San Jose State University
SJSU ScholarWorks

Mineta Transportation Institute Publications

7-2022

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

Pitiporn Asvapathanagul
California State University, Long Beach

Leanne Deocampo
California State University, Long Beach

Nicholas Banuelos
California State University, Long Beach

Follow this and additional works at: https://scholarworks.sjsu.edu/mti_publications

 Part of the Sustainability Commons, and the Transportation Commons

Recommended Citation

Pitiporn Asvapathanagul, Leanne Deocampo, and Nicholas Banuelos. "Biological Hydrogen Gas Production from Food Waste as a Sustainable Fuel for Future Transportation" *Mineta Transportation Institute Publications* (2022). https://doi.org/10.31979/mti.2022.2141

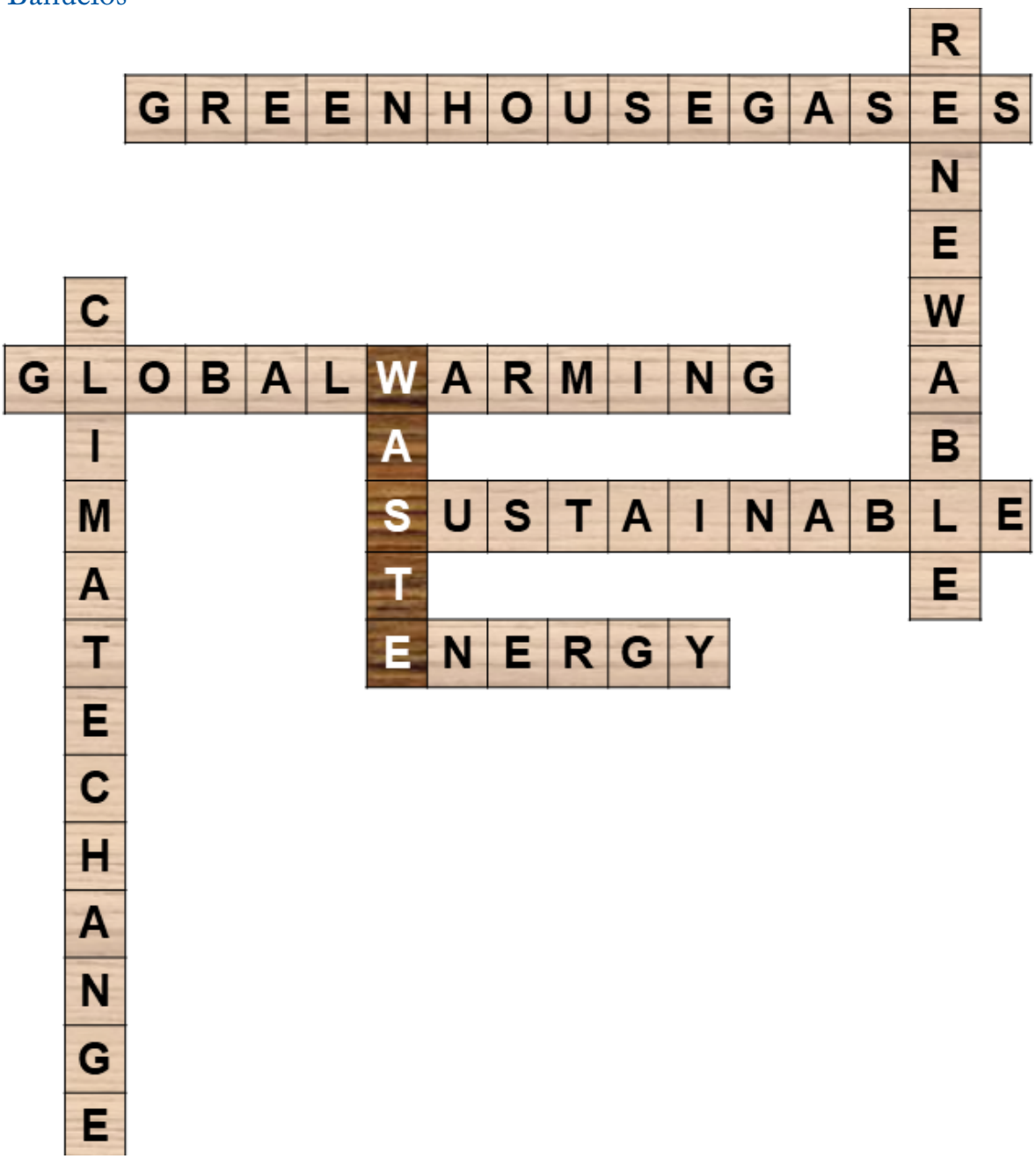
This Report is brought to you for free and open access by SJSU ScholarWorks. It has been accepted for inclusion in Mineta Transportation Institute Publications by an authorized administrator of SJSU ScholarWorks. For more information, please contact scholarworks@sjsu.edu.

SJSU SAN JOSÉ STATE UNIVERSITY

MTI MINETA TRANSPORTATION INSTITUTE

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

Pitiporn Asvapathanagul, PhD
Leanne Deocampo
Nicholas Banuelos



CSUTC California State University Transportation Consortium

CALIFORNIA STATE UNIVERSITY LONG BEACH

CSU TRANSPORTATION CONSORTIUM

transweb.sjsu.edu/csutc

Mineta Transportation Institute

Founded in 1991, the Mineta Transportation Institute (MTI), an organized research and training unit in partnership with the Lucas College and Graduate School of Business at San José State University (SJSU), increases mobility for all by improving the safety, efficiency, accessibility, and convenience of our nation's transportation system. Through research, education, workforce development, and technology transfer, we help create a connected world. MTI leads the Mineta Consortium for Transportation Mobility (MCTM) funded by the U.S. Department of Transportation and the California State University Transportation Consortium (CSUTC) funded by the State of California through Senate Bill 1. MTI focuses on three primary responsibilities:

Research

MTI conducts multi-disciplinary research focused on surface transportation that contributes to effective decision making. Research areas include: active transportation; planning and policy; security and counterterrorism; sustainable transportation and land use; transit and passenger rail; transportation engineering; transportation finance; transportation technology; and workforce and labor. MTI research publications undergo expert peer review to ensure the quality of the research.

Education and Workforce

To ensure the efficient movement of people and products, we must prepare a new cohort of transportation professionals who are ready to lead a more diverse, inclusive, and equitable transportation industry. To help achieve this, MTI sponsors a suite of workforce development and education opportunities. The Institute supports educational programs offered by the Lucas Graduate School of Business: a Master of Science in Transportation Management, plus graduate certificates that include High-Speed and Intercity Rail Management and Transportation Security Management. These flexible programs offer live online classes so that working transportation professionals can pursue an advanced degree regardless of their location.

Information and Technology Transfer

MTI utilizes a diverse array of dissemination methods and media to ensure research results reach those responsible for managing change. These methods include publication, seminars, workshops, websites, social media, webinars, and other technology transfer mechanisms. Additionally, MTI promotes the availability of completed research to professional organizations and works to integrate the research findings into the graduate education program. MTI's extensive collection of transportation-related publications is integrated into San José State University's world-class Martin Luther King, Jr. Library.

Disclaimer

The contents of this report reflect the views of the authors, who are responsible for the facts and accuracy of the information presented herein. This document is disseminated in the interest of information exchange. MTI's research is funded, partially or entirely, by grants from the California Department of Transportation, the California State University Office of the Chancellor, the U.S. Department of Homeland Security, and the U.S. Department of Transportation, who assume no liability for the contents or use thereof. This report does not constitute a standard specification, design standard, or regulation.

Report 22-35

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

Pitiporn Asvapathanagul, Ph.D.

Leanne Deocampo

Nicholas Banuelos

July 2022

A publication of the
Mineta Transportation Institute
Created by Congress in 1991
College of Business
San José State University
San José, CA 95192-0219

TECHNICAL REPORT DOCUMENTATION PAGE

1. Report No. 22-35	2. Government Accession No.	3. Recipient's Catalog No.	
4. Title and Subtitle Biological Hydrogen Gas Production from Food Waste as a Sustainable Fuel for Future Transportation		**5. Report Date** July 2022 **6. Performing Organization Code**	
7. Authors Pitiporn Asvapathanagul, Ph.D., P.E. Leanne Deocampo Nicholas Banuelos		**8. Performing Organization Report** CA-MTI-2141	
9. Performing Organization Name and Address Mineta Transportation Institute College of Business San José State University San José, CA 95192-0219		**10. Work Unit No.** **11. Contract or Grant No.** ZSB12017-SJAUX	
12. Sponsoring Agency Name and Address State of California SB1 2017/2018 Trustees of the California State University Sponsored Programs Administration 401 Golden Shore, 5th Long Beach, CA 90802		**13. Type of Report and Period Covered** **14. Sponsoring Agency Code**	
15. Supplemental Notes			

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 (H2) is well-known as one of the cleanest and most sustainable energy sources, one that mainly yields only water vapor as a byproduct. Additionally, H2 generates triple the amount of energy compared to hydrocarbon fuels. H2 can be synthesized from several technologies, but currently only 1% of H2 production is generated from biomass. Biological H2 production generated from anaerobic digestion is a fraction of the 1%. This study aims to enhance biological H2 production from anaerobic digesters by increasing H2 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. H2 content was also investigated throughout the study. The study's results demonstrate two critical outcomes, (i) food waste as substrate yielded the highest H2 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 H2 recovery due to H2 being taken up by other bacteria for methane (CH4) formation. Our experiment was operated under the most optimal condition for CH4 formation as suggested by wastewater operational manuals. Therefore, CH4-forming bacteria possessed more advantages than other microbial populations, including H2-forming groups, and rapidly utilized H2 prior to methane synthesis. This study demonstrates H2 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.

17. Key Words	18. Distribution Statement		
Renewable energy sources, Sustainable transportation, Greenhouse gases, Global warming, Climate change	No restrictions. This document is available to the public through The National Technical Information Service, Springfield, VA 22161.		
19. Security Classif. (of this report) Unclassified	**20. Security Classif. (of this page)** Unclassified	**21. No. of Pages** 31	**22. Price**

Form DOT F 1700.7 (8-72)

Copyright © 2022

by **Mineta Transportation Institute**

All rights reserved.

DOI: 10.31979/mti.2022.2141

Mineta Transportation Institute
College of Business
San José State University
San José, CA 95192-0219
Tel: (408) 924-7560
Fax: (408) 924-7565
Email: mineta-institute@sjsu.edu
transweb.sjsu.edu/research/2141

ACKNOWLEDGMENTS

We greatly appreciate the Mineta Transportation Institute, College of Business, San José State University (SJSU) for primary funding support, as well as the 2020 Presidents' Commission Scholars Grant Program from California State University-Program for Education and Research in Biotechnology (CSUPERB) and 2021-2022 CSU, Long Beach – Undergraduate Research Opportunities Program (CSULB-UROP). The research team would also like to express our deep gratitude to our College of Engineering Associate Dean for Research & Graduate Studies, CSULB, Dr. Hamid Rahai, for his guidance and assistance throughout the project campaign.

The research team would like to recognize Agricultural Research Services Culture Collection (ARS), Northern Regional Research Laboratory (NRRL), United State Department of Agriculture (USDA) for all pure cultures used in this study. Furthermore, we greatly thank board of directors, plant operators, and lab technicians at the Chiquita Water Reclamation plant, Santa Margarita Water District (SMWD), for providing wastewater samples and sludge for this project. We praise Tina Mow, College of Engineering (CSULB) fiscal analyst, for her help in materials and supplies acquisitions. Likewise, the Civil Engineering and Construction Engineering Management Department and College of Engineering at CSULB have provided tremendous necessary aids and services to assure the success of the project.

Finally, our research team would like to acknowledge Maggie Ly, Monica Robles, and Nora Mirza for their contributions during laboratory preparation and set up stages.

CONTENTS

LIST OF FIGURES

Executive Summary

Hydrogen gas (H_2) has received much enthusiasm as an alternative future fuel. Energy acquired from H_2 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 H_2 as energy source, for example, to power vehicles and rockets. Generally, H_2 can be synthesized from several technologies. However, only 1% of H_2 production is generated from biomass. Biological H_2 production generated from anaerobic digestion is accounted for within the 1%. Therefore, this project aimed to enhance H_2 gas generation and recovery from anaerobic digesters.

This project's hypothesis was: "H_2 gas generation and recovery can be enhanced when external H_2 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 (NH_4^+-N) concentrations were measured using a spectrophotometer, and H_2 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 H_2 content compared to other substrate types at day 4 and day 6. After day 6, the H_2 content decreased, possibly due to methanogens (methane forming microbes) consuming H_2 prior to methane formation. Furthermore, the difference in H_2 recovery between the control reactors and *C. acetobutylicum* reactors were not observed. Hence, our finding suggests a high abundance of H_2 forming microorganisms does not promote H_2 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 H_2 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 H_2 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 H_2 formation in anaerobic digesters. At day 5 and later, the H_2 recovery from three reactors decreased substantively.

Once room-temperature-digested sludge seeds were utilized and incubated, the H_2 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 H_2 in this circumstance. Nevertheless, this indicated that by using room-temperature-digested sludge seeds, H_2-forming bacteria in the seeds were active and yielded high H_2 gas content compared to the 4°C-digested sludge seeds. It also confirmed that an H_2-forming bacteria spike was not required to enhance H_2 production. On day 4 and after, the H_2 recovery was found at 0 or significantly lower than the H_2 content obtained on the second day. Therefore, a solids detention time of 2–4 day is suggested for enhanced H_2 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 (CO_2) 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 anthrophonic 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 CO_2 (greenhouse gas; GHG). Biogas is a mixture of gases, and hydrogen gas (H_2) 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 H_2-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, H_2 recovery from biomass/anaerobic digesters can potentially serve as our future renewable H_2 fuel source.

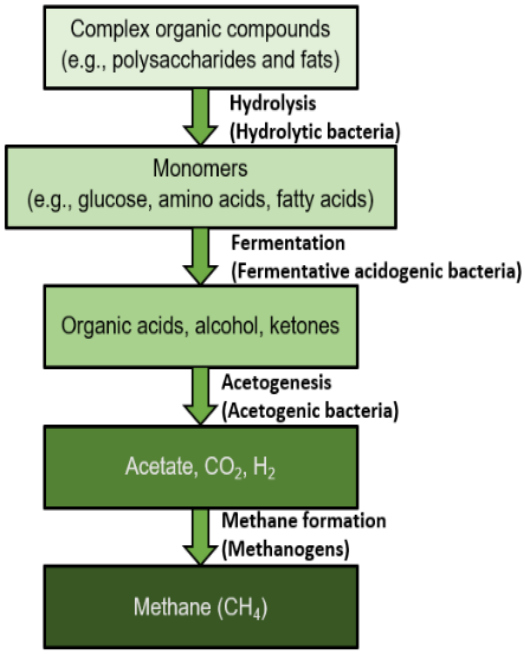
1.2 Food Waste Generation and its Impacts to the Environment

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 CO_2 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 (CH_4) and CO_2) 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 heath. 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—CO_2, 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. CH_4 is about 20 times more effective than CO_2 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 CH_4 gas percentage. Biogas is a mixture of gases, entailing CH_4, CO_2, N_2, H_2S, H_2, and H_2O, 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 H_2 gas formation by externally providing additional H_2-forming bacteria in existing anaerobic digested sludge. The goal aligns with 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 atSanta 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 H_2 Content Measurement

Chemical oxygen demand (COD), Volatile fatty acid (VFA), and alkalinity and ammonium (NH_4^+-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). H_2 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.

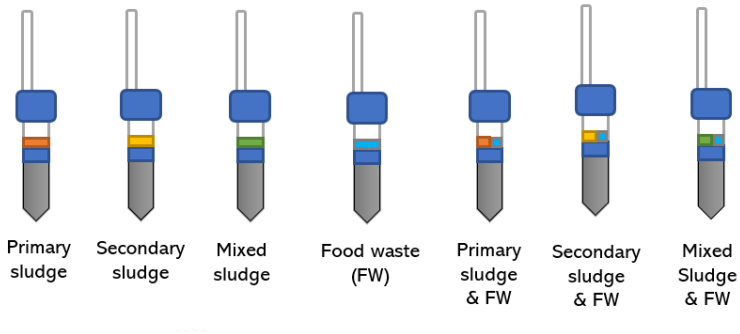
2.5 Statistical Analysis

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.

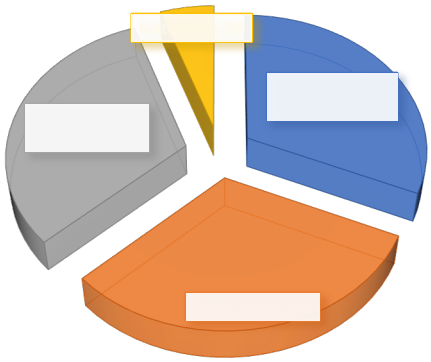
Figure 2. Batch Reactor Set up



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



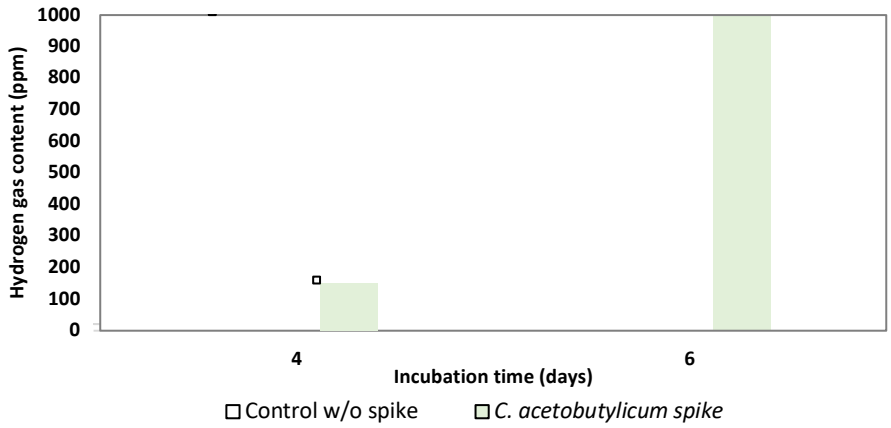
3. Results

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 H_2 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 H_2 production performance. Therefore, a spike is not essential for H_2 recovery enhancement. However, food waste was proofed as the most suitable substrate to enhance H_2 recovery compared to other tested substrates.

Our results show non-steady H_2 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-H_2-forming bacteria possessed high kinetics and reached their maximum rates before methane-forming bacteria. Therefore, H_2 was collected at higher contents at the early days. After day 6, H_2 content in all food waste reactors was below the minimum detection limit of the hydrogen gas analyzer. This suggests that H_2 was utilized for other bacterial groups as reactants or substrates or H_2 generation was deteriorated, which resulted in nondetectable H_2 percentage from the reactors.

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

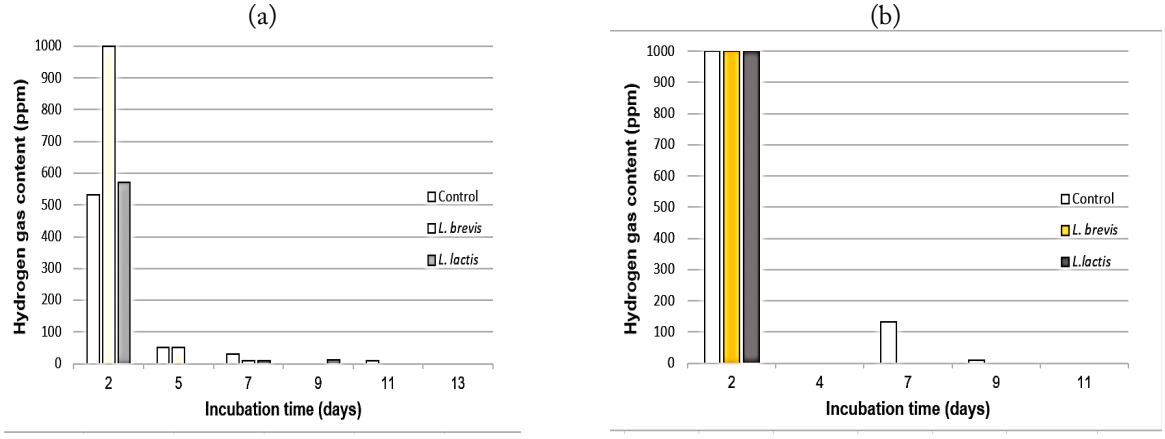


3.2 Hydrogen Recovery using Different Anaerobically Digested Sludge Seeds

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. H_2 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 H_2 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 H_2 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 H_2 content. Therefore, *L. brevis* possibly enhance H_2 formation in anaerobic digesters. At day 5 and later, the H_2 recovery from the three reactors decreased substantively. After seven days of incubation, H_2 contents were mostly measured at 0 ppm.

Once room-temperature digested sludge seeds were utilized and incubated, the H_2 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 H_2 in this circumstance, this indicated that using room temperature digested sludge seeds, the H_2-forming bacteria in the seeds were active and yielded high H_2 gas content compared to the 4°C digested sludge seeds and that the H_2-forming bacteria spike was not required to enhance H_2 production (Figure 5a). On day 4 and after, the H_2 recovery was found at 0 or significantly lower than the H_2 content obtained on the second day. Therefore, a solids detention time of 2–4 days is suggested for enhanced H_2 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

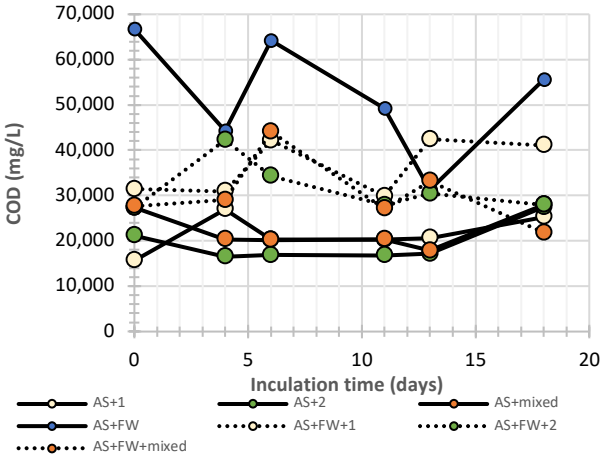


3.3 Change in Physicochemical Parameters Associated with H_2 Recovery

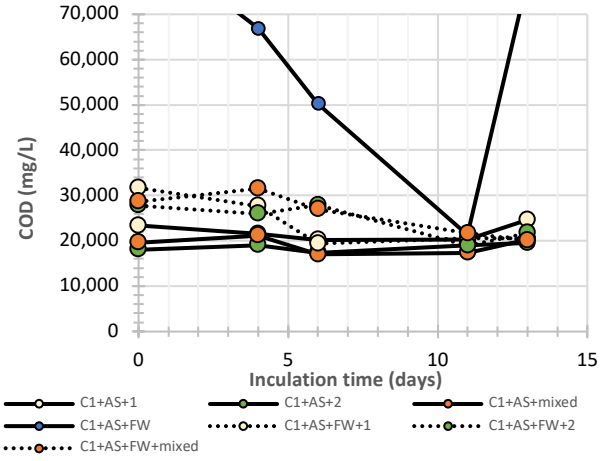
COD and VFA concentrations from reactors fed with food waste had the highest COD and VFA concentrations compared to other substrate types. Moreover, a decrease of COD and VFA overtime, except the spike at the end of the study, suggests that food waste was effectively utilized during anaerobic digestion. 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 H_2 gas recovery and had higher influences on H_2 recovery than additional *C. acetobutylicum*. Although, high COD and VFA were observed during high H_2 recovery, the H_2 contents were not detected after days 4 or 6. Therefore, H_2 may be further utilized for methane formation, or H_2 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

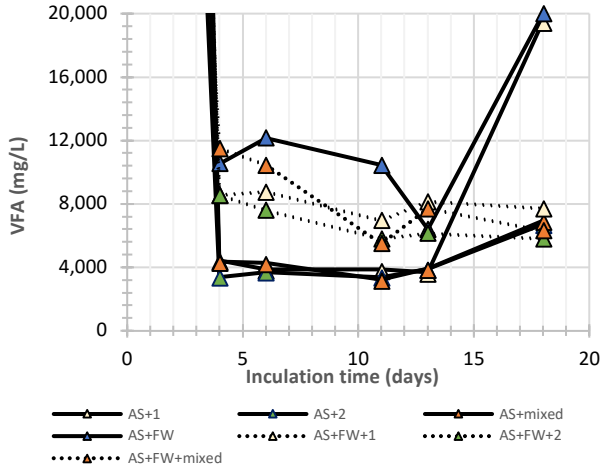
(a)



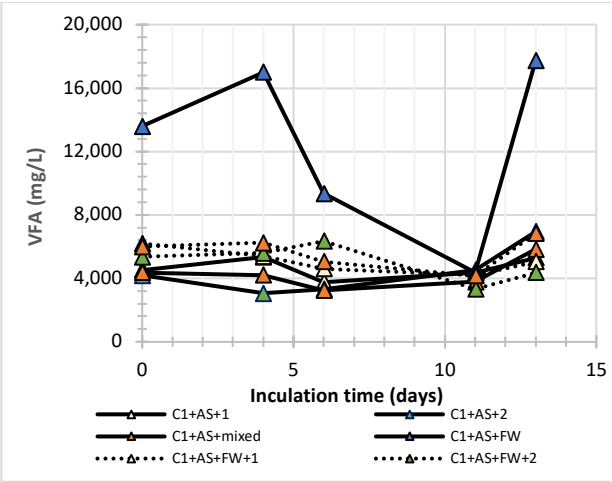
(b)

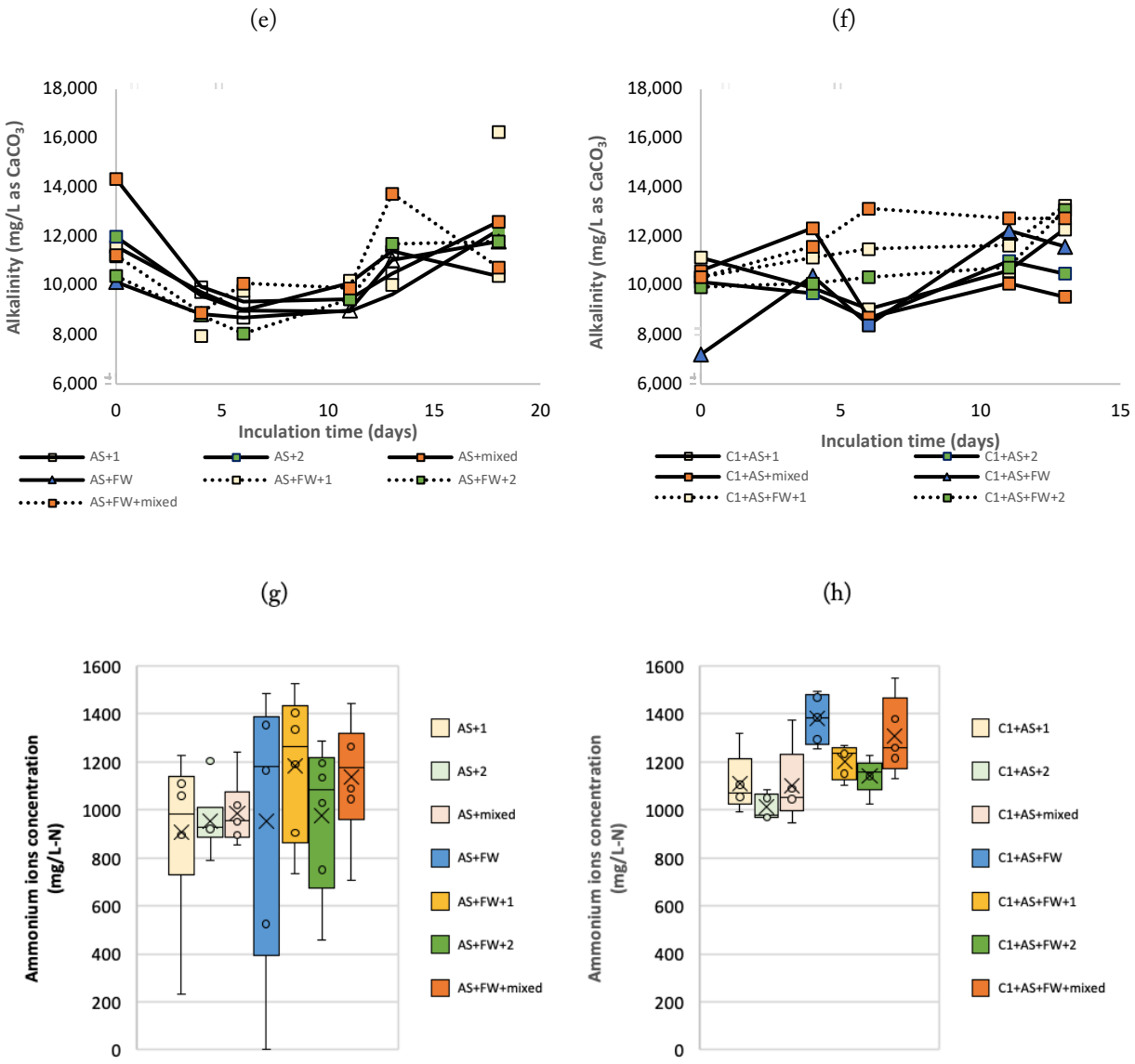


(c)



(d)





Alkalinity was initially prepared at greater than 10,000 mg/L as $CaCO_3$ (Figures 6e and 6f). However, initial alkalinity concentration of food waste reactor spike with *C. acetobutylicum* was lower at 8,000 mg/L as $CaCO_3$ (Figure 6f). Alkalinity varied within a similar range in all reactors. The alkalinity concentrations maintained in all reactors were sufficient to regulate neutral pH.

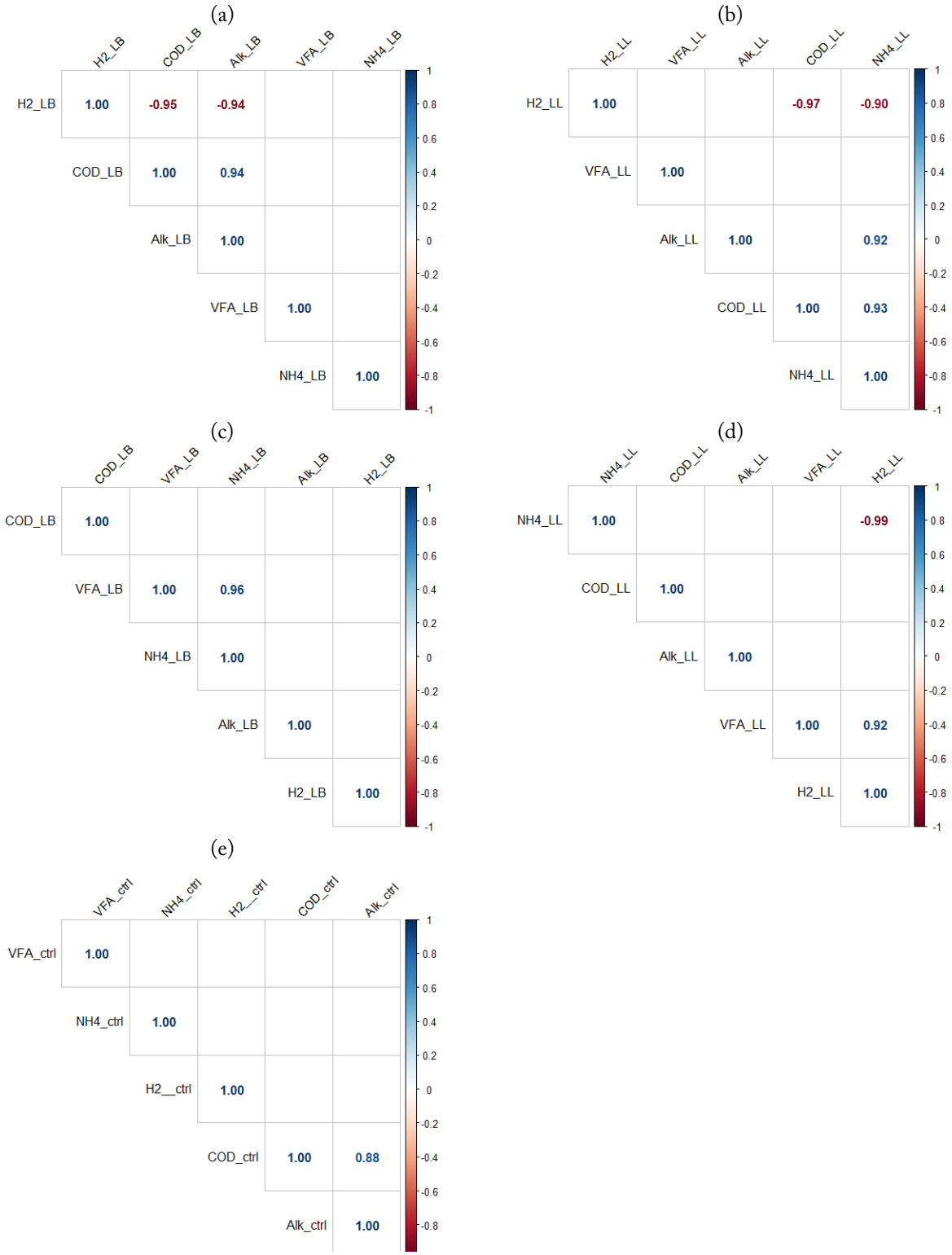
Figure 6g (control) and 6h (*C. acetobutylicum* spike) display ammonium concentrations in both reactors. The ammonium concentrations from the control reactors were varied more than the spike reactors. Ammonia/ammonium toxication was not observed in our study.

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 H_2 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 H_2

content only in the *L. brevis* spike reactor (r = -0.94), and ammonium had a negative relationship with the H_2 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 H_2 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

H_2 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 H_2 formation. In addition, there was no substantive difference in H_2 content measured among control and spike reactors, which suggests there were enough H_2-producing microorganisms in the digested sludge seeds. However, the H_2 contents that decreased after days 2, 4, or 6, varied by reactor types. 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. 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 H_2 content as soon as day 2. After two days, H_2 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 H_2 recovery and prohibit methanogens from taking up H_2 for methane synthesis in the food waste anaerobic digesters.

Bibliography

American Public Health Association/American Water Works Association/Water Environment Federation. (2017). Standard methods for the examination of water and wastewater, 23rd edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, D.C.

Appels, L., Lauwers, J., Degrève, J., Helsen, L., Lievens, B., Willems, K., Van Impe, J., Dewil, R. (2011). Anaerobic digestion in global bio-energy production: Potential and research challenges. *Renewable & Sustainable Energy Reviews*, *15*(9), 4295–4301. https://doi.org/10.1016/j.rser.2011.07.121

Arán Carrión, J., Espín Estrella, A., Aznar Dols, F., Zamorano Toro, M., Rodríguez, M., Ramos Ridao, A. (2008). Environmental decision-support systems for evaluating the carrying capacity of land areas: Optimal site selection for grid-connected photovoltaic power plants. *Renewable & Sustainable Energy Reviews*, *12*(9), 2358–2380. https://doi.org/10.1016/j.rser.2007.06.011.

Berry, G. D., Pasternak, A. D., Rambach, G. D., Ray Smith, J., Schock, R. N. (1996). Hydrogen as a future transportation fuel. *Energy (Oxford)*, *21*(4), 289–303. https://doi.org/10.1016/0360-5442(95)00104-2

Bitton, G. (2011). Wastewater Microbiology, Wiley, New Jersey, US.

California Air Resources Board (2018). AB 32 Global warming solutions Act of 2006. Retrieved November 27, 2020, from https://ww2.arb.ca.gov/resources/fact-sheets/ab-32-global-warming-solutions-act-2006

Das, Khanna, N., Veziroğlu, N. (2008). Recent developments in biological hydrogen production processes. *Chemical Industry and Chemical Engineering Quarterly*, *14*(2), 57–67. https://doi.org/10.2298/CICEQ0802057D

Energy Innovation Policy & Technology LLC (2022). California Climate Policy: California: a global climate leader. Retrieved November 27, 2020, from https://energyinnovation.org/policy-programs/california-climate-policy/

Emerson, C.W. (2008). Hydrogen energy: a study of the use of anaerobic digester gas to generate electricity utilizing stand-alone hydrogen fuel cells at wastewater treatment plants. Rochester Institute of Technology, N.Y.

Fisgativa, H., Tremier, A., Le Roux, S., Bureau, C., Dabert, P. (2017). Understanding the anaerobic biodegradability of food waste: Relationship between the typological, biochemical and microbial characteristics. *Journal of Environmental Management*, *188*, 95–107. https://doi.org/10.1016/j.jenvman.2016.11.058

Food Waste Reduction Alliance (2016) Analysis of U.S. Food Waste Among Manufacturers, Retailers, and Restaurants. Retrieved July 10, 2020, from http://www.foodwastealliance.org/wp-content/uploads/2013/05/FWRA-Food-Waste-Survey-2016-Report_Final.pdf

Hosseini, S. E. & Wahid, M. A. (2016). Hydrogen production from renewable and sustainable energy resources: Promising green energy carrier for clean development. *Renewable & Sustainable Energy Reviews*, *57*, 850–866. https://doi.org/10.1016/j.rser.2015.12.112

Huang, Z., Gedalanga, P. B., Asvapathanagul, P., Olson, B. H. (2010). Influence of physicochemical and operational parameters on *Nitrobacter* and *Nitrospira* communities in an aerobic activated sludge bioreactor. *Water Research (Oxford)*, *44*(15), 4351–4358. https://doi.org/10.1016/j.watres.2010.05.037

Hung, C. H., Chang, Y. T., Chang, Y.J. (2011). Roles of microorganisms other than Clostridium and Enterobacter in anaerobic fermentative biohydrogen production systems – A review. *Bioresource Technology*, *102*(18), 8437–8444. https://doi.org/10.1016/j.biortech.2011.02.084

Kalghatgi Levinsky, H., Colket, M. (2018). Future transportation fuels. *Progress in Energy and Combustion Science*, *69*, 103–105. https://doi.org/10.1016/j.pecs.2018.06.003

Markets & Markets (2020). Hydrogen generation market by generation and technology, forecast to 2023 | COVID-19 impact analysis | MarketsandMarkets.

Momirlan, M., Veziroglu, T. (2005). The properties of hydrogen as fuel tomorrow in sustainable energy system for a cleaner planet. *International Journal of Hydrogen Energy*, *30*(7), 795–802. https://doi.org/10.1016/j.ijhydene.2004.10.011

Monteiro Lunardi, M., Alvarez-Gaitan, J. P., Bilbao, J. I., Corkish, R. (2018). A review of recycling processes for photovoltaic modules. In B. Zaidi (Ed.), *Solar Panel and Photovoltaic Materials* (pp. 10). intechOpen. London.

"NIST Chemistry Webook". Webbook.nist.gov.

Parthiba, O. P., Karthikeyan, Trably, E., Mehariya, S., Bernet, N., Wong, J. W. C., Carrere, H. (2018). Pretreatment of food waste for methane and hydrogen recovery: A review. *Bioresource Technology*, *249*, 1025–1039. https://doi.org/10.1016/j.biortech.2017.09.105

Posmanik, R., Labatut, R. A., Kim, A. H., Usack, J. G., Tester, J. W., Angenent, L. T. (2017). Coupling hydrothermal liquefaction and anaerobic digestion for energy valorization from model biomass feedstocks. *Bioresource Technology*, *233*, 134–143. https://doi.org/10.1016/j.biortech.2017.02.095

R Core Team. (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

RStudio Team. (2015). RStudio: Integrated Development Environment for R, Boston.

Salemdeeb, R., Vivanco, D. F., Al-Tabbaa, A., zu Ermgassen, E. K. H. J. (2017). A holistic approach to the environmental evaluation of food waste prevention. *Waste Management (Elmsford)*, *59*, 442–450. https://doi.org/10.1016/j.wasman.2016.09.042

S.B. 697, 2021 as amended, Hueso. Cap-and-Trade Program: Green Hydrogen Credit, 2021 Legislative Counsel's Digest. (CA. 2021). Bill Text - SB-697 Cap-and-Trade Program: Green Hydrogen Credit Program.

Slopiecka, K., Liberti, F., Massoli, S., Bartocci, P., Fantozzi, F. (2022) Chemical and physical characterization of food waste to improve its use in anaerobic digestion plants. *Energy Nexus*, 5, 100049–, https://doi.org/10.1016/j.nexus.2022.100049.

USDA. (2015). U.S. Food Waste Challenges. Retrieved July 12, 2020, from https://www.usda.gov/oce/foodwaste/faqs.htm

US EPA. Overview of the Clean Air Act and Air pollution: Clean Air Act Text. Retrieved November 27, 2020, from: https://www.epa.gov/clean-air-act-overview/clean-air-act-text

USEPA. (2019). Sustainable Management of Food: United States 2030 Food Loss and Waste Reduction Goal. Retrieved July 12, 2020, from https://www.epa.gov/sustainable-management-food/united-states-2030-food-loss-and-waste-reduction-goal#:~:text=The%202030%20FLW%20reduction%20goal%20aims%20to%20reduce%20food%20waste,to%20109.4%20pounds%20per%20person

Yu, Z. T., Mohn, W. W. (1999). Killing two birds with one stone: simultaneous extraction of DNA and RNA from activated sludge biomass. *Canadian Journal of Microbiology*, *45*(3), 269–272. https://doi.org/10.1139/cjm-45-3-269

About the Authors

Pitiporn Asvapathanagul, Ph.D., P.E.

Dr. Pitiporn Asvapathanagul is an Associate Professor of Environmental Engineering in the Department of Civil Engineering and Construction Engineering Management at California State University, Long Beach. Her research interests are molecular biology for enhancing the understanding of biological wastewater treatment processes, including nitrification, denitrification, bulking, foaming, anaerobic digesters, and aeration diffuser biofilms as well as microbial source tracking, biological hydrogen gas production, biohealing of construction materials, biocementation, and chemical micropollutants coupled with microplastics removal in water and wastewater.

Leanne Deocampo

Leanne Deocampo is currently a third-year student at California State University, Long Beach working towards a B.S. in Civil Engineering. She aims to specialize in Environmental Engineering and has been assisting Dr. Pitiporn Asvapathanagul with her project titled, "Microbial populations shift during mesophilic and thermophilic anaerobic digestion" since Spring 2021. She is interested in researching the viability of alternative energy sources to improve the environment. Leanne also has experience in construction and works with Kiewit as a Field/Office Engineer Intern.

Nicholas Banuelos

Nicholas Banuelos is an undergraduate student at California State University, Long Beach studying Environmental Science and minoring in Biology. Nicholas' research interests involve creating cleaner, more efficient wastewater treatments to combat microplastics for a healthier and more sustainable environment in developing communities, but also for wildlife. This ties into his other interests in coastal ecology, studying ways to sustain marine life in drastically changing environments due to climate change. Nicholas has contributed to research involving the biohealing of construction materials, biocementation, and chemical micropollutants (pollutants of emerging concern) coupled with microplastics removal in water and wastewater.

MTI FOUNDER

Hon. Norman Y. Mineta

MTI BOARD OF TRUSTEES

Founder, Honorable Norman Mineta*
Secretary (ret.),
US Department of Transportation

Chair, Will Kempton
Retired Transportation Executive

Vice Chair, Jeff Morales
Managing Principal
InfraStrategies, LLC

Executive Director, Karen Philbrick, PhD*
Mineta Transportation InstituteSan José State University

Winsome Bowen
Transportation Executive

David Castagnetti
Co-Founder
Mehlman Castagnetti Rosen & Thomas

Maria Cino
Vice President, America & U.S. Government Relations
Hewlett-Packard Enterprise

Grace Crunican**
Owner
Crunican LLC

Donna DeMartino
Managing Director
Los Angeles-San Diego-San Luis Obispo Rail Corridor Agency

John Flaherty
Senior Fellow
Silicon Valley American Leadership Forum

Stephen J. Gardner *
President & CEO
Amtrak

Rose Guilbault
Board Member
Peninsula Corridor Joint Power Board

Kyle Holland
Senior Director, Special Projects, TAP Technologies, Los Angeles County Metropolitan Transportation Authority (LA Metro)

Ian Jefferies*
President & CEO
Association of American Railroads

Diane Woodend Jones Principal & Chair of Board
Lea & Elliott, Inc.

Steven Keck*
Acting Director
California Department of Transportation (Caltrans)

Therese McMillan
Executive Director
Metropolitan Transportation Commission (MTC)

Abbas Mohaddes
President & COO
Econolite Group Inc.

Stephen Morrissey
Vice President – Regulatory and Policy
United Airlines

Dan Moshavi, PhD*
Dean
Lucas College and GraduateSchool of Business, San José State University

Toks Omishakin*
Secretary
California State Transportation Agency (CALSTA)

Takayoshi Oshima
Chairman & CEO
Allied Telesis, Inc.

Greg Regan
President
Transportation Trades Department, AFL-CIO

Paul Skoutelas*
President & CEO
American Public Transportation Association (APTA)

Kimberly Slaughter
CEO
Systra USA

Beverley Swaim-Staley
President
Union Station Redevelopment Corporation

Jim Tymon*
Executive Director
American Association of State Highway and Transportation Officials (AASHTO)

* = Ex-Officio
** = Past Chair, Board of Trustees

Directors

Karen Philbrick, PhD
Executive Director

Hilary Nixon, PhD
Deputy Executive Director

Asha Weinstein Agrawal, PhD
Education Director
National Transportation Finance Center Director

Brian Michael Jenkins
National Transportation Security Center Director