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Hydrogen Production in the Anaerobic Treatment of Domestic-Grade Synthetic Wastewater

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Abstract: The aim of this study was to evaluate the potential of domestic wastewater for anaerobic hydrogen production. High-strength and ordinary-strength organic loadings of synthetic wastewater, *i.e.*, real-time domestic wastewater with and without a mixture of food waste, were tested. During operation at a high strength loading, the initial pH was maintained at 7 and then gradually decreased, and a pH of 5–5.5 was observed as the best experimental condition. A pH of 5–5.5 was controlled during the operation at an ordinary-strength loading. Maximum hydrogen yields of 1.125 mol H_2/mol glucose and 1.01 mol H_2/mol glucose were observed during operation at high (48 g COD/L·day) and ordinary (3 g COD/L·day) strength loadings in terms of chemical oxygen demand (COD), respectively, with hydrogen contents of 42%–53%. The operating environment of the hydrogen production system was found to be very crucial because the metabolic pathway of the microorganism and production of intermediates were found to be dynamic with the controlled environment. Smaller COD removals of 30% and 26% were observed in high-strength and ordinary-strength loadings, respectively. Organic mass balance in terms of COD described the distribution of organics in the system via reactor byproducts. The findings of this study can be applied during the design of onsite domestic wastewater and energy recovery systems.

Keywords: anaerobic; decentralized treatment; domestic wastewater; hydrogen; energy recovery

1. Introduction

In various under-developed, developing and developed countries, water quality and sanitation are key public concerns. Especially in the case of developing and under-developed countries, a lack of sewage management is leading to increasingly negative effects on economic growth, social welfare and the world's eco-system. Various centralized waste treatment plants and sewerage systems have been employed in developed countries and a few even in developing and under-developed countries [1]. These centralized systems of collection and treatment are currently perceived to be expensive in terms of cost and energy. Moreover, a centralized system also increases the per capita cost due to expensive sewer line installation, construction and the waste/wastewater collection system. The traditional centralized system of wastewater treatment often reuses/disposes far from the point of generation, which creates difficulty in returning treated wastewater to the watershed of origin [2]. Therefore, to increase the reuse of wastewater, constructions of reliable, simple and cost-

effective decentralized treatment systems have to be adopted in developing countries and even in developed countries for newly planned towns and urban modification. Particularly, decentralized systems are preferable for communities with improper zoning, such as scattered low-density populated rural areas [3]. The large capital investments of sewage systems and pumping costs associated with centralized systems can be reduced in decentralized treatment systems. However, the construction and development of numerous decentralized plants in urban areas can be tedious and expensive. Decentralized treatment systems should be targeted in newly developed towns and communities, where the development costs of treatment units could be overcome by valorizing the nutrients and energy extracted. This could assist in an eradication/minimization of the cost of expensive sewer line installation.

Domestic wastewater is water that has been used by a community and contains all the materials added to the water during its use. It is thus composed of human bodily waste (feces and urine) together with the water used for flushing toilets and also wastewater resulting from personal washing, laundry, food preparation and cleaning of kitchen utensils. Domestic wastewater organic contents usually range from less than 400 to more than 1500 mg COD/L. A high concentration of domestic wastewater discharge occurs when brown water is mixed with food waste via the use of kitchen sink disposers, thus leading to an organic strength of more than 10,000 mg COD/L [4].

Among the various advanced and sophisticated technologies available for treatment of waste and wastewater, the bioremediation technique is considered simple and easy for applications in decentralized treatments and thus advantageous for the environment, health, life and the world's economy [5]. Bioprocess technologies, such as anaerobic digestion, photo fermentation and microbial fuel/electrolysis cells, are widely studied and tested in applications [5]. Anaerobic treatment is regarded as a proven and core technology for the recovery of energy and nutrients from waste and wastewater because it converts organic matter to hydrogen and methane, which can be used to produce electricity and heat, while at the same time anaerobic treatment yields low amounts of excess sludge [6]. Organic wastewater is a potential bioenergy source for the anaerobic fermentation technology. Wastewater is perceived as a vast resource as the paradigm shifts from treatment only to treatment along with energy and valuable nutrient recovery. Bioenergy generation in terms of methane or hydrogen from wastewater costs less and can be carried out using local feedstock [7]. Biohydrogen, a high-energy and clean fuel, is perceived as an appealing future energy carrier due to its conversion to energy yielding only pure water. It is a promising substitute for antediluvian energy sources such as fossil fuels because biohydrogen has the potential to obviate problems created due to the extensive use of fossil fuels. Biohydrogen has been deliberated and proposed as an ultimate transport fuel for vehicles and vessels because of its non-polluting aspect, and it also enables the use of highly efficient fuel cells to convert chemical energy to electricity [8]. Several technologies for biohydrogen production have been proposed over the last decade, such as bio-photolysis, photo fermentation, microbial fuel/electrolysis cells (MECs and MFCs) and dark fermentation; the latter appears as the most feasible and pragmatic [9–11].

Dark fermentation in the acidogenic phase utilizing obligate and facultative anaerobes leads to H_2 production. Previous studies depicted four fermentation reactions taking place in anaerobic H_2 production systems, *i.e.*, acetic acid fermentation, butyric acid fermentation, propionic acid fermentation and ethanol fermentation. Among these reactions, H_2 is generated from acetic, butyric and ethanol fermentations. Operational physical and biochemical environments are crucial factors to be considered for anaerobic hydrogen fermentation. As dark fermentation of hydrogen occurs in the acidogenic phase, the adjustment of pH is important. Factors such as temperature, OLR (Organic Loading Rate) and HRT (Hydraulic Retention Time) are also important [12]. Anaerobic sludge, sewage sludge and soil have been used as mixed inoculum for fermentative hydrogen production. However, hydrogen-consuming bacteria in mixed cultures may consume the hydrogen produced by hydrogen-producing bacteria. Various works have been performed to minimize the hydrogen consumers. *Clostridium* spp., the main hydrogen-producing bacterium, has been reported to be enriched in this environment [13].

A number of studies have been carried out on the production of biohydrogen using soybean protein wastewater, cassava wastewater, alcohol wastewater, rice winery wastewater and olive mill wastewater. Studies show that industrial wastewater has been extensively applied for hydrogen production. However, very few attempts have been performed to produce hydrogen from domestic wastewater. The treatment of domestic wastewater with low energy consumption and high sustainability is needed today. Moreover, H_2 production is just one stage for the extraction of fuel from the acidogenic stage. Effluent from the acidogenic stage should be further treated using methanogenic reactor systems or followed by aeration or membrane treatments for organic removal. This study is intended to evaluate the biohydrogen production potential of domestic-grade synthetic wastewater in anaerobic conditions and to analyze the governing factors and the efficacy of the system in terms of energy production.

2. Materials and Methods

2.1. Inoculum and Feedstock

The seed sludge (mixed culture) was taken from an anaerobic digestion tank at a local wastewater treatment plant in Changwon, South Korea. The seed sludge was subjected to heat pretreatment at 90 °C for 30 minutes to impoverish hydrogen consumers and augment hydrogen-producing bacteria by curtailing obligate non-spore-forming methanogens because hydrogen-producing bacteria such as *Clostridium* sp. can form protective spores under extremely strict living environments (high temperature, extreme alkalinity and acidity) [14]. Before the pretreatment of seed sludge, it was screened through a 200 μm sieve to remove large particulate matter and sand contamination. Two types of wastewater were prepared as modified domestic wastewaters: ordinary domestic wastewater and one containing high-strength wastewater. The two synthetic wastewaters were prepared at COD concentrations of 1 g/L and 16 g/L, which were analogous to real-time domestic wastewater. Wastewaters with different organic strengths were prepared to provide high and low organic loadings to the system, which resemble the real-time domestic wastewater strength from low concentration to high concentration depending on the situation of use. The growing attention to the use of food waste disposers provides relatively high strengths in domestic wastewater. The rationale behind the selection of different organic loadings by concentration is due to variance in the organic concentration released from the domestic level. Ordinary wastewater released from the domestic level provides organic concentrations of 400 mg/L to 1000 mg/L, which originate from showers, baths, whirlpool tubs, washing machines, dishwashers and sinks (aside from kitchen sinks) [15]. On the other hand, high-concentration wastewater is also released from the domestic level with organic concentrations of more than 10000 mg/L, which can be defined as blackwater (mixture of food waste and feces). Definition of blackwater varies from state to state; in this study, this type of water is categorized under high-strength wastewater. Sufficient inorganic nutrients were provided in the synthetic wastewater, *i.e.*, (in mg/L) NH_4Cl 1300; KH_2PO_4 250; $MgCl_2 \cdot 6H_2O$ 125; $FeSO_4 \cdot 7H_2O$ 5; $ZnCl_2$ 0.5; $NiCl_2 \cdot 6H_2O$ 0.5; H_3BO_4 0.5; $Na_2MoO_4 \cdot 2H_2O$ 0.5; $MnCl_2 \cdot 6H_2O$ 2.5; KI 2.5; $CoCl_2 \cdot 6H_2O$ 2.5.

2.2. Experimental Setup

Schematic of the experimental apparatus is shown in Figure 1. The type of reactor used in this experiment is a continuous stirred tank reactor (CSTR). Heat-pretreated anaerobic sludge having an initial biomass concentration of 8000 mg/L was seeded to a 5 L anaerobic reactor with a working volume of 4 L. High-strength organic loading of 48 g COD/L·day (denoted as Run 1) and ordinary-strength loading of 3 g COD/L·day (denoted as Run 2) were operated consecutively. The initial pH of the system was found to be neutral and was not controlled in Run 1. However, the pH in Run 2 was maintained between 5 and 5.5 using 1 N NaOH solution based on the result obtained from Run 1. The operational temperature of the system reactor was maintained at 37 °C throughout the experiment using a circulating water bath (Wisetherm). The hydraulic retention time (HRT) of the reactor system was kept at eight hours.

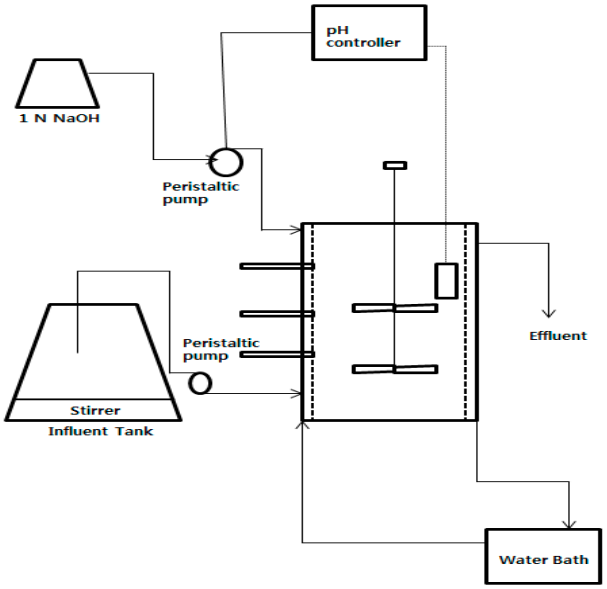

Figure 1. Schematic of the reactor configuration.

2.3. Assays Conducted

The gas produced was quantified daily using a water displacement method. The content of the produced gas was analyzed using a gas chromatograph (GC-2010 plus; Shimadzu, Kyoto, Japan) equipped with a thermal conductivity detector (TCD) and a 30 m × 0.53 mm, 50 μm MolSeive 5A open tabular capillary column (Restek Co., Bellefonte, DE, USA). The operational temperatures of the injection port, column oven and detector were 50, 35 and 120 °C, respectively. The carrier gas used in this assay was 99.99% helium at a flow rate of 30 mL/min. Volatile fatty acids (VFAs) were analyzed using a high performance liquid chromatograph (HPLC) (LC-20A; Shimadzu, Kyoto, Japan) equipped with an UV detector (210 nm) and a 300 m × 7.8 mm Aminex HPX-87H column. The samples were centrifuged at 12000 rpm for 5 minutes, and the supernatants were filtered through a membrane with a pore size of 0.45 μm prior to the VFA test. Sulfuric acid (0.005 M) was used as the mobile phase solution at a flow rate of 0.6 ml/min. The procedures described in the Standard Methods were applied to determine the COD [16].

3. Results and Discussion

3.1. Hydrogen Production at Different Organic Loadings

Among the assorted factors determining the process performance for anaerobic hydrogen fermentation, the substrate concentration is regarded as an important factor. In this experiment, synthetic wastewaters with COD concentrations of 16 g/L and 1 g/L were used, corresponding to a high-strength wastewater from the domestic level (mixture of food waste and brown water) and an ordinary-strength wastewater, respectively. Figure 2 illustrates the hydrogen yields of the anaerobic system when high-strength and ordinary-strength organic loadings were applied in the reactor. Maximum hydrogen yields of 1.125 mol H_2/mol glucose and 1.01 mol H_2/mol glucose (with hydrogen contents in the biogas from 42% to 53%) were obtained from organic loadings of 48 g COD/L·day and 3 g COD/L·day, respectively.

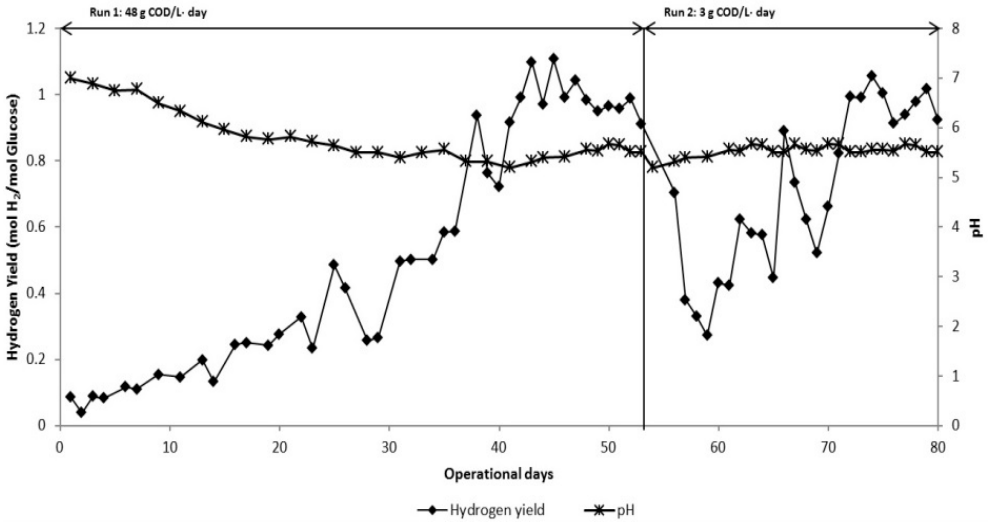

Figure 2. Hydrogen yields at high-strength and ordinary-strength organic loadings.

The result shows that a maximum hydrogen yield of 1.125 mol H_2/mol glucose was obtained in Run 1 when the system reached the steady-state condition. For the first 20 days of reactor operation in Run 1, the hydrogen yield was observed to be lower and fluctuating, but after day 30 of operation, the hydrogen production increased abruptly. This tendency of hydrogen production could be associated with the non-acclimatization of the microbial population and the delay in THF (time to reach full hydrogen-genesis regime) [17]. Yu *et al.* [18] reported a THF of 20 days in a mesophilic laboratory-scale reactor. The change in pH of the system from an initial pH of 7 to 6 and 5 might be the reason behind the delay. When the system experienced an appropriate operating environment, hydrogen production was higher. The process with ordinary domestic wastewater was operated at an organic loading rate of 3 g COD/L·day and at a pH of 5–5.5 after determining the favorable pH value from the high-strength organic loading. In the initial stage of Run 2, hydrogen production first decreased and then started increasing. This trend of hydrogen production could be explained by the sudden decrease in organic concentration. When the system operated at a higher organic concentration in Run 1, the accumulation of a higher protein content sometimes occurred, which was dominant in the reactor system during the initial phase of Run 2. When the carbohydrate content in the reactor system was slightly higher than or overcame the protein content, the hydrogen yield increased. Yu and Fang [19] studied the anaerobic acidification of synthetic wastewater using glucose and asserted that hydrogen came exclusively from carbohydrate degradation, which was more rapid than protein degradation that produced less hydrogen. Similarly, a hydrogen yield of 1.01 mol H_2/mol glucose was found during the operation of the anaerobic reactor system with an ordinary organic loading, referred to as Run 2. The result of the high-strength organic loading of this study was analogous to the result obtained by Lima *et al.* [20], who used glucose as a substrate with a 2 g/L COD concentration in upflow anaerobic fixed biofilm reactor (UAFBr) at a shorter retention time of 2 hrs. The results in this study were found to be slightly lower than those found by Chen *et al.* [21], who used a higher substrate concentration and a longer retention time in a CSTR system. However, the results of this study were also found to be slightly higher than the results of Hu *et al.* [22] using glucose as a substrate with a COD concentration of 20 g/L in upflow anaerobic sludge blanket reactor (UASBr). The hydrogen yields from the anaerobic system at both organic concentrations (16 and 1 g/L COD; Run 1 and Run 2) were not significantly different. Table 1 shows the comparisons made with other similar studies at different applied conditions. The comparisons of the results obtained by various other works and this work suggest that hydrogen production is not a function of substrate concentration; rather, it is a function of the operational environment and the system employed. The result in this study shows that at two different organic loadings, the hydrogen yield of the system at steady state does not vary. Organic loading is dependent on two independent variables, *i.e.*, HRT and COD concentrations. The OLR in this study was dependent on the COD concentration. Certain

specific ranges of OLR could be maintained in the system depending on the reactor type used and other operational parameters. Higher OLR does not necessarily lead to higher hydrogen production [12]. As shown in Table 1, using the same substrate at different concentrations, vast differences in the hydrogen yield were not observed. At a certain substrate loading, the hydrogen production rate increased until a maximum specific growth rate was reached, and then production declined slowly. The reported values for the hydrogen yield have no definitive range or optimum OLR in fermentative hydrogen production. This phenomenon could be due to the diverse environmental or operating conditions applied. When hydrogen production is not high, even at a higher organic loading rate, some refractory fraction of the COD content in wastewater was accumulated inside the reactor system. Cubillos *et al*. [23] also observed similar hydrogen yields with different substrate concentrations and noted that all of the glucose fractions were not removed. Therefore, to enhance or optimize hydrogen production from domestic-level wastewater, other operational environments should be taken into consideration.

Table 1. Comparison of maximum H_2 yields obtained in various types of H_2-production reactors.

Reactor Type	Substrate	Concentration (COD)	OLR (g COD/L·day)	Temp	HRT (Hrs)	Maximum Yield	Ref
CSTR	Glucose	7 g/L	28	36 °C	6	2.1 mol H_2/mol glucose	[24]
CSTR	Glucose	20 g/L	36	35 ± 1 °C	13.3	1.63 mol H_2/mol glucose	[21]
UASBr	Glucose	20 g/L	90	35 ± 1 °C	5.3	0.99 mol H_2/mol glucose	[22]
AFBR *	Glucose	4 g/L	48	30 ± 1 °C	2	2.49 mol H_2/mol glucose	[25]
UAFBr	Glucose	2 g/L	24	25 °C	2	1.51 mol H_2/mol glucose	[20]
CSTR	Glucose	16 g/L	48	37 °C	8	1.12 mol H_2/mol glucose	[This study]
CSTR	Glucose	1 g/L	3	37 °C	8	1.01 mol H_2/mol glucose	[This study]

* AFBR (Anaerobic fluidlized bed reactor)

3.2. Role of Operating pH on Hydrogen Production

The pH in a hydrogen-producing system is the most important parameter for consideration because the hydrogen-producing *Clostridium*-rich bioprocess is highly dependent on the pH of the system. The initial pH of the reactor system when operated at a high-strength organic loading (Run 1) was 7. Figure 2 shows the variation in the hydrogen yield during reactor operation. With a decrease in the initial pH from 7 to 6 and 5, the hydrogen yield increased significantly. When the pH decreased from 6 to 5, increments in the hydrogen production were noticed. Higher pH in a hydrogen fermentation system using wastewater is not favorable because a consistently high pH not only rapidly neutralizes produced acids but also depletes bacterial metabolism [26]. At a pH of 6, the high hydrogen production was no longer observed when acetic and butyric acids were present in high quantities. The lower hydrogen production even with a high acetate production could be explained by the microbial transformation of glucose into acetate, as shown in equation 1, explained by Gavala *et al.* [27]. When the system was tested at a pH lower than 5, slightly lower hydrogen production was observed. The lower hydrogen production at a low pH could be explained by the accumulation of propionic acid, which is difficult for microorganisms to further convert. At various pH values of the system, various bacterial systems are activated and intermediates produced might be changed, which are important for hydrogen fermentation. Moreover, in the case of wastewater and wastewater with food waste, some inhibitory or enhancing effect could be observed due to the presence of indigenous microorganisms.

$$C_6H_{12}O_6 \rightarrow 3\ CH_3COOH \quad (1)$$

The process with ordinary domestic wastewater (Run 2) was operated at an organic loading rate of 3 g COD/L·day and a pH of 5–5.5 after determining the favorable pH from the high-strength organic loading. Various studies revealed that a pH of 5–6 is ideal in avoiding methanogenesis and solventogenesis, which is a key factor for effective hydrogen generation [21,22]. The result of this study was similarly observed by Fang *et al*. [24] who found maximum hydrogen production at a pH of 5–5.5 with a hydrogen content of 50%–60%. Maintaining pH in the acidophilic range (5–6) is ideal for effective H_2 production due to repression in the methanogenic bacteria, thus indirectly promoting H_2 producers within the system. Furthermore, a moderately low pH (5.0–6.0) induces a process, *i.e*., an acid tolerance response, that protects cells from subsequent difficulties at a lower pH [28]. Various other works reported a moderately low pH of 5–6 as the optimum for hydrogen fermentation.

3.3. Production of Volatile Fatty Acids at Different Organic Loading

Hydrogen fermentation is always accompanied by a large amount of volatile fatty acids (VFAs) and other soluble microbial products (SMPs). The VFA composition is important as it can provide information regarding hydrogen production and fermentation pathways. VFA production during anaerobic hydrogen production at the two organic loadings is illustrated in Figure 3. The average values of acetic, propionic and butyric acid concentrations in the reactor system during steady-state conditions in Run 1 and Run 2 are 3.78 ± 0.015, 0.68 ± 0.005, 2.57 ± 0.02 and 0.3 ± 0.003, 0.050 ± 0.001, 0.3 ± 0.001 g/L, respectively. The specific yields of acetate, propionate and butyrate in Run 1 and Run 2 were found to be 0.75, 0.10, 0.34 mol/mol glucose and 1, 0.12, 0.62 mol/mol glucose, respectively.

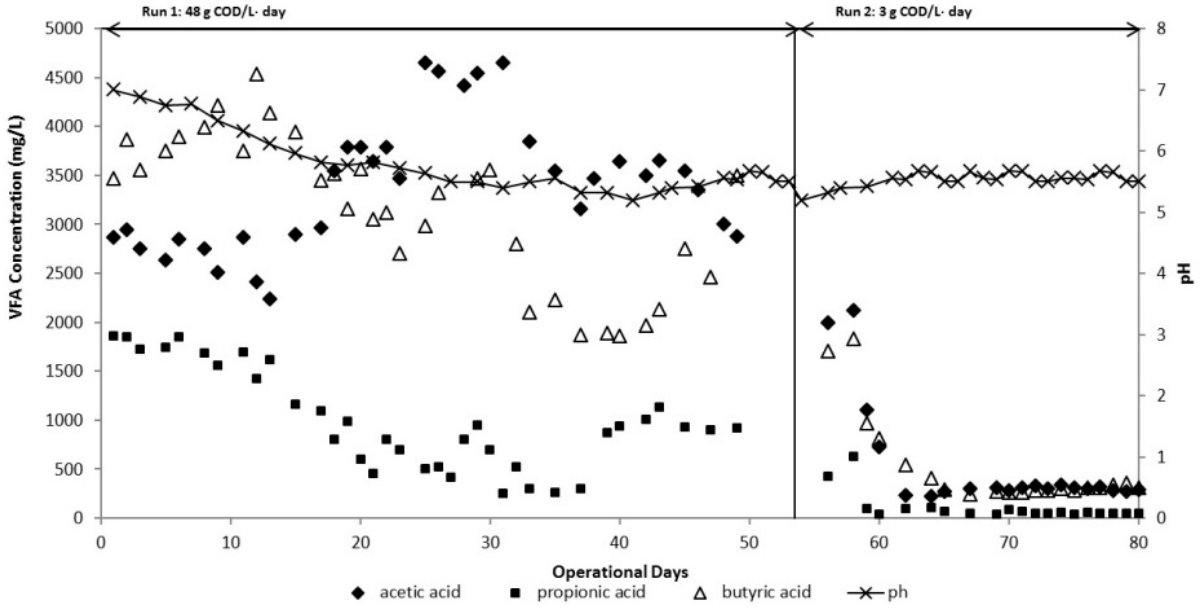

Figure 3. Variation of intermediates produced at high-strength and ordinary-strength loadings.

Butyric and acetic acids were found to be dominant. Propionic acid concentration was found to be lower when the system was operated with both organic loadings (Run 1 and Run 2). This signifies that hydrogen fermentation followed either the butyric or acetic acid pathway. This result supports the theory that for higher hydrogen production, the conversion of glucose follows the acetate pathway. The biochemical metabolic route from glucose is presented in Equations (2) and (3) below. However, Guo *et al.* [29] noted that the biochemical theory of acetate to hydrogen production gives an illusion that a higher accumulation of acetate would lead to higher hydrogen production. Guo *et al.* [29] noticed that the accumulation of acetate was a bad indicator for hydrogen production. The butyrate pathway is also linked with hydrogen production, and no direct hydrogen consumption pathway related to butyrate production has been reported [30]. Guo *et al*. and Hawkes *et al.* [29,30]

evoked that the butyrate/acetate (B/A) ratio might be used as a quantitative indicator of microbial metabolism associated with hydrogen production. The B/A ratio during the initial days of operation was found to be higher, but hydrogen production was not observed to be higher as shown in Figures 2 and 3. Low hydrogen during the initial days of operation could also be attributed to the non-acclimatization of microbes. The converse ratio was tested after day 30 when the system reached the steady state, where acetic acid concentration and hydrogen production were observed to be higher (Figures 2 and 3, Run 1). Butyrate and acetate ratios provided contradictory results; hence, the butyrate-to-propionate B/P ratios were studied. Arooj *et al.* [31] stated that the B/P ratio is an important governing factor for hydrogen production, with the highest butyrate-to-propionate ratio providing a higher hydrogen yield. The B/A ratios in Run 2 during the initial days (organic load shifting period) were higher; however, the hydrogen yield was low. This further shows that the use of the B/A ratio as an indicator is contradictory. The B/P ratios in Run 2 were found to be similar to those in Run 1 and were higher than four. Arooj *et al.* [31] also observed a higher hydrogen production with higher B/P ratios. Other reported values of hydrogen production and B/P ratios for equivalent pH values were also higher [20,32]. Vavilin *et al.* [33] stated that the limiting substrate for butyrate production was glucose, while the limiting substrate for propionate production was H_2, and these end products were balanced in the microbial consortium producing H_2 (Figures 2 and 3).

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

$$C_6H_{12}O_6 \rightarrow 2\ H_2 + 2\ CO_2 + CH_3CH_2CH_2COOH \quad (3)$$

VFAs produced within the reactor system are regulated by the pH of the system. The VFA production at different pH values for hydrogen production was monitored (Figure 4). The production of these intermediates reflects changes in the metabolic pathways of the microorganisms involved. As shown in Figure 4, different acid concentrations were found to be dominant at pH levels of 4, 5 and 6. When the reactor system was operated at a pH of 6, the butyric acid concentration was found to be dominant; however, propionic acid was also found to be high at this pH level. In an exploratory study in our laboratory by Jeong *et al*. [34] prior to this study, it was concluded that the high propionic acid accumulation was due to the presence of *C. pasteurianum.* The same reason could be argued for the presence of propionic acid even at a pH of 6 compared to other studies. Moreover, the result shows that there might be a presence of *C. thermoaceticum*, *C. thermoautotrophicum,* and *C. magnum* in the mixed cultures, which have abilities to convert glucose or sucrose to acetate [35]. VFA production at pH levels of 5 and 4 also followed similar trends but with acetic acid being dominant. Butyric acid concentration was found to be relatively higher in the system at pH 4 and 5 compared to propionic acid, which provides evidence for the presence of butyric acid–producing *Clostridium* sp., e.g., *C. butyricum*. The trend of VFA production suggests that a specific *Clostridium* sp. is activated in specific pH ranges, but the identity of the bacterial community is still contradictory. The results of this study and the assumption of Costello *et al.* [36] provide support for the results found by Guo *et al*. [29], who concluded that the accumulation of butyric acid has no direct hydrogen consumption pathway.

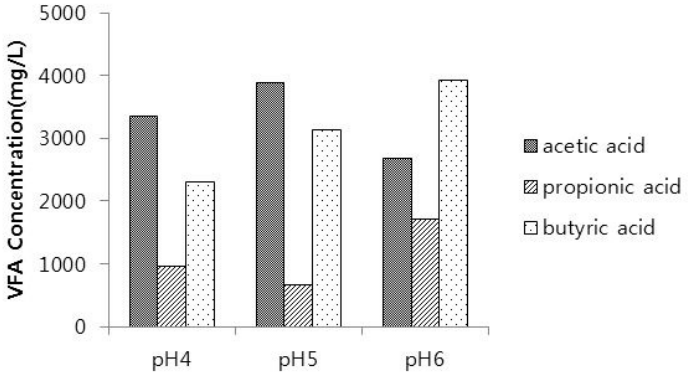

Figure 4. VFAs at different pH conditions (high strength loading).

3.4. COD Variation in the System

Anaerobic hydrogen fermentation itself is an acidogenic conversion of the carbon substrate to gas. Higher degrees of COD removal could not be achieved; instead, the influent substrate was converted into various intermediate liquids.

Figure 5 shows the variation in influent and effluent COD concentrations, which depicts COD removals of approximately 30% in the case of the high-strength organic loading and 26% in case of the ordinary loading rate. As the reactor is operated at a moderately low pH, the COD removal efficiency is not very significant. A neutral pH is ideal for wastewater treatment, while an acidophilic pH is useful for effective H_2 production [37]. Even with a high operating pH during the initial days of reactor operation, the COD removal was low. This was due to the non-acclimatization of microbes. Few other works have been performed to study the COD removal from H_2-producing systems with COD removal efficiencies ranging from 17% to 52% [37]. Lower COD degradation in the ordinary loading system could be explained by the refractory fraction of the COD content accumulated in the reactor system during operation with high-strength organic loading. The substrates utilized by the microbes to produce EPS (Extracellular Polymeric Substances) and biomass-associated (use-associated and degradation-associated) SMPs were not accounted for in this study. Although a higher COD removal could not be achieved during hydrogen fermentation, the fermented supernatant could be recycled or mixed with other low-strength wastewater/grey water streams for further treatment and reuse. When these types of acidogenic systems for the treatment of real-time wastewater are applied, differences in the results may occur, exhibiting higher or lower gas production or organic removal. This may happen in real cases due to the presence of indigenous microflora in the wastewater, which may compete with or enhance the performance of inoculated microorganisms.

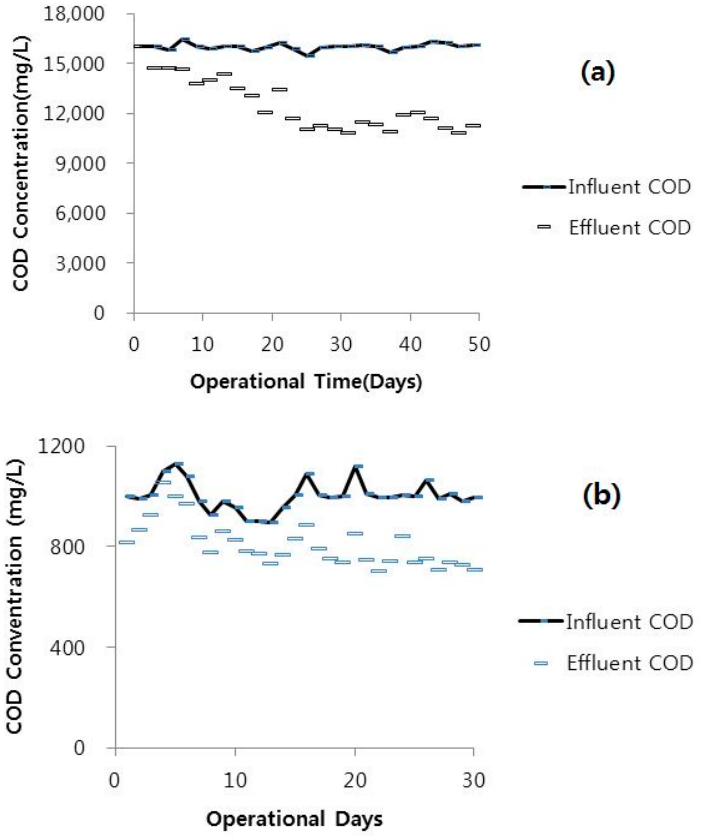

Figure 5. COD variations in the reactor system for (**a**) high-strength organic loading and (**b**) ordinary organic loading.

3.5. Organic Mass Balance

System mass balance was performed in terms of the COD balance and gas produced per day during 14 days of observation under steady-state conditions. The organic balance for the system can be represented with the following equation:

$$C_I = C_O + C_G + C_{Biom} \quad (4)$$

where C_I represents the influent COD loading in the reactor, C_O represents the output COD, C_{Biom} represents the COD assimilated for biomass growth and C_G is the COD converted to produce gas. According to the calculated mass balance, a mass flow diagram was prepared in Figure 6 for both operational runs in the reactor, *i.e.*, Run 1 for the high-strength wastewater and Run 2 for the ordinary-strength wastewater. The COD transferred to the gas phase was calculated based on the theoretical coefficient of hydrogen production via glucose to be 89.62 L H_2/192 g COD (0.46 L H_2/g COD) and 1 kg CO_2/kg COD. Technically, the COD is not transferred to the hydrogen gas, but rather carbon sources are transferred to the metabolic intermediates produced during hydrogen fermentation. A factor of 1.42 representing the COD of cell tissues [38] and the Volatile suspended solids (VSS) concentration of the reactor were taken into account for the calculation of COD transferred to the biomass and sludge.

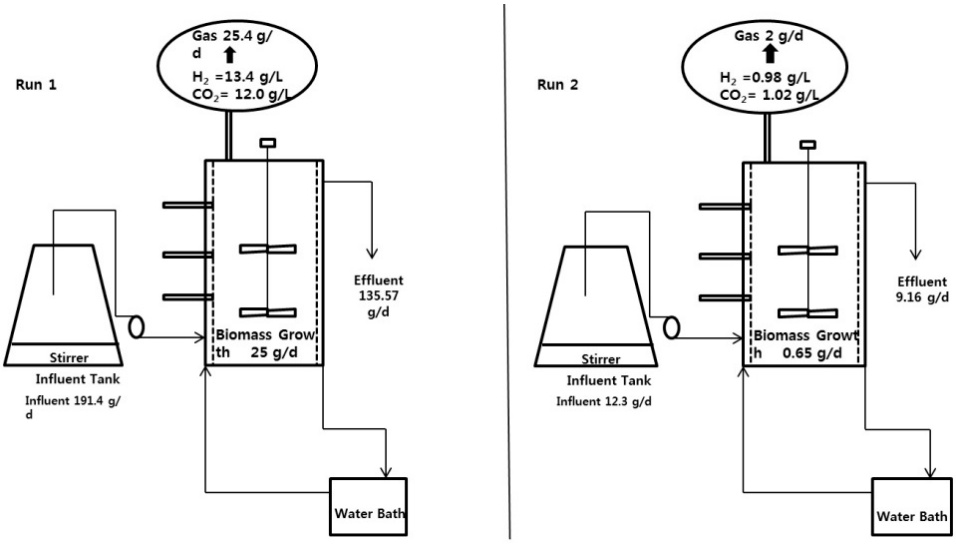

Figure 6. COD mass balance in the anaerobic reactor system.

In Run 1, the incoming COD was 191.4 g/d out of which approximately 70% of the COD was found outgoing with a value of 135.5 g/d. Also, 25 g/d of the incoming COD was consumed by microorganisms for biomass growth, which was approximately 13% of the total incoming COD. A total of 25.5 g/d of COD was converted to gas produced in the reactor system, which constituted COD consumptions of 13.43 g/L and 12.03 g/L for hydrogen and carbon dioxide productions, respectively. This shows that 13.2% of the incoming COD was utilized for gaseous outcomes. The difference between incoming and outgoing COD was found to be approximately 4%. This unbalanced COD could be ascribed to the transformation to alcohols, residual gelatins, unknown metabolites, EPS, SMPs and other biomass-forming agents and also may be due to assumptions made and instrumental/experimental errors [39]. In Run 2, the total incoming COD was 12.3 g/d, out of which 9.16 g/d of COD was found in the effluent, making up 74% of the incoming COD. COD utilized for biomass growth and gas production were 0.65 and 2 g/d, respectively, which corresponded to 5.28% and 16.33% of the total incoming COD and showed a 96% balance in Run 2. Using a high-concentration substrate in Run 1 resulted in the growth of the biomass, but the biomass concentration in the same reactor system was not significantly decreased when an ordinary-strength substrate was used over the same biomass. This is due to a huge concentration difference between the incoming

substrates in the same reactor system. Similar maximum hydrogen yields in this study for Run 1 and Run 2 can also be related to the biomass concentration of the system. This signifies the importance of the operating environmental conditions of an anaerobic hydrogen reactor system. The result of this study shows a minimum of 4628 J to a maximum of 91000 J of energy could be generated using domestic wastewater (energy content of hydrogen = 130 kJ/g) [40]. However, during the treatment of wastewater, input energy is still a major portion of the energy that will be consumed by the reactor system when operated in mesophillic conditions at 37 °C. Further study is required to assess the input energy and energy balance of the system.

4. Conclusions

The study result shows that anaerobic hydrogen production is a potent technology for decentralized energy recovery using domestic wastewater. Maximum hydrogen yields of 1.125 and 1.014 mol H_2/mol glucose were found using substrates of high- and ordinary-strength wastewater. This result implies that the substrate load does not significantly affect hydrogen production but rather that the operational environment should be taken into account. Hydrogen gas production was found to be favorable at a pH of approximately 5.0–5.5 when intermediates produced within the system could maintain appropriate ratios and activate the metabolism of the specific microbes responsible for hydrogen fermentation. The organic removal from the hydrogenic reactor system was not significant; the effluent from the hydrogenic reactor could be further treated using a methanogenic reactor system or any other method of operational ease. A minimum 5 kJ/day to a maximum of 91 kJ/day of energy could be generated using domestic wastewater as the substrate. To apply these systems for wastewater treatment the energy balance should be clear enough. Future studies should focus on input energy and energy balance of the system to fully evaluate the applicability of this system. The findings of this study show that domestic wastewater possesses a great potential for energy recovery via hydrogen fermentation. Further works could be carried out to enhance hydrogen production, hydrogen energy transference and storage, and organic removal using domestic wastewater.

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