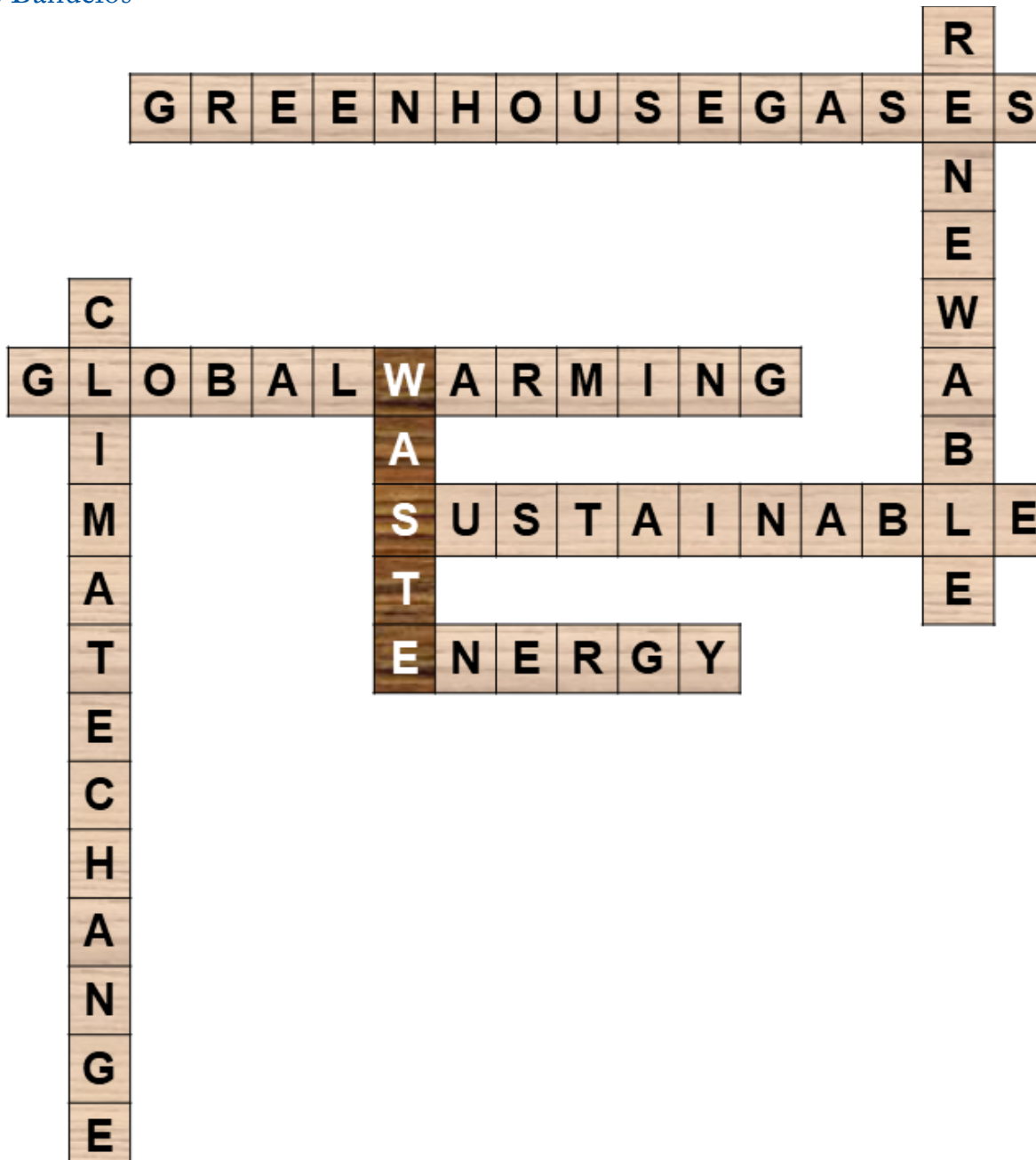


Biological Hydrogen Gas Production from Food Waste as a Sustainable Fuel for Future Transportation

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16. Abstract In the global search for the right alternative energy sources for a more sustainable future, hydrogen production has stood out as a strong contender. Hydrogen gas (H ₂) is well-known as one of the cleanest and most sustainable energy sources, one that mainly yields only water vapor as a byproduct. Additionally, H ₂ generates triple the amount of energy compared to hydrocarbon fuels. H ₂ can be synthesized from several technologies, but currently only 1% of H ₂ production is generated from biomass. Biological H ₂ production generated from anaerobic digestion is a fraction of the 1%. This study aims to enhance biological H ₂ production from anaerobic digesters by increasing H ₂ forming microbial abundance using batch experiments. Carbon substrate availability and conversion in the anaerobic processes were achieved by chemical oxygen demand and volatile fatty acids analysis. The capability of the matrix to neutralize acids in the reactors was assessed using alkalinity assay, and ammonium toxicity was monitored by ammonium measurements. H ₂ content was also investigated throughout the study. The study's results demonstrate two critical outcomes, (i) food waste as substrate yielded the highest H ₂ gas fraction in biogas compared to other substrates fed (primary sludge, waste activated sludge and mixed sludge with or without food waste), and (ii) under normal operating condition of anaerobic digesters, increasing hydrogen forming bacterial populations, including Clostridium spp., Lactococcus spp. and Lactobacillus spp. did not prolong biological H ₂ recovery due to H ₂ being taken up by other bacteria for methane (CH ₄) formation. Our experiment was operated under the most optimal condition for CH ₄ formation as suggested by wastewater operational manuals. Therefore, CH ₄ -forming bacteria possessed more advantages than other microbial populations, including H ₂ -forming groups, and rapidly utilized H ₂ prior to methane synthesis. This study demonstrates H ₂ energy renewed from food waste anaerobic digestion systems delivers opportunities to maximize California's cap-and-trade program through zero carbon fuel production and utilization.			
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Executive Summary

Hydrogen gas (H₂) has received much enthusiasm as an alternative future fuel. Energy acquired from H₂ combustion presents three main advantages compared to other energies: 1) it has the least emission of greenhouse gases, 2) produces water as an end product, and 3) 2.75 more heat value compared to other fossil fuels. In addition, several implications successfully demonstrate the utilizations of H₂ as energy source, for example, to power vehicles and rockets. Generally, H₂ can be synthesized from several technologies. However, only 1% of H₂ production is generated from biomass. Biological H₂ production generated from anaerobic digestion is accounted for within the 1%. Therefore, this project aimed to enhance H₂ gas generation and recovery from anaerobic digesters.

This project's hypothesis was: "H₂ gas generation and recovery can be enhanced when external H₂ forming microbial abundance was added to the system and operated under mesothermic conditions." Three pure cultures, including *Clostridium acetobutylicum* (B-527), *Clostridium*, *Lactobacillus brevis* (B-1835), and *Lactococcus lactis* subspecies *lactis* (B-1232), were experimented with using batch reactors at 37°C for at least 10 days. Throughout the study, chemical oxygen demand (COD), Volatile fatty acid (VFA), alkalinity and ammonium (NH₄⁺-N) concentrations were measured using a spectrophotometer, and H₂ content were detected using hydrogen gas analyzer. DNA was extracted and subjected to next generation DNA sequencing.

The first set of the experiment employed two different sludge seed types, including wastewater digested sludge (control) and wastewater digested sludge spike with *C. acetobutylicum*. Seven substrates were fed into different reactors, entailing (i) primary sludge, (ii) waste activated sludge (WAS), (iii) mixed primary and WAS, (iv) food waste (FW), (v) mixed FW and primary sludge, (vi) mixed FW and WAS, and (vii) mixed FW and mixed sludge. Therefore, seven reactors for each feed were tested. The results showed FW substrate yielded the highest H₂ content compared to other substrate types at day 4 and day 6. After day 6, the H₂ content decreased, possibly due to methanogens (methane forming microbes) consuming H₂ prior to methane formation. Furthermore, the difference in H₂ recovery between the control reactors and *C. acetobutylicum* reactors were not observed. Hence, our finding suggests a high abundance of H₂ forming microorganisms does not promote H₂ recovery in biogas. We also observed different microbial kinetics from this experiment. All bacterial seeds/sludge were stored at 4°C prior to the experiment. Lag phase and exponential growth phase of different microorganisms resulted in high H₂ utilization for methane synthesis after six days.

Similar experiments were carried out using other pure cultures with a reported ability to produce hydrogen gas, *Lactobacillus brevis* and *Lactococcus lactis* subspecies *lactis*. The H₂ recovery from 4°C-digested sludge feed measured the highest on the second day after incubation at 533, 1,000+, and 570 ppm, respectively. Therefore, *L. brevis* possibly enhances H₂ formation in anaerobic digesters. At day 5 and later, the H₂ recovery from three reactors decreased substantively.

Once room-temperature-digested sludge seeds were utilized and incubated, the H₂ recovery from all reactors was measured above the maximum detection limit of the hydrogen gas analyzer (> 1,000 ppm = 0.1%);). Therefore, our study could not specify which reactors produced highest H₂ in this circumstance. Nevertheless, this indicated that by using room-temperature-digested sludge seeds, H₂-forming bacteria in the seeds were active and yielded high H₂ gas content compared to the 4°C-digested sludge seeds. It also confirmed that an H₂-forming bacteria spike was not required to enhance H₂ production. On day 4 and after, the H₂ recovery was found at 0 or significantly lower than the H₂ content obtained on the second day. Therefore, a solids detention time of 2–4 day is suggested for enhanced H₂ production from food waste anaerobic digesters. This project resulted in two poster presentation as shown below (red indicating CSULB students).

Deocampo, L., Ly, M., Asvapathanagul, A. Microbial populations shift during mesophilic and thermophilic anaerobic digestion. 2022 CSU-Program for Education and Research in Biotechnology (CSUPERB) symposium (CSUPERB January 12–15, 2022) Virtual.

Deocampo, L., Banuelos, N., Asvapathanagul, A. Microbial populations shift during mesophilic and thermophilic anaerobic digestion phase 1: Biological hydrogen gas production from lab-scale batch anaerobic digester using various substrates. 2022 American Society for Microbiology (ASM Microbe) Conference (ASM Microbe June 9–13, 2022) at Washington, D.C.

1. Introduction

1.1 Hydrogen Gas as Future Energy Source

Approximately, a large amount of 11-billion liters liquid petroleum is required to globally supply transportation each day, which accounts for 99% of the total fuels consumed by the transportation sector (Kalghatgi et al., 2018). This results in 14% of total greenhouse gas (GHG) emissions from carbon dioxide (CO₂) and other GHGs (Kalghatgi et al., 2018). With rapid population growth, the 2050 projected climate impact illustrates that the use of petroleum fuels be consistent (Kalghatgi et al., 2018). Therefore, the significant GHG emissions from automobiles must be promptly minimized prior to mitigate impacts to global warming. As a result, regulations and policies, mainly in Europe and America, provide initiatives for alternative, low-carbon, and carbon-neutral fuels such as electric batteries, plug-in hybrids, and fuel-cell systems using (renewable) hydrogen, which will immediately reduce anthropogenic GHG emissions from fossil fuels combustion. Specifically, California aims to achieve 40% carbon reduction by 2030, compared to 2016 (Senate Bill 32) (Energy Innovation, 2022). In addition, the state has a goal of 100% carbon-emission free electricity (carbon neutrality via Senate Bill 100, The 100 Percent Clean Energy Act of 2018; (Energy Innovation, 2022)). After 2030, newly manufactured carbon-fueled vehicles will no longer be traded in California.

While solar energy has been widely adopted, the aged solar panels need special disposal and recycling (Monteiro Lunardi et al., 2018). Furthermore, wind energy is limited by regional and climate factors (Carrion et al., 2008). Hydrogen gas is well-known as one of the most clean and sustainable energies with a yielded heating value nearly 3 times higher than petroleum fuels (Momirlan et al., 2005). Comparing combustion heat among several fuels, hydrogen has the highest heating value of 61,000 British Thermal Unit (BTU)/lb (Hung et al., 2011; NIST, 2022). Hydrogen gas can be synthesized from several technologies, but only 1% of hydrogen gas production is generated from biomass (Das et al., 2008). Biological hydrogen gas production during anaerobic digestion is accounted for within that 1% (Hosseini et al., 2016). Currently, most energy utilization from biogas is methane oxidization, which contributes to global warming because its combustion significantly emits CO₂ (greenhouse gas; GHG). Biogas is a mixture of gases, and hydrogen gas (H₂) has the highest heating value compared to other gases in the biogas mixture (Hung et al., 2011). When hydrogen gas reacts with oxygen gas, energy and water are generated with minimum GHG gas emission (Hosseini et al., 2016). Therefore, hydrogen energy is a promising alternative fuel for H₂-powered electric vehicles (Berry et al., 1996). Emerson (2008) reports the economic potential of hydrogen-fuel-cell utilization for large-scale applications. Furthermore, several countries encourage hydrogen fuel for transportation (Market et al., 2020). All of the benefits of hydrogen energy satisfy opportunities to maximize California's cap-and-trade program, which is designed to reduce the impact of transportation on climate change (Senate Bill 697, 2021). Hence, H₂ recovery from biomass/anaerobic digesters can potentially serve as our future renewable H₂ fuel source.

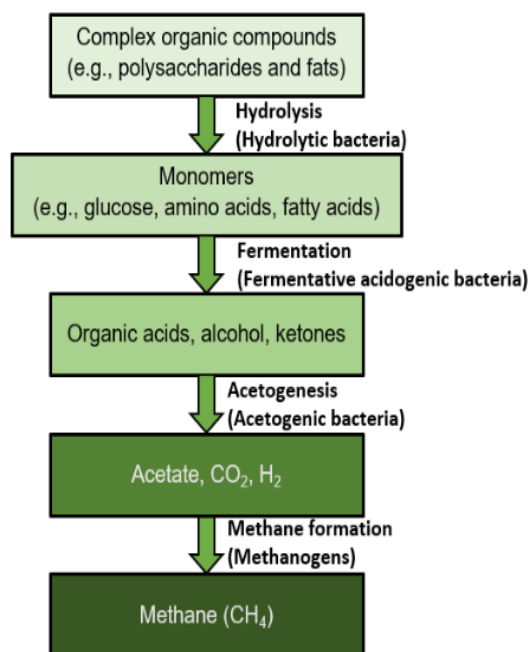
1.2 Food Waste Generation and its Impacts to the Environment

One third of the world's annual food production (approximately 1.3 billion tons) is wasted and disposed in landfills, contributing the equivalent of about 3.3 billion tons CO₂ emissions per year (Salemdeeb et al., 2017; Fisgativa et al., 2017). Specifically, in the U.S., the amount of food waste (FW) has increased by 50% since 1974 (Posmanik et al. 2017). Nevertheless, only 2% of FW in the U.S. is currently anaerobically digested (Food Waste Reduction Alliance, 2016), while 22% of FW are landfilled, and 22% of FW are combusted with energy recovery in 2017 (USEPA, 2019). By 2025, the world's annual amount of FW is projected to be almost 2.5 billion tons (Karthikeyan et al., 2018). These numbers highlight potential environmental concerns regarding FW disposals, especially fugitive greenhouse gas (GHG; i.e., methane (CH₄) and CO₂) from landfills. Hence, FW must be handled more effectively by reducing the amount of FW that is created, thus preventing fugitive GHG during the disposal processes, and by recovering renewable bioenergy and sustainable materials, such as a product that can improve soil health. In September 2015, the Environmental Protection Agency (EPA), along with the U.S. Department of Agriculture (USDA), announced the 2030 food waste and loss reduction goal that aims to reduce FW disposed to combustion with energy recovery and landfills by 50%, to 109.4 lbs per person by the year 2030 (USEPA, 2019; USDA, 2015). This will substantially minimize climate change impacts because 20% of total U.S. methane emissions originates from landfills (USEPA, 2019).

Evaluation of sustainable approaches to FW disposal, including landfilling, incineration, FW composting, and FW anaerobic digestion (FW-AD), has showed FW-AD as a relatively cost-effective technology due to its generation of renewable energy. Moreover, AD can accommodate a much wider range of substrates and can be operated in different bioreactor scales at various locations (Appels et al., 2011). Incineration and composting mostly convert useful organic contents into GHG—CO₂, not methane gas. Although the reaction in landfills is anaerobic, it is not feasible to provide favorable operational factors to promote methane formation. Hence, methane gas is directly emitted to the atmosphere. CH₄ is about 20 times more effective than CO₂ in destroying the ozone layer, therefore, FW, as a major carbon resource delivered to landfills, must be properly minimized.

1.3 Anaerobic Digestion Pathways

Figure 1. Metabolic Bacterial Groups Involved in Anaerobic Digestion of Wastes (Bitton, 2011)



Anaerobic digestion of waste consists of four main processes: hydrolysis, fermentation/acidogenesis, acetogenesis, and methane formation (Figure 1). Anaerobic digesters (ADers) at water resource recovery facilities (WW-ADers) and food waste and wastewater anaerobic co-digesters (FW-WW-ADers) currently share the same microbial seeds to begin, but the substrates are different. Most ADers are operated to achieve the highest biogas volume at the maximum CH₄ gas percentage. Biogas is a mixture of gases, entailing CH₄, CO₂, N₂, H₂S, H₂, and H₂O, for example. Analyzing the microbial pathways of biogas formation (Figure 1), hydrogen gas is an intermediate compound for methane production (see red arrow). Therefore, the system must provide favorable conditions to promote hydrogen-forming bacteria and to restrict methane-producing bacteria. As a result, hydrogen gas is not converted to methane gas, and a large fraction of hydrogen gas will be retained in biogas.

The goal of this project is to enhance H₂ gas formation by externally providing additional H₂-forming bacteria in existing anaerobic digested sludge. The goal aligns with California's cap-and-trade program to reduce the impact of transportation on climate change (Senate Bill 697, 2021). The project goal associated with this paper's objectives deliver environmental and transportation solutions. While electric vehicles require electricity produced from coal, natural gas, etc., and hybrid automobiles are partially fueled by gasoline, hydrogen energy is known as nearly-zero carbon emissions. In addition, this project's outcomes will help reduce the amount of food waste disposed in landfills and incinerators, which directly supports the 2030 EPA's and USDA's

food waste and loss reduction goals (USEPA, 2019; USDA, 2015). Moreover, once fugitive methane gas from landfills is reduced, climate change impacts will also substantially be minimized because 20% of total U.S. methane emissions originate from landfills, which also satisfies Assembly Bill 32 (AB 32) and the EPA's Clean Air Act on climate change mitigation.

2. Materials and Methods

2.1 Pure Cultures and Growth Conditions

Clostridium acetobutylicum (B-527), *Lactobacillus brevis* (B-1835), and *Lactococcus lactis* subspecies *lactis* (B-1232) were kindly provided by the Agricultural Research Services Culture Collection (ARS), the Northern Regional Research Laboratory (NRRL), and the United State Department of Agriculture (USDA). All pure cultures were grown using liver infusion broth at 28°C in an orbital incubator for 24 hours.

2.2 Wastewater Sludge

Primary sludge, waste activated sludge, thickened sludge, and anaerobic digested sludge were obtained from the Chiquita Water Reclamation Plant at Santa Margarita Water District (SMWD). All sludge samples were collected in one-gallon sterilized containers, stored at 4°C, transported to the laboratory, and kept at 4°C until used.

2.3 Physiochemical Analysis of Sludge and H₂ Content Measurement

Chemical oxygen demand (COD), Volatile fatty acid (VFA), and alkalinity and ammonium (NH₄⁺-N) concentrations were measured using a DR 3900 spectrophotometer (Hach, Loveland, CO) with TNT822, TNT872, TNT833, and TNT870 kits. Suspended solids analysis was performed in accordance with the Standard Method (American Public Health Association/American Water Works Association/Water Environment Federation, 2017). H₂ content was detected using a hydrogen gas analyzer.

2.4 DNA Extraction and Molecular Analysis

DNA was subjected to DNA extraction using modified bead-beating protocol (Huang et al., 2010; Yu and Mohn, 1999) and purified using phenol/chloroform and chloroform. The extracts were then precipitated in isopropanol at -20°C before being further washed with 70% ethanol and eluted with high performance liquor chromatography (HPLC) water. DNA extracts were measured for DNA concentration and purity using a nanodrop™ lite spectrophotometer (Thermo Scientific). DNA extracts were diluted to 10 ng/μL for next generation DNA sequencing analysis.

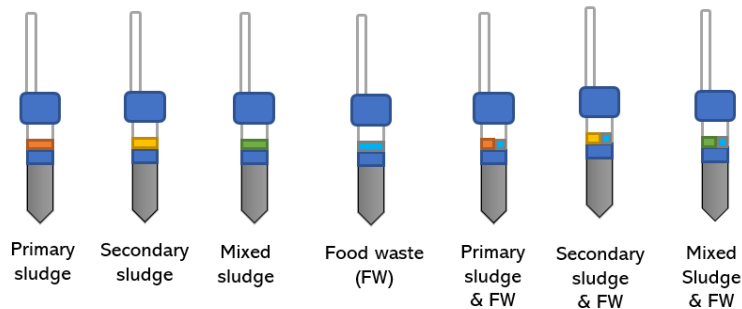
2.5 Statistical Analysis

Pearson correlation coefficient, t-test, f-test, and degree of significance were determined using Microsoft Excel. RStudio (RStudio Team, 2015) and R v.3.5.1 (R Core Team, 2018) were employed for data analysis for multivariable analysis-Redundancy Discriminant Analysis (RDA).

2.6 Reactor Preparation and Incubation Condition

50 mL sterilized conical tubes were added with 25 mL digested sludge, 5 mL sodium bicarbonate (NaHCO_3), and 5 mL of each substrate (Figure 2). The mixed feeds were prepared from 2.5 mL of each feed mixture. All reactors were incubated in an orbital heating bath at 37°C and 30 rpm for approximately two weeks. Pure cultures were individually spiked to each reactor after supernatant broth was removed.

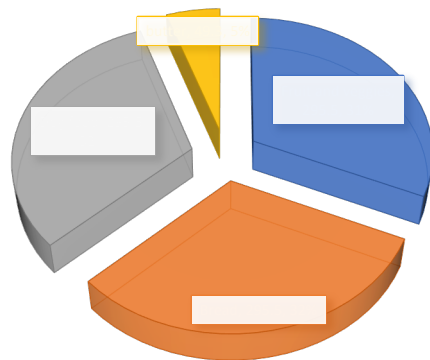
Figure 2. Batch Reactor Set up



2.7 Food Waste Preparation

Food waste was prepared from fresh groceries, which contained 49.3 g of butter (5%) as fat, 295.5 g of fruit and vegetables (31.6%) as fiber, 295.5 g of bread (31.6%) as carbohydrate, 295.5 g of dog food (31.6%) as protein (Figure 3). All groceries were ground using a cooking blender and food processor. All processed food waste was equally portioned at 30 g in a sterilized container and kept frozen at -20°C until use. 30 mL deionized water was added to each 30 g of thawed and processed food waste prior to the experiment. Food waste composition was prepared according to recommended publications (Slopiecka et al., 2022).

Figure 3. Food Waste Composition



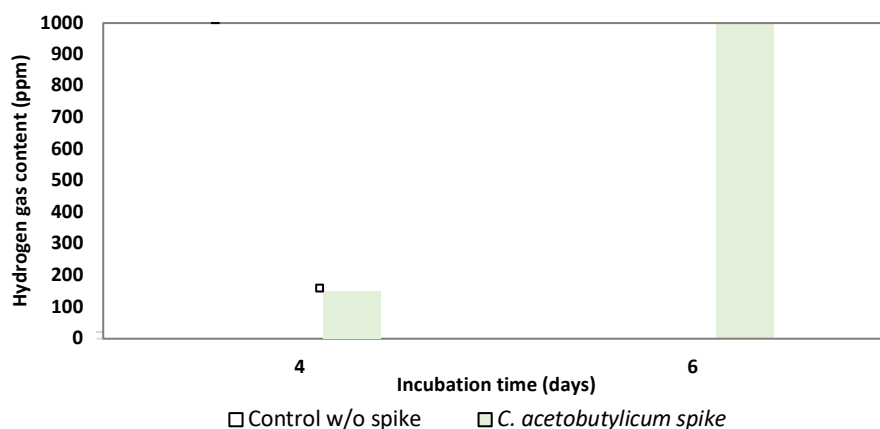
3. Results

3.1 Hydrogen Recovery by *C. Acetobutylicum*

C. acetobutylicum was spiked into anaerobic digestion reactors, which were separately fed with seven different types of feed, including (i) primary sludge, (ii) waste activated sludge (WAS), (iii) mixed primary and WAS, (iv) food waste (FW), (v) mixed FW and primary sludge, (vi) mixed FW and WAS, and (vii) mixed FW and mixed sludge. The H₂ gas recovery was measured at day 4 and 6 from food waste anaerobic digesters with spike and control (Figure 4), as well as with mixed food waste and primary sludge control reactor at day 4 at 900 ppm (data not shown). The 4°C-digested sludge was used to seed all reactors. Our findings indicate no significant advantages of a *C. acetobutylicum* spike in H₂ production performance. Therefore, a spike is not essential for H₂ recovery enhancement. However, food waste was proofed as the most suitable substrate to enhance H₂ recovery compared to other tested substrates.

Our results show non-steady H₂ recovery patterns. When the 4°C-digested sludge was operated anaerobically at 37°C, microbial populations adjusted their kinetics for higher-temperature activities. Our data implied that the high-H₂-forming bacteria possessed high kinetics and reached their maximum rates before methane-forming bacteria. Therefore, H₂ was collected at higher contents at the early days. After day 6, H₂ content in all food waste reactors was below the minimum detection limit of the hydrogen gas analyzer. This suggests that H₂ was utilized for other bacterial groups as reactants or substrates or H₂ generation was deteriorated, which resulted in nondetectable H₂ percentage from the reactors.

Figure 4. Hydrogen Gas Recovery from Control and *C. Acetobutylicum* Spike Food Waste Anaerobic Digestion Reactors

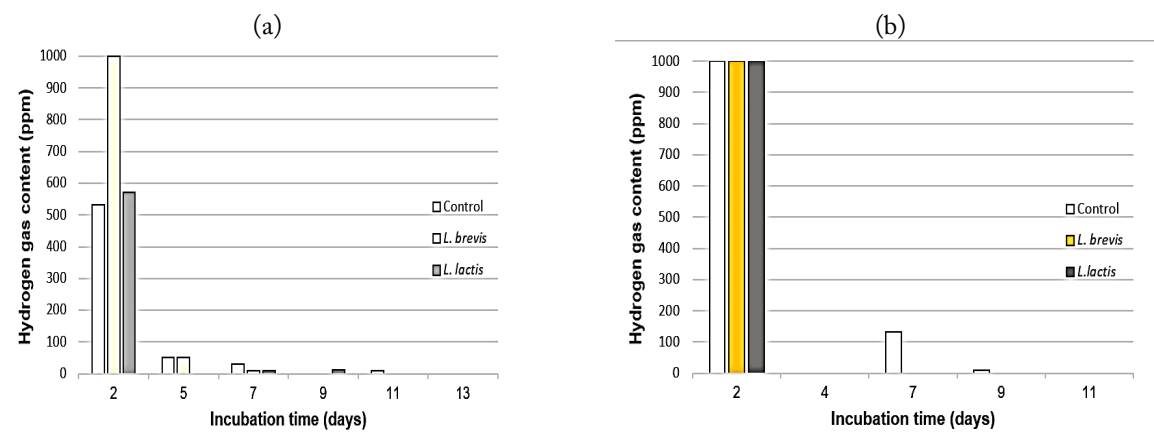


3.2 Hydrogen Recovery using Different Anaerobically Digested Sludge Seeds

Similar experiments were carried out with other pure cultures based on their reported ability to produce hydrogen gas, *Lactobacillus brevis* and *Lactococcus lactis* subspecies *lactis*. H₂ content generated from batch anaerobic digesters fed with food waste with *L. brevis*, *L. lactis* subspecies *lactis* spike and control are displayed in Figure 5. The experiments utilized two different digested sludge, whose temperature were at 4°C (Figure 5a) and at room temperature (Figure 5b) prior to the start of incubation. The H₂ recovery from 4°C-digested-sludge feed measured the highest on the second day after incubation at 533, 1,000+ and 570 ppm. While the H₂ contents from the control and the *L. lactis* subspecies *lactis* spike reactors were assessed approximately at equal concentrations, the *L. brevis* spike reactor produced nearly twice as much H₂ content. Therefore, *L. brevis* possibly enhance H₂ formation in anaerobic digesters. At day 5 and later, the H₂ recovery from the three reactors decreased substantively. After seven days of incubation, H₂ contents were mostly measured at 0 ppm.

Once room-temperature digested sludge seeds were utilized and incubated, the H₂ recovery from all reactors was measured above the maximum detection limit of the hydrogen gas analyzer (> 1,000 ppm = 0.1%; Figure 5b). While our study could not specify which reactors produced the highest H₂ in this circumstance, this indicated that using room temperature digested sludge seeds, the H₂-forming bacteria in the seeds were active and yielded high H₂ gas content compared to the 4°C digested sludge seeds and that the H₂-forming bacteria spike was not required to enhance H₂ production (Figure 5a). On day 4 and after, the H₂ recovery was found at 0 or significantly lower than the H₂ content obtained on the second day. Therefore, a solids detention time of 2–4 days is suggested for enhanced H₂ production from food waste anaerobic digesters.

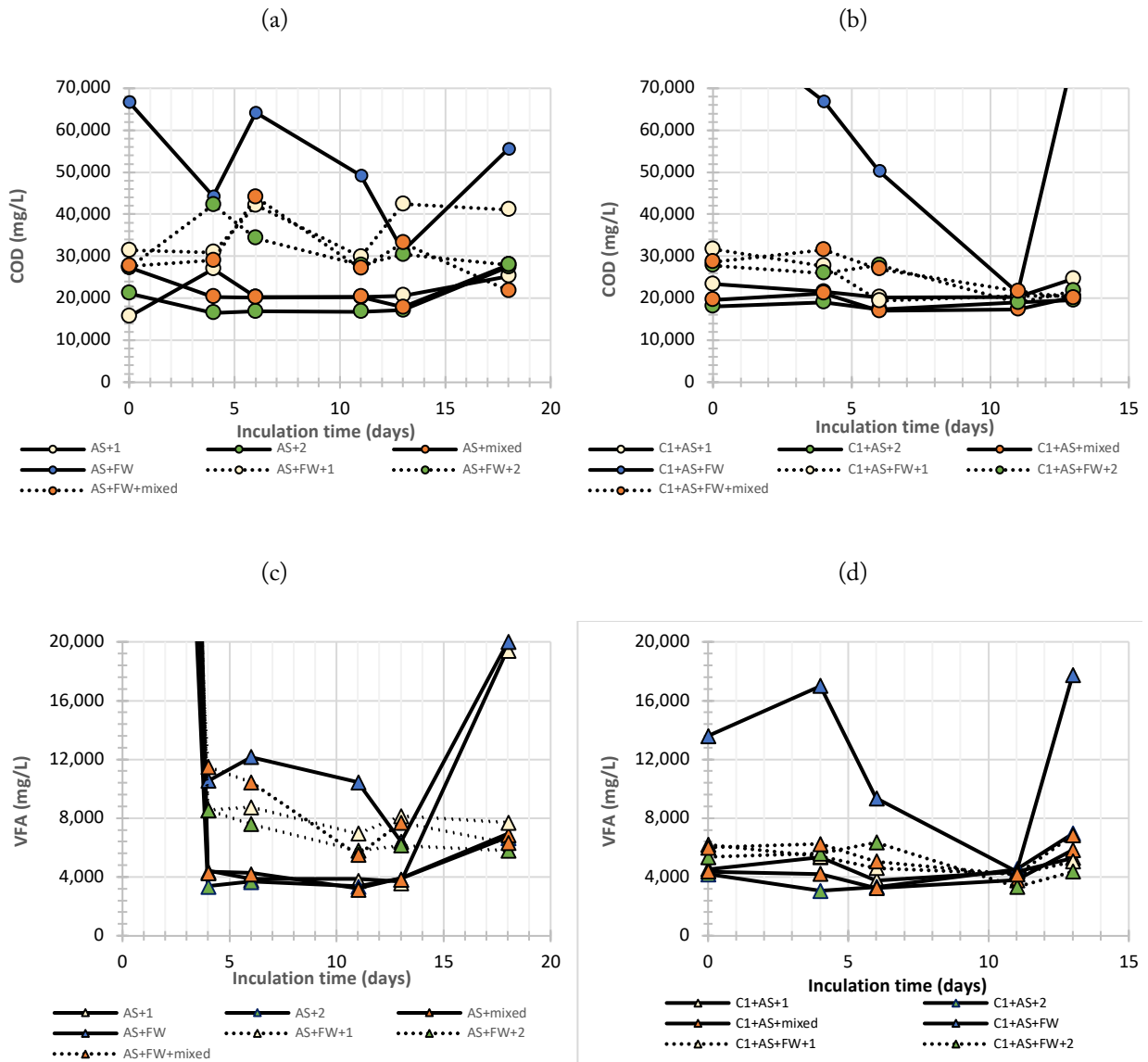
Figure 5. Hydrogen Gas Recovery from Control, *L. Brevis* Spike and *L. Lactis* Subspecies *Lactis* Spike Reactors (a) 4°C Digested Sludge Seed and (b) Room Temperature Digested Sludge Seed

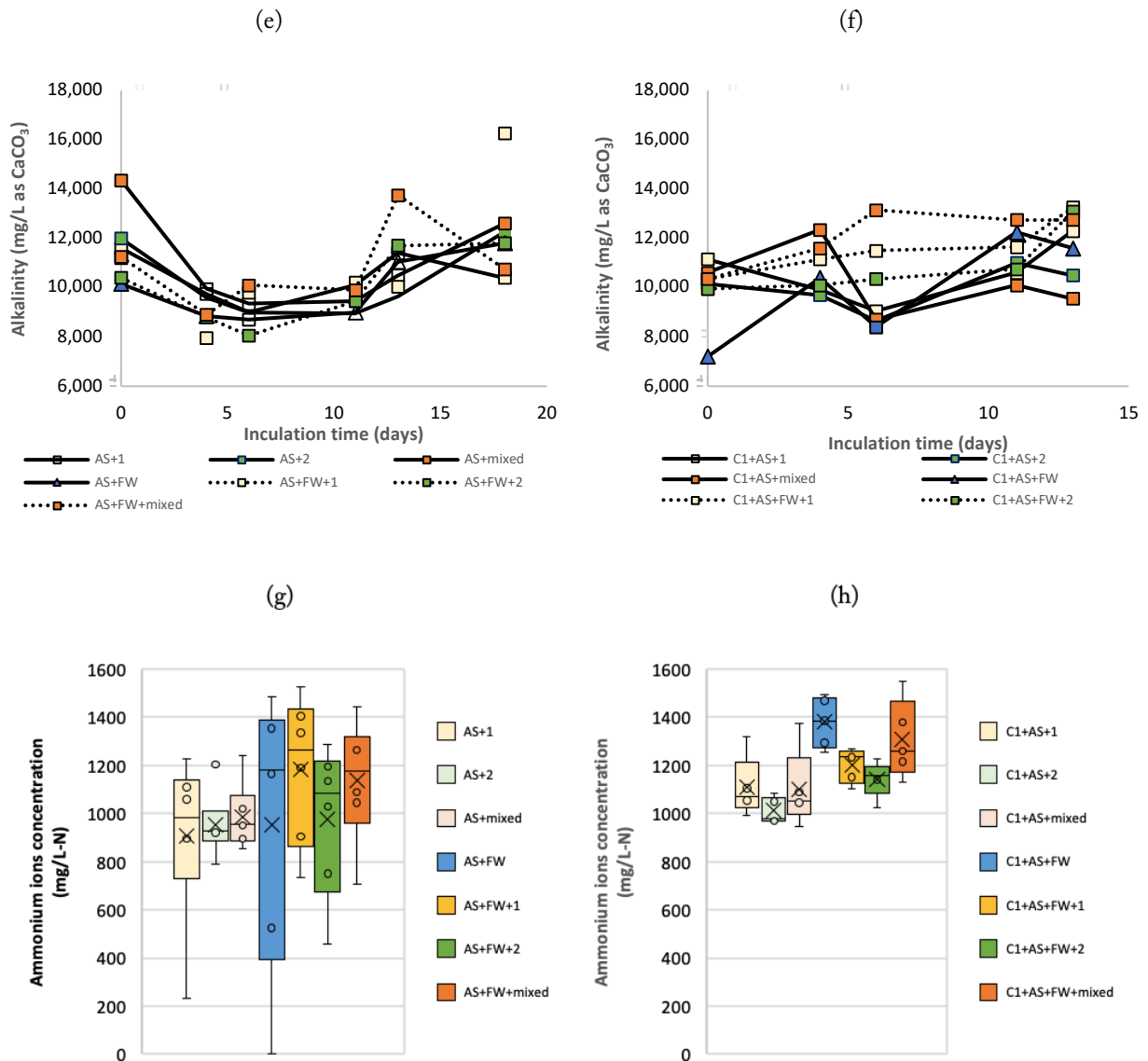


3.3 Change in Physicochemical Parameters Associated with H₂ Recovery

COD and VFA concentrations from reactors fed with food waste had the highest COD and VFA concentrations compared to other substrate types. Moreover, a decrease of COD and VFA overtime, except the spike at the end of the study, suggests that food waste was effectively utilized during anaerobic digestion. Compared to other reactors fed with different substrates, different patterns of COD and VFA concentration shifts were observed, which implies that high COD and VFA in food waste reactors in spike and control reactors offered advantages for H₂ gas recovery and had higher influences on H₂ recovery than additional *C. acetobutylicum*. Although, high COD and VFA were observed during high H₂ recovery, the H₂ contents were not detected after days 4 or 6. Therefore, H₂ may be further utilized for methane formation, or H₂ formation was deteriorated.

Figure 6. Physicochemical Parameters Shift (a) COD – Control, (b) COD – Spike, (c) VFA – Control, (d) VFA – Spike, (e) Alkalinity – Control, (f) Alkalinity – Spike, (g) Ammonium – Control, and (h) Ammonium – Spike





Alkalinity was initially prepared at greater than 10,000 mg/L as CaCO₃ (Figures 6e and 6f). However, initial alkalinity concentration of food waste reactor spike with *C. acetobutylicum* was lower at 8,000 mg/L as CaCO₃ (Figure 6f). Alkalinity varied within a similar range in all reactors. The alkalinity concentrations maintained in all reactors were sufficient to regulate neutral pH.

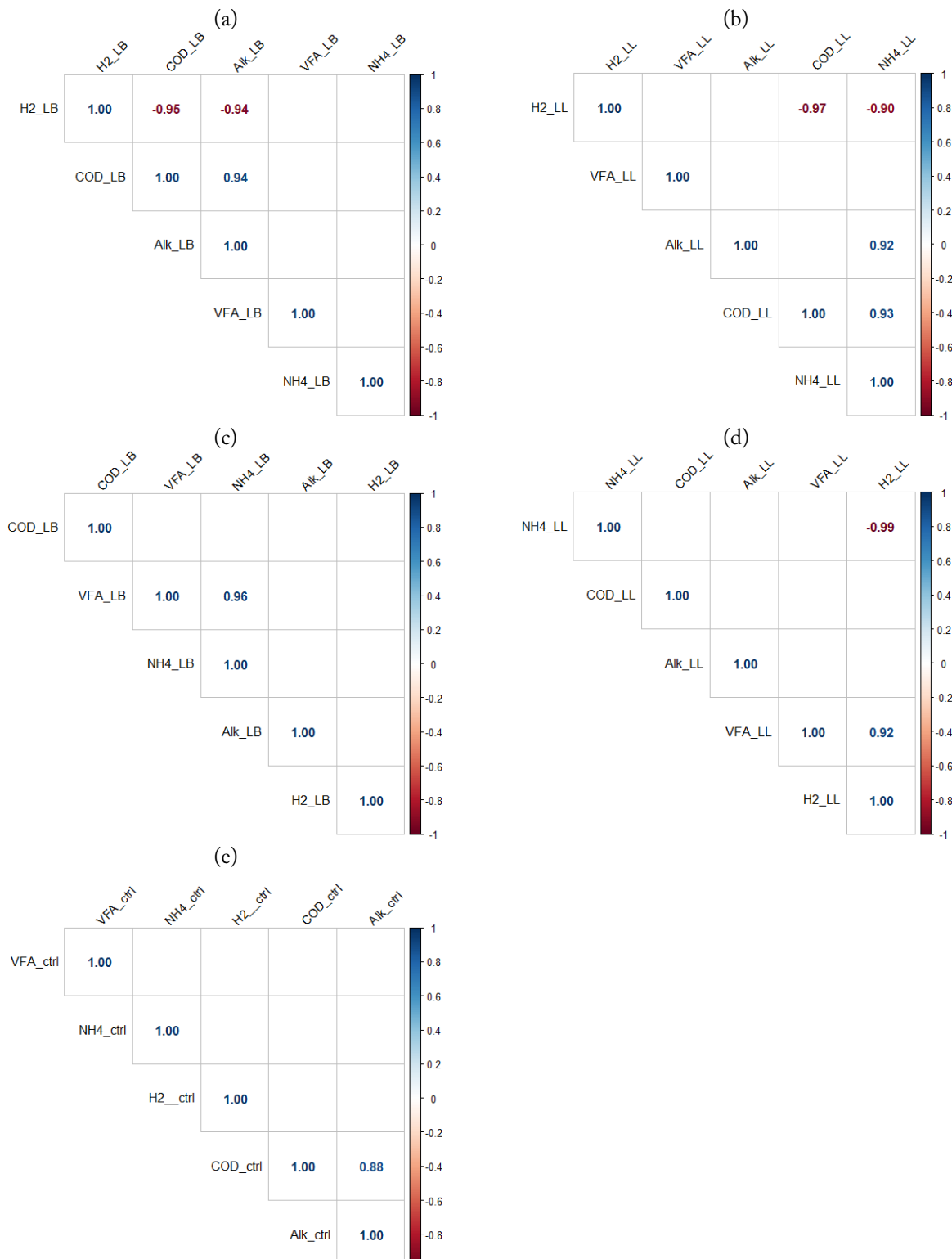
Figure 6g (control) and 6h (*C. acetobutylicum* spike) display ammonium concentrations in both reactors. The ammonium concentrations from the control reactors were varied more than the spike reactors. Ammonia/ammonium toxication was not observed in our study.

Pearson correlation coefficients with degree of significance (*P*) greater than 0.05 among physicochemical parameters in the same food waste reactor are illustrated in Figure 7. When cold digested sludge seeds were spiked with *L. brevis* spike and *L. lactis* subspecies *lactis* in different reactors, the H₂ content had an inverse relationship with COD concentration at $r = -0.95$ and -0.97 , respectively (Figures 7a and 7b). Alkalinity had a negative correlation with the H₂

content only in the *L. brevis* spike reactor ($r = -0.94$), and ammonium had a negative relationship with the H₂ content only in the *L. lactis* subspecies *lactis* spike reactor ($r = -0.90$). No significant correlations were found for sludge characteristics obtained from the control reactor.

Similar statistical analysis was tested among sludge characteristics for the room-temperature digested sludge seeds (Figures 7c, 7d, and 7e). Among the three reactors, *L. brevis* spike and *L. lactis* subspecies *lactis* and control, positive Pearson correlation coefficient was found in the *L. lactis* subspecies *lactis* reactor fed with food waste, while there was no statistically significant correlation between the H₂ content parameters determined in the other two reactors.

Figure 7. Correlation Matrix of Sludge Characteristics (a) *L. brevis* with 4°C Seeds, (b) *L. Lactis* Subspecies *Lactis* with 4°C Seeds, (c) *L. Brevis* with RT Seeds, (d) *L. Lactis* with RT Seeds, and (e) Control with RT Seeds



Note: RT denoting room temperature.

Our study had small sample sizes to test degree of significance ($n = 6$). Furthermore, the H_2 contents were mostly below the minimum detection limits of the hydrogen gas analysis. Hence, most H_2 contents were reported as 0 or 1,000 ppm, which impacted statistical analysis. Our findings suggest that a spike of H_2 -forming bacterial populations is not necessary. Moreover, the H_2 contents measured were not maintained at high or detectable value. This implies that H_2 was utilized by other microbial groups or that H_2 synthesis was terminated, which could not be concluded in this study because only H_2 was measured, not other biogases such as CH_4 , CO_2 , H_2O , H_2S , N_2 , or O_2 . Here, all reactors were operated at the optimal environmental conditions to maximize methane generation. Therefore, it is likely that H_2 was taken up for methane formation. In addition, solids retention time in the reactor must be minimized to inhibit methanogens in future studies.

4. Summary & Conclusions

H₂ recovery in three pure culture reactors and one control were measured between days 2–6, depending on reactor types. Food waste was observed as the best substrate for enhancing H₂ formation. In addition, there was no substantive difference in H₂ content measured among control and spike reactors, which suggests there were enough H₂-producing microorganisms in the digested sludge seeds. However, the H₂ contents that decreased after days 2, 4, or 6, varied by reactor types. This implies that H₂ was utilized by other microbial groups or that H₂ synthesis was terminated, which could not be concluded in this study. Once the digested sludge seeds were prepared at room temperature prior to 37°C incubation, all three reactors, *L. brevis* spike and *L. lactis* subspecies lactis and control, produced equivalent amounts of H₂ content as soon as day 2. After two days, H₂ was nearly below the minimum detection limit of the hydrogen gas analyzer. Consequently, our data suggests a solids detention time of two days is the most suitable to enhance H₂ recovery and prohibit methanogens from taking up H₂ for methane synthesis in the food waste anaerobic digesters.

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