in order to resist a pH drop from VFA accumulation in the methanogenic process and maintain stability. The main buffer in the anaerobic digester is bicarbonate (HCO_3^-), which is usually added to carbohydrate rich substrates before feeding them to the first stage of H_2 fermentation because the first stage needs to be controlled with pH within the favorable range of 5–6 for H_2 -producing bacteria [123, 124]. Lee et al. [25] found that the pH drop below 6.4 caused by the accumulation of 122 mM VFA in the attached growth reactor operated at 55°C and fed with 11.0 gVS/L·d (5.13 d HRT) of the food waste fermentation. The pH could inhibit the bioactivity of methanogenesis. Meanwhile, the maximum CH_4 production rate of 2100 mL CH_4 /L·d with a CH_4 content of 65% was obtained at pH around 7.5, where the reactor was operated at a 7.7 day HRT (7.9 gVS/L·d OLR) and almost VFA degradation was achieved. For the high rate anaerobic reactor, UASB reactor was operated at double OLR comparing to CSTR at thermophilic temperature (55°C) which providing better VFAs degradation than mesophilic temperature (35°C) [125]. This is mainly attributed to the increase of chemical and biological reaction rates for operating temperature of thermophilic condition and the organic acid oxidation reactions become more energetic at higher temperature [126, 127]. Because the H_2 reactor effluents are in soluble form of organic matters as the consequence of hydrolysis and acidogenesis in the first stage, the reactor type used to convert these soluble organic matters to CH_4 in the second stage are based on high rate biofilm systems as reviewed by Demirel et al. [27]. Cell mass is retained well in the biofilm/granular aggregates in biofilm systems, leading to have much higher sludge retention time (SRT) compared to HRT, which provides the advantage that the reactor can run at a higher flow rate and can tolerate higher toxic concentrations [128]. Various types of high rate biofilm systems such as UASB, ABR, and SAB can be operated by continuous feeding with the H_2 reactor effluent, with HRTs of less than 5 days [114, 125, 129, 130]. Among the high rate reactor types, the UASB is the most popular for anaerobic treatment of soluble organic matters

due to the large surface area of granular sludge, which provides fast biofilm development and improves methanogenesis. Also clogging and channeling occur less in the UASB reactor than other biofilm systems [121].

6. Application of biohythane process

Methane is being commonly used, not only in the chemical industry but also in transport as compressed natural gas (CNG), which has been regarded as the clean energy carrier in comparison to gasoline or diesel. By combining the advantages of H_2 and CH_4 , biohythane is considered one of the important fuels involved in achieving the transition of technical models from a fossil fuel-based society to renewable-based society. CH_4 used as a fuel for vehicle has weak points on its narrow range of flammability, slow burning speed, poor combustion efficiency as well as requirement for high ignition temperature of CNG-powered vehicles. Interestingly, H_2 perfectly complements the weak points of CH_4 such as the hydrogen/carbon ratio which is increased by adding H_2 , which reduces greenhouse gas emissions. Adding H_2 , thus, improves the fuel efficiency and can extend the narrow range of flammability of CH_4 . The flame speed of CH_4 can be greatly increased by adding H_2 , eventually reducing combustion duration and improving heat efficiency. The quenching distance of CH_4 can be reduced by the addition of H_2 , making the engine easy to ignite with less input energy. A two-stage process technique, combining acidogenesis and methanogenesis appears to give more efficient waste treatment and energy recovery than a single methanogenic process [13]. As the results reported by Kongjan and Angelidaki [129], mixed gas of CH_4 , CO_2 , and H_2 with the volumetric content of 44.8, 38.7, and 16.5%, respectively, containing approx. 10% H_2 on energy basis could be achieved. This specification was found to be most suitable for burning directly in the internal combustion engines [131] and could be biohythane. In addition to economical concern, the two-stage thermophilic anaerobic process has been previously evaluated that the payback time is around 2–6 years, depending on the disposal costs of organic wastes/residues [28].

Various types of organic wastes can be used as substrate for biohythane production such as starch wastewater, palm oil mill effluent (POME), biowaste, sugarcane syrup, olive pulp, desugared molasses, food waste, and organic solid waste [13, 18, 19]. H_2 and CH_4 yield from two-stage biohythane production of palm oil mill effluent (POME) was 201 mL H_2 /gCOD and 315 mL CH_4 /gCOD, respectively [13], which were higher than those of starch wastewater (130 mL H_2 /gCOD and 230 mL CH_4 /gCOD, respectively) [18], sugarcane syrup (88 mL H_2 /gCOD and 271 mL CH_4 /gCOD, respectively) [111], and biowaste (21 mL H_2 /gCOD and 55 mL CH_4 /gCOD, respectively) [112]. H_2 and CH_4 yield from two-stage biohythane production of olive pulp (190 mL H_2 /gVS and 160 mL CH_4 /gVS, respectively) [110] was lower than that of food waste (205 mL H_2 /gVS and 464 mL CH_4 /gVS, respectively) [21]. Successful biohythane production from POME by two-stage thermophilic H_2 reactor and mesophilic CH_4 reactor was achieved with biohythane production rate of 4.4 L/L·d with biogas composition of 51% CH_4 , 14% H_2 , and 35% CO_2 [13]. POME is a suitable substrate for H_2 production in terms of high biogas production volume. Energy analysis of two-stage anaerobic fermentation

process has greater net energy recovery than the single stage H_2 production and single stage CH_4 production process. O-Thong et al. [15] applied two-stage thermophilic fermentation and mesophilic methanogenic process with methanogenic effluent recirculation to H_2 reactor for biohythane production from POME. The pH two-stage reactor was control by recirculation of methanogenic effluent with H_2 and CH_4 yield of 135 mL H_2 /gVS and 414 mL CH_4 /gVS, respectively. Flow diagram of successful thermophilic two-stage anaerobic fermentation for biohythane from POME at lab scale 5 L CSTR and 25 L UASB, semi-pilot scale 50 L CSTR and 250 L UASB and industrial scale 5 m³ CSTR and 25 m³ UASB are shown in **Figure 3**.

Improvement methods such as effluent recirculation to mix with feedstock in H_2 reactor, bio-methane gas recirculation to H_2 reactor, and the combined effluent recirculation to H_2 reactor with biomethane gas sparging to CH_4 reactor were reported to enhance biohythane production (**Figure 4**). The two-stage anaerobic fermentation process with methanogenic sludge recirculation (two-stage recirculation process) could be successfully operated and maintained at pH around 5.5 in H_2 reactor without any alkaline addition [21]. The recirculation of part of the methanogenic sludge to a H_2 reactor was provided as the buffer for the first stage. Kim et al. [132] also reported the recycling of a methanogenic effluent to a H_2 reactor with H_2 production increased from 1.19 to 1.76 m³ H_2 /m³·d, and decreased the requirement for alkali addition. H_2 yield from the two-stage anaerobic fermentation with the recirculation process was 2.5–2.8 mol/mol hexose [25], which was relatively high comparing to 4 mol/mol hexose from the maximum theoretical H_2 yield. The recirculation of the CH_4 effluent to hydrogen reactor could protect the H_2 fermentation process from a sharp drop in pH or organic overloading. Operations with the circulation of heat-treated sludge performed considerably better than those with the recirculation of raw sludge with respect to both the H_2 production rate and yield [19]. Lee et al. [25] improved two-stage anaerobic fermentation for biohythane production by biomethane gas sparging to second stage and recirculation biomethane effluent for pH adjustment in H_2 reactor. The gas yields were 2.3 mol H_2 /mol hexose and 287 L CH_4 /kg COD, respectively, while TS of food waste was kept at 10%. The recirculation of methanogenesis effluent provides ammonia-rich buffer, which flavors H_2 -producing bacteria eventually and improves the performance of the H_2 reactor. Liu et al. [34] were the first group to develop a two-stage CSTR-CSTR system for mesophilic H_2 and CH_4 production using household solid waste as both inoculum and substrate. The yields of H_2 and CH_4 were 43 and 500 L/kg VS, respectively, while the TS of the H_2 CSTR was maintained at 10%. CH_4 production was over 20% higher than that in single-stage CH_4 fermentation. Cavinato et al. [120] established a two-stage CSTR-CSTR reactor under thermophilic condition for biohythane production from municipal solid waste. The H_2 and CH_4 gas yields were 52 L H_2 /kg VS and 410 L CH_4 /kg VS, respectively. Willquist et al. [113] proposed a biohythane process from wheat straw including pretreatment, H_2 production using *Caldicellulosiruptor saccharolyticus*, CH_4 production using a methanogenic consortium, and gas upgrading using an amine solution. The first reactor was extreme thermophilic CSTR and the second reactor was mesophilic UASB applying for biohythane production. A biohythane gas with the composition of 46–57% H_2 , 43–54% CH_4 , and 0.4% CO_2 could be produced at high production rates (2.8–6.1 L/L·d), with 93% chemical oxygen demand (COD) reduction, and a net energy yield of 7.4–7.7 kJ/g dry straw. The CO_2

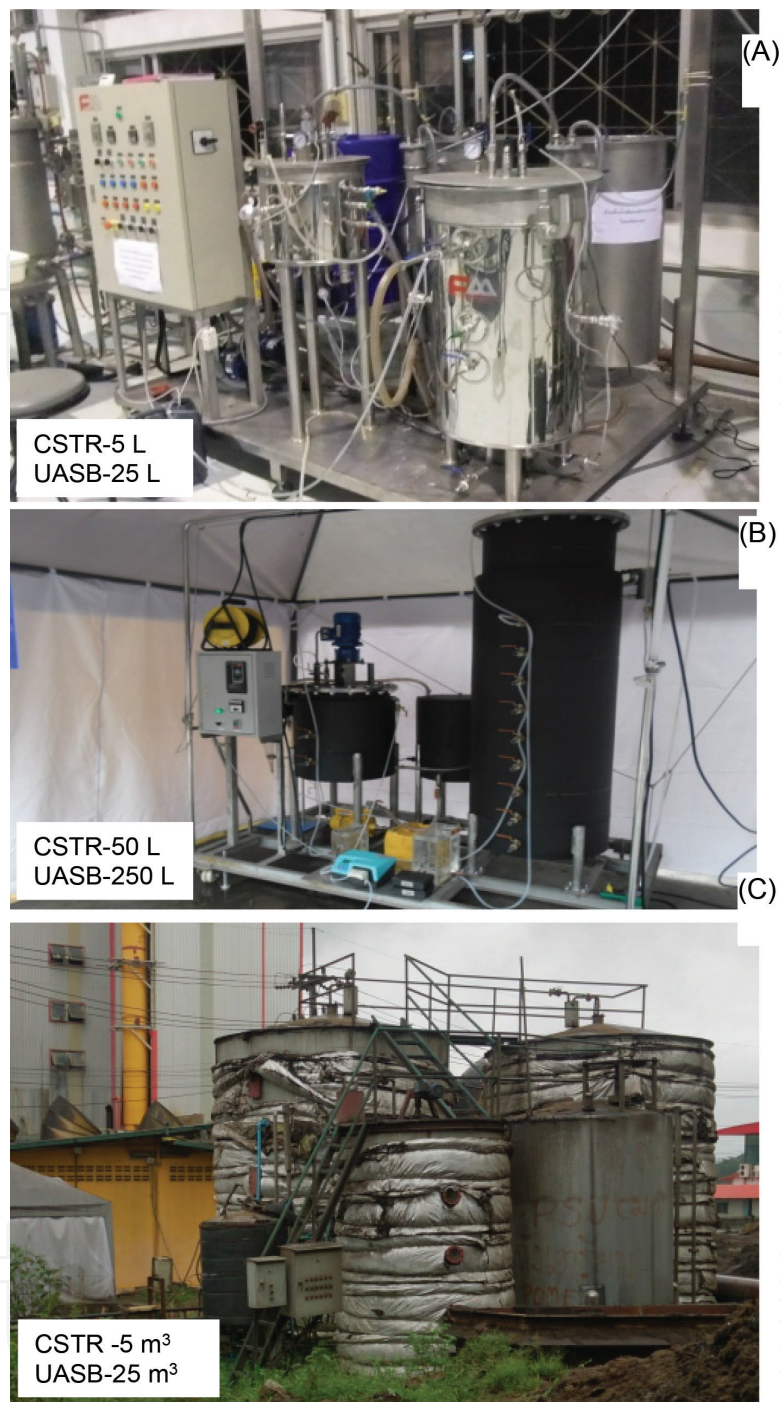


Figure 3. Flow diagram of scaling-up of the two-stage anaerobic fermentation for biohythane production from POME; a lab scale 5 L CSTR and 25 L UASB (A), semi-pilot scale 50 L CSTR and 250 L UASB (B), industrial scale 5 m³ CSTR and 25 m³ UASB (C).

has to be removed before the biogas can be used as hythane by an amine solution, consisting of a mixture of 40% N-methyldiethanolamine (MDEA), 10% piperazine (PZ) and 50% water, by weight. This is a solvent commonly used in industry for the removal of CO₂ in various mixtures of gases, including biogas.

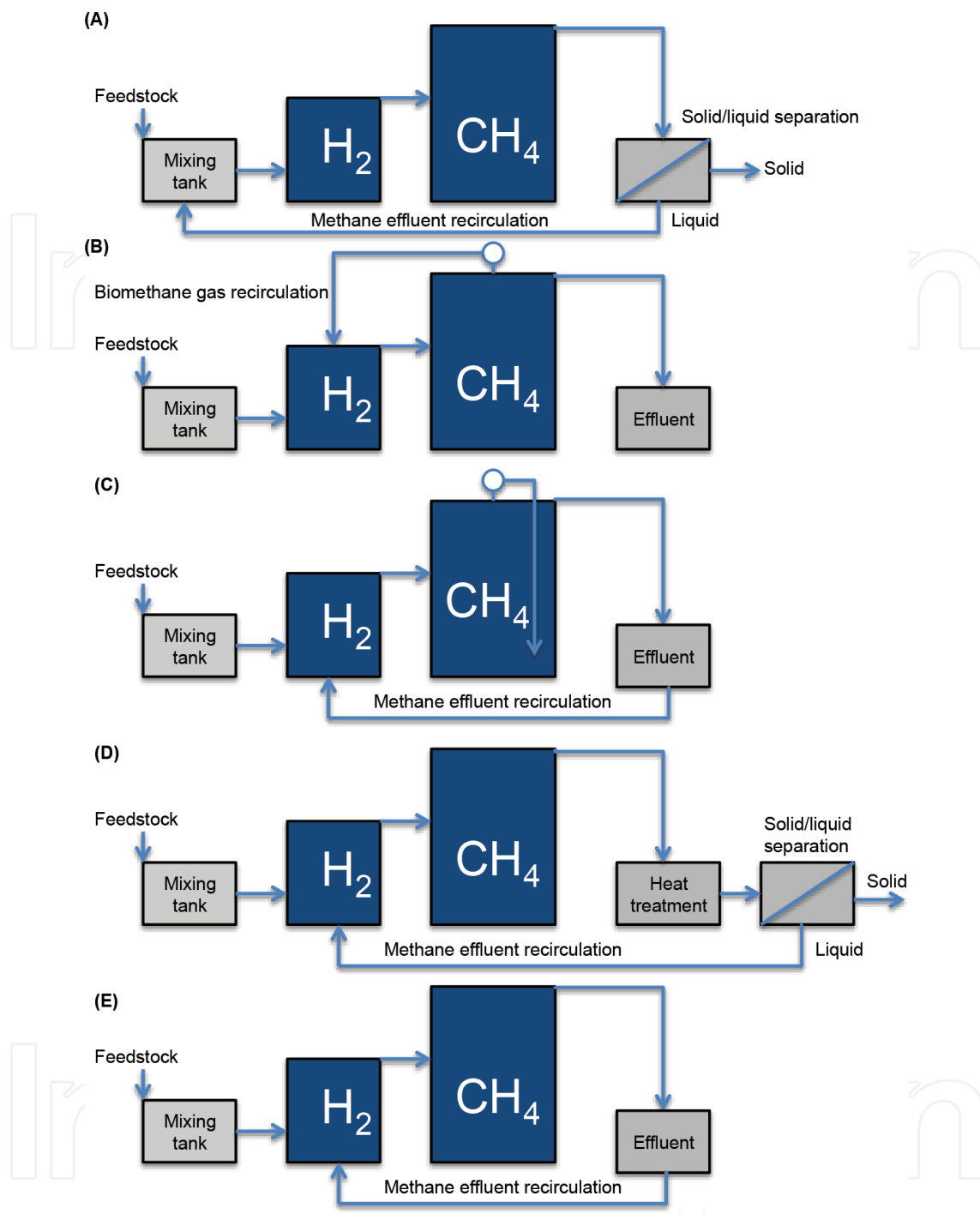


Figure 4. Schematic flow diagrams of gas yield improving for two-stage anaerobic fermentation for biohythane production by liquid methane effluent recirculation method (A), biomethane gas recirculation method (B), the combine liquid methane effluent recirculation and biomethane mixing method (C), liquid methane effluent heated recirculation method (D), and mixed solid and liquid methane effluent recirculation (E).

7. Conclusions

Biohythane via two-stage anaerobic fermentation using organic waste could be a promising technology for higher energy recovery and a cleaner transport biofuel than the biogas.

The H_2/CH_4 ratio of range 0.1–0.25 is suggested for biohythane. A flexible and controllable H_2/CH_4 ratio afforded by two-stage fermentation is of great importance in making biohythane. Biohythane can be achieved by two-stage anaerobic fermentation; in the first stage, organic wastes is fermented to H_2 , CO_2 , VFA, lactic acid and alcohols. Effluents from first stage containing VFA, lactic acid, and alcohols are converted to CH_4 in the second stage by methanogens under a neutral pH range of 7–8 and HRT of 10–15 days. The pH of 5–6 and an HRT of 2–3 days are optimized for first stage that favor acidogenic bacteria to convert organic wastes to H_2 . *Clostridium* sp., *Enterobacter* sp., *Caldicellulosiruptor* sp., *Thermotoga* sp., and *Thermoanaerobacterium* sp., are efficient H_2 producers in the first stage. *Methanosarcina* sp. and *Methanoculleus* sp. played an important role in the second stage CH_4 production. The combination of biohydrogen and biomethane production from organic wastes via two-stage anaerobic fermentation could yield a gas with a composition like hythane (10–15% of H_2 , 50–55% of CH_4 , and 30–40% of CO_2) called biohythane. Biohythane could be upgraded to bio-based hythane by removing CO_2 . The two-stage anaerobic fermentation could increase COD degradation efficiency, increase net energy balance, increase CH_4 production rates as well as high yield and purity of the products. In addition, the two-stage process has advantages of improving negative impacts of inhibitive compounds in feedstock, increased reactor stability with better control of the acid production, higher organic loading rates operation, and significantly reducing the fermentation time.

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References

- [1] Hallenbeck PC, Ghosh D. Advances in fermentative biohydrogen production: The way forward? Trends in Biotechnology. 2009;27:287-297. DOI: 10.1016/j.tibtech.2009.02.004

- [2] Angenent LT, Karim K, Al-Dahhan MH, Wrenn BA, Espinosa RD. Production of bioenergy and biochemicals from industrial and agricultural wastewater. *Trends in Biotechnology*. 2004;**22**:477-485. DOI: 10.1016/j.tibtech.2004.07.001
- [3] Hawkes FR, Dinsdale R, Hawkes DL, Hussy I. Sustainable fermentative hydrogen production: Challenges for process optimization. *International Journal of Hydrogen Energy*. 2002;**27**:1339-1347. DOI: 10.1016/S0360-3199(02)00090-3
- [4] Levin DB, Pitt L, Love M. Biohydrogen production: Prospects and limitation to practical application. *International Journal of Hydrogen Energy*. 2004;**29**:171-185. DOI: 10.1016/S0360-3199(03)00094-6
- [5] Kapdan IK, Kargi F. Bio-hydrogen production from waste materials. *Enzyme and Microbial Technology*. 2006;**38**:569-582. DOI: 10.1016/j.enzmictec.2005.09.015
- [6] van Ginkel SW, Oh SE, Logan BE. Biohydrogen gas production from food processing and domestic wastewaters. *International Journal of Hydrogen Energy*. 2005;**30**:1535-1542. DOI: 10.1016/j.ijhydene.2004.09.017
- [7] Pan J, Zhang R, El-Mashad HM, Sun H, Ying Y. Effect of food to microorganism ratio on biohydrogen production from food waste via anaerobic fermentation. *International Journal of Hydrogen Energy*. 2008;**33**:6968-6975. DOI: 10.1016/j.ijhydene.2008.07.130
- [8] Zhang T, Liu H, Fang HHP. Biohydrogen production from starch in wastewater under thermophilic condition. *Journal of Environmental Management*. 2003;**69**:149-156. DOI: 10.1016/S0301-4797(03)00141-5
- [9] O-Thong S, Prasertsan P, Intrasingkha N, Dhamwichukorn S, Birkeland NK. Improvement of biohydrogen production and treatment efficiency on palm oil mill effluent with nutrient supplementation at thermophilic condition using an anaerobic sequencing batch reactor. *Enzyme and Microbial Technology*. 2007;**41**:583-590. DOI: 10.1016/j.enzmictec.2007.05.002
- [10] Liu Z, Zhang C, Lu Y, Wu X, Wang L, Wang L, Han B, Xing XH. States and challenges for high-value biohythane production from waste biomass by dark fermentation technology. *Bioresource Technology*. 2013;**135**:292-303. DOI: 10.1016/j.biortech.2012.10.027
- [11] Kongjan P, O-Thong S, Angelidaki I. Performance and microbial community analysis of two-stage process with extreme thermophilic hydrogen and thermophilic methane production from hydrolysate in UASB reactors. *Bioresource Technology*. 2011;**102**:4028-4035. DOI: 10.1016/j.biortech.2010.12.009
- [12] Luo G, Talebnia F, Karakashev D, Xie L, Zhou Q, Angelidaki I. Enhanced bioenergy recovery from rapeseed plant in a biorefinery concept. *Bioresource Technology*. 2011;**102**:1433-1439. DOI: 10.1016/j.biortech.2010.09.071
- [13] Mamimin C, Singkhala A, Kongjan P, Suraraksa B, Prasertsan P, Imai T, O-Thong S. Two-stage thermophilic fermentation and mesophilic methanogen process for biohythane production from palm oil mill effluent. *International Journal of Hydrogen Energy*. 2015;**40**:6319-6328. DOI: 10.1016/j.ijhydene.2015.03.068

- [14] Kongjan P, O-Thong S, Angelidaki I. Hydrogen and methane production from desugared molasses using a two-stage thermophilic anaerobic process. *Engineering in Life Sciences*. 2013;**13**:118-125. DOI: 10.1002/elsc.201100191
- [15] O-Thong S, Suksong W, Promnuan K, Thipmunee M, Mamimin C, Prasertsan P. Two-stage thermophilic fermentation and mesophilic methanogenic process for biohythane production from palm oil mill effluent with methanogenic effluent recirculation for pH control. *International Journal of Hydrogen Energy*. 2016;**41**:21702-21712. DOI: 10.1016/j.ijhydene.2016.07.095
- [16] Si B-C, Li J-M, Zhu Z-B, Zhang Y-H, Lu J-W, Shen R-X, Zhang C, Xing X-H, Liu Z. Continuous production of biohythane from hydrothermal liquefied cornstalk biomass via two-stage high-rate anaerobic reactors. *Biotechnology for Biofuels*. 2016;**9**:1-15. DOI: 10.1186/s13068-016-0666-z
- [17] Liu Z, Si B, Li J, He J, Zhang C, Lu Y, Zhang Y, Xing XH. Bioprocess engineering for biohythane production from low grade waste biomass: Technical challenges towards scale up. *Current Opinion in Biotechnology*. 2018;**50**:25-31. DOI: 10.1016/j.copbio.2017.08.014
- [18] Xie L, Dong N, Wang L, Zhou Q. Thermophilic hydrogen production from starch wastewater using two-phase sequencing batch fermentation coupled with UASB methanogenic effluent recycling. *International Journal of Hydrogen Energy*. 2014;**39**:20942-20949. DOI: 10.1016/j.ijhydene.2014.10.049
- [19] Kobayashi T, Xu KQ, Li YY, Inamori Y. Effect of sludge recirculation on characteristics of hydrogen production in a two-stage hydrogen-methane fermentation process treating food wastes. *International Journal of Hydrogen Energy*. 2012;**37**:5602-5611. DOI: 10.1016/j.ijhydene.2011.12.123
- [20] Pawar SS, Nkemka VN, Zeidan AA, Murto M, Van Niel EWJ. Biohydrogen production from wheat straw hydrolysate using *Caldicellulosiruptor saccharolyticus* followed by biogas production in a two-step uncoupled process. *International Journal of Hydrogen Energy*. 2013;**38**:9121-9130. DOI: 10.1016/j.ijhydene.2013.05.075
- [21] Chu CF, Li YY, Xu KQ, Ebie Y, Inamori Y, Kong HN. A pH-and temperature-phased two-stage process for hydrogen and methane production from food waste. *International Journal of Hydrogen Energy*. 2008;**33**:4739-4746. DOI: 10.1016/j.ijhydene.2008.06.060
- [22] Nathao C, Sirisukpoka U, Pisutpaisal N. Production of hydrogen and methane by one and two stage fermentation of food waste. *International Journal of Hydrogen Energy*. 2013;**38**:15764-15769. DOI: 10.1016/j.ijhydene.2013.05.047
- [23] O-Thong S, Khongkliang P, Mamimin C, Singkhala A, Prasertsan P, Birkeland N. Draft genome sequence of *Thermoanaerobacterium* sp. strain PSU-2 isolated from thermophilic hydrogen producing reactor. *Genomics Data*. 2017;**12**:49-51. DOI: 10.1016/j.gdata.2017.02.012
- [24] Wang J, Yin Y. Principle and application of different pretreatment methods for enriching hydrogen-producing bacteria from mixed cultures. *International Journal of Hydrogen Energy*. 2017;**42**:4804-4823. DOI: 10.1016/j.ijhydene.2017.01.135

- [25] Lee HS, Vermaas WFJ, Rittmann BE. Biological hydrogen production: Prospects and challenges. *Trends in Biotechnology*. 2010;**28**(5):262-271. DOI: 10.1016/j.tibtech.2010.01.007
- [26] Si B, Li J, Li B, Zhu Z, Shen R, Zhang Y, Liu Z. The role of hydraulic retention time on controlling methanogenesis and homoacetogenesis in biohydrogen production using upflow anaerobic sludge blanket (UASB) reactor and packed bed reactor (PBR). *International Journal of Hydrogen Energy*. 2015;**40**:11414-11421. DOI: 10.1016/j.ijhydene.2015.04.035
- [27] Capson-Tojo G, Rouez M, Crest M, Steyer J-P, Delgenès J-P, Escudie R. Food waste valorization via anaerobic processes: A review. *Reviews in Environmental Science and Biotechnology*. 2016;**15**:499-547. DOI: 10.1007/s11157-016-9405-y
- [28] Wang X, Zhao YC. A bench scale study of fermentative hydrogen and methane production from food waste in integrated two-stage process. *International Journal of Hydrogen Energy*. 2009;**34**:245-254. DOI: 10.1016/j.ijhydene.2008.09.100
- [29] De Mes TZD, Stams AJM, Reith JH, Zeeman G. Methane production by anaerobic digestion of wastewater and solid wastes. In: Reith JH, Wijffels RH, Barten H, editors. *Bio-Methane & bio-Hydrogen: Status and Perspectives of Biological Methane and Hydrogen Production*. The Netherlands: Dutch Biological Hydrogen Foundation-NOVEM; 2003. pp. 58-102
- [30] Lee DY, Ebie Y, Xu KQ, Li YY, Inamori Y. Continuous H₂ and CH₄ production from high-solid food waste in the two-stage thermophilic fermentation process with the recirculation of digester sludge. *Bioresource Technology*. 2010;**101**:S42-S47. DOI: 10.1016/j.biortech.2009.03.037
- [31] Nandi R, Sengupta R. Microbial production of hydrogen: An overview. *Critical Reviews in Microbiology*. 1998;**24**:61-84. DOI: 10.1080/10408419891294181
- [32] Demirel B, Scherer P, Yenigun O, Onay T. Production of methane and hydrogen from biomass through conventional and high-rate anaerobic digestion processes. *Critical Reviews in Environmental Science and Technology*. 2010;**40**:116-146. DOI: 10.1080/10643380802013415
- [33] Bolzonella D, Pavan P, Zanette M, Cecchi F. Two-phase anaerobic digestion of waste activated sludge: Effect of an extreme thermophilic prefermentation. *Industrial and Engineering Chemistry Research*. 2007;**46**:6650-6655. DOI: 10.1021/ie061627e
- [34] Liu D, Liu D, Zeng RJ, Angelidaki I. Hydrogen and methane production from household solid waste in the two-stage fermentation process. *Water Research*. 2006;**40**:2230-2236. DOI: 10.1016/j.watres.2006.03.029
- [35] Arreola-Vargas J, Flores-Larios A, González-Álvarez V, Corona-González RI, Méndez-Acosta HO. Single and two-stage anaerobic digestion for hydrogen and methane production from acid and enzymatic hydrolysates of *Agave tequilana* bagasse. *International Journal of Hydrogen Energy*. 2015;**41**:897-904. DOI: 10.1016/j.ijhydene.2015.11.016
- [36] Zhong J, Stevens DK, Hansen CL. Optimization of anaerobic hydrogen and methane production from dairy processing waste using a two-stage digestion in induced bed

- reactors (IBR). International Journal of Hydrogen Energy. 2015;**40**:15470-15476. DOI: 10.1016/j.ijhydene.2015.09.085
- [37] Demirel B, Yenigün O. Two-phase anaerobic digestion processes: A review. Journal of Chemical Technology and Biotechnology. 2002;**77**:743-755. DOI: 10.1002/jctb.630
- [38] Kamalaskar LB, Dhakephalkar PK, Meher KK, Ranade DR. High biohydrogen yielding *Clostridium* sp. DMHC-10 isolated from sludge of distillery waste treatment plant. International Journal of Hydrogen Energy. 2010;**35**:10639-10644. DOI: 10.1016/j.ijhydene.2010.05.020
- [39] Khetkorn W, Rastogi RP, Incharoensakdi A, Lindblad P, Madamwar D, Pandey A, Larroche C. Microalgal hydrogen production-a review. Bioresource Technology. 2017;**243**: 1194-1206. DOI: 10.1016/j.biortech.2017.07.085
- [40] Taguchi F, Mizukami N, Taki TS, Hasegawa K. Hydrogen-production from continuous fermentation of xylose during growth of *Clostridium* sp strain no.2. Canadian Journal of Microbiology. 1995;**41**:536-540. DOI: 10.1139/m95-071
- [41] Maintinguer SI, Lazaro CZ, Pachiega R, Varesche MBA, Sequinel R, Oliveira JE. Hydrogen bioproduction with *Enterobacter* sp. isolated from brewery wastewater. International Journal of Hydrogen Energy. 2017;**42**:152-160. DOI: 10.1016/j.ijhydene.2016.11.104
- [42] Jung GY, Kim JR, Park J, Park S. Hydrogen production by a new chemoheterotrophic bacterium *Citrobacter* sp. Y19. International Journal of Hydrogen Energy. 2002;**27**:601-610. DOI: 10.1016/S0360-3199(01)00176-8
- [43] Maeda T, Sanchez-Torres V, Wood TK. Enhanced hydrogen production from glucose by metabolically engineered *Escherichia coli*. Applied Microbiology and Biotechnology. 2007;**77**:879-890. DOI: 10.1007/s00253-007-1217-0
- [44] Kalia VC, Purohit HJ. Microbial diversity and genomics in aid of bioenergy. Journal of Industrial Microbiology & Biotechnology. 2008;**35**:403-419. DOI: 10.1007/s10295-007-0300-y
- [45] Kotay SM, Das D. Microbial hydrogen production with *Bacillus coagulans* IIT-BT S1 isolated from anaerobic sewage sludge. Bioresource Technology. 2007;**98**:1183-1190. DOI: 10.1016/j.biortech.2006.05.009
- [46] O-Thong S, Prasertsan P, Karakashev D, Angelidaki I. Thermophilic fermentative hydrogen production by the newly isolated *Thermoanaerobacterium thermosaccharolyticum* PSU-2. International Journal of Hydrogen Energy. 2008;**33**:1204-1214. DOI: 10.1016/j.ijhydene.2007.12.015
- [47] O-Thong S, Prasertsan P, Intrasingkha N, Dhamwichukorn S, Birkeland NK. Optimization of simultaneous thermophilic fermentative hydrogen production and COD reduction from palm oil mill effluent by *Thermoanaerobacterium*-rich sludge. International Journal of Hydrogen Energy. 2008;**33**:1221-1231
- [48] Liu Y, Yu P, Song X, Qu Y. Hydrogen production from cellulose by co-culture of *Clostridium thermocellum* JN4 and *Thermoanaerobacterium thermosaccharolyticum* GD17. International Journal of Hydrogen Energy. 2008;**33**:2927-2933. DOI: 10.1016/j.ijhydene.2008.04.004

- [49] Cao GL, Ren NQ, Wang AJ, Guo WQ, Xu JF, Liu BF. Effect of lignocellulose-derived inhibitors on growth and hydrogen production by *Thermoanaerobacterium thermosaccharolyticum* W16. *International Journal of Hydrogen Energy*. 2010;**35**:13475-13480. DOI: 10.1016/j.ijhydene.2009.11.127
- [50] Khamtib S, Reungsang A. Biohydrogen production from xylose by *Thermoanaerobacterium thermosaccharolyticum* KCU19 isolated from hot spring sediment. *International Journal of Hydrogen Energy*. 2012;**37**:12219-12228. DOI: 10.1016/j.ijhydene.2012.06.038
- [51] Roy S, Vishnuvardhan M, Das D. Improvement of hydrogen production by newly isolated *Thermoanaerobacterium thermosaccharolyticum* IIT BT-ST1. *International Journal of Hydrogen Energy*. 2014;**39**:7541-7552. DOI: 10.1016/j.ijhydene.2013.06.128
- [52] Mamimin C, Thongdumyu P, Hniman A, Prasertsan P, Imai T, O-Thong S. Simultaneous thermophilic hydrogen production and phenol removal from palm oil mill effluent by *Thermoanaerobacterium*-rich sludge. *International Journal of Hydrogen Energy*. 2012;**37**:15598-15606. DOI: 10.1016/j.ijhydene.2012.04.062
- [53] Levin D, Islam R, Cicek N, Sparling R. Hydrogen production by *Clostridium thermocellum* 27405 from cellulosic biomass substrates. *International Journal of Hydrogen Energy*. 2006;**31**:1496-1503. DOI: 10.1016/j.ijhydene.2006.06.015
- [54] Zeidan A, van Niel EWJ. Developing a thermophilic hydrogen-producing co-culture for efficient utilization of mixed sugars. *International Journal of Hydrogen Energy*. 2009;**34**:4524-4528. DOI: 10.1016/j.ijhydene.2008.07.092
- [55] van Niel EWJ, Budde MAW, De Haas GG, Van Der Wal FJ, Claassen PAM, Stams AJM. Distinctive properties of high hydrogen producing extreme thermophiles, *Caldicellulosiruptor saccharolyticus* and *Thermotoga elfii*. *International Journal of Hydrogen Energy*. 2002;**27**:1391-1398. DOI: 10.1016/S0360-3199(02)00115-5
- [56] Pradhan N, Dipasquale L, d'Ippolito G, Panico A, Lens PNL, Esposito G, Fontana A. Hydrogen production by the Thermophilic bacterium *Thermotoga neapolitana*. *International Journal of Molecular Sciences*. 2015;**16**:12578-12600. DOI: 10.3390/ijms160612578
- [57] Finkelstein M, McMillan DJ, Davison BH, editors. *Biotechnology for Fuels and Chemicals*. New Jersey: Humana Press; 2002. p. 1015
- [58] O-Thong S, Prasertsan P, Birkeland N. Evaluation of methods for preparing hydrogen-producing seed inocula under thermophilic condition by process performance and microbial community analysis. *Bioresource Technology*. 2009;**100**:909-918. DOI: 10.1016/j.biortech.2008.07.036
- [59] Ueno Y, Kawai T, Sato S, Otsuka S, Morimoto M. Biological hydrogen production of hydrogen from cellulose by natural anaerobic microflora. *Journal of Fermentation and Bioengineering*. 1995;**79**:395-397. DOI: 10.1016/0922-338X(95)94005-C
- [60] Lauerer G, Kristjansson JK, Langworthy TA, König H, Stetter KO. *Methanothermus sociabilis* sp. nov., a second species within the *Methanothermaceae* growing at 97°C. *Systematic and Applied Microbiology*. 1986;**8**:100-105. DOI: 10.1016/S0723-2020(86)80156-4

- [61] Huber H, Thomm M, Knig H, Thies G, Stetter KO. *Methanococcus thermolithotrophicus*, a novel thermophilic lithotrophic methanogens. Archives of Microbiology. 1982;**132**:47-50. DOI: 10.1007/BF00690816
- [62] Asakawa S, Nagaoka K. *Methanoculleus bourgensis*, *Methanoculleus olentangyi* and *Methanoculleus oldenburgensis* are subjective synonyms. International Journal of Systematic and Evolutionary Microbiology. 2003;**53**:1551-1552. DOI: 10.1099/ ijs.0.02508-0
- [63] Zellner G, Neudörfer F, Diekmann H. Degradation of lactate by an anaerobic mixed culture in a fluidized-bed reactor. Water Research. 1994;**28**:1337-1340. DOI: 10.1016/0043-1354(94)90299-2
- [64] Chang J, Chen W, Shih S, Yu S, Lay J, Wen F, Huang C. Molecular detection of the clostridia in an anaerobic biohydrogen fermentation system by hydrogenase mRNA-targeted reverse transcription-PCR. Applied Microbiology and Biotechnology. 2006;**70**:598-604. DOI: 10.1007/s00253-005-0106-7
- [65] Compton R, Perkin S, Gamblin D, Davis J, Marken F, Padden A, John P. *Clostridium isatidis* colonised carbon electrodes: Voltammetric evidence for direct solid state redox processes. New Journal of Chemistry. 2000;**24**:179-181. DOI: 10.1039/A909172F
- [66] Yokoyama H, Moriya N, Ohmori H, Waki M, Ogino A, Tanaka Y. Community analysis of hydrogen-producing extreme thermophilic anaerobic microflora enriched from cow manure with five substrates. Applied Microbiology and Biotechnology. 2007;**77**:213-222. DOI: 10.1007/s00253-007-1144-0
- [67] Weiss A, Jérôme V, Freitag R, Mayer HK. Diversity of the resident microbiota in a thermophilic municipal biogas plant. Applied Microbiology and Biotechnology. 2008;**81**:163-173. DOI: 10.1007/s00253-008-1717-6
- [68] Kraemer JT, Bagley DM. Supersaturation of dissolved H₂ and CO₂ during fermentative hydrogen production with N₂ sparging. Biotechnology Letters. 2006;**28**:1485-1491. DOI: 10.1007/s10529-006-9114-7
- [69] Chen CC, Lin CY, Lin MC. Acid-base enrichment enhances anaerobic hydrogen production process. Applied Microbiology and Biotechnology. 2002;**58**:224-228. DOI: 10.1007/s002530100814
- [70] Hawkes FR, Hussy I, Kyazze G, Dinsdale R, Hawkes DL. Continuous dark fermentative hydrogen production by mesophilic microflora: Principles and progress. International Journal of Hydrogen Energy. 2007;**32**:172-184. DOI: 10.1016/j.ijhydene.2006.08.014
- [71] Fang HHP, Yu HQ. Acidification of lactose in wastewater. Journal of Environmental Engineering. 2001;**127**:825-831. DOI: 10.1061/(ASCE)0733-9372(2001)127:9(825)
- [72] Li C, Fang HHP. Fermentative hydrogen production from wastewater and solid wastes by mixed cultures. Critical Reviews in Environmental Science and Technology. 2007;**37**:1-39. DOI: 10.1080/10643380600729071
- [73] Khanal SK. Anaerobic Biotechnology for Bioenergy Production: Principle and Application. New Jersey: John Wiley & Sons; 2008. p. 320

- [74] Ueno Y, Fukui H, Goto M. Operation of a two-stage fermentation process producing hydrogen and methane from organic waste. *Environmental Science & Technology*. 2007; **41**:1413-1419. DOI: 10.1021/es062127f
- [75] Zahedi S, Solera R, Micolucci F, Cavinato C, Bolzonella D. Changes in microbial community during hydrogen and methane production in two-stage thermophilic anaerobic co-digestion process from biowaste. *Waste Management*. 2016; **49**:40-46. DOI: 10.1016/j.wasman.2016.01.016
- [76] Zhu H, Stadnyk A, Beland M, Seto P. Co-production of hydrogen and methane from potato waste using a two-stage anaerobic digestion process. *Bioresource Technology*. 2008; **99**:5078-5084. DOI: 10.1016/j.biortech.2007.08.083
- [77] Okamoto M, Miyahara T, Mizuno O, Noike T. Biological hydrogen potential of materials characteristic of the organic fraction of municipal solid wastes. *Water Science and Technology*. 2000; **41**:25-32
- [78] Chang FY, Lin CY. Biohydrogen production using an up-flow anaerobic sludge blanket reactor. *International Journal of Hydrogen Energy*. 2004; **29**:33-39. DOI: 10.1016/S0360-3199(03)00082-X
- [79] Lay JJ, Fan KS, Chang JJ, Ku CH. Influence of chemical nature of organic wastes on their conversion to hydrogen by heat-shock digested sludge. *International Journal of Hydrogen Energy*. 2003; **28**:1361-1367. DOI: 10.1016/S0360-3199(03)00027-2
- [80] Noike T, Mizuno O. Hydrogen fermentation of organic municipal wastes. *Water Science and Technology*. 2000; **42**:155-162
- [81] Mamimin C, Prasertsan P, Kongjan P, O-Thong S. Effects of volatile fatty acids in biohydrogen effluent on biohythane production from palm oil mill effluent under thermophilic condition. *Electronic Journal of Biotechnology*. 2017; **29**:78-85. DOI: 10.1016/j.ejbt.2017.07.006
- [82] Hniman A, O-Thong S, Prasertsan P. Developing a thermophilic hydrogen producing microbial consortia from geothermal spring for efficient utilization of xylose and glucose mixed substrates and oil palm trunk hydrolysate. *International Journal of Hydrogen Energy*. 2011; **36**:8785-8793. DOI: 10.1016/j.ijhydene.2010.09.067
- [83] Lay CH, Wu JH, Hsiao CL, Chang JJ, Chen CC, Lin CY. Biohydrogen production from soluble condensed molasses fermentation using anaerobic fermentation. *International Journal of Hydrogen Energy*. 2010; **35**:13445-13451. DOI: 10.1016/j.ijhydene.2009.11.128
- [84] Kan E. Effects of pretreatments of anaerobic sludge and culture conditions on hydrogen productivity in dark anaerobic fermentation. *Renewable Energy*. 2013; **49**:227-231. DOI: 10.1016/j.renene.2012.01.026
- [85] Li C, Fang HHP. Inhibition of heavy metals on fermentative hydrogen production by granular sludge. *Chemosphere*. 2007; **67**:668-673. DOI: 10.1016/j.chemosphere.2006.11.005
- [86] O-Thong S. Microbial population optimization for control and improvement of dark hydrogen fermentation. In: Jozala AF, editor. *Fermentation Process*. Rijeka: INTECH; 2017. pp. 119-144

- [87] Valdez-Vazquez I, Rios-Leal E, Carmona-Martinez A, Munoz-Paez K, Poggi-Varald H. Improvement of biohydrogen production from solid wastes by intermittent venting and gas flushing of batch reactors headspace. *Environmental Science & Technology*. 2006;**40**:3409-3415. DOI: 10.1021/es052119j
- [88] Willquist K, van Niel EdWJ. Lactate formation in *Caldicellulosiruptor saccharolyticus* is regulated by the energy carriers pyrophosphate and ATP. *Metabolic Engineering* 2010;**12**:282-290. DOI: 10.1016/j.ymben.2010.01.001
- [89] Kyazze G, Dinsdale R, Guwy AJ, Hawkes FR, Premier GC, Hawkes DL. Performance characteristics of a two-stage dark fermentative system producing hydrogen and methane continuously. *Biotechnology and Bioengineering*. 2007;**97**:759-770. DOI: 10.1002/bit.21297
- [90] Lay JJ. Biohydrogen generation by mesophilic anaerobic fermentation of microcrystalline cellulose. *Biotechnology and Bioengineering*. 2001;**74**:281-287. DOI: 10.1002/bit.1118
- [91] Zhang ZP, Show KY, Tay JH, Liang DT, Lee DJ. Biohydrogen production with anaerobic fluidized bed reactors - a comparison of biofilm-based and granule-based systems. *International Journal of Hydrogen Energy*. 2008;**33**:1559-1564. DOI: 10.1016/j.ijhydene.2007.09.048
- [92] Kim SH, Han SK, Shin HS. Performance comparison of a continuous-flow stirred tank reactor and an anaerobic sequencing batch reactor for fermentative hydrogen production depending on substrate concentration. *Water Science and Technology*. 2005;**52**:23-29
- [93] Angelidaki I, Karakashev D, Batstone DJ, Plugge CM, Stams AJM. Biomethanation and its potential. *Methods in Enzymology*. 2011;**494**:327-351. DOI: 10.1016/B978-0-12-385112-3.00016-0
- [94] Dohanyos M, Zabranska J. Anaerobic digestion. In: Spinosa L, Vesilind PA, editors. *Sludge into Biosolids: Processing, Disposal, and Utilization*. London: IWA publishing; 2001. pp. 223-241
- [95] Cai G, Jin B, Saint C, Monis P. Metabolic flux analysis of hydrogen production network by *Clostridium butyricum* W5: Effect of pH and glucose concentrations. *International Journal of Hydrogen Energy*. 2010;**35**:6681-6690. DOI: 10.1016/j.ijhydene.2010.04.097
- [96] van Niel E, Claassen P, Stams A. Substrate and product inhibition of hydrogen production by the extreme thermophile, *Caldicellulosiruptor saccharolyticus*. *Biotechnology and Bioengineering*. 2003;**81**:255-262. DOI: 10.1002/bit.10463
- [97] Parkin G, Owen WF. Fundamentals of anaerobic digestion of wastewater sludges. *Journal of Environmental Engineering*. 1986;**112**:867-920. DOI: 10.1061/(ASCE)0733-9372(1986)112:5(867)
- [98] Lo HM, Liu MH, Pai TY, Liu WF, Lin CY, Wang SC, Banks CJ, Hung CH, Chiang CF, Lin KC, Chen PH, Chen JK, Chiu HY, Su MH, Kurniawan TA, Wu KC, Hsieh CY, Hsu HS. Biostabilization assessment of MSW co-disposed with MSWI fly ash in anaerobic bioreactors. *Journal of Hazardous Materials*. 2009;**162**:1233-1242. DOI: 10.1016/j.jhazmat.2008.06.028

- [99] Vindis P, Mursec B, Janzekovic M, Cus F. The impact of mesophilic and thermophilic anaerobic digestion on biogas production. *Journal of Achievements in Materials and Manufacturing Engineering*. 2009;**36**:192-198
- [100] van Groenestijn JW, Hazewinkel JHO, Nienoord M, Bussmann PJT. Energy aspects of biological hydrogen production in high rate bioreactors operated in the thermophilic temperature range. *International Journal of Hydrogen Energy*. 2002;**27**:1141-1147. DOI: 10.1016/S0360-3199(02)00096-4
- [101] Gavala HN, Skiadas IV, Ahring BK. Biological hydrogen production in suspended and attached growth anaerobic reactor systems. *International Journal of Hydrogen Energy*. 2006;**31**:1164-1175. DOI: 10.1016/j.ijhydene.2005.09.009
- [102] Watts S, Hamilton G, Keller J. Two-stage thermophilic-mesophilic anaerobic digestion of waste activated sludge from a biological nutrient removal plant. *Water Science and Technology*. 2006;**53**:149-157. DOI: 10.2166/wst.2006.245
- [103] Speece RE. Anaerobic biotechnology for industrial wastewater treatment. *Environmental Science & Technology*. 1983;**17**:416A-427A. DOI: 10.1021/es00115a001
- [104] Romero-Güiza MS, Vila J, Mata-Alvarez J, Chimenos JM, Astals S. The role of additives on anaerobic digestion: A review. *Renewable and Sustainable Energy Reviews*. 2016;**58**:1486-1499. DOI: 10.1016/j.rser.2015.12.094
- [105] Lee YJ, Miyahara T, Noike T. Effect of iron concentration on hydrogen fermentation. *Bioresource Technology*. 2001;**80**:227-231. DOI: 10.1016/S0960-8524(01)00067-0
- [106] Ferchichi M, Crabbe E, Gil G-H, Hintz W, Almadidy A. Influence of initial pH on hydrogen production from cheese whey. *Journal of Biotechnology*. 2005;**120**:402-409. DOI: 10.1016/j.jbiotec.2005.05.017
- [107] Wang XJ, Ren NQ, Xiang WS, Guo WQ. Influence of gaseous end-products inhibition and nutrient limitations on the growth and hydrogen production by hydrogen producing fermentative bacteria B49. *International Journal of Hydrogen Energy*. 2007;**33**:1153-1163. DOI: 10.1016/j.ijhydene.2006.08.003
- [108] Buchenau B, Kahnt J, Heinemann IU, Jahn D, Thauer RK. Heme biosynthesis in *Methanosarcina barkeri* via a pathway involving two methylation reactions. *Journal of Bacteriology*. 2006;**188**:8666-8668. DOI: 10.1128/JB.01349-06
- [109] Hafez H, Nakhla G, Nagggar HE. An integrated system for hydrogen and methane production during landfill leachate treatment. *International Journal of Hydrogen Energy*. 2010;**35**:5010-5014. DOI: 10.1016/j.ijhydene.2009.08.050
- [110] Koutrouli EC, Kalfas H, Gavala HN, Skiadas IV, Stamatelatou K, Lyberatos G. Hydrogen and methane production through two-stage mesophilic anaerobic digestion of olive pulp. *Bioresource Technology*. 2009;**100**:3718-3723. DOI: 10.1016/j.biortech.2009.01.037
- [111] Nualsri C, Reungsang A, Plangklang P. Biochemical hydrogen and methane potential of sugarcane syrup using a two-stage anaerobic fermentation process. *Industrial Crops and Products*. 2016;**82**:88-99. DOI: 10.1016/j.indcrop.2015.12.002

- [112] Cavinato C, Bolzonella D, Fatone F, Cecchi F, Pavan P. Optimization of two-phase thermophilic anaerobic digestion of biowaste for hydrogen and methane production through reject water recirculation. *Bioresource Technology*. 2011;**102**:8605-8611. DOI: 10.1016/j.biortech.2011.03.084
- [113] Willquist K, Nkemka VN, Svensson H, Pawar S, Ljunggren M, Karlsson H, Murto M, Hultberg C, Van Niel EWJ, Liden G. Design of a novel biohythane process with high H₂ and CH₄ production rates. *International Journal of Hydrogen Energy*. 2012;**37**:17749-17762. DOI: 10.1016/j.ijhydene.2012.08.092
- [114] Elreedy A, Tawfik A, Kubota K, Shimada Y, Harada H. Hythane (H₂+CH₄) production from petrochemical wastewater containing mono-ethylene glycol via stepped anaerobic baffled reactor. *International Biodeterioration and Biodegradation*. 2015;**105**:252-261. DOI: 10.1016/j.ibiod.2015.09.015
- [115] Wu SY, Lin CY, Lee KS, Hung CH, Chang JS, Lin PJ, Chang FY. Dark fermentative hydrogen production from xylose in different bioreactors using sewage sludge microflora. *Energy & Fuels*. 2008;**22**:113-119. DOI: 10.1021/ef700286s
- [116] Kotsopoulos T, Zeng R, Angelidaki I. Biohydrogen production in granular up-flow anaerobic sludge blanket (UASB) reactors with mixed cultures under hyper-thermophilic temperature (70°C). *Biotechnology and Bioengineering*. 2006;**4**:296-302. DOI: 10.1002/bit.20844
- [117] Kim J, Kim Y, Yeom S, Song B, Kim I. Enhancing continuous hydrogen gas production by the addition of nitrate into an anaerobic reactor. *Process Biochemistry*. 2006;**41**:1208-1212. DOI: 10.1016/j.procbio.2005.11.017
- [118] Kumar N, Das D. Continuous hydrogen production by immobilized *Enterobacter cloacae* IIT-BT 08 using lignocellulosic materials as solid matrices. *Enzyme Microb Technol*. 2001;**29**:280-287. DOI: 10.1016/S0141-0229(01)00394-5
- [119] Lee K, Lo Y, Lin P, Chang J. Improving biohydrogen production in a carrier-induced granular sludge bed by altering physical configuration and agitation pattern of the bioreactor. *International Journal of Hydrogen Energy*. 2006;**31**:1648-1657. DOI: 10.1016/j.ijhydene.2005.12.020
- [120] Cavinato C, Bolzonella D, Pavan P, Cecchi F. Two-phase thermophilic anaerobic digestion of biowaste for bio-hythane production: Yields and feasibility of the process. *Journal of Biotechnology*. 2010;**150**:162. DOI: 10.1016/j.jbiotec.2010.08.421
- [121] Parawira W, Murto M, Zvauya R, Mattiasson B. Comparative performance of a UASB reactor and an anaerobic packed-bed reactor when treating potato waste leachate. *Renewable Energy*. 2006;**31**:893-903. DOI: 10.1016/j.renene.2005.05.013
- [122] Lay J, Li Y, Noike T, Endo J, Ishimoto S. Analysis of environmental factors affecting methane production from high-solids organic waste. *Water Science and Technology*. 1997;**36**:493-500. DOI: 10.1016/S0273-1223(97)00560-X
- [123] Forbes C, Hughes D, Fox J, Ryan P, Colleran E. High-rate anaerobic degradation of 5 and 6 carbon sugars under thermophilic and mesophilic conditions. *Bioresource Technology*. 2010;**101**:3925-3930. DOI: 10.1016/j.biortech.2010.01.019

- [124] Venetsaneas N, Antonopoulou G, Stamatelatou K, Kornaros M, Lyberatos G. Using cheese whey for hydrogen and methane generation in a two-stage continuous process with alternative pH controlling approaches. *Bioresource Technology*. 2009;**100**:3713-3717. DOI: 10.1016/j.biortech.2009.01.025
- [125] Lepistö R, Rintala J. Extreme thermophilic (70 °C), VFA-fed UASB reactor: Performance, temperature response, load potential and comparison with 35 and 55 °C UASB reactors. *Water Research*. 1999;**33**:3162-3170. DOI: 10.1016/S0043-1354(99)00034-2
- [126] Batstone DJ, Keller J, Angelidaki I, Kalyuzhnyi SV, Pavlostathis SG, Rozzi A, Sanders W, Siegrist H, Vavilin VA. The IWA anaerobic digestion model no 1 (ADM1). *Water Science and Technology*. 2002;**45**:65-73
- [127] van Lier J. Limitations of thermophilic anaerobic wastewater treatment and the consequences for process design. *Antonie Leeuwenhoek*. 1996;**69**:1-14. DOI: 10.1007/BF00641606
- [128] Saravanan V, Sreekrishnan T. Modelling anaerobic biofilm reactors-a review. *Journal of Environmental Management*. 2006;**81**:1-18. DOI: 10.1016/j.jenvman.2005.10.002
- [129] Kongjan P, Angelidaki I. Extreme thermophilic biohydrogen production from wheat straw hydrolysate using mixed culture fermentation: Effect of reactor configuration. *Bioresource Technology*. 2010;**101**:7789-7796. DOI: 10.1016/j.biortech.2010.05.024
- [130] Tatara M, Yamazawa A, Ueno Y, Fukui H, Goto M, Sode K. High-rate thermophilic methane fermentation on short-chain fatty acids in a down-flow anaerobic packed-bed reactor. *Bioprocess and Biosystems Engineering*. 2005;**27**:105-113. DOI: 10.1007/s00449-004-0387-8
- [131] Porpatham E, Ramesh A, Nagalingam B. Effect of hydrogen addition on the performance of a biogas fuelled spark ignition engine. *International Journal of Hydrogen Energy*. 2007;**32**:2057-2065. DOI: 10.1016/j.ijhydene.2006.09.001
- [132] Kim SH, Cheon HC, Lee CY. Enhancement of hydrogen production by recycling of methanogenic effluent in two phase fermentation of food waste. *International Journal of Hydrogen Energy*. 2012;**37**:13777-13782. DOI: 10.1016/j.ijhydene.2012.03.112