2023년 8월

석사학위논문

Biohydrogen Production from a Mixture of Swine Wastewater and Food Waste Leachate by Bioelectrochemical Acid Fermentation

조선대학교 대학원

환경공학과

전 휘 서

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생물전기화학적 산발효를 통한 양돈폐수 및 음폐수 탈리액의 혼합액으로부터 바이오수소 생산

2023년 8월 25일

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이 논문을 공학 석사학위신청 논문으로 제출함

2023년 4월

조선대학교 대학원

환경공학과

전 휘 서

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Contents

List of tables

List of figures

Abstract

Biohydrogen Production from a Mixture of Swine Wastewater and Food Waste Leachate by Bioelectrochemical Acid Fermentation

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바이오 가스 생산의 중요성이 커짐에 따라 최근 유기성 폐기물 처리와 동시에 바이오가스 생산이 가능한 혐기성 소화 기술이 주목을 받고 있으며 이에 관한 연구 또한 지속되어지고 있다. 실증 혐기성 소화 시설은 산발효조와 메탄발효조가 분리되어 운영되는 이상형 혐기성소화(Two-phased anaerobic digestion) 시설이 대다수이다. 대부분 연구들은 최종 산물인 메탄발효조의 효율적인 운영과 동시에 메탄생성균의 활성화에 대한 연구와, 전단의 산발효조는 율속단계로서의 연구만 진행되고 물질의 대사를 통해 중간 과정에서 발생하는 수소에 대한 연구는 소수의 연구만 진행되어지고 있다.

본 연구에서는 양돈폐수와 음폐수 탈리액을 기질로 사용하여 산발효조 슬러지 접종을 통해 고부가물질인 바이오 수소생산 연구를 진행하였으며, 고효율의 수소생산을 위해 생물전기화학 기술 중 하나인 미생물 전기분해 (microbieal electrolysis cell, MEC) 기술과 융합한 생물전기화학 혐기성소화 시스템(Hydrogen productin-Bioelectrochemical anaerobic digestion system, HP-BEAD)을 구동시킨 연구를 진행하였다.

실험은 3단계로 나누어 진행하였으며 유기성 폐기물을 이용한 바이소수소 생산 이후 생물전기화학 시스템을 도입한 바이오수소 생산, 마지막으로 최적의 조건들을 도입한 연속식 HP-BEAD 시스템을 이용한 수소 생산 순으로 진행하였다.

첫 단계에서 유기성 폐기물 양돈폐수와 음폐수 탈리액의 혼합 비율별 수소 생산 비교 분석 결과, 양돈폐수와 음폐수 비율 7:3, 5:5에서 높은 수소 생산성을 확인하였으며 이때의 초기 pH 5.5-6.1와 초기 COD/N 비율 50-56의 중요성을 확인하였다. 이에 따라 양돈폐수와 음폐수 탈리액으로부터 각기 단일기질의 초기 pH를 조

절하여(pH 7.0, 6.0, 5.5, 5.0, 4.5) 수소 생산성을 확인해보았다. 음폐수 탈리액으로부터는 초기 24시간 내에 pH의 급격한 하락과 동시에 가스생산을 확인할 수 없었으나, 양돈폐수로부터는 pH 6에서 24시간 이내로 급격한 수소 생산 속도 증가와 함께 48시간에서 66.52±1.82mL 발생량을 기록하였고 이후 pH 5.5에서 79.91±1.67mL의 발생량을 확인하였다. 수소 발생량은 pH 5.5 조건에서 최대치를 기록하였으며 수소수율과, 단위 체적당 수소 발생 속도 비교 결과는 pH 6의 조건에서 우세함을 확인하였다.

두 번째 단계로서 수소생산을 위한 생물전기화학 시스템(HP-BEAD) 운전을 위해 COD/N비율과 초기 pH의 중요성을 바탕으로 양돈폐수 음폐수 탈리액 혼합액으로부터 최적의 인가전압 조건과, 최적의 전극 전처리 방법을 탐색하였다.

HP-BEAD를 적용한 다양 전압 범위 별 운전 결과 0.3V에서 단위 체적 당 수소 생산 속도 368.0±68.1mL/L-d의 결과를 확보하였고 Gompertz 모델 적용 누적수소 생산량 695.28±12.3mL, 수소 수율 35.04±5.35mL/g·VS를 달성하였으며 이는 모든 조건들 중 최고효율을 보였다. 이외에 인가 전압이 높을수록(0.6V, 0.9V, 1.2V) 다소 느린 균체들의 지체기(lag phase) 양상을 확인하였고 높은 범위의 전압 인가에서 균체들의 활성을 저하와 동시에, 0.3V의 낮은 조건에서 수소생산의 증대를 확인할 수 있었다.

그 외에 전압 인가가 안된, 전극 삽입 유무에 따른 조건들에서 수소 생산 효율에 차이가 있었는데 이는 전극에 대한 미생물들의 활성 저하로 판단되어, 생체친화적인 전극 사용을 위한 다양한 전극 전처리 방법을 탐색해보았다. 결과적으로 5mM의 황산 전처리방법과 가열 전처리 방법은 단순 산발효조 반응기 대비 낮은 수소 생산량을 보여 미생물 균체들의 활성 저하를 일으켰다고 판단되었으며, pH 6으로 조절한 인산염완충용액(PBS) 방법과, 아세톤 전처리방법이 산발효조 반응기 대비 누적 수소생산량 약 43% 향상된 효율을 보여 전처리 방법으로서 적합함을 확인하였다.

최종적으로 대조군인 단순 혐기성 산발효 반응기와 PBS와 아세톤으로 전극 전처리를 진행한 0.3V의 전압이 인가된 HP-BEAD 반응기를 연속운전을 통해 수소 생산성을 비교 평가하였다. 유입 기질 pH 조건 5.8-6.0으로 연속식 운전을 진행해보았으며, 수리학적 체류시간 3일 운전 결과, 6일 만에 HP-BEAD시스템에서 단위 체적 당 수소생산율 723.75mL/L-d를 기록하였으며 이후 7일에서 단순 산발효 반응기는 509.52mL를 기록하였다. 이는 HP-BEAD 시스템이 다소 빠른 생산과 동시에

산발효 반응기 대비 42.04% 증가와, 수리학적 체류시간 2일 변경 후 HP-BEAD 반응기에서 산발효 반응기 대비 63.15% 증가된 발생량을 확인하였으며, 약 15일간 연속적으로 수소 발생을 확인하였다.

결론적으로 수소 생산을 위한 최적 조건(초기 pH, 기질의 COD/N비)을 만족시켜 운전한 결과 0.3V인가 생물전기화학 시스템 (HP-BEAD)에서 단순 산발효조 대비 높은 수소생산을 확인하였으며, 이를 통해 바이오 수소 생산을 위한 산발효조 공정 도입 시, 단순 혐기성 산발효 공정 대비 높은 효율의 수소 생산이 가능할 것으로 판단된다.

Chapter Ⅰ. Introduction

1.1 Background and Objective

As economic growth increases energy consumption, the importance of developing renewable energy sources increases. For decades, energy systems typically in the industrial, transportation, housing, and heating (Arsad et al., 2022) have been heavily dependent on petroleum and coal, which have limited stores and are rapidly depleting. Additionally, combustion of fossil fuels release deleterious gases, including carbon dioxide (CO_2), carbon monoxide (CO), oxides of sulfur (SO_x), oxides of nitrogen (NO_x), and smoke in to atmosphere. These gases are major causes of environmental deterioration, global warming and climate change through greenhouse gases production (Singh et al., 2022). According to the Paris Agreement, the increase in global temperatures should not exceed 1.5°C above pre-industrial levels. It was ratified by 196 countries in 2015. To address these issues, we need discussing on reducing fossil fuels dependence when and energy consumption by transitioning from fossil fuels to energy.

Various renewable energy sources including such as solar, wind, biodiesel, geothermal, and hydrogen technology are known (Brar et al., 2022). Among them, hydrogen is particularly important energy source. As the Korean government announced a roadmap for revitalizing the hydrogen economy, highlighting the promote producing of hydrogen as a fossil fuel alternative. It has an advantage of high energy yield of 122 kJ/g (Singh & Wahid, 2015). Furthermore, hydrogen combustion does not release harmful air pollutants or GHG and only generates water as a clean byproduct.

Hydrogen can be generated through physical processes like the thermal dissociation of water, chemical processes such as reacting with water or oxygenated hydrocarbons, as well as through biological process is possible by utilizing microorganisms. The noted process is Biological hydrogen, transforms organic materials to hydrogen under standard pressure and temperature conditions. By employing organic waste or wastewater as substrates, it not only produces energy but also lessens certain negative effects of

pollution (Chu et al., 2013; Das & Veziroğlu, 2001). Biohydrogen can be produced through photosynthesis, fermentation, and microbial electrolysis cells (MECs) techniques, which are derived from carbon-neutral and renewable resources (Lee et al., 2010).

Moreover, it is generated through renewable and carbon-neutral means. For biohydrogen production, numerous techniques are available, including photocatalysis, photo-fermentation, dark fermentation, and microbial electrolysis cells. Dark fermentation, a technology that does not require an external light source, has been steadily researched, and recently, MECs, a modification of microbial fuel cells (MFCs) capable of current production from wastewater, have gained attention for hydrogen production.

MECs is an advanced energy-producing wastewater treatment system that simultaneously removes organic matter and generates biogas. In the oxidation reaction, electron-releasing microorganisms breakdown organic matter, releasing electrons onto the electrode surface of the anode. The liberated electrons in the reduction process combine with cations in the cathode to create hydrogen gas. Although this reaction requires external power supply owing to its non-spontaneous nature, it exhibits high energy efficiency, reaching up to 400%. Undoubtedly, it is a crucially important technology for both renewable energy production and wastewater treatment.

In line with these developments, in South Korea, there is a growing emphasis on the energy conversion of organic waste from composting and feed production to energy resources. The development of biological treatment methods for organic waste is also becoming more crucial as interest in the biological generation of biogas under anaerobic circumstances grows.

Consequently, the purpose of this study aims to evaluate the influencing factors of hydrogen production and propose a new strategy for energy conversion through the development of the microbial electrochemical process, for converting organic waste into hydrogen, which has a high added value.

Chapter Ⅱ. Literature Survey

1. Organic Waste

1.1 Organic Waste

Organic matter is significantly important for soil, agriculture, and source of energy production (Chavan et al., 2022) and it can also serve as an indicator of water pollution. Organic waste is mainly waste from the life cycles of humans, plants and animals. However, improper organic waste management causes numerous problems, such as environmental contamination, eutrophication, aesthetic destruction to urban landscapes, release of greenhouse gases, and adverse health effects. Organic waste includes livestock waste, food waste, sewage sludge, animal and plant residues, and others. High concentrations of organic wastewater, such as livestock and food wastewater, emit stink and toxic gases such as methane, sulfur oxides, and ammonia. In addition, discharging organic wastewater into water systems can result in high biological oxygen demand (BOD) levels. In South Korea, most organic waste (sewage sludge, food and livestock wastewater, and others) used to be openly dumped into oceans. However, since 2012, abandoning organic waste dumping in the ocean has been prohibited as part of compliance with international agreements by the London Convention (이헌모& 양병수, 1993). Organic waste has the potential to be recycled as a source of energy and has several applications, such as soil amendment and fertilizer, energy recovery (heat, electricity, biogas, or liquid fuels), and the production of various chemicals (volatile organic acid, the ammonium products, and alcohols) (Westerman & Bicudo, 2005). Additionally, due to their biochemical properties, they can be biodegraded naturally by microorganisms, which allows decomposing of natural origin wastes (Ashokkumar et al., 2022). Only organic waste conversion into renewable bioenergy will culminate in zero-waste disposal (Ashokkumar et al., 2022; Dhanya et al., 2020). Usually, biodegradable organic waste can be processed with or without air access, compositing is

an aerobic process, and digesting is an anaerobic process to produce biogas (Westerman & Bicudo, 2005). Therefore, it is important to explore environmentally sustainable methods for organic waste (Das & Veziroğlu, 2001).

(1) Generation of Organic Waste and Utilization Status

Figure 2-1, as shown the of organic waste generation in South Korea, which amount of to 67.21 million tons per year in 2019 (환경부, 2022b), representing a 14% increase from the level in 2010. It has increased by 18.6 million tons in the last ten years and will probably increase in the future. The most common waste generated by year was livestock waste, accounting for approximately 82.3-84.5% of the total organic waste generated. which is about 10 times greater than the amount of food waste or sewage waste generated. As shown in table 2-1, various methods are currently used in Korea for treating and utilizing organic waste, such as feed conversion, composting, purification, and biogas production in 2019 (환경부, 2022b). The most utilized process was composting about 75.5% of the total in 2019, while biogas production accounted for only 5.7%. In particular, almost 86.1% of livestock waste was treated by composting. Food waste was also the most common composting method about 38.1% , followed by feed conversion with similar percentage (36.2%). However, composting has limitations as it can generate methane and nitrous oxide, which can contribute to soil and water pollution if over-sprayed as nitrogen fertilizer (강성수, 2023). In addition, issues such as declining demand and illegal dumping are driving the South Korean government to shift organic waste treatment from composting to energy production. In 2023, the government introduced a new law called ‘Biogasification law’ to promote the production and utilization of biogas from organic waste resources and increase the number of biogas plants.

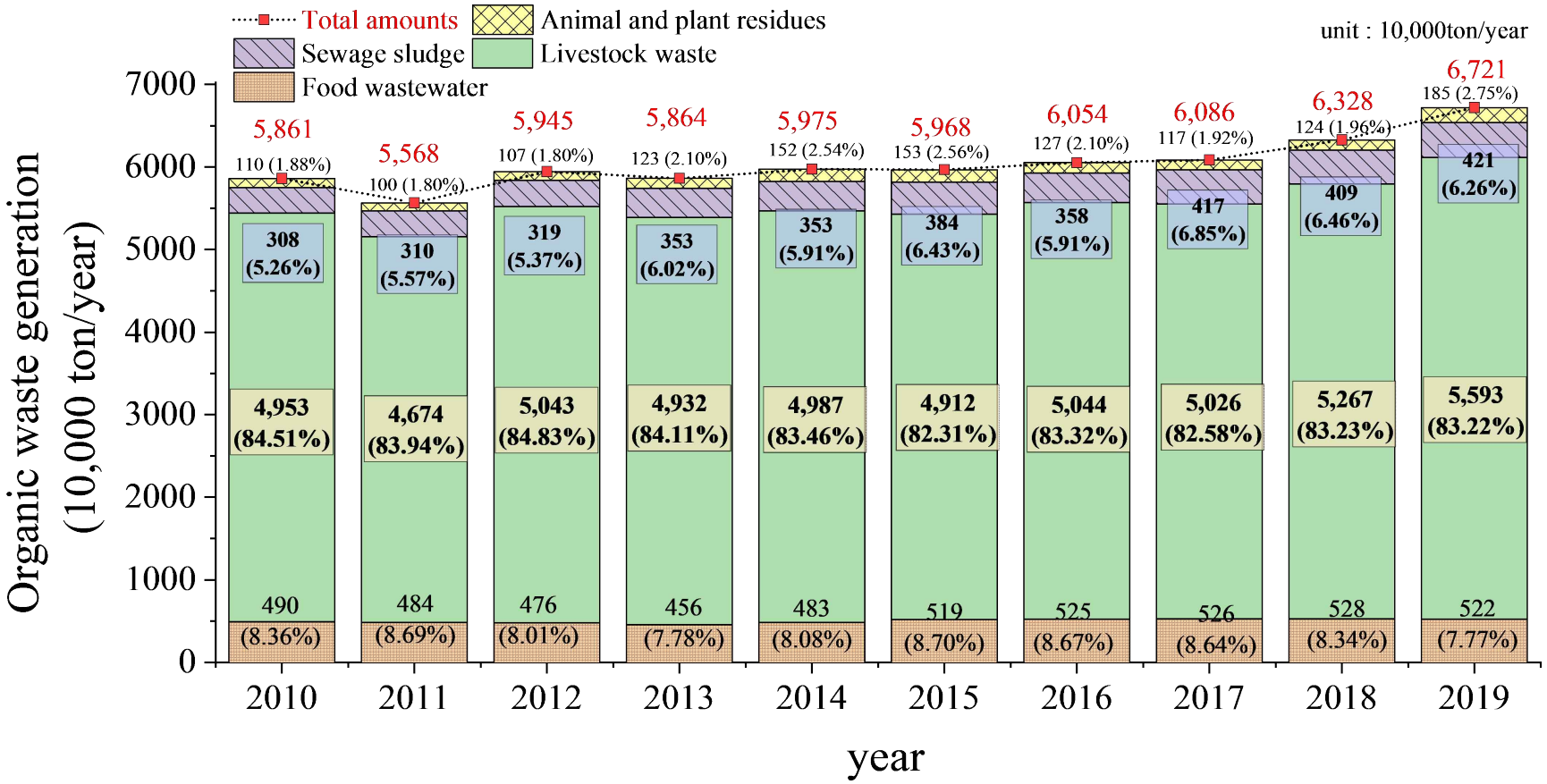


Figure 2-1. Organic waste generation in Korea (환경부 2022b).

Table 2-1. Status of organic waste treatment and utilization in Korea in 2019 (환경부 2022b).

[unit:10,000ton/year, %]

	Total	Feed conversion	Composting	Purification	Biogas	Other (incineration)
Total	6537 (100)	189 (2.9)	5015 (75.7)	680 (10.4)	375 (5.7)	278 (4.3)
Food waste	522 (100)	189 (36.2)	199 (38.1)	-	65 (12.5)	69 (13.2)
Livestock waste	5593 (100)	-	4816 (86.1)	680 (12.2)	92 (1.6)	5 (0.1)
Sewage sludge	422 (100)	-	-	-	218 (51.7)	204 (48.3)

1.2 Status of Biogas Facilities for Organic Waste

The relevance of ecologically friendly organic waste usage is rising with the passage of the Korean Biogas Production Promotion Act in 2022. According to the Ministry of Legislation, biogas refers to gas (excluding landfill gas) generated by converting organic matter into renewable energy and a biogas production facility that produces biogas using organic waste. The relevance of ecologically friendly organic waste usage is rising with the passage of the Korean Biogas Production Promotion Act in 2022. According to the Ministry of Legislation, biogas refers to gas (excluding landfill gas) generated by converting organic matter into renewable energy, and a biogas production facility that produces biogas using organic waste (국가법령정보센터, 2022).

The expansion of bio-gasification facilities in Korea has been carried out in order to utilize high-calorific organic waste as a resource and recycle it into energy. Table 2-2 illustrates that, in 2018, the number of livestock waste and sewage sludge facilities decreased, while the number of integrated facilities increased from 35 to 43. Currently, in 2021, there are about 110 bio-gasification facilities operating in Korea, including 25 for food waste or food wastewater, 3 for livestock waste, 28 for sewage sludge, and 4 for integrated organic waste (환경부, 2021).

Table 2-2. Status of organic waste bio-gasification facilities (환경부, 2021).

[unit: number]

Year	Total	Food waste·Food wastewater	Livestock waste	Sewage sludge	Integration	Etc.
2008	38	5	6	17	10	
2009	49	7	9	20	13	
2010	50	8	7	20	15	
2011	55	11	7	20	17	
2012	57	11	7	20	19	
2013	61	16	7	20	1	
2014	71	20	6	21	24	
2015	88	20	6	32	30	
2016	90	20	7	33	30	
2017	98	21	7	35	35	
2018	100	21	4	32	43	
2019	101	21	4	32	44	
2020	110	26	5	33	46	
2021	110	25	3	28	53	

2. Anaerobic Digestion

2.1 Fundamental of Anaerobic Digestion

Nowadays, the escalation in fossil fuels consumption has caused in a serious energy crisis and environmental challenges, there is an immediate need to develop and improve sustainable alternative renewable energy systems in order to address environmental concerns and the energy crisis (Wang et al., 2023). Anaerobic digestion (AD) is a promising sustainable biotechnology that can produce biogas through the decomposing organic waste in the absence of oxygen using anaerobic microorganisms.

AD has been widely employed in the processing of organic wastes, including agricultural wastes, animal excrements, sewage sludge, and urban wastes, with millions of anaerobic digesters erected and established across the world for this purpose (de Lemos Chernicharo, 2007). With the design septic tank system by Jean-Louis Mouras in 1870, the idea of AD was first proposed (Grando et al., 2017). For the past 100 years, it has been extensively utilized for treating industrial, municipal, and agricultural wastes since it is one of the most efficient waste treatment methods (Kadam et al., 2022). In particular, in Europe, numerous AD plants producing biogas are being operated due to strengthened organic waste treatment policies. The USA had around 174-240 large-scale AD plants, more over Europe supported over 244 such facilities for the treatment of organic waste (Linville et al., 2015; Zappi et al., 2021) AD plants in size from hundreds to thousands of m^3 and are mainly operated in developed countries. Through the AD process, CO_2 and CH_4 gas are produced as the final products. The gas contains about 60% methane and 40% carbon dioxide (Wilkie, 2005), which can be used as an alternative energy source to fossil fuels for heat and electricity in houses and industries. Facilities for producing biogas may use manure, municipal solid waste, food waste, sewage, effluent, and other industrial or agricultural waste with a high organic content. When on substance is mixed with another, it might require to go through a pretreatment step or be co-digested to increase process effectiveness and synergistic effects (Grando et al., 2017).

Aerobic methods, which are commonly employed to treat wastewater, involve biological treatment. Nevertheless, their energy demands are high, making them unsustainable. There are have been found to be ineffective in degrading more than 50% of the waste, whereas anaerobic methods have demonstrated a high level of activity in stabilizing up to 80-90% of the biodegradable portion of the waste (Bella & Rao, 2021). AD has various advantages over aerobic biological treatments, including a higher rate of organic loading, lower nutrient need, less sludge generation, lower treatment cost, less energy usage, better stink control, recovery of energy, and a decrease in emissions of greenhouse gases (Deng et al., 2023).

The microorganisms present in AD can be categorized into four groups based on their distinct roles: fermentative bacteria, acidogenic bacteria, acetogenic bacteria, and methanogenic bacteria. Each group is responsible for specific functions such as hydrolysis, acidogenesis, and methanogenesis, respectively (Wang et al., 2023). It is vital to comprehend the associated biological processes in order to enhance the AD process, as the interaction of microorganisms in the AD pathway is predominantly mutual and syntrophic (Zhang et al., 2023). Figure 2-2 illustrated the metabolic pathway in AD systems, the following process. An overview of metabolic pathways, AD start with the hydrolysis of complex polymeric substance such as cellulose and protein. Then hydrolyzed products are then transformed to volatile fatty acids (VFAs) and H_2/CO_2 through acidogenesis and acetogenesis, followed by methane gas production from acetate or H_2/CO_2 through methanogenesis (McCarty, 1982; Sasaki et al., 2011; Zappi et al., 2021).

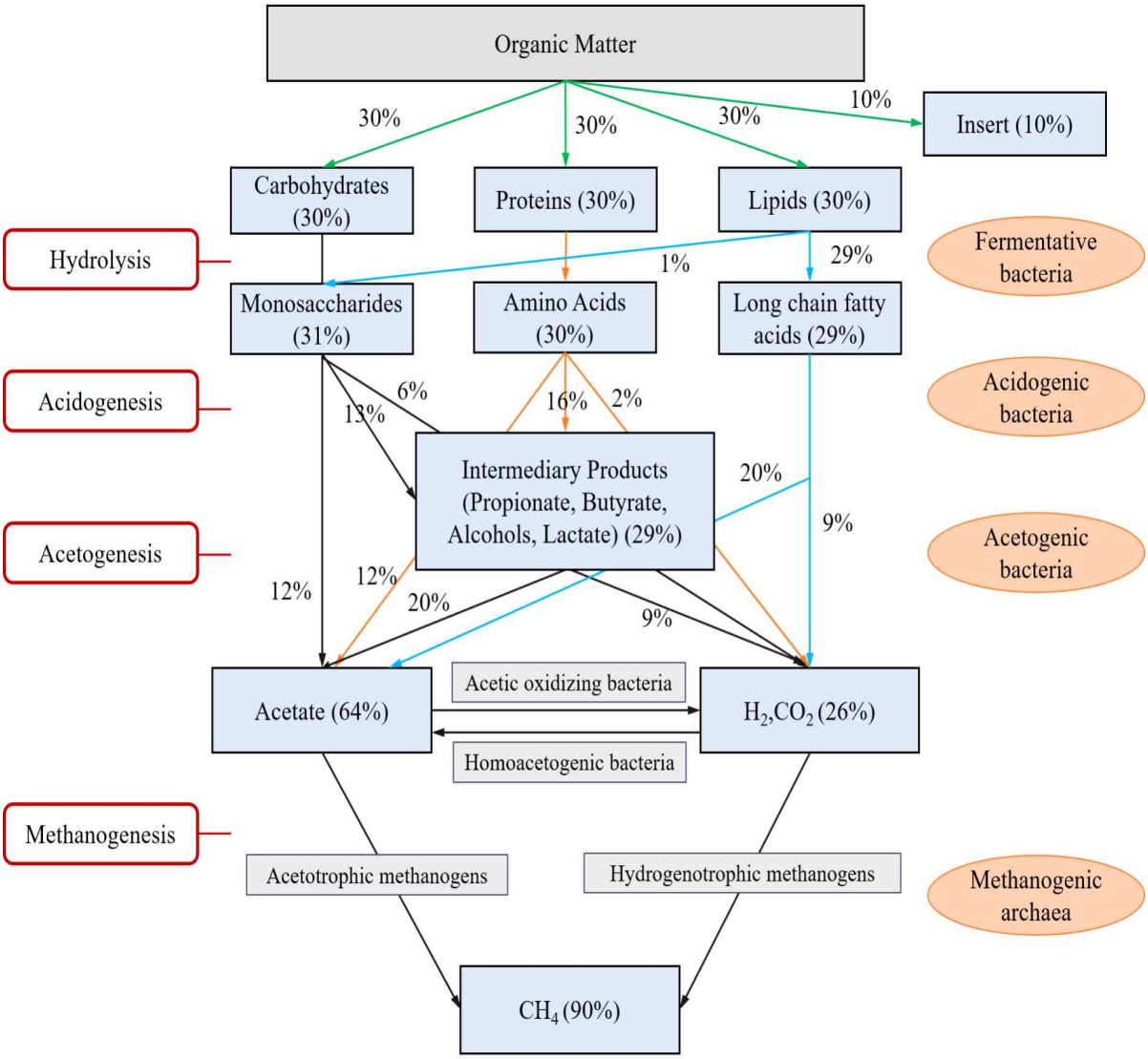


Figure 2-2. Metabolic pathway of anaerobic digestion (Li et al., 2019; Wang et al., 2018).

2.2 Two-Stage Anaerobic Digestion System

There are two main types of anaerobic digestion (AD) systems. One is a single-stage anaerobic digestion system (Single-AD) in which all four stages hydrolysis, acidogenesis, acetogenesis, and methanogenesis) occurred in one reactor. The one kind of AD system, known as a two-stage AD system, partitions the acidogenic fermenter from the methanogenic fermenter. In the first acidogenic fermenter, hydrolysis, acidogenesis, and acetogenesis take place enabling biohydrogen production. Then, connected with the secondary of methanogenic fermenter, the methanogenesis step takes place producing methane gas. Two-stage AD and Single-AD are shown in a simple diagram in Figure 2-3.

Chatterjee and Mazumder (2019) reported that single-stage systems are simple to manufacture and manage in terms of operational parameter monitoring and the potential for sharing of nutrients and other necessary growth components among all the viable bacterial populations participating in an AD process since all key stage functions in a Single-AD (Chatterjee & Mazumder, 2019). However, the Single-AD faces various challenges, including a low organic loading rate, long hydraulic retention time, and acidification, which leads to decreased efficiency in fermentation (Cremonez et al., 2021). Additionally, it is difficult to satisfy the stabilizing conditions of acidogenic bacteria and methanogen archaea in their entirety. Two-stage AD is possible to optimize the conditions of the reactor for different microorganisms by operating them independently since the hydrolysis/acidogenesis have significantly different growth characteristics. Two-stage AD is an effective process for producing sequential and directed substrate conversion at various phases, with rapid breakdown of practically all organic materials regardless of their source, as well as better product yield and quality (Cremonez et al., 2021). In addition, (Andrea Cristina Luongo Malave, 2015) studied that, using Two-stage AD was 24% more energy efficient than Single-AD when using glucose and 17% higher when using lignocellulose. Therefore, Two-stage AD is a suitable instrument for energy production from organic waste greater than Single-AD (Malave et al., 2015). According to the key function of H_2 in AD, the microbial

consortia employed to create acidogenic fermenter and methanogenic fermenter may be split into two primary groups: hydrogen-producing bacteria (HPB) and hydrogen-consuming bacteria (HCB) (Gómez Camacho et al., 2019; Malave et al., 2015).

As Figure 2-3. shows, a comparison between a Single-AD and Two-stage AD. Single-AD adjusts pH to about 6.5-7.5 and hydraulic retention time (HRT) is reported about 20~30 days (Srisowmeya et al., 2020). Two-stage AD system at the acidogenesis step is fast and operates well around pH 5.0 to pH 6.0, it is frequently operating at 2-4 days of low HRT (Srisowmeya et al., 2020; Zappi et al., 2021). And the methanogenesis step is operated at longer HRT, generally 8 to 10 days, which to encourage the growth of slow-growing methanogens. It is also more suited to pH 6.0-8.0 (Srisowmeya et al., 2020). While the acidogenic fermenter at Two-stage AD is under acidic conditions, an abrupt decrease in pH to values below pH 4 can compromise the process's efficacy. The effluent from the methanogenesis reactor (usually pH 6.0-8.0 conditions) an abrupt decrease in pH to values below pH 4 can compromise the process's efficacy. as a pH control (5.0-6.0) for the acid reactor. Also, methanogens cannot perform the conversion of hydrogen into methane when the pH level drops below 6.2 and it dies under a pH below 6.0 (Zappi et al., 2021).

Effective removal of total solids (TS) and volatile solids (VS) during the acidogenic stage increases methane yield during the methanogenic stage (Srisowmeya et al., 2020). Many researchers on the AD process have focused on increasing methane production rates in the methanogenic stage and improving biogas yield and removing organic matter as a step in stabilizing the process. However, research on the development and utilization of acidogenic fermenter that can produce hydrogen is currently inadequate.

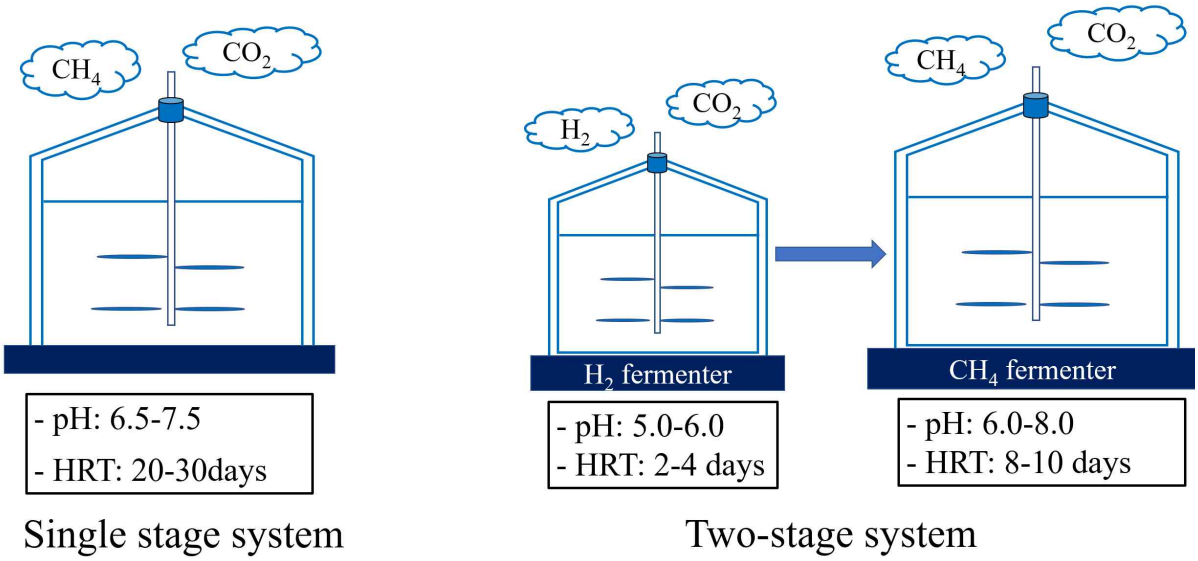


Figure 2-3. Comparison between a single-stage AD and two-stage AD system. Derived from Cremonez et al., 2021; Srisowmeya et al., 2020.

3. Biohydrogen

3.1 Biohydrogen

As humanity continues to industrialize, urbanize, and demand more energy, pollution of the environment has become one of the most significant environmental issues confro nting contemporary society. The Paris Agreement set the goals of limiting the increase in global average temperature to 1.5°C and attempting to keep it below 2°C above preindustrial levels (Arregi et al., 2018). There has been a lot of study into the creation of alternative energy sources as a result of the impending depletion of fossil fuels and growing environmental concerns.

Among them, hydrogen is regarded as an alternative energy source due to its high calorific value (140.4MJ/kg), which is 2.75 times higher than hydrocarbon fuels (Ramachandran & Menon, 1998). As a pure energy source, hydrogen does not produce hazardous byproducts (Aiken et al., 2019), and it is economical, storable, and renewable. In addition, hydrogen emits no greenhouse gases and does not contribute to air or water pollution (Park et al., 2020; Sillero et al., 2022).

Unfortunately, non-renewable sources that have a substantial carbon footprint are used to produce a sizable amount of the world's hydrogen supply. These sources include hydrocarbon steam reforming of natural gas (50%), petroleum refining (30%), and gasification of coal (20%) (Sahrin et al., 2022).

In order to achieve the goals set for the use of fossil fuels and the reduction of CO_2 emissions, it is necessary to create new sustainable processes that produce hydrogen from renewable sources, such as the thermochemical or biological process that utilizes biomass as a feedstock (Arregi et al., 2018).

One of these renewable and sustainable energy sources is biohydrogen, which is hydrogen created from biomass. It is considered more environmentally and uses less energy than physicochemical processes (Kim et al., 2011). Moreover, the two primary p

rocesses for producing biohydrogen from biomass-based feedstock are thermochemical and biochemical processes (Sahrin et al., 2022). The thermochemical process of biomass is comparable to the gasification or pyrolysis of fuels, but the biological process of biomass has recently received more scientific attention because of its energy-efficient and ecologically friendly features (Sahrin et al., 2022). In general, biological technologie s for producing hydrogen can be categorized; bio-photolysis of water involving algae and cyanobacteria, photo fermentation by photosynthetic bacteria (Mohan et al., 2007), and dark fermentation in anaerobic fermentation for producing hydrogen from organic compounds. A schematic diagram of the hydrogen production methods illustrated in Figure 2-4 (Tran & Kim, 2023). The generation of biohydrogen can be divided down into two categories: light-independent procedures, such as dark fermentation and the microbial electrolysis cell (MEC), and light-dependent processes, such as photo fermentation and bio-photolysis (Tran & Kim, 2023).

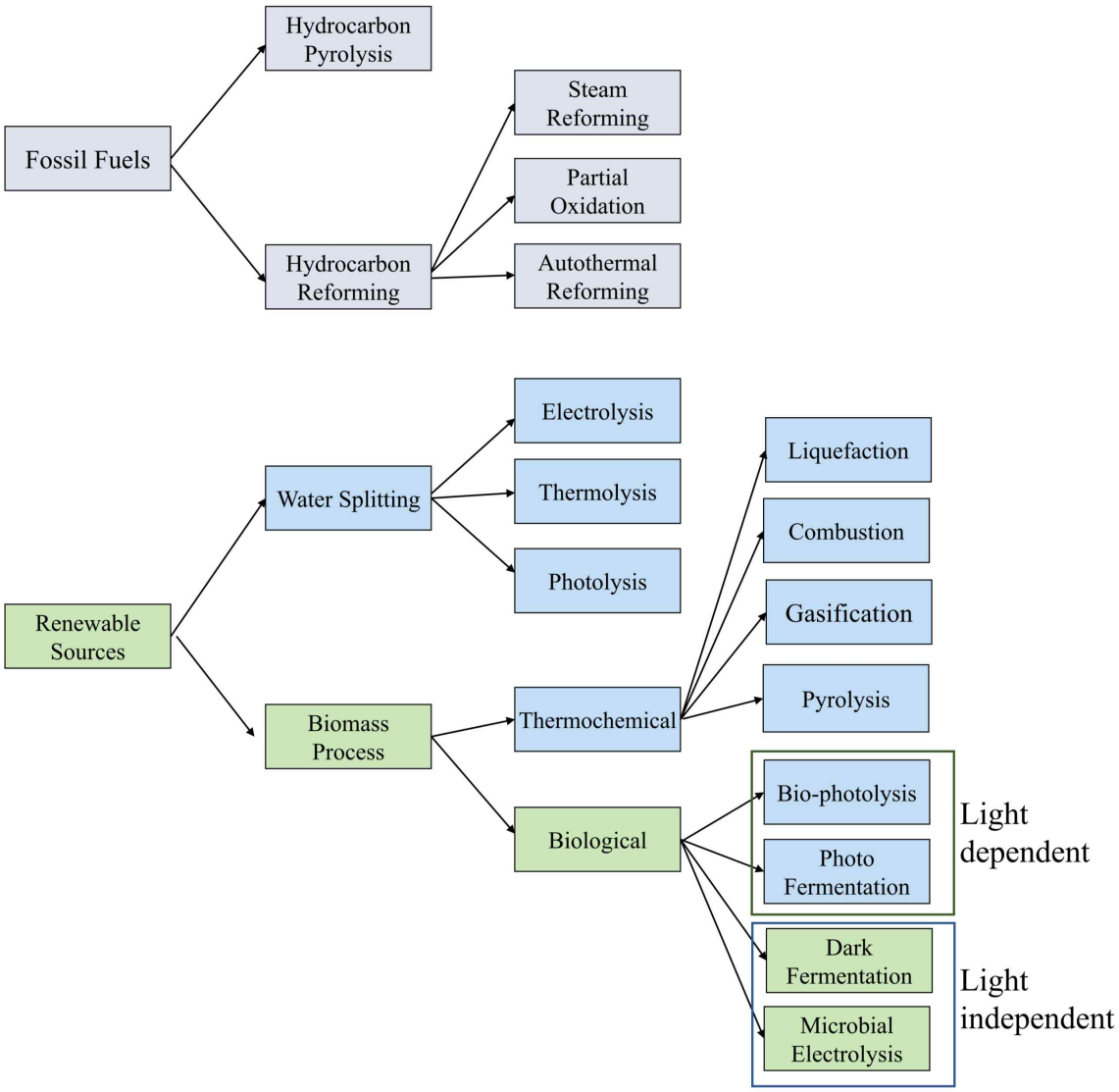


Table 2-4. Hydrogen production methods (Tran & Kim, 2023).

3.2 Dark-Fermentation

Dark fermentation (DF) is the anaerobic process of converting organic substrate (organic wastes) and biomass materials to biohydrogen when it is dark and anerobic (Azwar et al., 2014). It is one of the anaerobic fermentation processes. During the fermentation, microorganisms transform organic waste into hydrogen, compared to photo-fermentation, the most promising technique for producing hydrogen is DF. Its capacity to produce H_2 continuously even in the absence of light, its higher hydrogen production rate, its simplicity of procedure, its reduced energy input, and its usage of low-value waste as raw materials are some of its distinguishing characteristics (Azwar et al., 2014; Sarangi & Nanda, 2020). DF requires organic substrates, high-carbohydrate source materials such as sugar and cereal starch, organic residues from municipal waste, and wastewater from food and industrial effluents (Sarangi & Nanda, 2020). Biohydrogen is predominantly produced through the fermentative activity of bacteria, including *Escherichia* coli, facultative anaerobic *Enterobacter* species, and strictly anaerobic *Clostridium* species. These microorganisms are capable of generating biohydrogen under strictly anaerobic or facultative anaerobic conditions during DF (Mudhoo et al., 2011; Ntaikou et al., 2010).

Hydrogenases are the primary enzymes regulating the hydrogen metabolism. [Fe-Fe]-hydrogenase and [NiFe]-hydrogenase are two fundamental hydrogenases that are phylogenetically distinct and have distinct active sites. These enzymes catalyze reversible chemical reactions (Łukajtis et al., 2018). Moreover, [Fe-Fe]-hydrogenases are more efficient at producing molecular hydrogen than [NiFe]-hydrogenases, which predominantly catalyze molecular hydrogen oxidation and [Fe-Fe]-hydrogenases are usually sensitive to oxygen (Łukajtis et al., 2018).

Figure 2-5 shows the metabolic pathway for hydrogen production through DF glycolysis of glucose model (Łukajtis et al., 2018). Also reaction the theoretical maximal yield of biohydrogen from glucose fermentation is 4mol hydrogen per mol of consumed glucose present of equation (1.1) (Ntaikou et al., 2010).

$$C_6H_{12}O_6 + 4H_2O \rightarrow 2CO_3COO^- + 2HCO_3^- + 4H^+ + 4H_2 \quad \Delta G^0 = -206.3 kJmol^- \quad (1.1)$$

According to Ghimire et al. (2015), H_2-producing bacteria in Figure 2-7 use glycolic pathways to convert glucose to pyruvate, where it is then converted to adenosine triphosphate (ATP) using adenosine diphosphate (ADP) and the reduced form of nicotinamide adenine dinucleotide (NADH). Pyruvate is further oxidized by pyruvate ferredoxin oxidoreductase and hydrogenase into acetyl coenzyme A (acetyl-CoA), CO_2, and H_2. A variety of the microorganisms and conditions in the environment, pyruvate can be transformed to acetyl-CoA and formate, which can be converted into H_2 and CO_2. In the presence of [BiFe]-hydrogenases or [Fe-Fe]-hydrogenases (Łukajtis et al., 2018), formate can be transformed to H_2 and CO_2. Additionally, acetyl-CoA could be converted into acetate, butyrate, and ethanol (Figure 2-5) (Ghimire et al., 2015).

The hydrogen fermentation reaction involves the conversion of glucose into acetic acetate and butyrate, as present in equation (1.2) (1.3) respectively. Approximately 4 moles of hydrogen are generated during the production of acetate, and butyrate around 2 moles of hydrogen (Khanna & Das, 2013; Sarangi & Nanda, 2020). That indicated the ratio of butyrate to acetate can determine the glucose hydrogen ability (Sarangi & Nanda, 2020). In case of *Clostridium butyricum*, a species of *clostridia* that has been the subject of extensive research, butyric acid is produced as the predominant fermentation by product, along with acetate and hydrogen (Hawkes et al., 2007).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad \Delta G^0 = -206\, kJmol^- \quad (1.2)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad \Delta G^0 = -254\, kJmol^- \quad (1.3)$$

Kim et al., 2006, found that as mol H_2 mol^{-1} hexose was usded during the operation of sucrose, butyrate to acetate (B/A) ratios were excatly proportional to H_2 production. Butyrate to acetate ratios can determine the glucose hydrogen ability (Sarangi & Nanda, 2020). The butyrate pathway was more favorable for anaerobes from a thermodynamic standpoint due to its lower Gibbs free energy, despite its lower hydrogen yield

(2mol/mol) than the acetate path way (4mol/mol) (Zhang et al., 2021). When a B/A ratio 3:2 is generally found in DF with mixed cultures, and H_2 yield of 2.5 mol H_2 per pole of fermented hexose is produced, as shown by the equation (1.4) (Hawkes et al., 2007).

$$4C_6H_{12}O_6 + 2H_2O \rightarrow 3CH_3CH_2CH_2COOH + 2CH_3COOH + 8CO_2 + 10H_2 \quad (1.4)$$

Moreover, the hydrogen-consuming route that *Clostridium* spp. uses to produce propionate equation (1.5) (Ghimire et al., 2015). Similarly, metabolic pathway leading to the production of only ethanol and latic acid by *Clostridia barkeri* sp. do not produce hydrogen equation (1.6-1.7) (Ghimire et al., 2015).

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (1.5)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (1.6)$$

$$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + 2CO_2 \quad (1.7)$$

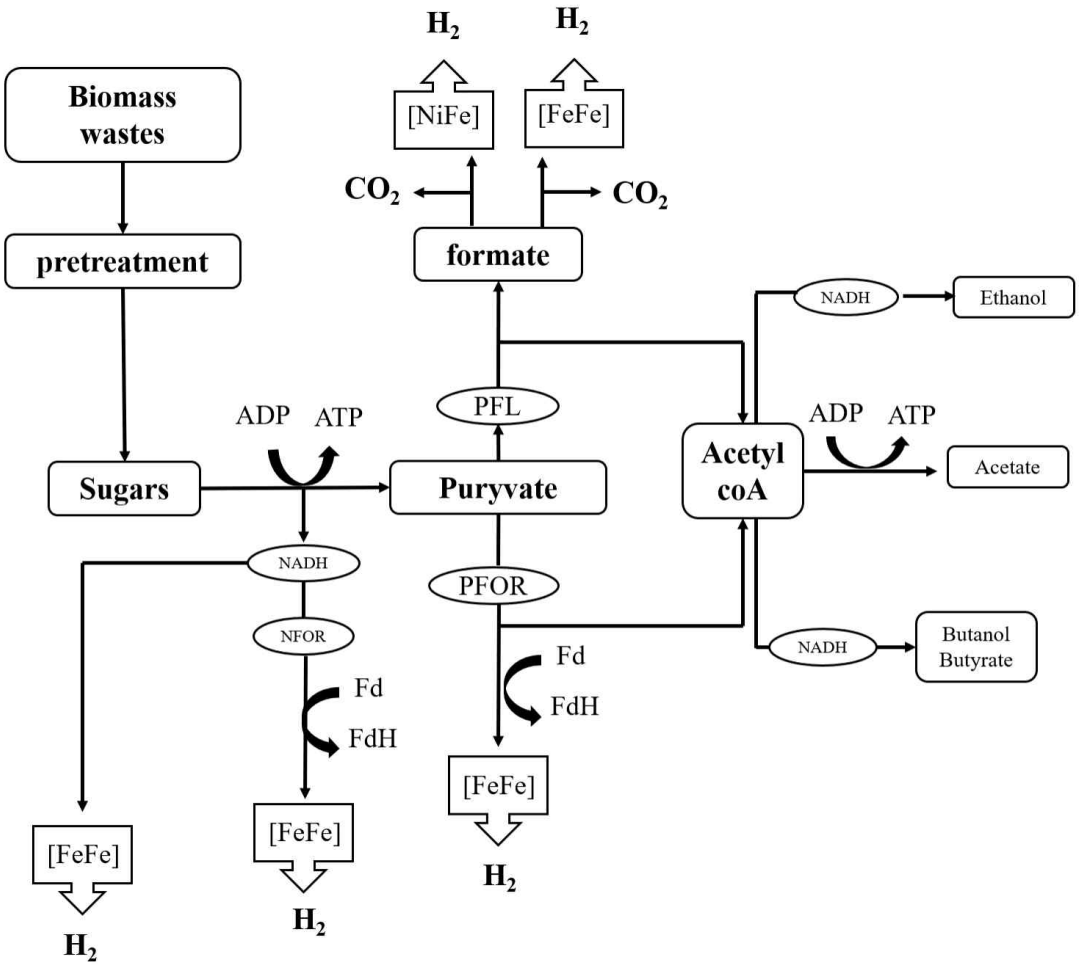


Figure 2-5. Metabolic pathways during dark fermentation (Ghimire et al., 2015).

4. Biohydrogen Production Parameter

4.1 Substrate

The substrate has a significant impact on the hydrogen yield, the efficacy of hydrogen generation, and the overall economics of the process. These are determined primarily by the number of carbohydrates present in the substrate, as well as their accessibility and rate of degradation (Ghimire et al., 2015). The synthesis of biohydrogen requires an abundant supply of renewable substrates, which may be supported by second-generation biomass source such as waste biomass, which are abounding (Ghimire et al., 2015; Show et al., 2011). Many organic wastes include complex particle structures like proteins and lipids in addition to carbohydrates. Food waste(Han & Shin, 2004), Kitchen waste (Jayalakshmi et al., 2009), Cheese whey (Castelló et al., 2009), Rice slurry (Fang et al., 2006), Liquid swine manure (Wu et al., 2009), Palm oil mill effluent (Vijayaraghavan & Ahmad, 2006), Fruit and vegetable waste (Ruggeri & Tommasi, 2012), etc., which are rich in carbohydrates, were used as substrates for hydrogen production. Due to the microflora's capacity to convert sucrose to hydrogen under these nutritional circumstances, a C/N ratio of around 47 permits a respectable quantity of hydrogen generation (Ruggeri et al., 2015).

4.2 Pretreatment of Inoculum

H_2-producing bacteria are widely distributed and can be found in various environments, including compost, wastewater sludge, and soil. Therefore, these materials can be utilized as inoculum for hydrogen fermentation. They can be sourced from diverse places such as cow dung, anaerobic sludge, municipal solid waste, soil, and compost (Ghimire et al., 2015). However, pretreatment is required to enhance selective biohydrogen production while inhibiting hydrogen-consuming microorganisms such as

methanogens and homoacetogens commonly present in mixed inoculum (Peiris et al., 2006).

Physical (heat shock, chilling, and aeration) and chemical (chloroform, acids, bases, sodium 2-bromoethane sulfate) pretreatment techniques are frequently used to obtain a viable bacterial inoculum for enhanced biohydrogen production (Ananthi et al., 2022). Heat shock is the most common pretreatment for hydrogen-producing microbes among these methods.

In anaerobic hydrogen fermentation, spore-forming bacteria, particularly *Clostridium* species (Lay et al., 2003) are the key microorganisms of choice. Heating the inoculum eliminates methanogens and other non-spore producing, hydrogen-consuming bacteria, while promoting the spore production of *Clostridia* through changes in germination receptors (Goud et al., 2014). The literature reports varying control temperatures for heat-shock treatments, ranging from 90 to 121°C, with exposure periods between 20 and 120 minutes (Luo et al., 2010). The primary differences among hydrogen producing bacteria and hydrogen consuming bacteria are that hydrogen consuming bacteria have a narrower pH range (around 7-8) than hydrogen producing bacteria (4.5-7.0). In addition, hydrogen producing bacteria withstand tough environmental circumstance due to the development of protective spores, whereas hydrogen consuming bacteria are extremely sensitive and lack this capacity (Ruggeri et al., 2015).

4.3 Temperature

In mixed cultures, temperature is frequently regarded as one of the most influential factors influencing both biohydrogen production yields and microbial metabolisms. Changes in temperature have a substantial effect on the rate of generation of hydrogen, substrate consumption, formation of metabolites including VFAs, and biohydrogen yield (Ananthi et al., 2022). The effect of a range of operational temperatures, including mesophilic (35℃), thermophilic (55℃), on biohydrogen production has been investigatedThe impact of mesophilic (35°C) and thermophilic (55°C) operational temperatures on biohydrogen production has been studied (Ghimire et al., 2015). Wongthanate et al., 2014, tested hydrogen productivity by temperature conditions using food and raw starch waste, hydrogen was highest achieved 0.28L/L under mesophilic temperatures. Valdez-Vazquez et al., 2005, test hydrogen production using organic portio n of municipal solid waste at lab scale, thermophilic reactor produced the greatest yields and percentages of hydrogen. Moreover, during thermophilic digestion, acetic acid predominant, whereas butyrate predominated during mesophilic digestion (Valdez-Vazquez et al., 2005). The optimal operational temperature varies depending on the proportion of readily biodegradable materials in the substrate and the type of inoculum used (Ghimire et al., 2015). *Clostridium* and *Enterobacter* sp. have the highest hydrogen production rate among mesophiles, whereas *Thermobacterium* sp. has the highest yield among thermophiles. The entropy of the system increases with temperature, which thermodynamically favors hydrogen generation, however, extremely thermophilic environments are not economically viable due to the amount of energy required to maintain the temperature (Usman et al., 2019).

4.4 pH

One of the environmental parameters that has been shown to have the greatest impact on metabolic pathways and the creation of biohydrogen is the system pH (Show et al., 2011). Altering the pH affects enzyme activity and may prefer alternate metabolic pathways over the original one. It is sometimes difficult to understand the metabolic pathways when there are more than two contemporaneous products in the medium (Ruggeri et al., 2015). One of the primary variables in the inhibition of methanogenic processes in mixed culture systems is acidic pH, which has an impact on the activity of the hydrogenase enzyme (Ghimire et al., 2015). As a result of hydrogen fermentation using sucrose and starch as a substrate in Khanal et al., 2004, the optimal pH range for biohydrogen was determined to be between 5.5-5.7, and it was discovered that the initial pH impacts both probability of hydrogen production and its rate (Khanal et al., 2004). Lay et al., 2000, investigated the highest specific biohydrogen production at pH 5.2; no action was seen below pH 4, and output fell off at pH 6. The pH had an impact on the composition of the byproducts of fermentation; the concentration of butyrate, the main component of volatile fatty acids (VFAs), decreased with rising pH (Lay, 2000). Fan et al., 2006, demonstrated that the maximum hydrogen production at pH 5.5 as a result of pH tests from 5 to 6.5 in hydrogen production using brewery wastewater (Fan et al., 2006). pH also affects the dominant degradation pathways that determine the VFAs of a process (Arimi et al., 2015). In a anaerobic process, only the exponential growth step produces hydrogen; once the stationary step is attained, the processes shift from producing hydrogen/acid to producing solvent (Khanal et al., 2004). Most researchers have seen this alteration when the pH drops below 4.5. In addition, the accumulation of VFAs may result in a sudden decrease in the pH of the reactor, thereby prohibiting the production of biohydrogen (Khan et al., 2018). According to a investigation of a continuous acidogenic bioreactor of biohydrogen production, the optimal pH for butyric, propionic, and ethanol fermentation is 5.5, >6 and 4.5, respectively (Arimi et al., 2015; Ren et al., 2007). Thus, the pH limits bacterial growth and also provides information about the concentrations of all solvents.

4.5 Hydraulic retenteion time

The hydraulic retention time (HRT) may influence substrate hydrolysis and, as a result, the formation of intermediates and end products, impacting hydrogen production. In addition HRT may also be used to control methanogenic activity. (Ghimire et al., 2015). Methane-producing bacteria generally exhibit a specific growth ranging from 0.0167 to 0.02 per hour, which is considerably slower compared to hydrogen-producing bacteria with a rate of 0.172 per hour. Consequently, by adjusting the HRT, it is possible to retain hydrogen-producing bacteria while effectively removing methane-producing bacteria (Kumar et al., 2022). The optimal HRT for hydrogen production is maintained between 8 and 14 hours (Usman et al., 2019). Aguilar et al., 2013, as a result of impact of various HRT on municipal solid waste in continuous stirred-tank reactor, the maximum H_2 production was obtained at 1.9 days of HRT. In study, when utilizing cheese whey and cow manure as the substrate, the maximum rate of hydrogen production reached 1.72L/L-d and a hydrogen yield of 0.54mol/carbohydrate consumed at a HRT of 0.75 days (Dareioti & Kornaros, 2014). It has been demonstrated that shorter HRTs are advantageous for hydrogen production, although the specific operational parameters may vary depending on the substrate type. The optimal HRT in a hydrogen fermentation procedure is determined by factors such as the concentration of the input substrate, the operational temperature, the concentration of the sludge, and the microorganisms present in the sludge (Arimi et al., 2015).

5. Bioelectrochemical System

Bioelectrochemical systems (BES) are an emerging field that utilizes microbial electrochemical technologies (MET) to efficiently convert organic or inorganic waste into valuable resources such as energy, nutrients, metals, or biochemicals. This innovative approach involves employing microbial biocatalysts to facilitate the conversion process (Chatterjee et al., 2019). Using microorganisms attached to bioelectrodes to catalyze the oxidation process for bioanode and/or the reduction reaction for biocathode (Hamelers et al., 2010). BES uses microbes that are electrochemically active to produce chemicals, process waste for environmental remediation, and the recovery of renewable energy. In terms of renewable energy recovery, microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) have demonstrated their viability as effective approaches (Kumar et al., 2017).

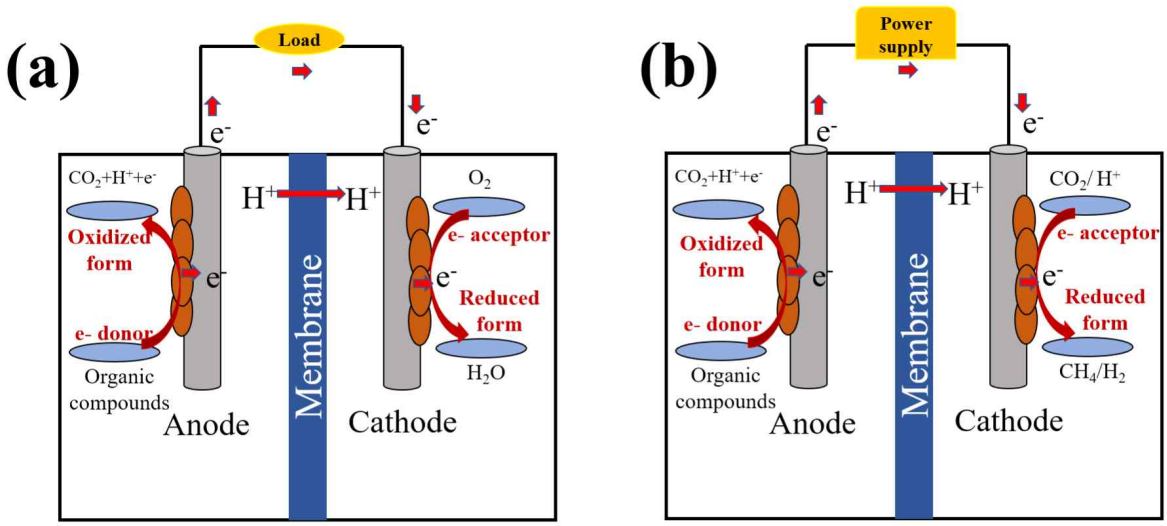
MFCs and MECs consist of an oxidation chamber with an anode, a reduction chamber with a cathode, a selectively ion-permeable membrane for ion transfer, and an external circuitry that facilitates electron movement. Organic materials are broken down into protons (H^+), electrons (e^-), and carbon dioxide (CO_2) in the anodic compartment, and extracellular electron transfer (EET) transporting the release electrons from microbes to the cathode. In the cathodic compartment, the microorganisms connected to the electrodes use the generated electrons to produce products through a reduction reaction (Wang et al., 2022). In MFC, anaerobic respiration is used to directly produce energy from organic molecules as electrons go from the anode to the cathode through an external circuit (Chatterjee et al., 2019), which reaction was spontaneously. Whereas, MEC was in the absence of oxygen, a non-spontaneous reaction occurs when microorganisms at the anode of MECs release electrons that combine with protons to produce hydrogen at the cathode (Chatterjee et al., 2019). By adding a small quantity of electricity to the cathode of an MFC, it is possible to convert it into a MEC that generates products like hydrogen gas, thereby providing additional benefits (Sangeetha et al., 2020). Figure 2-6, illustrated the MFC and MEC reactor of BES systems.

In MEC systems, provide the energy necessary for the reduction of H^+ to H_2 (-0.412V) is supplied by a microbial electrical supply (-0.289V) and an externally applied voltage. When overpotentials and ohmic loss of the electrodes are considered, the applied voltage is approximately 0.14V in theory and over 0.2V in practice (Jafary et al., 2015). This voltage is significantly lower than the 2.3 V required for water electrolysis (2.3V). Utilizingsing electrochemically active microbes to decompose organic matter and incorporating a low voltage (>0.2V in practical applications) into the MEC system, offers several of benefits : 1) overcoming the limitation of the fermentation barrier' impediment, 2) achieving high rates of hydrogen production, and 3) enabling comprehensive utilization of the carbon source (Cheng et al., 2022). *Geobacter*, *Shewanella* and *Pseudomonas* sp. are typical exoelectrogenic microorganisms capable of electron transfer to an electrode (anode), whereas the function and community composition of the microorganisms at the cathode are unkown (Ghimire et al., 2015). When acetate is employed as substrate in MEC, the following reactions occur in chamb ers equation (1.22) and (1.23) (Kadier et al., 2014):

$$Anode: CH_3COO^- + 4H_2O \rightarrow 2HCO^{3-} + 9H^+ + 8e^- \text{ (-0.28V)} \qquad (1.22)$$

$$Cathode: 8H^+ + 8e^- \rightarrow 4H_2 \text{ (-0.42)} \qquad (1.23)$$

These two reactions take place under the standard biological conditions of 1 atm pressure, 25℃ temperature, pH 7 (Kadier et al., 2014). Transferring electrons from cell to anode is a crucial process that is determined by a number of variables. The composition of material, surface area, charge, conductivity, and stability of the electrodes, as well as other physical and chemical characteristics, are crucial for an efficient MEC system (Moo-Young, 2019).



Microbial fuel cell (MFC)

Microbial electrolysis cell (MEC)

Figure 2-6. Comparison of bioelectrochemical system of microbial fuels cells (a) and micr obial electrolysis cells (b).

6. Hydrogen Production-Bioelectrochemical Anaerobic Digestion (HP-BEAD)

Bioelectrochemical anaerobic digestion (BEAD) systems, which integrate bioelectrochemical systems with anaerobic digestion (AD), provide a robust solution that surpasses the limitations of traditional AD systems. In recent years, the microbial electrolysis cell (MEC) has emerged as a notable AD reactor among these systems. It has significant attention as a promising technology for enhancing hydrogen yield from organic materials (Huang et al., 2020). In this context, the concept of hydrogen production in a bioelectrochemical AD system (HP-BEAD) has gained considerable interest. HP-BEAD, by integrating AD with MEC, it is possible to reduce the buildup of VFA, decrease the hydraulic retention time (HRT), improve the rates of hydrolysis and acidogenesis, and increase the amount of exoelectrogen acitivties, therefore enhancing the performance of AD (Wang et al., 2022). Huang et al, studied integrating single-chamber MEC with AD using food waste, when compared to AD, the amount of hydrogen generated by HP-BEAD (or AD-MEC) was much higher than the amount produced by AD. Moreover the energy recovery and hydrogen recovery rates for HP-BEAD (AD-MEC) reached as high as 238.7±5.8% and 96%, respectively (Huang et al., 2020). Hassanein et al., 2020, evaluated the impact of combining a MEC with AD in a single chamber (HP-BEAD) using refuse activated sludge as substrate. During the 23-day HP-BEAD test, cumulative hydrogen production reached 3.39L higher than AD 0.2L H2 (Hassanein et al., 2017). Despite the advantages in increasing hydrogen production efficiency, the BEAD system has predominantly focused on methane generation research, with limited studies on hydrogen production. This is primarily due to the ease with which hydrogen produced at the electrode can be readily removed by methane-producing microorganisms, homoacetogens, or electron scavengers within a single chamber. As a result, there is a need to enhance research on hydrogen production by incorporating strategies to inhibit methane-producing bacteria within the single chamber, thereby improving the efficiency of hydrogen generation in MECs.

Chapter Ⅲ. Biohydrogen Production from Swine Wasteawater and Food Waste Leachate in Anaerobic Digestion

1. Introduction

Hydrogen's potential as a renewable and purified energy source is immense. Hydrogen has the highest energy density, and it can be converted to other forms of energy via electrochemical and combustion procedures without emitting carbon-based contaminants that contribute to pollution and global warming (Levin et al., 2004). The production of hydrogen from biomass is a renewable and sustainable energy source, and the combustion product poses no harmful effects on the environment. The biohydrogen production process occurs at ambient temperatures and pressures, which makes it more energy-efficient and environmentally friendly (Brar et al., 2022).

Biomass includes vegetation, energy crops, biosolids, animal, forestry, and agricultural resides, municipal waste, agriculture waste, and domestic waste. It is attractive because of its potential global availability, conversion efficincy, and capacity to be produced and consumed in a CO_2-free way (Iakovou et al., 2010). In addition to producing clean electricity, waste-to-energy facilities also provide environmentally friendly waste management and disposal.

Anaerobic digestion (AD) is conversion of various organic wastes into biogas, which is composed of methane and carbon dioxide. Two-stage anaerobic digestion process involves separate acidogenic fermenter and methanogenic fermenter, with a focus primarily on researching the production of methane gas, the final product of the process. However, research on the acidogenic fermenter, which is located at the beginning of the process, has been limited to enhancing hydrolysis efficiency to enhance methane gas production.

Biological anaerobic hydrogen fermentation has been shown to be less energy and

environmentally damaging than conventional thermo- and electro-chemical hydrogen procedures (Wu et al., 2009). There have been many research studies about biological hydrogen production from organic waste, such as swine manure with fruit and vegetable waste (Tenca et al., 2011), brewery industrial wastewater (Kumar et al., 2022), cassava wastewater (Amorim et al., 2014), cheese whey (Rosa et al., 2014), food waste (Alian et al., 2021), municipal solid waste (Ebrahimian et al., 2022). Among them, in South Korea, most studies have been conducted on hydrogen fermentation using food waste leachate (FW) (김동훈 et al., 2011; 오세은 et al., 2013; 유정숙 et al., 2011; 장수진 et al., 2015; 장해남 et al., 2016; 조경민& 오세은, 2022). Whereas, research on the utilization of swine wastewater (SW), which accounts for about 83% of domestic organic waste generation (환경부, 2022a) has mainly focused on methane production. Since the carbon source is relatively insufficient in SW, research on hydrogen production using SW, such as by adding glucose or adding a small amount of SW to FW (장수진 et al., 2015; 장해남, 2016). There is little information about the use of SW as the substrate for hydrogen fermentation.

This study considers the potential for hydrogen production by adding different ratios of FW to SW. Evaluated the effect parameters for hydrogen production (pH, temperature, carbon-to-nitrogen ratio of organic matter, feedstock characteristics, and alkalinity) are highly diverse for different substrates, we assessed the hydrogen production potential by adding various ratios of FW to SW to produce bio-hydrogen. Based on the results, we determined that pH had the greatest impact on hydrogen fermentation, so we conducted additional experiments to produce hydrogen by adjusting the pH of SW and FW, respectively.

: Experiment step

(1) Operation of mixed swine wastewater and food waste leachate for optimization of hydrogen production

(2) Operation of anaerobic digestion at different initial pH for optimization of hydrogen production

2. Materials and Methods

2.1 Substrate

This study used two types of substrates, swine wastewater (SW), and food waste leachate (FW) collected from anaerobic digestion (AD) treatment plant (Nonsan Gyeryoung Livestock Cooperative Federation, Nonsan, Korea). After collecting wastewater, it was subdivided into 1L bottles and stored at -20℃ temperature in the freezer and then thawed at room temperature before use.

Before conducting the experiment, analysis of wastewater parameters about pH, total solids (TS), Volatile solids (VS), total chemical oxygen demand (TCOD), soluble chemical oxygen (SCOD), total phosphorus (T-P), total nitrogen (T-N), ammonia nitrogen (NH_3-N), alkalinity (mg/L as $CaCO_3$) were analyzed. Table 3-1 is the characteristics of substrate SW and FW.

Following are the characteristics of the SW used in the experiment: The pH of SW at 7.2-7.4 was higher than FW. TCOD; 28,000-39,100 mg/L, SCOD; 13,300-19,000 mg/L. T-N and NH_3-N of 3,900-4,200 mg/L and 2,723-3,173 mg/L, T-P of 1,280-1,400 mg/L, TS and VS of 1,93-2.54% and 1.24-1.75% respectively, and alkalinity of 10,550~11,650 mg/L as $CaCO_3$.

Characteristics of the FW used in the experiment: pH value 4.21-4.63, TCOD; 135,600-144,600 mg/L, SCOD 99,500-109,800 mg/L, T-N; 3200-3350 mg/L, NH_3-N; 543-644mg/L, T-P; 1,845-2,400mg/L, TS and VS; 11.35-11.94% and 10.77-12.02%, respectively.

Table 3-1. Characteristics of swine wastewater and food waste leachate.

Parameter	Swine wastewater	Food waste leachate
pH	7.2~7.4	4.2~4.6
TCOD (mg/L)	28,000~39,100	135,600~144,600
SCOD (mg/L)	13,300~19,000	99,500~109,800
T-N (mg/L)	3,900~4,200	3,200~3,350
NH_3-N (mg/L)	2,723~3,173	543~644
T-P (mg/L)	1,280~1,400	1,845~2,400
TS(%)	1.93~2.54	11.35~11.94
VS(%)	1.24~1.75	10.77~12.02
VS/TS (%)	0.64~0.68	0.94~0.99
Alkalinity (mg/L as $CaCO_3$)	10,550~11,650	

2.2 Seed sludge

Seed sludge used anaerobic acid sludge was obtained from and acidogenic fermenter in Nonsan, Korea, as part of a two-stage anaerobic digestion treatment. After sampling acidogenic fermenter sludge, high-purity nitrogen gas (99.999% N_2) was purged to make an anaerobic environment. A continuously-stirred tank reactor (CSTR) with a total volume of 8L bottles was operated on a laboratory scale. The reactor was maintained at a constant temperature in a room with mesophilic conditions (35±5℃). The digester was configured with an inlet, outle, sludge sampling port, and a gas bag. The pH of the sludge in the acidogenic fermenter was analyzed around pH 5.5, which is within the typical range for acidogenic fermenter (pH 5.5-6.0) (Cremonez et al., 2021; Srisowmeya et al., 2020). A mixture of swine and food waste leachate was added in a certain proportion to adjust the influent pH to the range of 5.5-6.0, and the mixture was discharged at a certain ratio. The mixing ratio varied slightly depending on the characteristics of the waste collected each season, but a ratio of approximately SW and FW 8:2 or 7.5:2.5 was found to meet the condition of pH 5.5-6.0.

CSTR that was set up for to maintaining the sludge from the acidogenic fermenter tank active. To pervent oxygen from entring the anaerobic reactor during experiment preparation, the seed sludge was collected via the use of the sludge sample port. Table 3-2 shows the characteristics of the seed sludge used in the experiment: Seed sludge constant 1.97% of TS, 1.16% of VS, and pH 5.56. TCOD and SCOD followed 49,600 and 43,700 mg/L, and T-N and NH_3-N of 3,200 mg/L and 2,434 mg/L.

Table 3-2. Characteristics of seed sludge used in acidogenic fermenter.

Parameter	Sludge
pH	5.56
TCOD (mg/L)	49,600
SCOD (mg/L)	43,700
TS (%)	1.97
VS (%)	1.16
T-N (mg/L)	3,200
NH_3-N (mg/L)	2,434
T-P	1,210

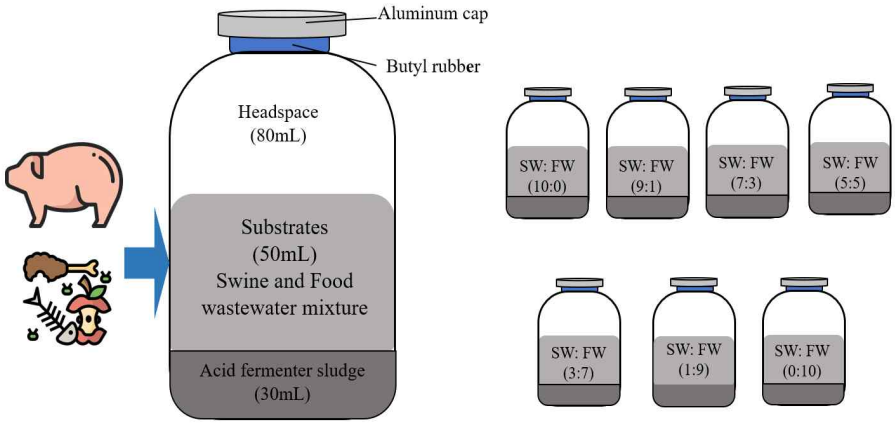
2.3 Experimental Setup and Operation Conditions

2.3.1 Operation of Mixed Swine Wastewater and Food Waste Leachate for Optimization of Hydrogen Production in Anaerobic Digestion

A batch experiment was conducted using substrates with different mixing ratios of swine wastewater (SW) and food waste leachate (FW) to find a suitable substrate for hydrogen production. SW and FW were mixed at various mixing ratios, including 10:0, 9:1, 7:3, 5:5, 3:7, 1:9, and 0:10 to make a 50mL substrate.

The batch test fermenter utilized 160mL serum bottles, with 50mL of substrate and 30mL of inoculum taken from the acidogenic fermenter sludge obtained laboratory CSTR digester. To make anaerobic conditions, high-purity nitrogen was injected (99.999% N_2 gas) and the bottle stoppers were made of butyl rubber and closed with an aluminum seal. Then reactors were incubated in a oven maintained at 35℃ and measuring biogas at 12 or 24 hour intervals using gas tight-syringe and sampling a small amount of substrate at 24-hour intervals using a syringe. Figure 3-1 shows a schematic diagram and photographs of the experimental setup.

(a)



(b)

Figure 3-1. Preparation of serum bottles with different mixing ratios of swine wastewater and food waste leachate (SW:FW = 10:0, 9:1, 7:3, 5:5, 3:7, 1:9, and 0:10).

2.3.2 Operation of Mixed Swine Wastewater (SW) and Food Waste Leachate (FW) for Optimization of Hydrogen Production in Anaerobic Digestion (AD)

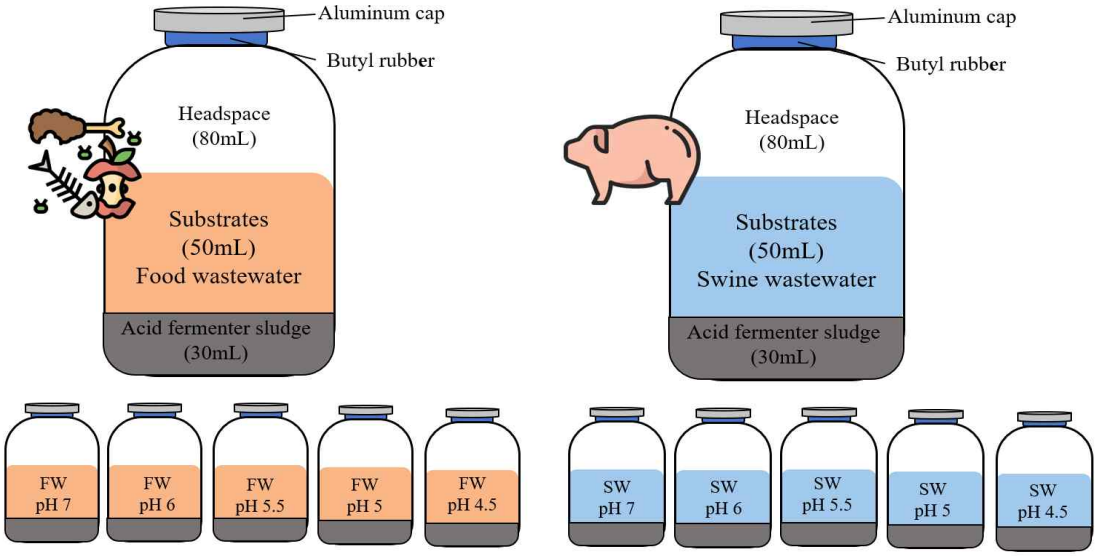
After assessing the potential for hydrogen production in a mixture of swine wastewater (SW) and food waste leachate (FW), we determined that the initial pH environment significantly affects hydrogen production. Therefore, we conducted anaerobic acid fermentation by adjusting the pH of SW and FW separately.

To achieve the pH levels of pH 7.0, pH 6.0, pH 5.5, pH 5.0, and pH 4.5 for the reactor substrate and sludge mixture, 10M H_3PO_4 and 5N NaOH were used to adjust the pH of wastewater. Considering that the pH of the sludges was 5.5 we ensured that the pH of the sludge-wastewater mixture fell within the pH ranges of each constituent.

In a 160mL serum bottle, we added 50mL of pH-adjusted substrate and 30mL of sludge from the anaerobic continuous stirred-tank reactor (CSTR) operated in the laboratory. To make anaerobic conditions, purged the bottle with high-purity nitrogen gas (99.99% N_2) and sealed the inlet with a stopper and butyl rubber, preventing external oxygen from entering the bottle. The total working volume of the reactor was 80mL, and the headspace of the bottle was approximately 80mL.

The operation was conducted at 35℃ in an oven for approximately 96 hours. Gas samples were collected at intervals of 12 or 24 hours for analysis, and wastewater samples were collected at 24-hour intervals. Figure 3-2 presents the photographs and schematics of the conducted experiment.

(a)



(b)

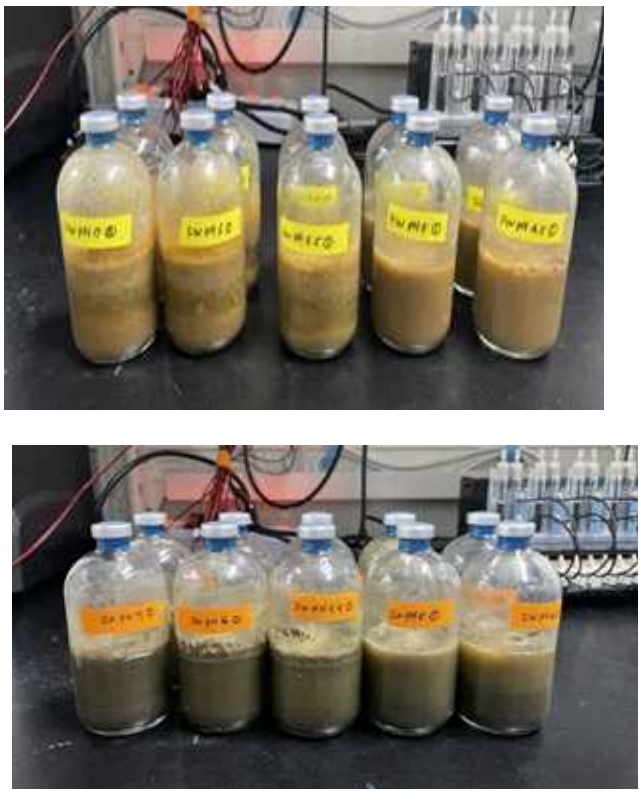


Figure 3-2. Preparation of serum bottles with different initial pH values of food waste leachate (FW: pH 7.0, pH 6.0, pH 5.5, pH 5.0, pH 4.5) and swine wastewater (SW: pH 7.0, pH 6.0, pH 5.5, pH 5.0, pH4.5) (a) a shematic diagram (b) photograph.

2.4 Analytical Methods

Gases concentrations were analyzed with a gas chromatography-GC (Young in Chromass, YL-6500) equipped with a thermal conductivity detector (GC-TCD) and a flame ionization detector (GC-FID/methanizer), and the column was used Molsieve 13X (3FTX1/8Inx2.MIM) and Porapak n (10FT X 1/8N x 2.1MM). When analyzing the gas, the operation conditions of GC oven temperature 40℃, injection temperature 150℃, TCD detector temperature 150℃, and FID detector temperature 250℃, and carrier gas was 15mL/min flow rate using nitrogen gas.

Volatile fatty acids (VFAs) were collected using a sterilized syringe and a certain amount of the sample was centrifuged (13000rpm, 10min) and then analyzed using gas chromatography (Shimadzu, GC-210) equipped with a flame ionization detector (GC-FIC). The colum used was AT-AQUAWAX (30m x 0.32mm x 0.25um). The operating conditions for the GC were an oven temperature 80℃, detector 220℃, and carrier gas flow was 30mL/hr using nitrogen gas.

Water quality parameters were analyzed from samples collected at 24-hour intervals. pH measurements were conducted using a pH meter (Metrohm 912). Total chemical ox ygen demand (TCOD) and ammonia (NH_3-N) were analyzed using a water quality analysis kit (Hach Company, USA) and using UV/VIS spectrophotometer (DR5000, Hach, USA). Total solids (TS) and volatile solids (VS) were measured following the standard method. For total solids, the evaporated dish method was employed, where the sample was heated in an oven (Jeio tech, FO-600M) at 105°C for 2 hours, cooled, and then weighed. VS were determined by heating the measured total solids in an electric furnace (Lab house, DY-6062-6) at 550°C for 30 minutes, followed by cooling and weighing to calculate the volatile solid content.

The formula used to calculate the TCOD removal efficiency (%) is as follows (2.1):

$$E = (\frac{COD_{(1)} - COD_{(2)}}{COD_{(1)}}) \times 100 \qquad (2.1)$$

$COD_{(1)}$: COD value in initial concentration in wastewater

$COD_{(2)}$: COD value in final concentration in wastewater

The formula used to calculate the VS removal efficiency (%) is as follows (2.2):

$$E = (\frac{VS_{(1)} - VS_{(2)}}{VS_{(1)}}) \times 100 \qquad (2.2)$$

$VS_{(1)}$: VS value in initial concentration in wastewater

$VS_{(2)}$: VS value in final concentration in wastewater

The volumetric hydrogen production rate (VHPR) was calculated according (2.3):

$$VHPR = \frac{Hydrogen\ prouction\ (L/d)}{Reactor\ volume\ (L)}$$

3. Results and discussion

3.1 Operation of Mixed Swine Wastewater and Food Waste Leachate for Optimization of Hydrogen Production in Anaerobic Digestion

3.1.1 Hydrogen production

A batch-type anaerobic acid fermentation was conducted by mixing swine wastewater (SW) with neutral pH 6.2-7.4 and food waste leachate (FW) with an acidic pH range of 4.2-4.6 in the proper proportions. The mixing ratios were as follows: SW:FW, 10:0, 9:1, 7:3, 5:5, 3:7, 1:9, 0:10. After inoculating the acid fermenter sludge, the initial pH values were 6.66±0.03 (10:0), 6.38±0.04 (9:1), 6.12±0.03 (7:3) , 5.64±0.03 (5:5), 5.35±0.02 (3:7). 5.07±0.01 (1:9), 4.98±0.01 (0:10). Figure 3-3 shows the pH value (a), hydrogen production (mL) (b), the volumetric hydrogen production rate (VHPR, mL/L-d) (c), the hydrogen yield (d) at different mixing ratios.

In Figure 3-1 (a), it can be observed that at the ratios of 10:0, 9:1, and 7:3, there was no significant change in pH over a period of 72 hours. Furthermore, in all ratios, there was no notable rapid increase or decrease in pH. However, when the ratio of FW gradually increases to 5:5, 3:7, 1:9, and 0:10, a decrease in pH of approximately 0.2-0.4 compared to the initial pH was observed at 48 hours. This decrease in pH can be attributed to the low alkalinity characteristics of FW (Figure 3-3 (a)).

Figures 3-3 (b) and (c) as show hydrogen production (mL) and volumetric hydrogen production rate (VHPR, mL/L-hr). During the operation, hydrogen of 26.5±2.5mL was produced at a 9:1 ratio, resulting in the highest VHPR of 20.1±3.6mL/L-d observed within 12 hours. However, after 12 hours, the VHPR gradually decreased at 9:1 and the increased VHPR at the 7:3 and 5:5 ratios. Moreover, both the hydrogen production (mL) and VHPR (mL/L-d) exhibited higher values at a 7:3 ratio during the experiment. At 56 hours, an abrupt maximum hydrogen production was observed at the 5:5 ratio, with the highest values being 167.7±32.4mL and 27.8±4.1mL/L-hr, which was similar to

values for the 7:3 ratio (156.3±9.6mL, 26.1±0.4mL/L-hr). As a result, the optimal mixing ratios of SW and FW were determined to be approximately 7:3 and 5:5.

On the other hand, in the reactor with only SW in the 10:0, no hydrogen production was observed, and only a small amount of methane gas (less than 2mL) was detected until the end of the operation. In comparison, 0:10 ratio and 1:9 ratios exhibited low VHPR values below 12mL/L-d, with H2 production(mL) also recording values below 70mL.

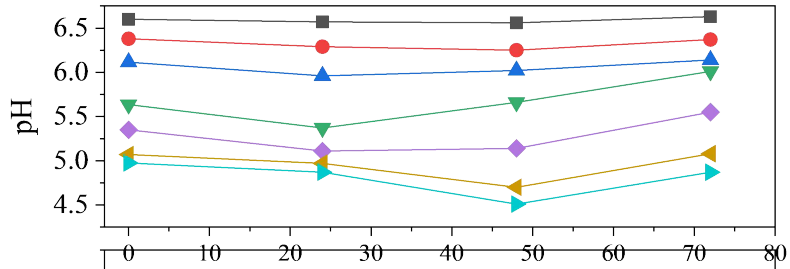
Figure 3-3 (d) a shows hydrogen yield (mL/g·VS) at 24hr, 48hr, 72hr. Within the initial 24 hours, a high yield was observed at 9:1. For both of 48hr and 72hr, the 7:3 ratio recorded the highest yield (44.88±11.04mL/g·VS, 85.68±4.70mL/g·VS), showing an increase of 36.8% and 46.3% higher than 5:5. Following that, the 9:1 and 3:7 ratios recorded yields of 46.50±4.05mL/g·VS and 25.66±1.54mL/g·VS, respectively. Conversely, the ratios of 1:9, 0:10 and 10:0 exhibited yields below 10mL/g·VS.

When comparing hydrogen production (mL), VHPR (mL/L-d), and yield, the ratio of 5:5 demonstrated higher maximum hydrogen production(mL) than 7:3. However, when comparing the VHPR as a whole, 5:5 exhibited a relatively slower VHPR than 7:3 (except 56hr). Overall, it can be concluded that the ratio of 7:3, which exhibited both fast production rate and high yield, is the most suitable for the mixed hydrogen production using a mixed SW and FW.

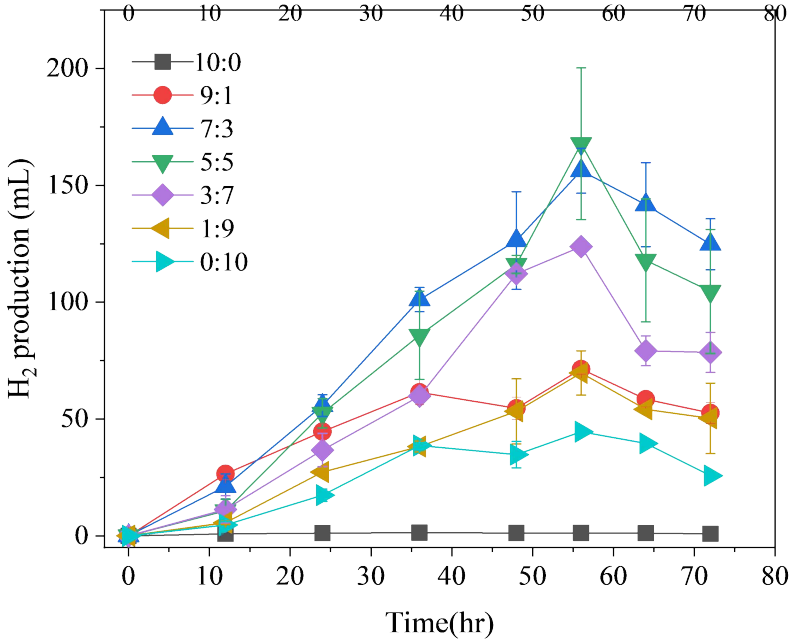
To maintain optimal pH levels and prevent the formation of pH gradients in a wastewater fermentation reactor, the interaction between substrates may play a vital role (Oduor et al., 2022). Additionally, the initial pH is an important factor in hydrogen production, and previous studies have reported that pH values ranging from 5.5-5.7 in sucrose (Khanal et al., 2004), 5.5-6.0 in food waste (Chu et al., 2008) are considered suitable.

Furthermore, the ratios of 7:3 and 5:5 indicate that an initial pH of 6.1 and 5.6, respectively, are conductive to hydrogen production. Moreover, a higher proportion of S W results in increased hydrogen production and a faster VHPR. Consequently, the fermentation process with an increased proportion of SW offers more advantages than using sole FW, as it allows for better pH balance maintenance.

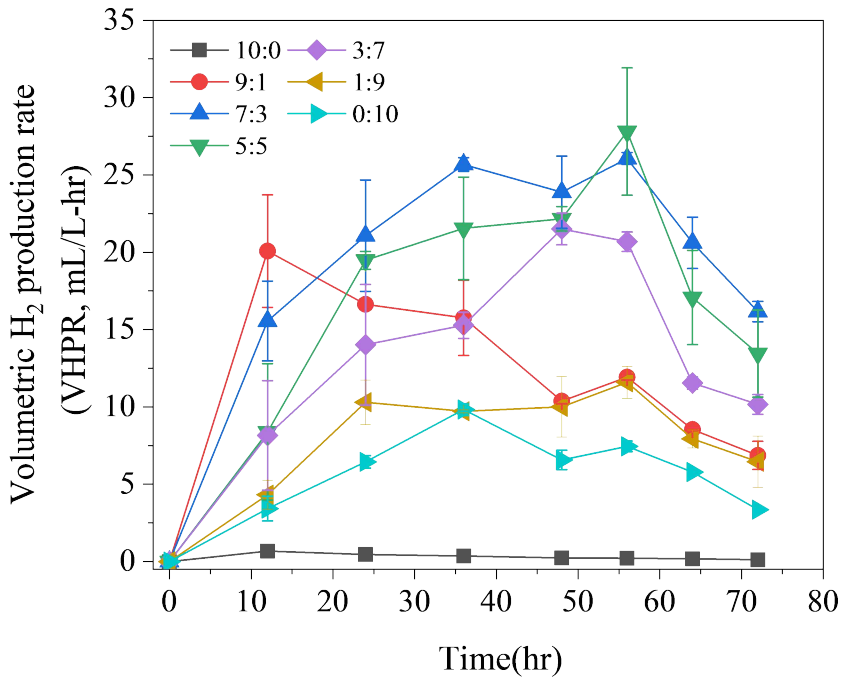
(a)



(b)



(c)



(d)

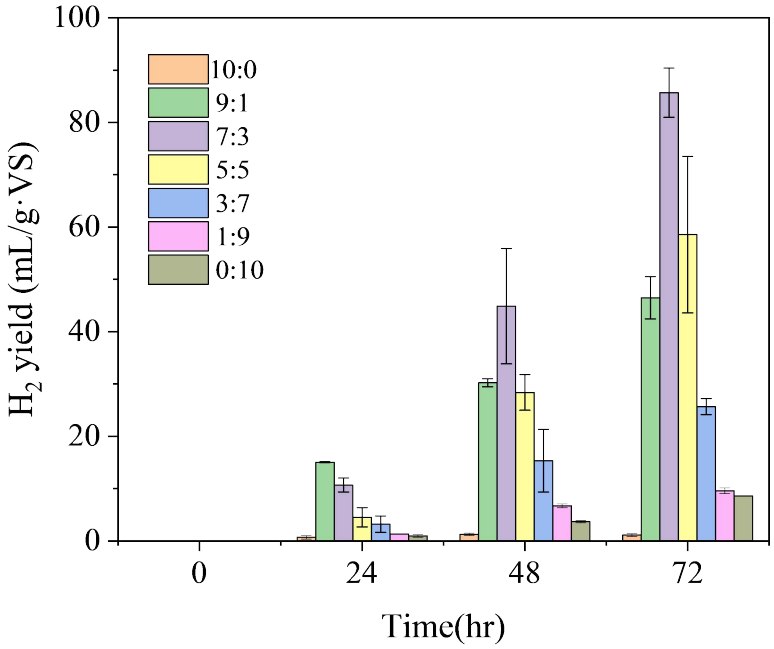


Figure 3-3. Time dependent changes of (a) pH value (b) hdrogen production (c) volumetric hydrogen production rate and (d) hydrogen yield in mixtures with different mixing ratios SW and FW (SW:FW).

3.1.2 TCOD, VS Removal and C/N Ratio

Figure 3-4 is show The chemical oxygen demand (COD) and volatile solid (VS), which are indicators of organic matter content. The removal rates of COD and VS at the beginning and end of the experiment were calculated to assess the degradation efficiency during anaerobic digestion. According to the characteristics of FW, it was found that FW has TCOD and VS concentrations approximately 4.17 times and 7.62 times higher than the swine wastewater (SW), respectively, indicating a higher organic matter content. Figure 3-4 presents the TCOD and VS removal efficiency (%) at start and end of the experiment, the SW: FW ratios of 10:0, 9:1, 7:3, 5:5, 3:7, 1:9, and 0:10, which were 22±2%, 23±3%, 48±3%, 29±1%, 33±8%, 23±2%, and 26±5%, respectively. As a result that the greatest amount of TCOD has been removed at a ratio of 7:3.

Similarly, the VS removal efficiency (%) followed the same order: 43±5%, 49±0%, 71±0%, 69±1%, 62±2%, 39±0%, and 40±1% for the respectively. The highest removal rate was also observed at a ratio of 7:3, followed by 5:5, 3:7, and 1:9. These results are consistent with the hydrogen production yields (mL/g·VS) presented in Figure 3-3 (d). Thus, the experiment demonstrated that using mixed ratios of 7:3, 5:5, 3:7, and 1:9 higher organic matter degradation rates and efficient hydrogen production compared to using a single substrate, either FW or SW.

The C/N ratio is an important factor for the stability of anaerobic processes. Carbon is consumed by microorganisms, and its utilization is proportional to the yield of the product and nitrogen is essential for the protein synthesis of microbes (Farghaly & Tawfik, 2017). Microorganisms require carbon sources at a ratio 25-30 times higher than nitrogen sources. Various studies have explored the optimal C/N ratios for hydrogen production, such as a straw and sewage sludge mixture with a C/N ratio of 25 (Kim et al., 2012), a food waste and sewage sludge mixture with COD/N ratios ranging from 26 to 30 (Zhou et al., 2013), and a coffee grounds and swine manure with a C/N ratio of 53 (Hernández et al., 2014). Observing the TCOD/N ratio in Figure 3-5, the ratios for SW: FW = 10:0, 9:1, 7:3, 5:5, 3:7, 1:9, and 0:10 were

26.9±0.8, 29.6±0.1, 50.7±5.3, 56.7±2.5, 74.1±2.5, 84.1±5, and 100.3±3.3, respectively, showing a significant decrease after 24 hours but maintaining a suitable range. The COD/N ratio at the end of the experiment was approximately 19.14±0.2, 22.29±0.1, 26.4±1.6, 36.45±2.7, 44.62±4.0, and 62.03±0.2 for the respective ratios mentioned above. It is crucial to select the appropriate initial COD/N ratio as it significantly decreases within the first 24 hours. Considering the hydrogen production yield results in Figure 3-5, the ratios of 7:3 and 5:5 exhibited the higher efficiency. Therefore, when using a mixture of FW and SW, it is beneficial to consider the COD/N ratio of approximately 50-56.

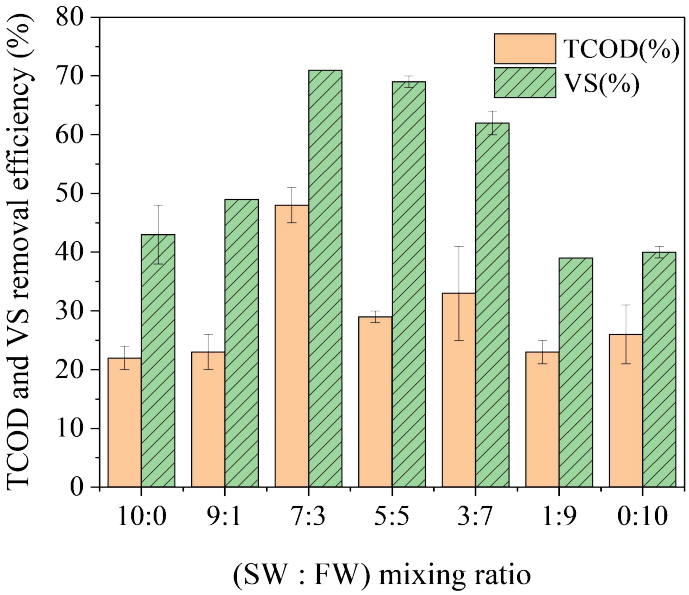


Figure 3-4. Removal efficiency (%) of TCOD and VS stages with different mixing ratios SW and FW.

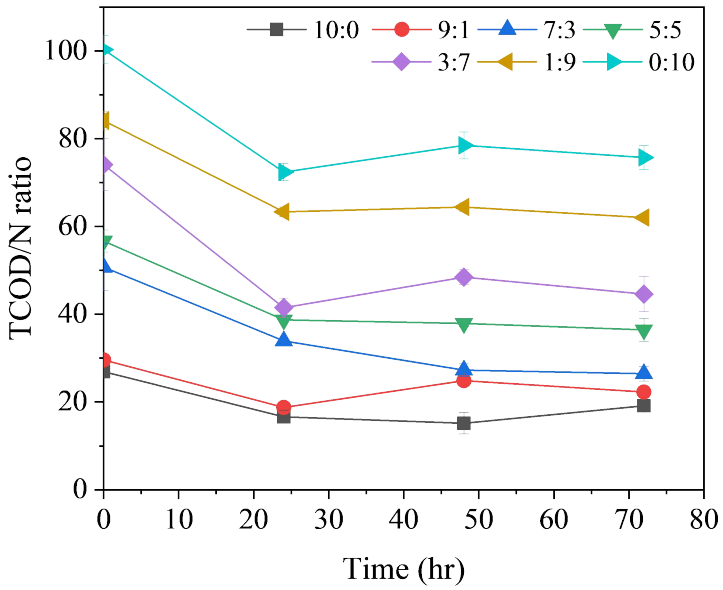


Figure 3-5. Changes of TCOD/N ratio during operation periods in different mixtures (SW:FW).

3.1.3 VFAs and Ethanol

Figure 3-6 illustrates the concentrations (g/L) of volatile fatty acids (VFAs) and ethanol, Figure 3-5 show the composition (%) of VFAs analyzed from samples collected every 24hours throughout the experiment period.

According to the concentrations of VFAs and ethanol in Figure 3-4, recorded after completion of experiment (72hr), the total VFA (TVFAs) and ethanol concentrations measured, 15.08 g/L, 15.83 g/L, 21.84 g/L, 24.62 g/L, 24.57 g/L, 29.22 g/L, and 28.92 g/L in the following order of SW:FW ratios: 10:0, 9:1, 7:3, 5:5, 3:7, 1:9, and 0:10. This observation confirms that higher TVFAs and ethanol production was achieved in reactors with a higher proportion of food waste, and specifically, a higher concentration of ethanol was observed with increasing food waste content. Additionally, when comparing the initial concentrations (mg/L) of ethanol at 0 hours for each ratio, it was found that the ethanol content a higher with a higher proportion of food waste. Comparing the SW:FW ratios of 10:0 and 0:10, the ethanol concentration in the 0:10 ratio (12.43 g/L) was approximately 3.79 times higher than that in the 10:0 ratio (7.27 g/L). Kim et al., reported that when the activity of hydrogenase is inhibited, reduced byproducts including ethanol and butanol are produced (Kim et al., 1984). Moreover Koutinas et al., suggested that solvents alter the functional capabilities of cells, leading to cell membrane disruption and cell death (Koutinas et al., 2014). Therefore, it means that, hydrogen did not produce due to the high ethanol content of FW and the decrease in activity of hydrogenase.

In addition to ethanol, the observation of propionic acid at 0 hours in SW revealed that a greater proportion of produced a small but elevated concentration of propionic acid (10:0 at 0.87 g/L, 0:10 at 0.34 g/L). The initial concentrations of other factors remained unchanged.

Examining the composition (%) of VFAs in Figure 3-7, acetic acid, butyric acid, and caproic acid were determined to be the predominant fermentation products in all ratios. In the cases of 7:3 and 5:5, which exhibited successful hydrogen production, an increase in the proportion of butyric acid was observed. Specifically, the proportion of

butyric acid increased from 18.6% to 25.35% at 7:3 and from 15.4% to 26.6% at 5:5 during experiment period. Conversely, a decrease in the proportion of acetic acid was observed at 5:5, which was consistent with the rapid decrease in acetic acid concentration at 48 and 72 hours and its comparison with hydrogen production (mL). Furthermore, based on the correlation between hydrogen production and butyric acid reported by Sarangi & Nanda (2020), it can be concluded that hydrogen production from the mixture of SW and FW is facilitated by the conversion to butyric acid.

In conclusion, it was confirmed that high concentration of ethanol was generated at high FW ratios of 3:7, 1:9, 0:10, and it was determined that a large amount of ethanol suppressed hydrogen production when compared to the hydrogen production results. In the ratio of 7:3 and 5:5, which recorded high hydrogen yields (figure 3-3 (d)), an increase in the concentration of butyric acid related to hydrogen production was confirmed.

In summary, it was determined that the increase in butyric acid in the mixture of SW and FW was closely related to the production of hydrogen.

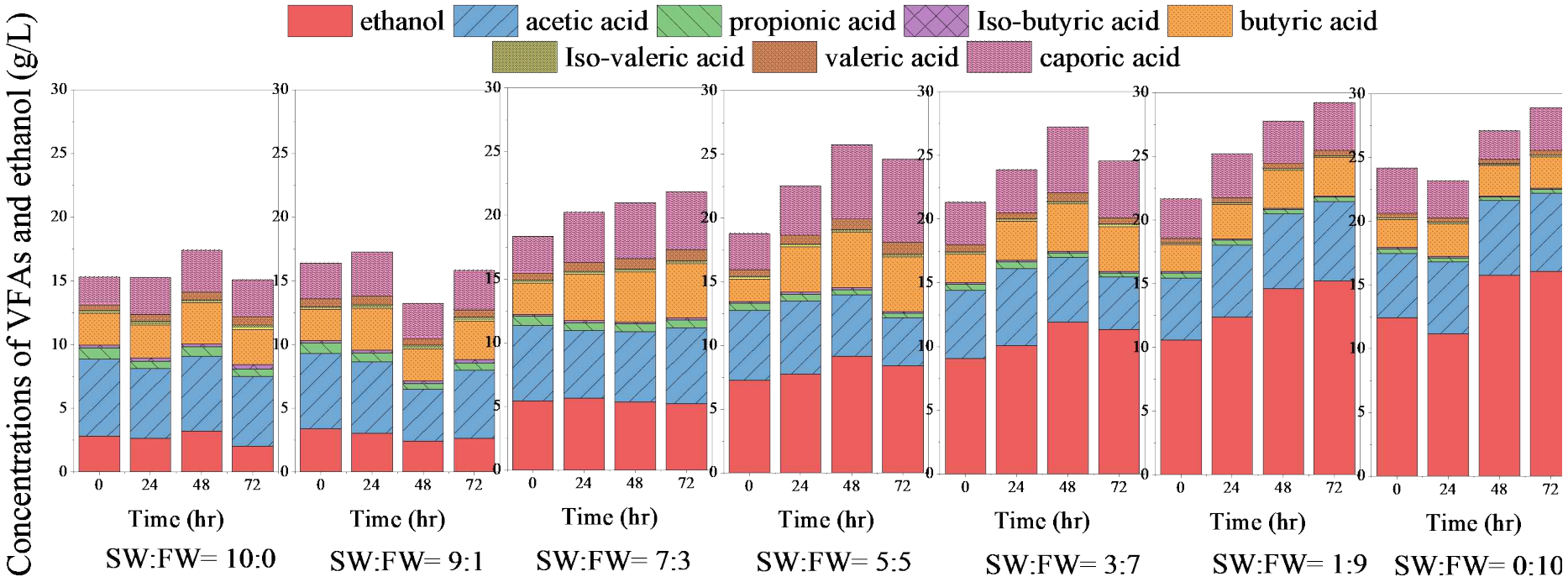


Figure 3-6. Concentration of VFAs and ethanol in different mixtures (SW:FW).

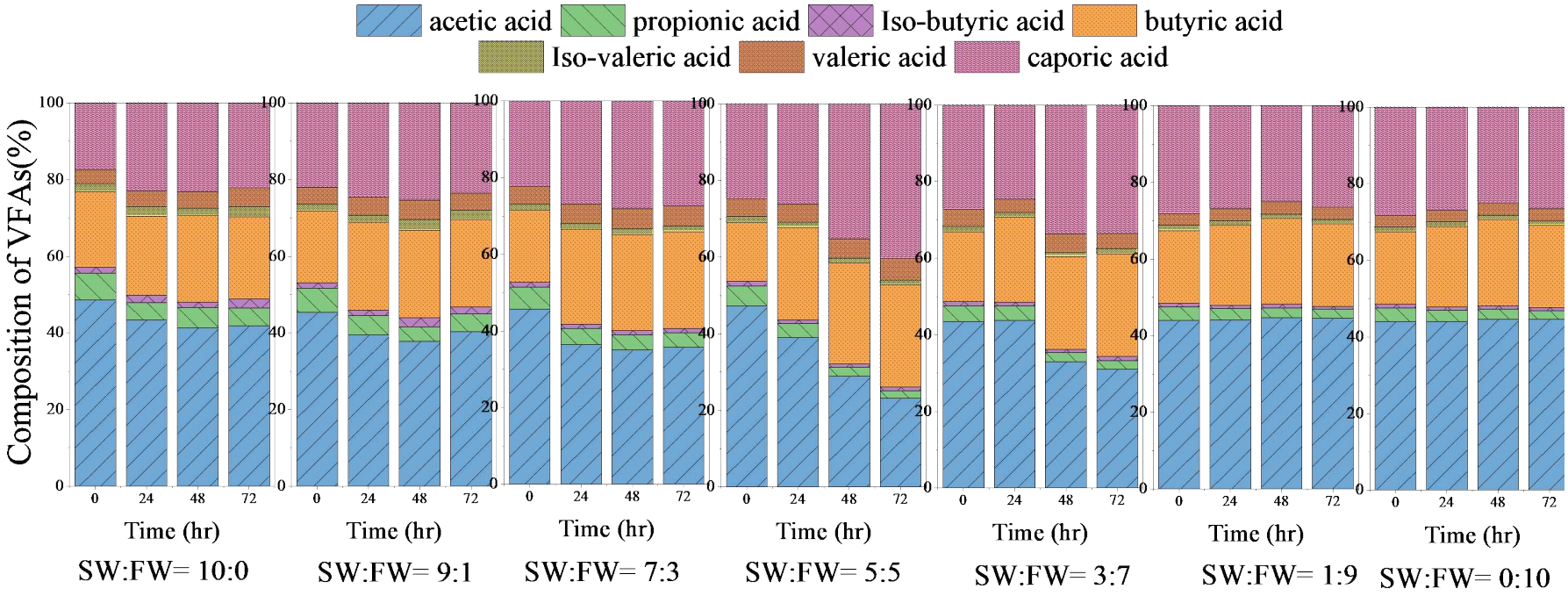


Figure 3-7. Composition of VFA (%) in different mixtures (SW:FW).

3.2 Effect of initial pH Conditions for Biohydrogen Production from Swine Wastewater and Food Waste Leachate

3.2.1 Hydrogen Production

(1) Swine wastewater

In order to investigate the possibility of hydrogen production in each wastewater without a mixture of SW and FW, anaerobic digestion was initiated through pH adjustment in various ranges of SW and FW.

In first, SW in anaerobic acid fermentation for 0-96 hours, Figure 3-8 shows pH value (a), hydrogen production (mL) (b), volumetric hydrogen production rate (VHPR, mL/L-hr) (c), methane production (mL) (e) during 0-96hr at different pH value (pH 7.0, pH 6.0, pH 5.5, pH 5.0, pH 4.5).

The pH changes are shown in Figure 3-8 (a). The initial pH levels were 6.89, 6.23, 5.57, 5.09, and 4.51 and after the end of 96hr, the pH levels were 6.49, 6.14, 5.58. 5.10, and 4.47, respectively. The observed pH changes were within ±0.4. Additionally, it was confirmed that if the pH was adjusted by adding artificial chemicals, it could be maintained without significant increase or decrease for 96 hours. It was determined that the reason for maintaining a constant pH is SW has high alkalinity than other types of wastewater. Alkalinity is a desired effect between reactors as it is a sign of the buffer effect of the mixture (신승구et al., 2015). Therefore, controlling the pH is critical as it has a major effect on the reactor behavior (신승구et al., 2015).

Figure 3-8 (b) demonstrated hydrogen production (mL) and Figure 3-8 (c) showed the volumetric hydrogen production rate (VHPR, mL/L-hr). The reactor with pH 6 produced 10.39±0.88mL of hydrogen within the initial 12 hours, the fastest rate among all pH levels within 12hr. At 24 hours of operation, with pH 6 had the fastest VHPR rate of all reactors at 1.71±0.20mL/L-hr. Following that, hydrogen production of 66.52±1.82mL was maximum production achieved in pH 6 reactor with 48hr and then gradually decreased. However, noted that although the VHPR was faster under pH 6 at

24hr, the maximum hydrogen production in all reactor of 79.91±1.67mL was observed in pH 5.5 at 60 hours of operation (b).

After 24 hour, a delayed increase hydrogen production was observed at pH 5.5, with a VHPR of 1.63±0.01mL/L-hr. Over the course of 96 hours, the VHPR at pH 6 reached 1.17±0.2 mL/L-hr after 24 hours, while at pH 5.5, it reached 1.63±0.01 mL/L-hr after 48 hours. Subsequently, hydrogen production began at a rate of 0.65±0.11 mL/L-hr at pH 5 after 72 hours (c). These findings indicate a slower initiation of hydrogen production in the order of pH 6, pH 5.5, and pH 5, suggesting that lower pH values result in longer lag phases for acidogenic bacteria.

Consequently, it can be concluded that the environmental conditions, specifically pH, determine the rate of hydrogen production by the microbial consortia. Moreover, pH 6 and pH 5.5 were found to reduce the lag time of microbial activity and promote hydrogen production compared to the pH 5 condition (Figure 3-6, (c)).

These results suggest that, when using and appropriate substrate for hydrogen production, maintaining the optimal pH increases hydrogen yield and accelerates the production of hydrogen. In conclusion, the experiment demonstrated that for optimal hydrogen production, the pH must be maintained between 5.5 to 6.0, and that hydrogen production is only possible by adjusting the pH with SW.

Whereas less than 10mL of hydrogen was generated at pH 7 and no hydrogen generation was observed at pH 4.5, indicating that pH 4.5 and pH 7 conditions are not suitable for hydrogen production in SW (Figure 3-8, (b)).

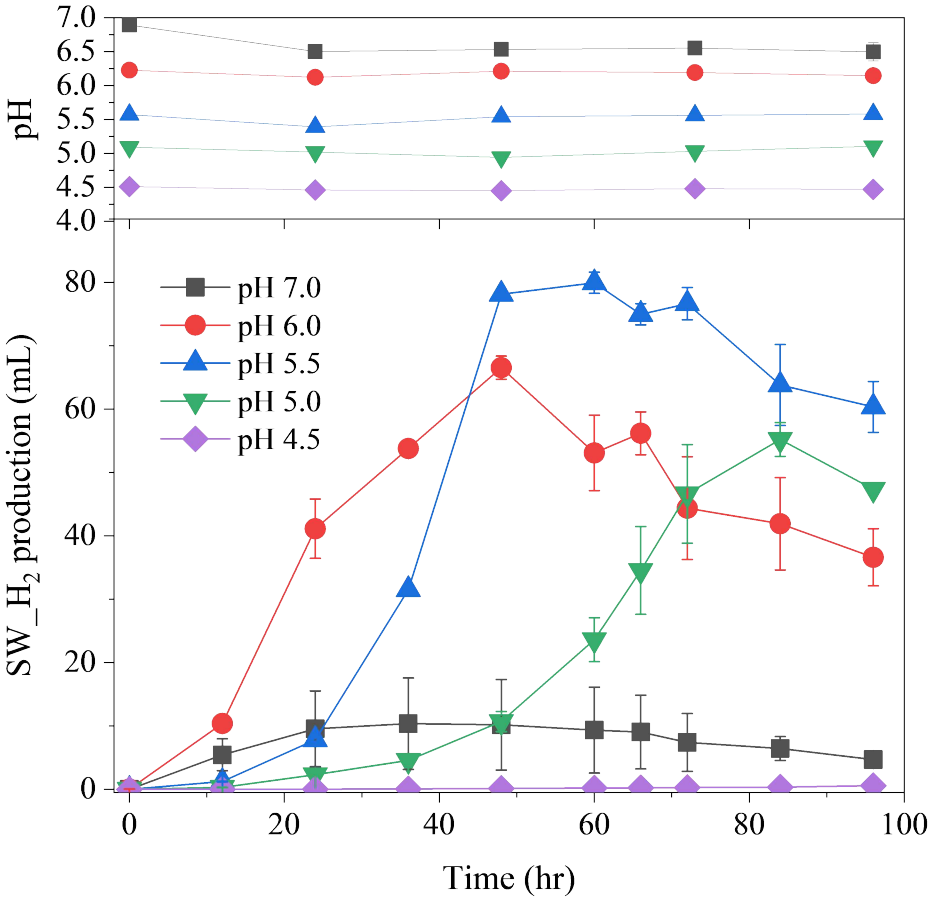
Figure 3-8 (e), presents methane production (mL) during the operate period (0~96hr). There was almost no methane production in all reactors, with only 4mL less of methane observed at pH 7.

The hydrogen yield (mL/g·VS) is shown in Figure 3-8 (d). pH 6 had the highest hydrogen production (mL) at 48 hours, and pH 5.5 had the highest hydrogen production (mL) at 60hr were compared. Among all reactors, the pH 6 achieved the highest hydrogen yield of 73.62±2.01 mL/g·VS at 48 hours, which was approximately 39.1% higher than that of pH 5.5. The pH 5.5 was attained high yield at 96 hours, recording a yield of 55.45±3.70 mL/g VS. These results indicate that pH 5.5 performed 50% better than pH 6 in terms of hydrogen production amount, but, in terms of VHPR

and hydrogen yield, pH 6 demonstrated more favorable outcomes. This consistent with the findings of the previous experiment (chapter 3.1) concerning the mixture of SW and FW. Similar patterns were observed, with pH 6.10 at the initial pH of the 7:3 (SW:FW) exhibiting rapid hydrogen production and the highest yield, and pH 5.6 at the initial pH of the 5:5 (SW:FW) exhibiting a slightly longer lag phase but the highest production (Figure 3-8, (d)).

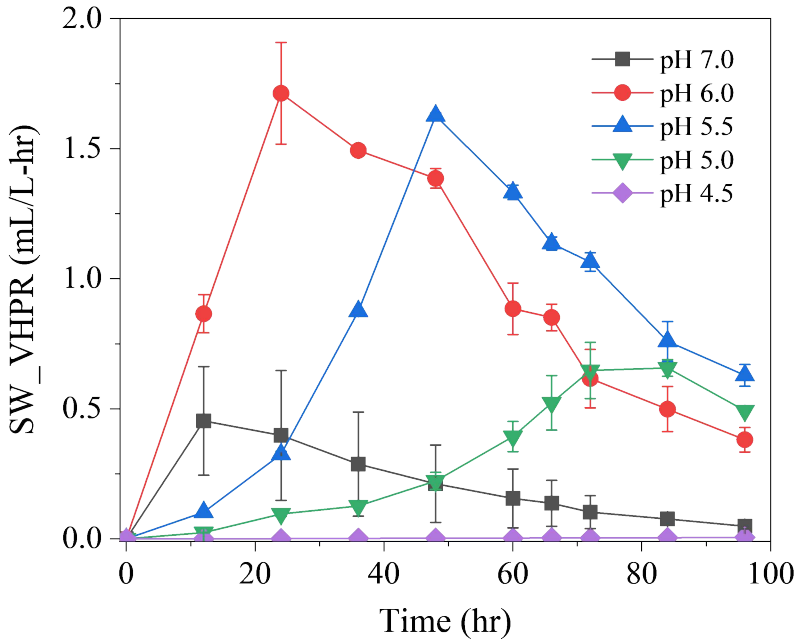
These results suggest that, when using and appropriate substrate for hydrogen production, maintaining the optimal pH increases hydrogen yield and accelerates the production of hydrogen. In conclusion, the experiment demonstrated that for optimal hydrogen production, the pH must be maintained between 5.5 to 6.0, and that hydrogen production is only possible by adjusting the pH with swine wastewater.

(a)

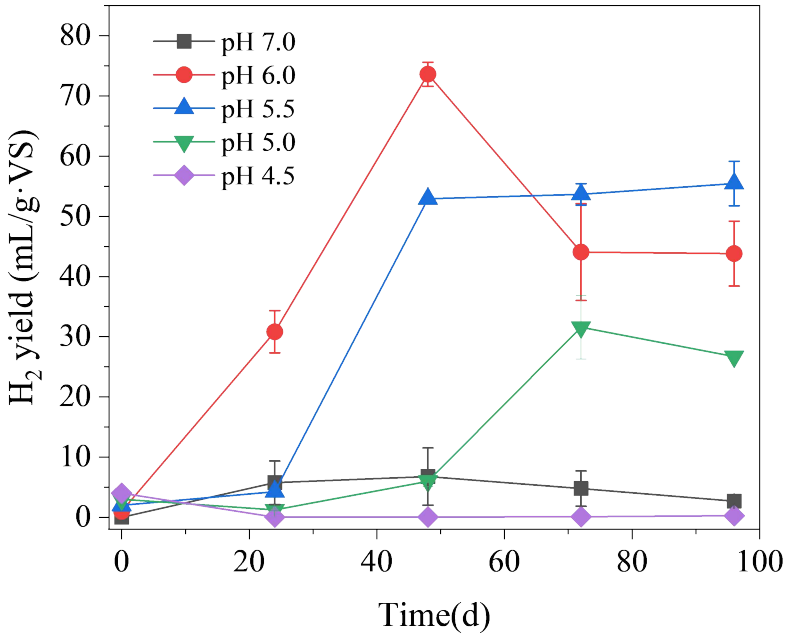


(b)

(c)



(d)



(e)

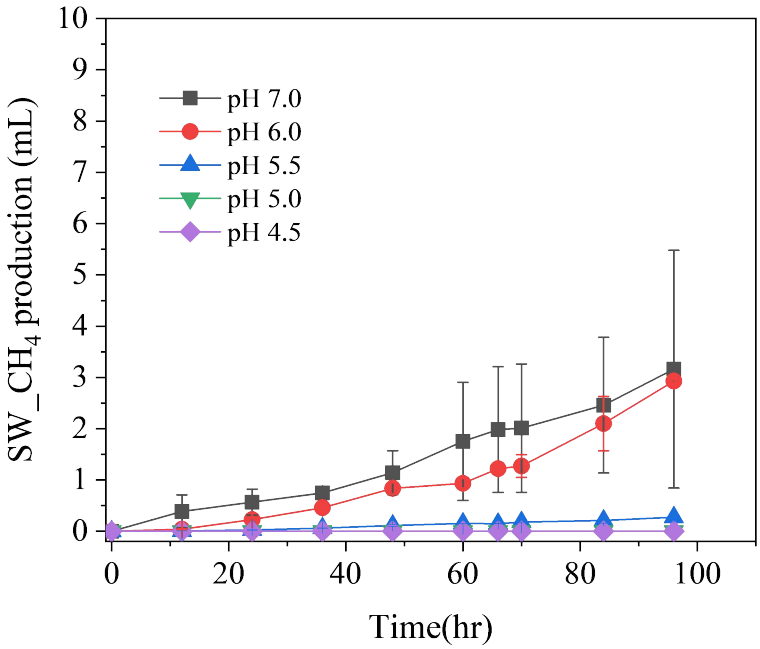


Figure 3-8. Time dependent changes of (a) pH value, (b) hydrogen production, (c) volu metric hydrogen production rate, (d) hydrogen yield, and (e) methane production with different pH values of SW.

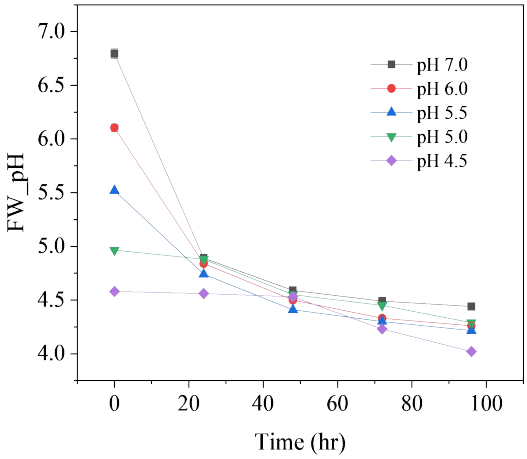
(2) Food Waste Leachate

Anaerobic acid fermentation was performed for about 96 hours by adjusting the pH in food waste leachate (FW). The initial pH was set at pH 7.0, pH 6.0, pH 5.5, pH 5.0, and pH 4.5 and the production of hydrogen and methane gas was measured during operation period.

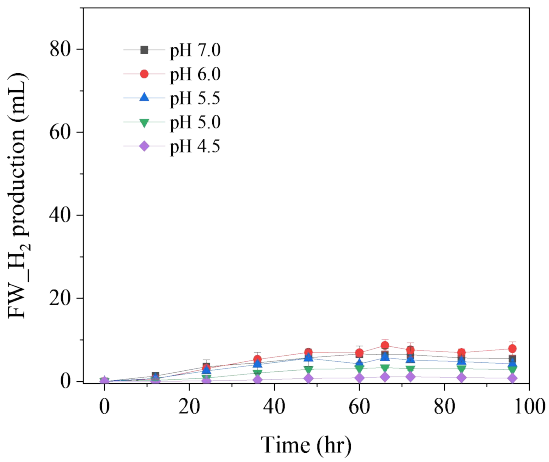
Figure 3-9 (a) shows the pH variation over time. After 24 hours of operation, a significant decrease in pH was observed from the initial pH values of 6.80, 6.11, 5.52, 4.97, and 4.58 to 4.89, 4.84, 4.74, 4.88 and 4.58, respectively. At the end of the 96 hour operation, the pH ranges from 4.00-4.40. The hydrogen production results at different pH value are shown in figure 3-9 (b). Up to 96hours, the reactor at pH 7 produced approximately 5.5±0.7mL of hydrogen, pH 6 produced 7.9±0.1mL, pH 5.5 produced 2.8±0.0mL, and pH 4.5 produced 0.8mL. Methane gas production was not observed.

Considering the differences in FW characteristics and the insufficient buffering capacity of FW, it is presumed that excessive acid fermentation led to pH reduction and microbial pathways shifted towards non-hydrogen-producing routes at excessively low pH levels. Previous studies on various FW utilization methods for hydrogen production have reported the use of acidogenic bacteria (*Clostridium* spp.) inoculation, alkaline pretreatment (김희영, 2022), pH adjustment to 8.0-9.0, and various pretreatment methods (이준표et al., 2017). It was challenging to expect hydrogen production using a single substrate without FW pretreatment in the pH range known to be suitable for acid fermentation, pH 5.5-6.0.

(a)



(b)



(c)

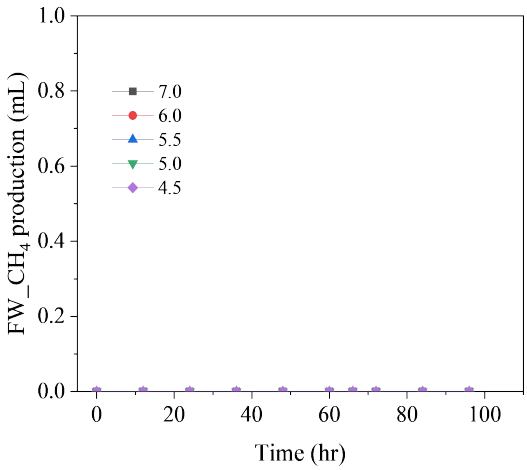


Figure 3-9. Changes of (a) pH value, (b) hydrogen production, and (c) methane production during operation time with different pH value of FW.

3.1.2 VFAs and Ethanol

Samples were collected every 24 hours during the anaerobic fermentation operation of swine wastewater (SW) with adjusted pH. The concentrations of volatile fatty acids (VFAs) and ethanol were analyzed for each VFAs composition were depicted in Figures 3-10 and 3-11.

From the initial VFA concentrations in each reactor, the concentration changes of VFA and ethanol were examined after the completion of the experiments (96hr). At pH 7.0, the recorded VFAs concentration ranged from 11.0 g/L to 16.43 g/L, at pH 6.0 ranged from 10.8 g/L to 17.0 g/L, at pH 5.5 ranged from 12.4 g/L to 14.1 g/L, at pH 5.0 ranged from 11.6 g/L to 13.6 g/L, and at pH 4.5 ranged from 12.6 g/L to 11.6 g/L. Except for pH 4.5, all ranges showed an increase in total VFAs concentration before and after the experiment. This can be attributed to the lack of fermentation activity due to the low pH at pH 4.5, which is evident from the absence of gas production in Figure 3-8 (b).

During the cultivation period, the ethanol concentration remained constant at 5.3-3.5 g/L in all reactors, indicating no significant increase or decrease in ethanol production or inhibition of hydrogen production caused by ethanol. At pH 6.0, which exhibited a high hydrogen yield, the total VFA concentration increased by more than 7.8 g/L compared to the initial concentration, while at pH 5.5, which recorded the maximum hydrogen production, the increase was only about 2 g/L.

When examining the composition ratios of VFAs during the cultivation period, acetic acid and butyric acid were the primary fermentation products, with propionic acid and caproic acid accounting for less than 20% of the composition. Over time, except for pH 7.0 and pH 4.5, a consistent pattern of acetic acid decrease, butyric acid increase, and caproic acid increase was observed. In pH 4.5, where metabolic activity was minimal, there was little variation in the composition of fermentation products over time.

The composition of VFAs at pH 6, where hydrogen production was rapid, showed a decrease from 39.6% to 33.0% in acetic acid and an increase from 27.1% to 35.5% in

butyric acid. Considering the maximum hydrogen production rate of 73.62±2.01 mL/g ·VS at pH 6 during anaerobic digestion, the increase in butyric acid content was deemed to be related to the enhanced activity of hydrogen-producing bacteria. Subsequently, from 48 hours, a decrease in hydrogen production accompanied by an increase in acetic acid and propionic acid was observed, suggesting that propionic acid consumption led to its increased proportion as it consumed hydrogen. A similar pattern of increased butyric acid ratio was observed when hydrogen production reached its maximum at pH 5.5 and pH 5.0, indicating a similar organic acid composition change.

Minor changes in propionic acid concentration were observed due to the production of methane gas at pH 7.0 and pH 6.0. At pH 7.0, propionic acid increased by approximately 1.8 times, from 1.15 g/L at 24 hours to 2.08 g/L at 96 hours, while at pH 6, it increased by 1.5 times, from 1.29.

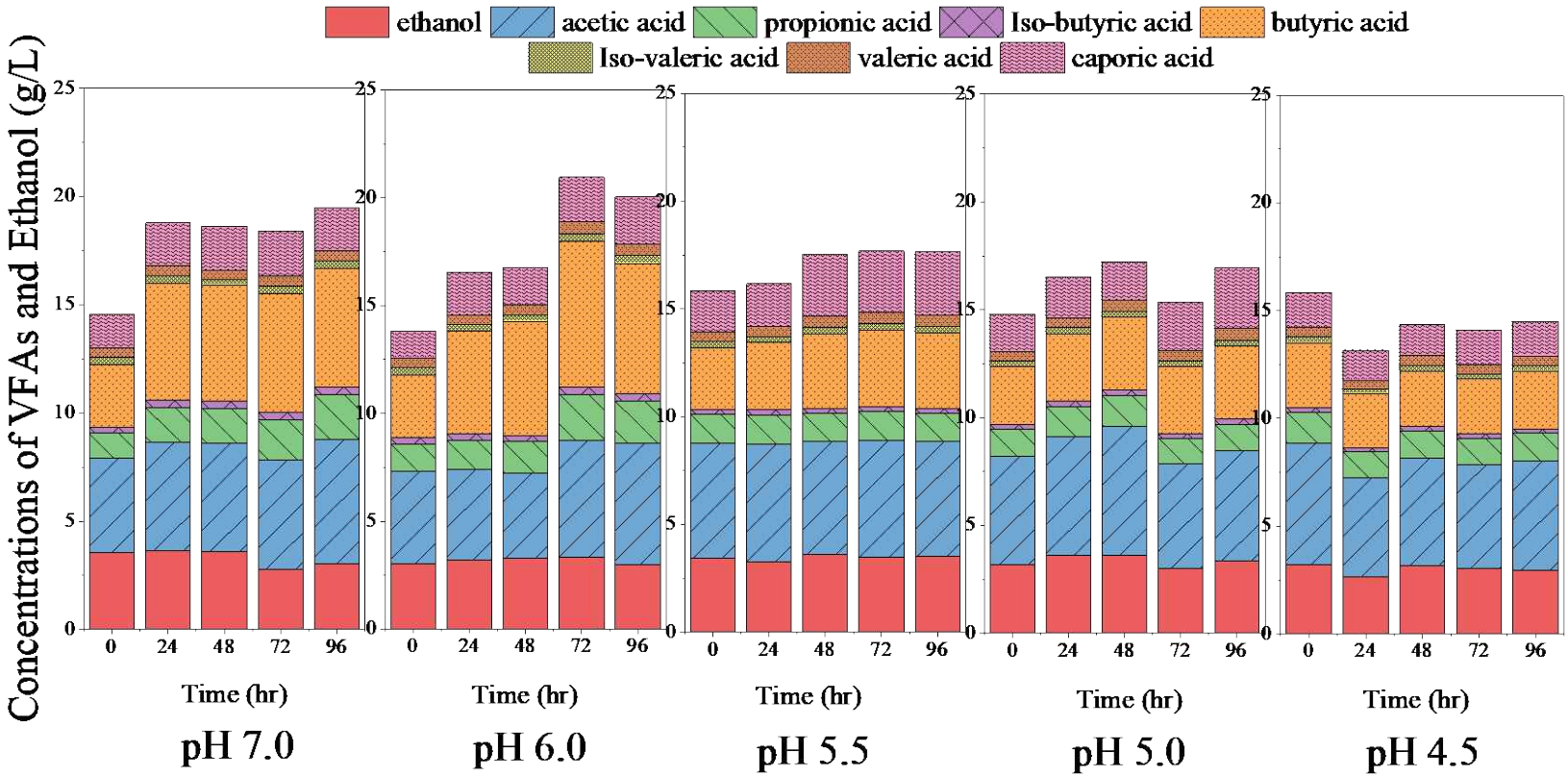


Figure 3-10. Concentrations of VFAs and ethanol after operation at different pH of SW.

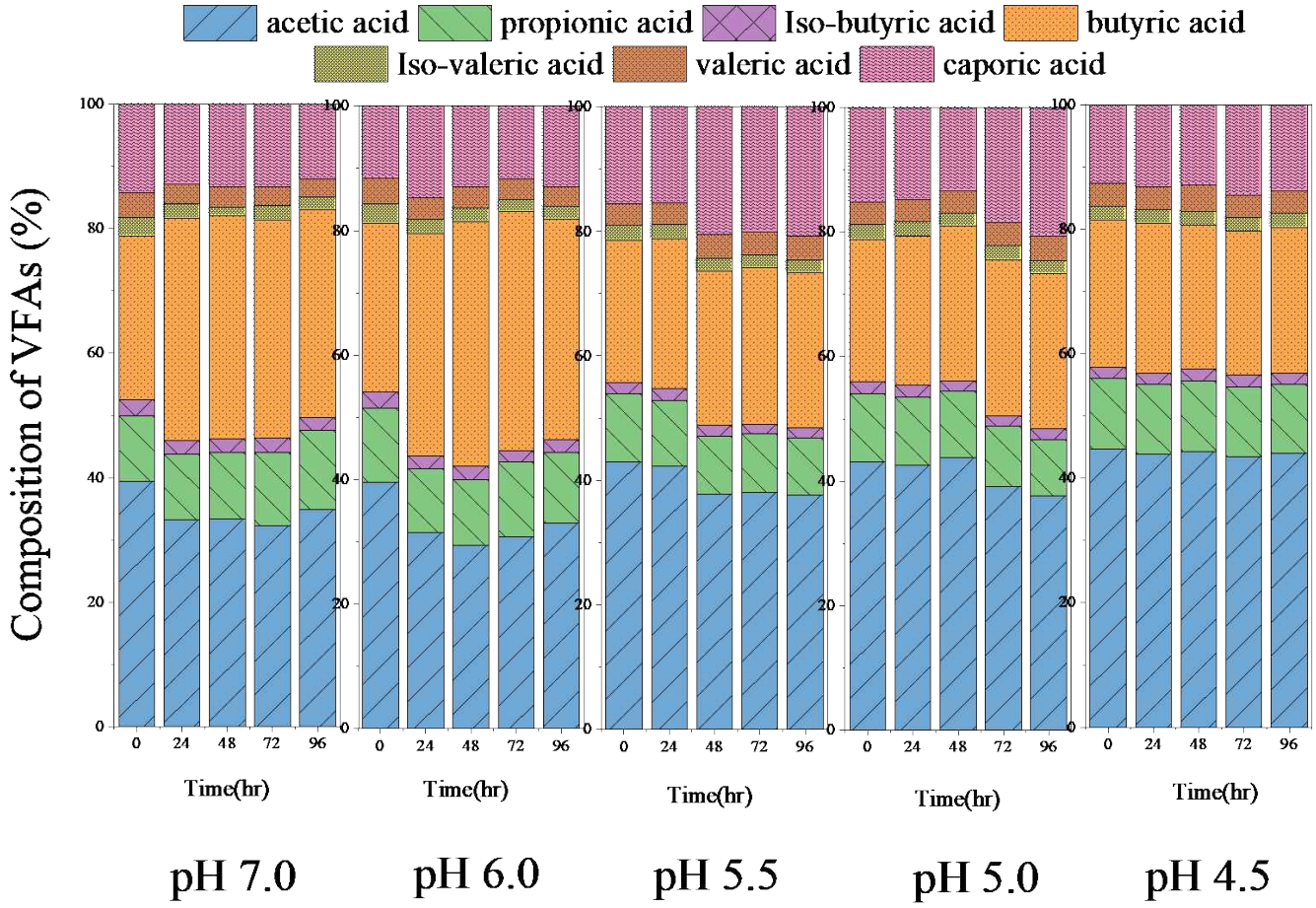


Figure 3-11. Composition of VFAs after operation at different pH of SW.

3.1.3 Microbial community

In the acid fermentation experiment using swine wastewater, observed microbial community changes in the settled sludge of pH 7.0, pH 6.0, pH 5.5, pH 5.0, and pH 4.5 after the end of operation (96 hours) and the acid fermenter sludge used in the experiment (Figure 3-12). In the phylum analysis, mainly *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* were detected, and more than 85% *Firmicute* dominance was observed in all conditions (Figure 3-12).

Firmicutes and *Bacteroidetes* are known for their role in the degradation of macromolecules such as proteins, fats, and polysaccharides into amino acids, fatty acids, and monosaccharides, as well as hydrolysis and acetic acid production during anaerobic digestion (Li et al., 2013; Yang et al., 2021). The results of Figure 3-12 suggest that the dominance of *Firmicutes* over 85% in all conditions contributed significantly to the organic matter degradation process during acid fermentation.

At the Ggenus level (Figure 3-13), identified *Clostridium*, *Anaerococcus*, *Terrisporobacter*, *Turicibacter*, *Bacteroides*, *Romboutsia*, *Peptoniphilaceae_uc*, *Peptostreptococcus*, and *Lactobacilus*, with a high percentage of *Clostridium* ranging from 26.38% to 62.45% in all conditions.

For the genus *Clostridium*, the proportion of hydrogen-producing bacteria was 60.5% at pH 5.5 and 45.24% at pH 6.0, where hydrogen production was fastest. This was followed by 62.45%, 62.01%, and 26.38% at pH 5.0, pH 4.5, and pH 7.0, respectively, while the percentage of *Clostridium* in the raw sludge used was 57.62%. The low percentage of 26.38% at pH 7.0, where no hydrogen was generated, and the highest percentage of 62.45% of *Clostridium* at pH 5.0, which had the slowest acid fermentation compared to the other conditions, could be attributed to the activity of the bacteria in generating hydrogen until the end of the experiment. The lower percentage of 45.24% at pH 6.0, which had the fastest acid fermentation, could be attributed to the consumption of hydrogen-producing bacteria by methanogenic bacteria due to methanogenesis.

Anaerococcus is known as an absolute anaerobic bacterium that produces butyric

acid, lactic acid, etc., by using peptone and amino acids as energy sources to decompose carbohydrate substances substances (Ezaki et al., 2001). As a result of genus level observation (Figure 4-13), was observed at pH 6.0 and pH 7.0, with a proportion was 11.39% at pH 6.0 and 13.88% at pH 7.0.

The redundancy analysis (RDA) of bacteria community at genus level related to environmental variables and hydrogen production and yield, is illustrate in Figure 3-14. Ammonia nitrogen showed a strong negative correlation with the genus *Clostridium* and *Lactobacillus* while hydrogen production rate had a positive correlation with *Clostridium* and *Lactobacillus*. It is likely responsible for the high hydrogen rate at pH of 5.5. the COD parameters had negative correlation with hydrogen production rate, which could be the reason why pH of 5.0 and 5.5 with relatively lower COD value accounted for the higher hydrogen production rate.

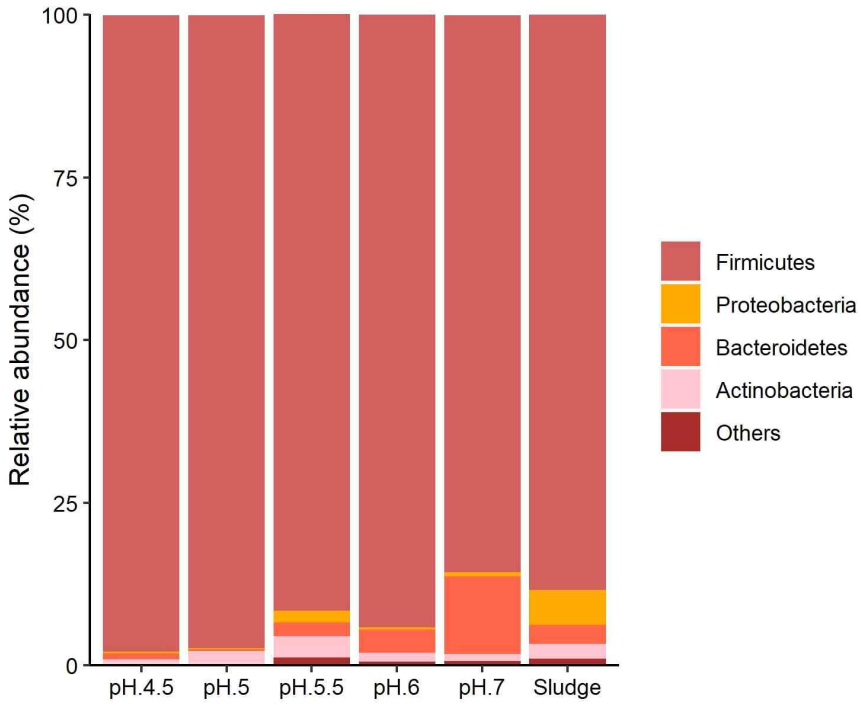


Figure 3-12. Relative abundance of microbial community in acid fermentation of SW in phylum level at different pH.

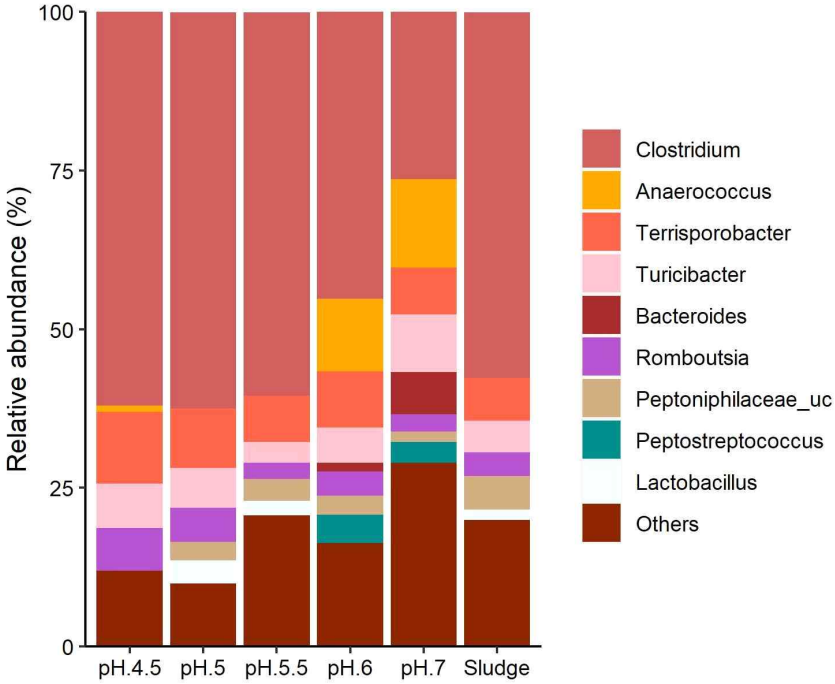


Figure 3-13. Relative abundance of microbial community in acid fermentation of SW in genus level at different pH.

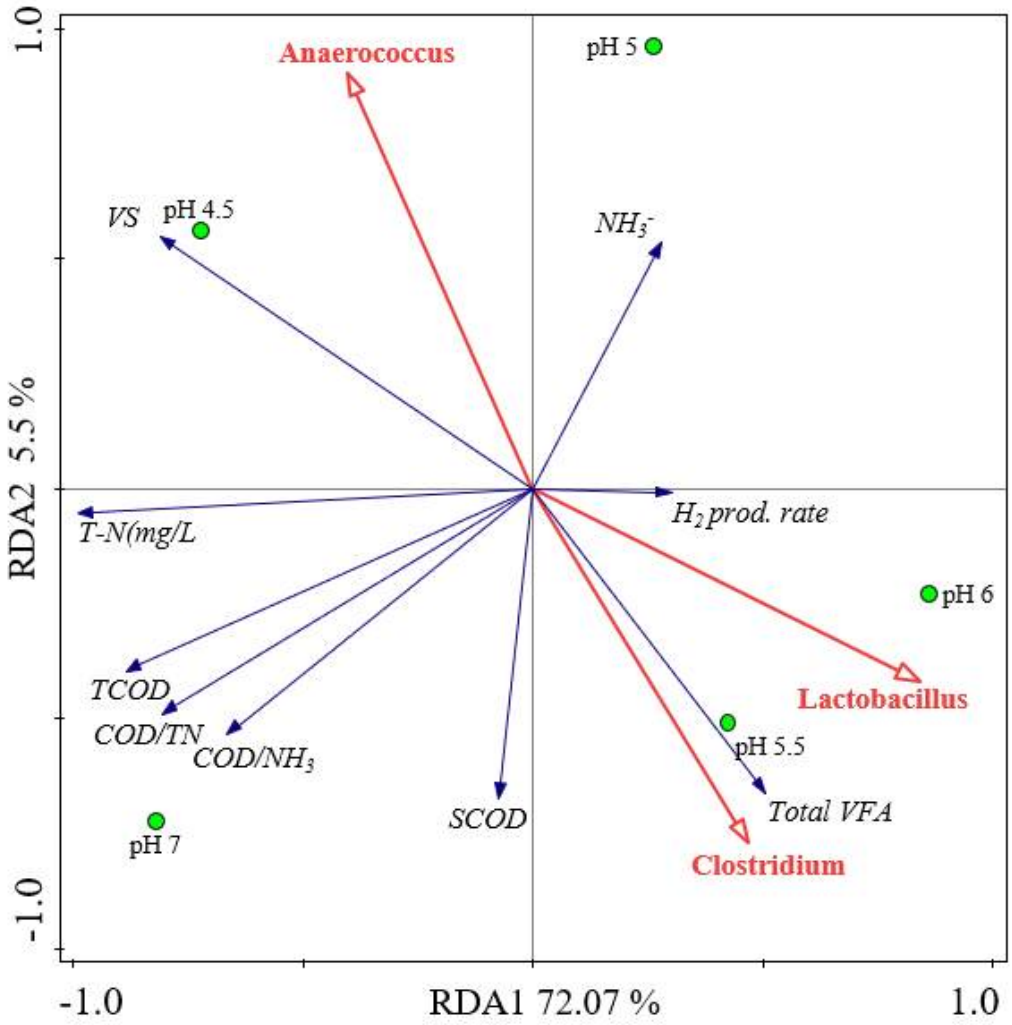


Figure 3-14. Redundancy analysis (RDA) of bacterial community at genus level related to environmental variable and hydrogen production and yield.

Chapter Ⅳ. Influence of Applied Voltage for Biohydrogen Production in Bioelectrochemical Anaerobic Digestion System (HP-BEAD)

1. Introduction

Increasing demand for energy derived from fossil fuels has caused a substantial increase in greenhouse gases in the environment. There is a need to develop renewable and eco-friendly alternative energy sources (Brar et al., 2022). The transition from a carbon economy to a hydrogen economy has led to a growing interest in biohydrogen, which emits no greenhouse gases. Additionally, a number of nations have begun promoting biohydrogen production as an alternative energy source due to its remarkable advantages, such as zero emissions from combustion, high calorific value, and variability of use (Sahrin et al., 2022).

Numerous technologies, such as water electrolysis, dark/photo fermentation, gasification, techniques based on microalgae, and microbial electrolysis cells (MECs), have been used for the generation of biohydrogen, with MEC being the most important due to its higher hydrogen yield (Rahimnejad, 2023). Moreover, hydrogen can be produced through the cathode by combining electrons and protons in the presence of a low applied voltage (>0.2V) in MEC (Cheng et al., 2022).

The MEC reactor can be designed with either a single or double chamber. In order to separate the anode and cathode in a double chamber MEC, ion exchange membranes (IEM) are typically employed. A Single chamber MEC can be operated without a membrane to decrease potential membrane loss and boost energy recovery in the process (Cheng et al., 2022). A single chamber MEC more straightforward design that cost less to construct. However, one drawback of this design is the microbial hydrogen loss to methanogens (Rahimnejad, 2023). In a study on single chamber MEC without a membrane conducted by Call and Logan et al., the maximum hydrogen production rate $3.12\pm0.02 m^3/H^2/m^3/d$ was observed when 0.8V was applied voltage using a graphite

fiber brush (Call & Logan, 2008). According to Lee et al., which involved a 32 hour experiment using acetate and an upflow single-chamber MEC, reported a cathode efficiency of 98±2% at converting coulombs to hydrogen, a coulombic efficiency of 60 ±1%, a hydrogen yield was 59.2 %, and the methane production was negligible (Lee et al., 2009).

Despite these studies, there are still many challenges that need to be addressed for the validation of methane-producing bacteria consuming hydrogen in a single-chamber reactor using complex substrates. These challenges include the tendency of methane-producing bacteria to consume hydrogen, the difficulty in controlling them, the economic feasibility of the reduction electrodes that constitute the reactor, and the need to secure power through external voltage application.

MEC operating under absence of oxygen conditions can be integrated with AD, an anaerobic process, which is called bioelectrochemical anaerobic digestion (BEAD). As improved anaerobic digestion process for organic waste, a new concept of anaerobic digestion when combined with bioelectrochemical technology, which includes an anode and a cathode inside the anaerobic digester, has recently received significant attention (Guo et al., 2013). BEAD reaction dependent on electrode potentials have been extensively studied to enhance biohydrogen production (HP-BEAD), electric energy storage in the form of methane, and biogas purity resulting from AD (Feng et al., 2016). However, the developed BEAD system primarily focuses on methane production (Carrillo-Peña et al., 2022; Xu et al., 2017). Research has primarily focused on optimizing methane production as the end product, given the relatively short duration of hydrogen generation before methane-producing bacteria consume it during the intermediate stages of AD. However, this can be overcome by pretreatment methods of the inoculum that inhibit methane-producing bacteria, with pretreatment methods such as thermal, alkaline, and other methods being utilized (Beegle & Borole, 2017; Hu et al., 2020).

The most critical factor in the HP-BEAD process is the applied voltage. Studies on the optimal applied voltage have yielded varying results across different research studies. In BEAD research focused on methane production, testing voltage ranges of 0.3-1.5V showed that higher applied voltages led to decrease in methane production,

with the best efficiency observed at 0.3V (Chen et al., 2016). Furthermore, it was found that the removal efficiency of COD and methane yield were optimized at 0.8V (Ding et al., 2016), experiment with a voltage of 0.9V applied to the BEAD (Hassanein et al., 2017). A wide range of voltages were used, but there were many opinions that excessive voltage inhibited microorganisms and reduced treatment efficiency (Chen et al., 2016).

In this study, we designed a reactor that combines AD integrated with MEC for hydrogen production in a bioelectrochemical anaerobic digestion system (HP-BEAD) to enhance hydrogen production by increasing the external electron transfer rate. Based on the research findings that operation is possible even at low voltages below 1.4V, we conducted various studies with different voltage range to determine the optimal voltage for driving HP-BEAD.

2. Materials and Methods

2.1 Substrate and inoculum

The wastewater used in this study was collected from the range specified in Table 3-1 of Chapter 3. As there are variations in the composition of wastewater inflow depending on the season and weather conditions at the anaerobic digestion pilot plant site, instead of manufacturing wastewater based on the optimal mixing ratio, the wastewater was mixed according to the optimal pH conditions (pH 5.5-6.0) based on previous hydrogen production experiments conducted at different initial pH values. The optimal mixing ratio of wastewater satisfy the pH conditions (pH 5.5-6.0) was 7.5:2.5 for swine wastewater to food waste, and the pH of the mixed wastewater was 6.10.

The sludge used in the study was obtained from the anaerobic digestion unit, and to ensure consistent microbial activity before usage, the sludge was subjected to a heat shock at approximately 90-100°C for about 15 minutes in a temperature-controlled water bath just prior to its use. This heat shock treatment aimed to equalize the initial microbial activity, considering the significant variations in microbial activity at the pilot plant site due to its operational period. The characteristics of the sludge are consistent with those presented in table 3-2 of chapter 3.

2.2 Reactor Setup and Operations

2.2.1 Applied Voltage for Biohydrogen Production in Bioelectrochemical Anaerobic Digestion (HP-BEAD)

A graphite felt electrode measuring 1.5cm × 1cm × 4cm (width × height × thickness) was utilized as the electrode material. The electrode was prepared by cutting it to the specified dimensions and then undergoing pretreatment with a 5mM sulfuric acid (H_2SO_4, 5mM) solution. It was stored in distilled water until the start of the experiment.

A 250 mL glass bottle was used as the reactor. To seal the reactor, a lid with holes was fitted with silicone and PTFE septa, and the electrodes were connected using platinum wire. Additionally, sample collection ports for wastewater and gas samples were made in the septa of the reactor lid. A wastewater sample collection line was connected at a position 5 cm above the bottom of the reactor, and a gas sample collection line, positioned at the top of the reactor where it was not in contact with the wastewater, was connected to a Tedlar gas bag to capture and collect the biogas samples.

A mixture of mixed wastewater (swine wastewater:food waste leachate = 7.5:2.5, pH 6.10) of 200mL and anaerobic digestion sludge of 50mL (substrate:seed sludge = 4:1) was inoculated. The electrodes within the reactor were positioned so that the anode and cathode were in contact with the side walls of the reactor as much as possible, and the distance between the electrodes was approximately 4cm. Furthermore, the distance between the reactor bottom and the electrodes was set at approximately 2cm in a vertical arrangement.

To enhance the electroactivity of hydrogen-producing microorganisms using the potential difference between the anode and cathode, a power supply device (RPS 305DU, Power supply, NDE) was connected to apply various external voltages. The following external voltage conditions were applied: a control group without electrodes and voltage (Control), a reaction group with only electrodes and no voltage (0V), and

reaction groups with applied voltages of 0.3V, 0.6V, 0.9V, and 1.2V. All conditions were subjected to batch-type experiments with an external resistance of 10Ω. The temperature was maintained at 30°C in a constant temperature chamber for 7days (168 hours) of operation (Figure 4-1).

Gas analysis (H_2, CH_4, CO_2) was conducted at intervals of 12 or 24 hours, and gas samples were collected and the biogas production was measured using Tedlar gas bags. Wastewater samples were also collected at 24-hour intervals, and the external voltage was monitored using a multimeter (DAQ 6510, Keithley).

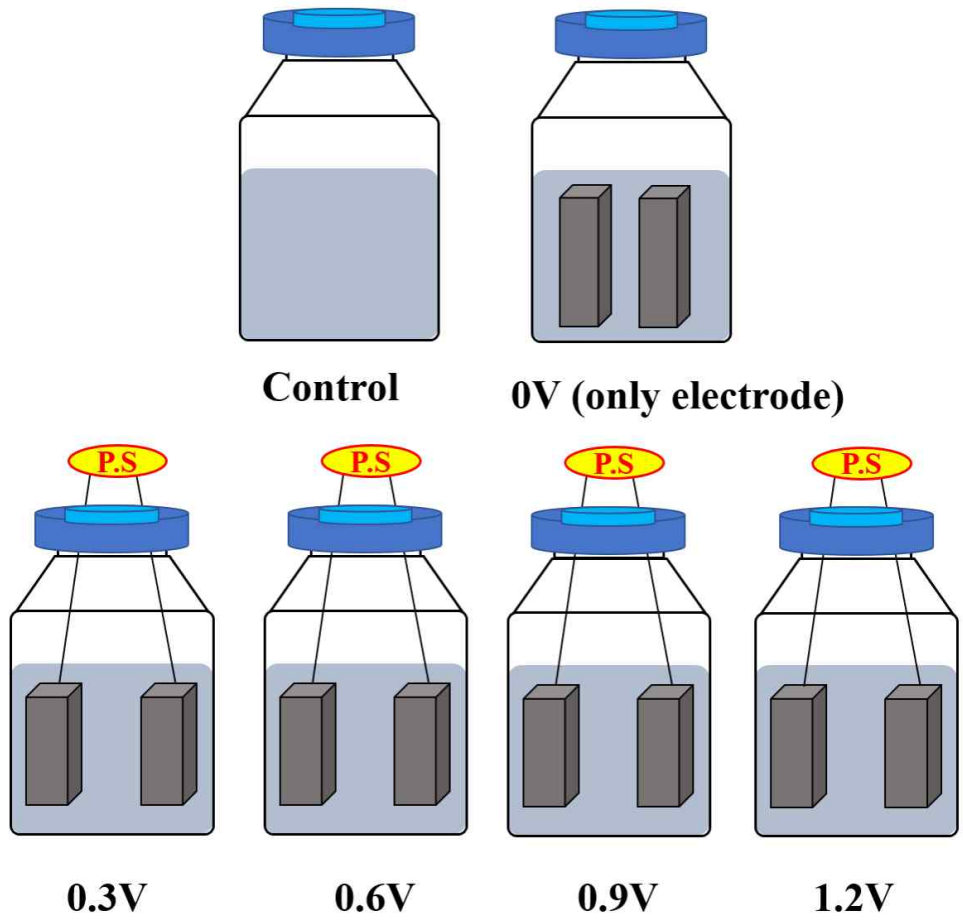


Figure 4-1. Schematic diagram of experimental reactors for various applied voltages of swine wastewater (SW) and food waste leachate (FW) mixture.

2.2.2 Impact of Electrode Pretreatment on Biohydrogen Production

In the HP-BEAD experiment, it was observed that microorganisms were hindered by the electrode. To evaluate the hydrogen production performance according to the electrode pretreatment methods, various pretreatment approaches were explored.

The reactor was operated under five different conditions: a control group, pretreatment with 5mM H_2SO_4, heat treatment, acetone treatment, and pH 6 of PBS treatment.

For the control group, a biological reactor without electrodes was used. In the case of the 5mM H_2SO_4 pretreatment, the electrode was cut to the specified dimensions, stored for about a day, and then rinsed with distilled water. For the heat treatment, the electrode was rinsed with distilled water and then heated in an oven at 450°C for 30 minutes before being dried. For the acetone treatment, the electrode was soaked in acetone for about 10 minutes to allow thorough penetration, rinsed with distilled water, and then dried at 55°C for 1 hour. For the PBS treatment, the electrode, which had been stored in 5mM H_2SO_4, was rinsed with deionized water. 1M phosphate buffer solutions, using Na_2HPO_4 and KH_2PO_4, with different pH values were prepared. The pH was adjusted to the desired pH of 6.0, and trace minerals and basal medium were added. The electrode was stored for approximately 30 minutes before conducting the experiment. These procedures were followed to evaluate the hydrogen production performance according to the electrode pretreatment methods. A simple schematic diagram of electrode pretreatment method is shown in Figure 4-2.

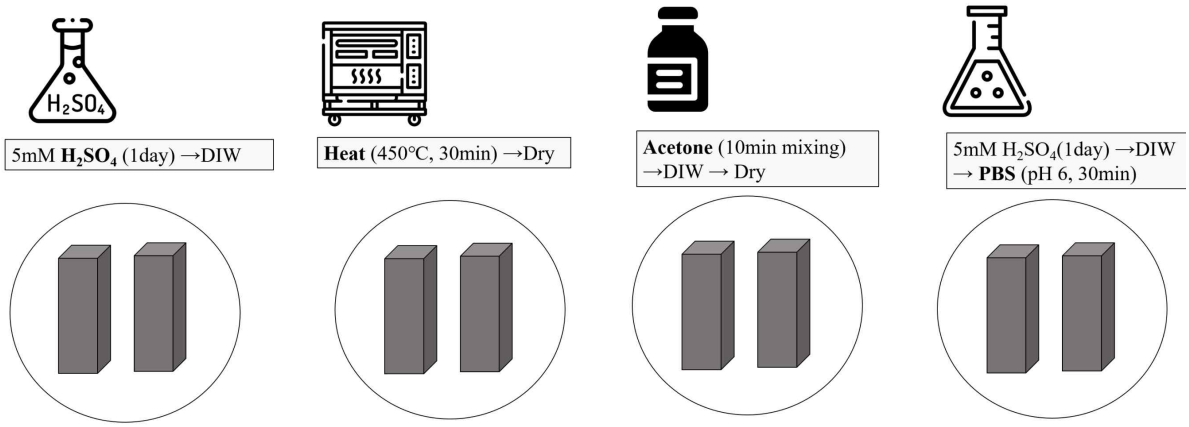


Figure 4-2. A simple schematic diagram of electrode pretreatment methods.

2.3 Analytical Methods

2.3.1 Modified Gompertz Model

This modified Gompertz model was used to describe the development of cumulative hydrogen production in batch experiment:

$$H = p \cdot exp\left\{-\exp\left[\frac{R_m}{p}(\lambda - t) + 1\right]\right\}$$

Where H is cumulative hydrogen production (mL), R_m is hydrogen production rate (mL/hour), p is maximum hydrogen , λ is the lag phase (hour), t is the time and e is exp (1) =2.718.

3. Results and discussion

3.1 Applied Voltage for Biohydrogen Production in Bioelectrochemical Anaerobic Digestion (HP-BEAD)

3.1.1 Hydrogen production

The applied voltage across the anode and cathode in a bioelectrochemical anaerobic digestion (BEAD) is a significant factor in determining the electrical potentials of the electrodes (Feng et al., 2016). We observed anaerobic acid fermentation under various applied voltages (0.3V, 0.6V, 0.9V, and 1.2V) for approximately 168 hours. Figure 4-3 (a) illustrates the pH variation over time, while Figure 4-3 (b) represents the volumetric hydrogen production rate (VHPR, mL/L-d). The experiment was conducted using six different conditions: a control reactor (as a biological reactor), a 0V reactor (with only an electrode inserted), and reactors with applied voltages of 0.3V, 0.6V, 0.9V, and 1.2V.

In Figure 4-3 (a), the pH changes were similar across all reactors over the 168 day operation. The pH initially ranged from 6.00 to 6.16 and gradually decreased to approximately 5.6-5.8 after 48 hours. Subsequently, it increased to around pH 6.5. This pH fluctuation suggests that the pH changes were primarily caused by the acidogenic fermentation rather than the applied voltage. The pH decrease shortly after hydrogen production commenced, indicating a delayed start of hydrogen production, and pH decrease estimated to the extended lag phase associated with sporulation of *Clostridium* spp. induced by thermal treatment.

Regarding the VHPR analysis in Figure 4-3 (b), hydrogen production occurred in all reactors, but the production rates varied with the applied voltage. The control and 0.3V reactors showed the fastest increase in VHPR from the 48-hour, reaching 29.0±20.9 mL/L-d and 15.7±8.6 mL/L-d, respectively. At 96 hours, the control reactor exhibited the highest maximum production rate of 289.4±15.6 mL/L-d, followed by the 0.3V

reactor with 368.0±68.1 mL/L-d. The VHPR of 0.3V was 27.3% higher than that of the control. The corresponding pH values were recorded as control pH 6.65±0.08 and 0.3V pH 6.62±0.04 (Figure 4-3 (a) and (b)).

In contrast, hydrogen production in the other reactors (0V, 0.6V, 0.9V, and 1.2V) began around 72 hours. The 0V reactor, which did not receive applied voltage but only had the electrode inserted, recorded a VHPR of 254.5±145.1 mL/L-d at 96 hours. The VHPR in the 0.6V, 0.9V, and 1.2V reactors increased at a slower rate, reaching the maximum rate at 120 hours. Among these three conditions, 0.6V (231.7±25.0 mL/L-d) exhibited higher efficiency than 0.9V and 1.2V, which showed similar values. This contradicts the prediction that higher applied voltages would result in increased hydrogen production due to enhanced activity of the electroactive microbial consortium. Instead, it suggests that the applied voltages may have inhibited the activity of the consortium, and the observed activity at 0.3V could be attributed to the activation of electrochemically active bacteria (EAB) communities even at very low voltage levels. This result is similar to the Feng et al, in which the efficiency of methane production was high under the low applied voltage condition of 0.3V when driving the BEAD system with a various range of voltages. (Feng et al., 2016).

Figure 4-4 and Table 4-1 present the predicted cumulative hydrogen production based on the modified Gompertz model. The obtained R^2 values, 0.995, 0.997, 0.996, 0.985, 0.993, and 0.996 for control, 0V, 0.3V, 0.6V, 0.9V, and 1.2V, respectively, indicate reliable results. Comparing the cumulative hydrogen production potential and the maximum hydrogen production rate obtained from the Gompertz function, the highest cumulative hydrogen production of 695.28±12.9 mL was observed at 0.3V, which is a 63.4% and 78.0% increase compared to control (425.60±7.15 mL) and 0V (390.57±5.8 mL), respectively. Additionally, the highest maximum production rate of 14.88±1.4 mL/hr was recorded at 0.3V (Figure 4-4, Table 4-1).

In accordance with the VHPR changes depicted in Figure 4-3 (b), the cumulative hydrogen production occurred in the following order: 0.3V, control, 0V, 0.6V, 0.9V, and 1.2V. Moreover, the lag phase was the shortest at 0.3V (59.80±1.4hr) and control (52.23±1.5hr), while 0.9V, 1.2V, and 0.6V started metabolism after 73 hours. This indicates that higher applied voltages prolonged the induction period of microbial

consortia. Methane gas production, excluding hydrogen gas, was not observed during the 0 to 180-hour period.

Figure 4-5 illustrated hydrogen yield (mL/g·VS), the highest yield was recorded at 96 hours in the 0.3V reactor (35.04±5.35 mL/g·VS), followed by the control reactor (31.63±1.87 mL/g·VS) and the 0V reactor (23.95±7.54 mL/g·VS). The yields increased at 120 hours in the 0.6V, 0.9V, and 1.2V reactors (Figure 4-5).

In conclusion, the evaluation of hydrogen production in anaerobic digestion with the integration of a bioelectrochemical system (HP-BEAD) showed that even a low applied voltage of 0.3V could achieve highly efficient hydrogen production, surpassing the performance of a simple fermentation process in the control and 0V reactors. When comparing hydrogen production between the control and 0V reactors, the 0V reactor exhibited 13.7% lower efficiency. This suggests that the electrode material, graphite felt, may have lower internal mass transfer capabilities, or the pretreatment method of the electrode may have resulted in inhibition of the microbial consortium. Further evaluations with different types of bioelectrodes and exploration of various pretreatment methods are necessary to better understand the hydrogen production process.

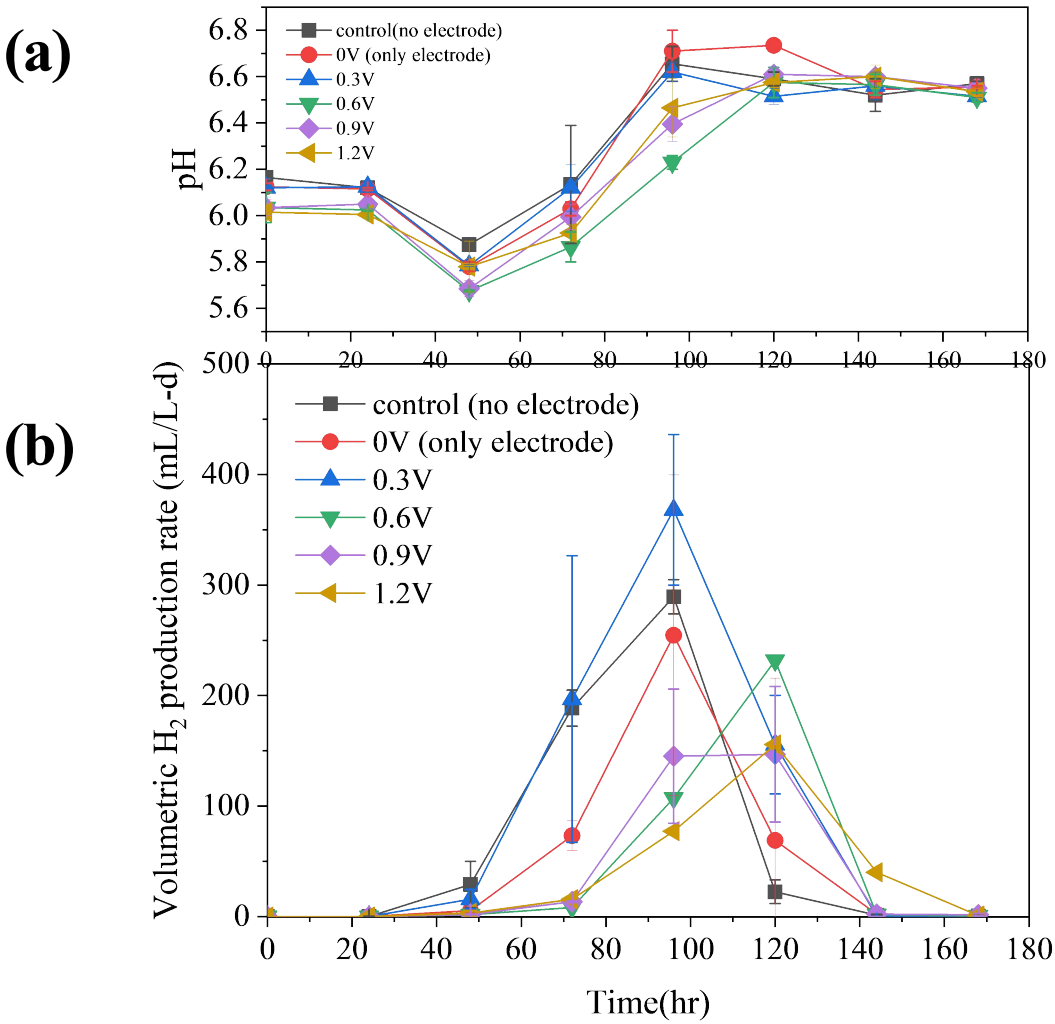


Figure 4-3, a) pH value and b) volumetric hydrogen production rate during operation at different applied voltages.

Table 4-1. Cumulative hydrogen production and model parameters estimated from modified Gompertz model at different applied voltage.

	P (mL)	R_m(mL/h)	λ(h)	R^2
Control	425.60±7.2	11.42±0.8	52.24±1.5	0.995
0V	390.57±5.9	9.12±0.5	62.15±1.2	0.997
0.3V	695.28±12.3	14.88±0.9	59.80±1.4	0.996
0.6V	383.69±16.3	9.45±1.3	83.38±2.7	0.985
0.9V	301.17±7.2	9.56±0.9	77.54±1.6	0.993
1.2V	371.20±8.5	7.23±0.4	73.22±1.5	0.996

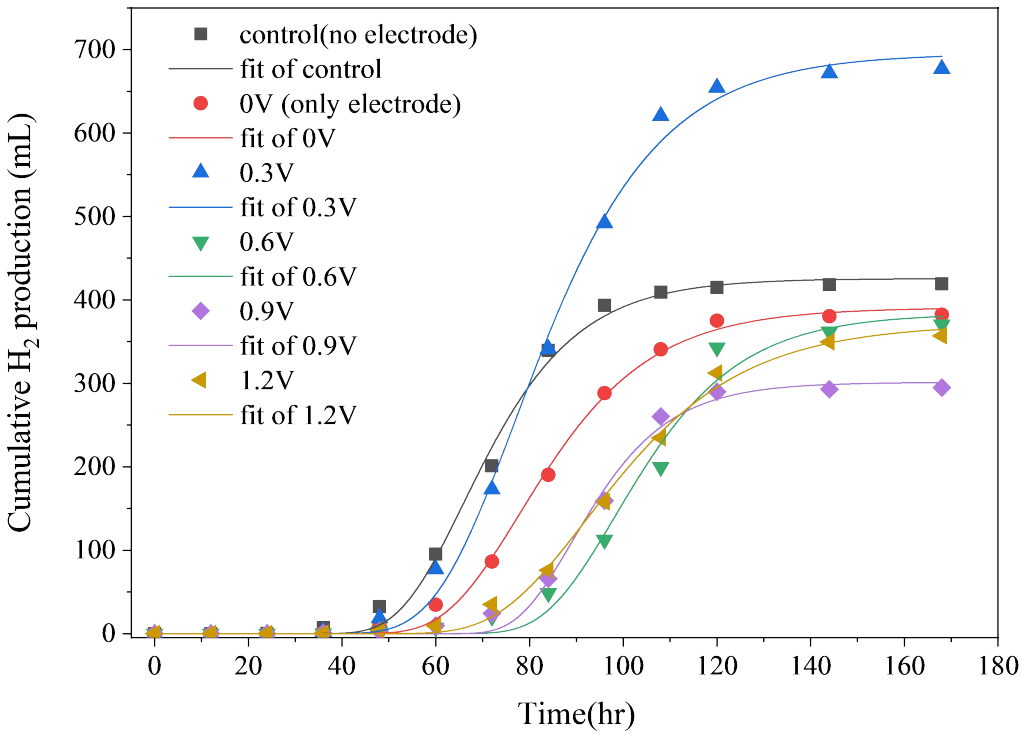


Figure 4-4. Cumulative hydrogen production at different applied voltages during operation (0h-168h). with modified Gompertz model.

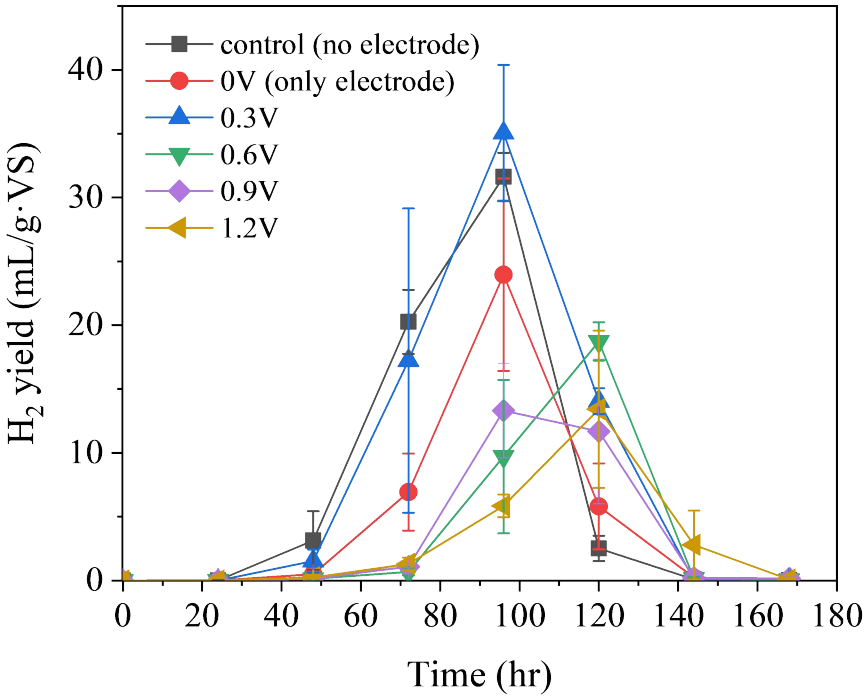


Figure 4-5. Hydrogen yield at different applied voltages during operation.

3.1.2 TCOD and VS removal efficiency

Figure 4-6 illustrates the TCOD concentration (mg/L) and removal efficiency (%) before and after operation. The initial TCOD concentration remained constant at 32-35 g/L, while the recorded concentration after operation completion was 28-29 g/L. It was observed that in all reactors, the TCOD concentration decreased compared to the initial concentration after operation, and the removal efficiencies were as follows: 10.73±5.7% for the control, 18.0±0.5% for 0V, 18.82±2.5% for 0.3V, 19.40±3.3% for 0.6V, 20.76±0.62% for 0.9V, and 15.58±2.1% for 1.2V. The control reactor exhibited the lowest TCOD removal rate, while the highest removal rate was observed at 0.9V. However, no significant TCOD removal effect was observed in any of the reactors.

Figure 4-7 presents the analysis results of TS and VS removal rates. The TS removal rates were highest in the order of 0.6V, 0.9V, control, 1.2V, 0.3V, and 0V, with the highest value of 39.23±15.58% recorded at 0.6V. The VS removal rates showed successful removal in the order of 0.9V, 1.2V, 0.6V, control, 0.3V, and 0V, with a VS removal rate of 40.02±7.07% recorded at 0.9V. The significant decomposition of organic matter under conditions of higher applied voltages suggests that electrolysis at relatively high voltage accelerates the hydrolysis rate.

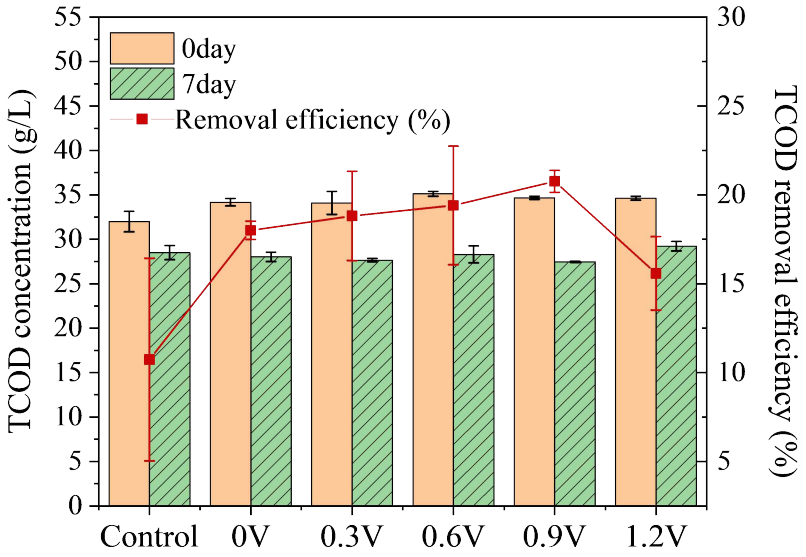


Figure 4-6. TCOD concentration and removal efficiency at different applied voltages

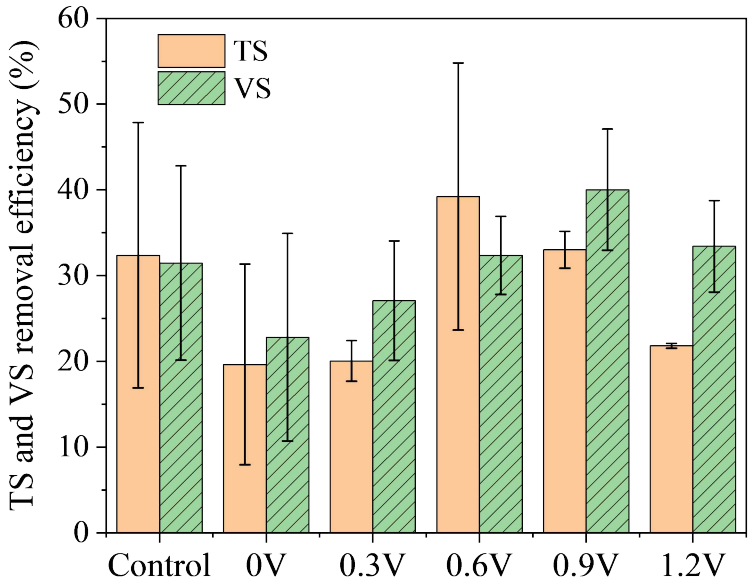


Figure 4-7. TS and VS removal efficiency at different applied voltages during operation.

3.1.3 VFAs and Ethanol

In Figure 4-8 and Figure 4-9, the concentrations of VFAs and ethanol before (0hr) and after (168hr) operation were presented for reactors with various applied voltages. Initially, all six reactors recorded a range of 9,239 mg/L to 10,121 mg/L for total VFAs and ethanol concentrations. After operation, a constant increase in VFAs and ethanol was observed in all reactors, with concentrations ranging from 11,756 mg/L to 12,435 mg/L. The percentage increase compared to the initial concentrations was 31.6% for the control, 20.1% for 0V, 25.4% for 0.3V, 17.6% for 0.6V, and 30.4% for 1.2V. The control and 1.2V reactors showed larger increases, while 0.6V exhibited a slower increase.

Examining the individual concentrations (Figure 4-8), ethanol concentrations initially ranged from 1,457 mg/L to 1,932 mg/L and increased to 1,844 mg/L to 2,058 mg/L after operation, without a significant increase observed. At the beginning of the experiment (0hr), acetic acid recorded concentrations of 4,843 mg/L to 5,423 mg/L, while butyric acid was in the range of 686 mg/L to 797 mg/L. However, after the operation, a sharp increase in butyric acid concentration to 3,196mg/L to 3,385mg/L was observed, while no significant changes were observed for other VFAs.

Analyzing the VFA composition (Figure 4-9), acetic acid and butyric acid were the major fermentation products after operation. Butyric acid, which accounted for 9.17% to 9.49% in all reactors, showed a consistent increase to 32% to 33.5% after the operation, while the proportion of acetic acid decreased from 64.16% to 64.93% initially to 45.11% to 45.52% after operation. It has been reported that the B/A ratio in hydrogen fermentation is proportional to hydrogen production (Fang & Liu, 2002), suggesting that a relatively higher production of butyrate is favorable for hydrogen generation. The observed increase in butyric acid, which was a prominent fermentation product in reactors with active hydrogen production as discussed in chapter 3, suggests successful acid fermentation in all reactors, without significant differences in the major fermentation products based on the applied voltage range.

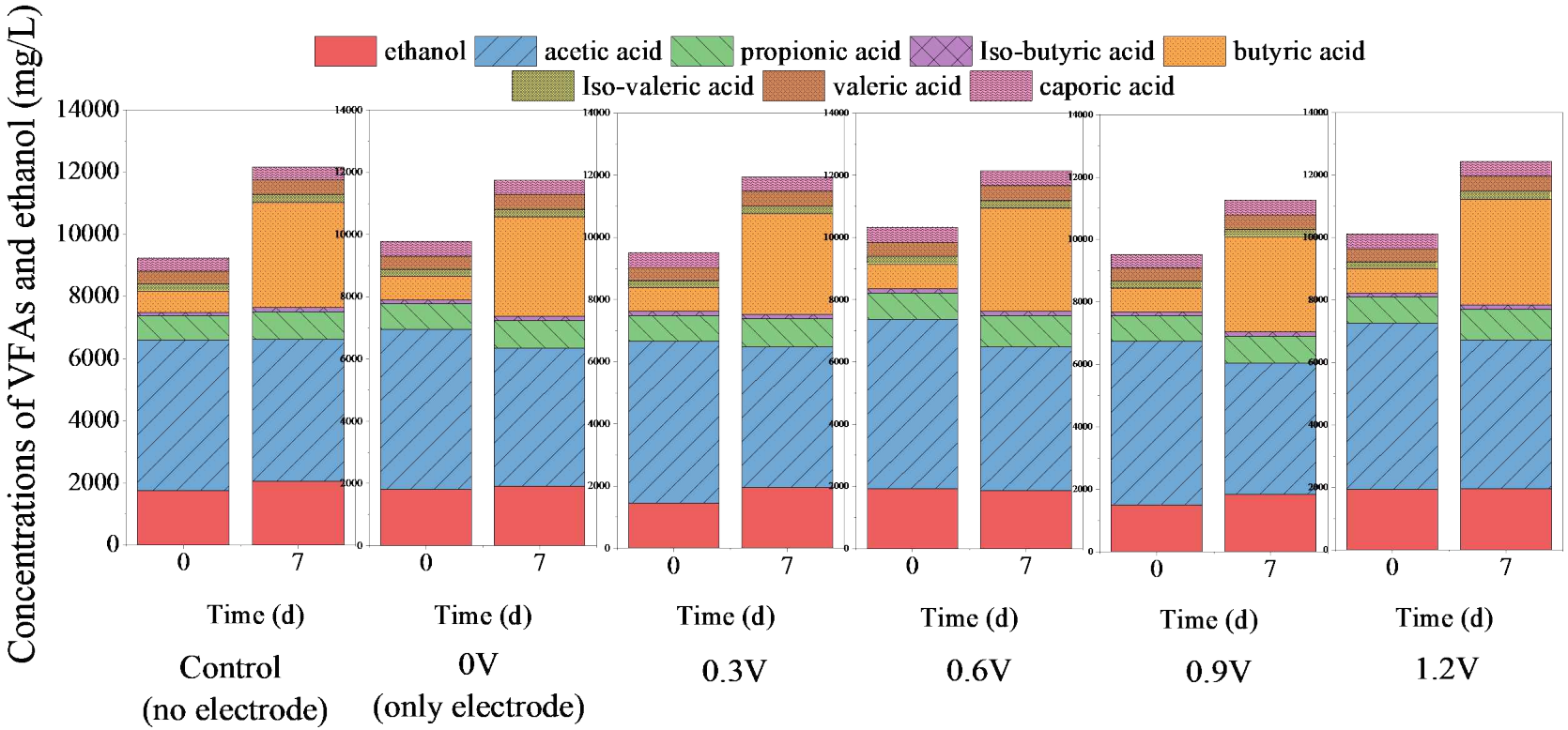


Figure 4-8. Concentration of VFAs and ethanol (mg/L) at different applied voltages during operation.

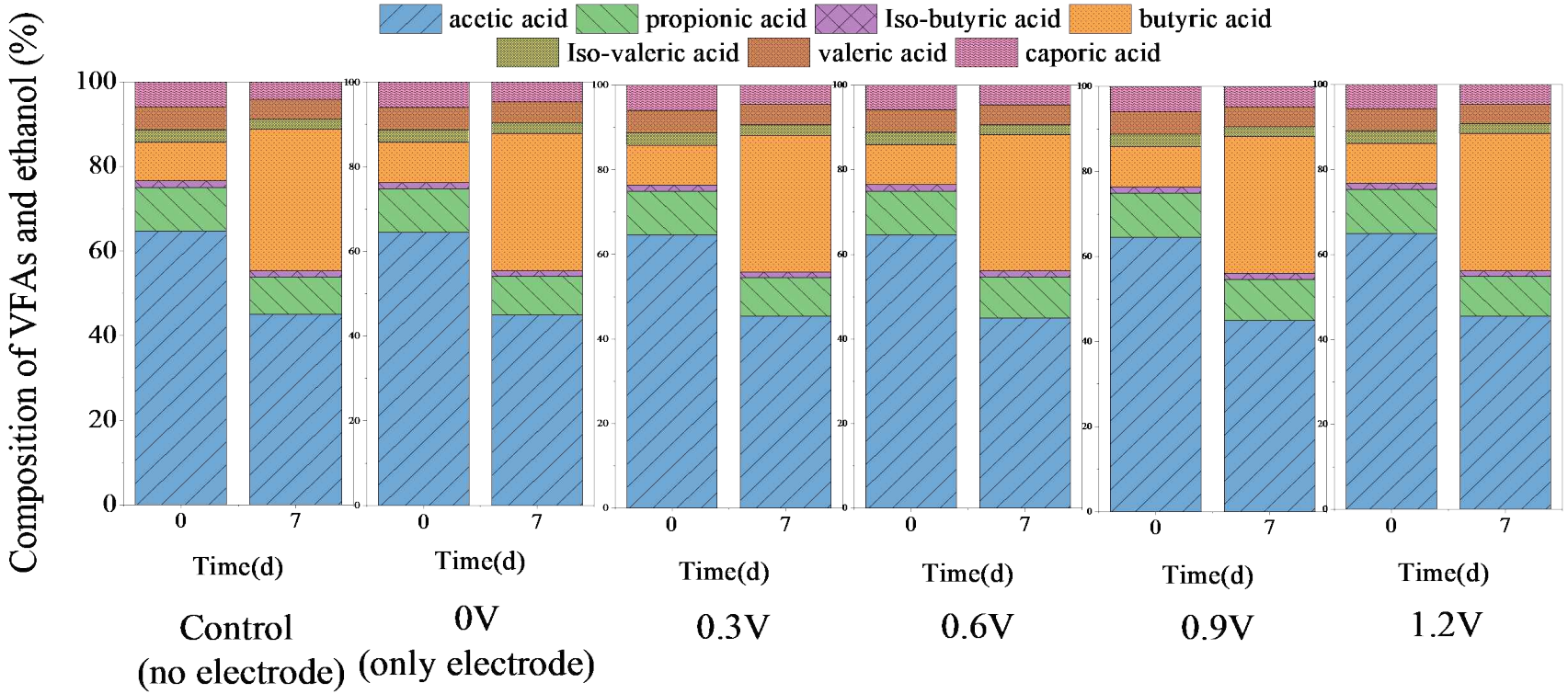


Figure 4-9. Composition of VFAs at different applied voltages during operation.

3.2 Impact of Electrode Pretreatment on Biohydrogen Production

3.2.1 Hydrogen production

In order to find the optimal electrode pretreatment method for the application of hydrogen production-bioelectrochemical anaerobic digestion (HP-BEAD), various pretreatment methods were conducted, including heating, 5mM H_2SO_4, acetone, and PBS (with 5mM H_2SO_4), for anaerobic acid fermentation experiments. The operation was carried out in batch mode for approximately 0 to 168 hours, and Figure 4-10 (a) illustr ates the pH changes, figure 4-10 (b) and table 4-2 cumulative hydrogen production (mL) with modified Gompertz model, and figure 4-11, indicates the hydrogen production yield (mL/g·VS).

Regarding the pH changes (a), all reactors exhibited an initial pH of 6.0, followed by a sharp decrease to pH 5.19-5.23 in all reactors after 24 hours of operation. The pH was maintained at 5.12-5.18 until 96 hours, and then a significant increase to approximately 5.50-5.95 was observed at 144 hours in all reactors. In contrast to the results in chapter 3 where no heat pretreatment was applied, the pH increased shortly after the beginning of hydrogen production, sililar to the pH change results in the sludge heat pretreatment experiment (Figure 4-3). This suggests that the prolonged lag phase was a result of the heat shock from the sludge pretreatment.

Regarding figure 4-10 (b) and Table 4-2, the cumulative hydrogen production predicted by the modified Gompertz model was 437.22 ±6.1mL in PBS, which was a 43% and 38% increase compared to the control and acetone reactors, respectively, and a 137% improvement compared to the 5mM H_2SO_4 pretreatment method used in previous applied voltage experiments.

Comparing the cumulative hydrogen production between the 5mM H_2SO_4 pretreatment method used in previous experiments, the control without electrodes, and PBS, the control recorded 304.83±7.6mL, while the 5mM H_2SO_4 pretreatment recorded 184.04±5.03mL. This clearly indicates that the H_2SO_4 used in the pretreatment method negatively affected the microbial cell activity.

Regarding the hydrogen production rate (mL/hr) of the R_m(mL/hr) parameter in Gompertz model, acetone exhibited the highest production rate, followed by PBS, heat, control, and H_2SO_4.

The estimated lag phase showed consistent values of 122-124 hours for control, H_2SO_4, heat, and acetone, while PBS exhibited a lag phase of approximately 112.45±0.9 hours, which was approximately 10 hours shorter than the other methods.

The hydrogen production yield (mL/g·VS) also showed that PBS achieve the highest yield, recording 100.66±46.10 mL/g·VS at 144 hours. Acetone followed with a yield of 96.07±14.90mL/g·VS, and control exhibited a high yield of 79.27±12.05mL/g·VS. On the other hand, heat and H_2SO_4 methods recorded lower values than the control, indicating that PBS and acetone pretreatment methods are more favorable for enhancing hydrogen production compared to H_2SO_4 pretreatment (Figure 4-11).

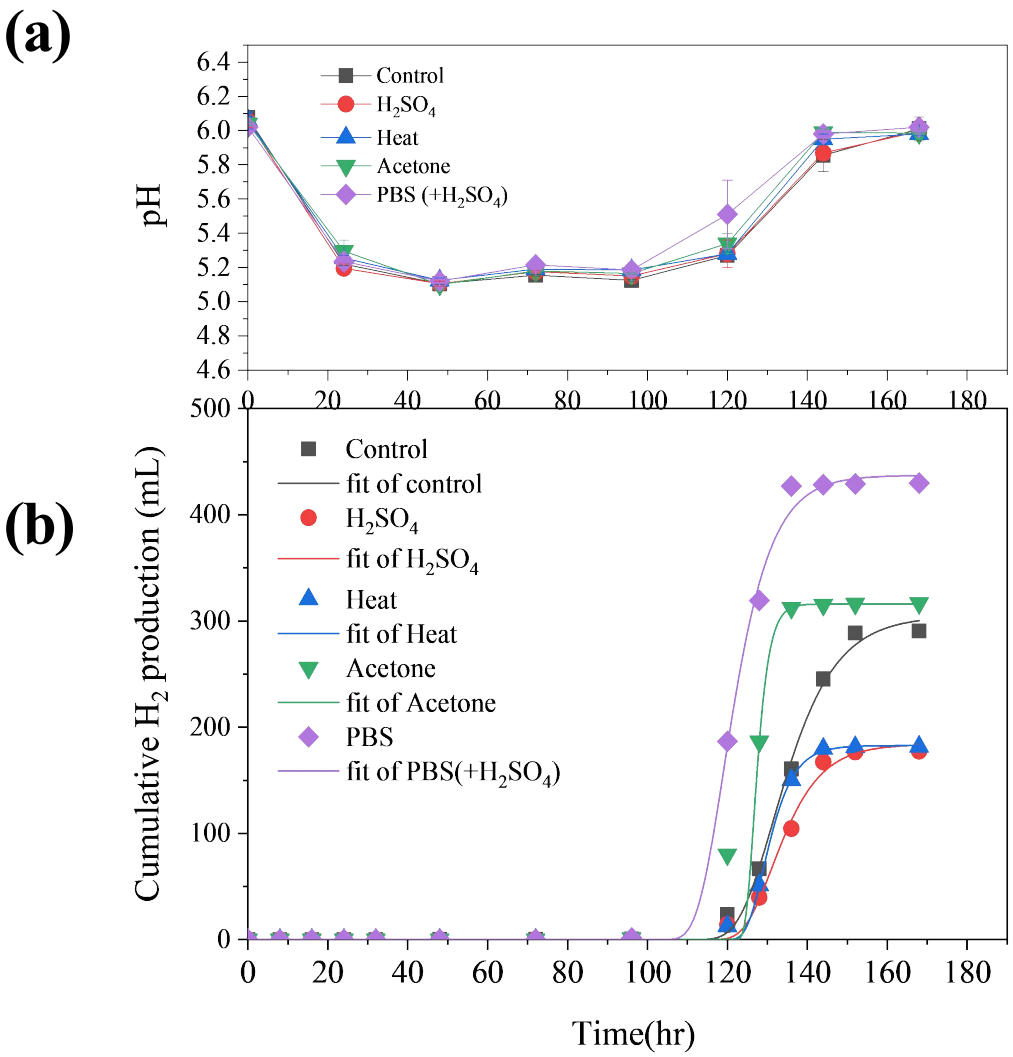


Figure 4-10. a) pH value and b) cumulative hydrogen production at different pretreatment methods during operation period.

Table 4-2. Cumulative hydrogen production and model parameters estimated from modified Gompertz model at different pretreatment methods.

	P (mL)	R_m(mL/h)	λ(h)	R^2
Control	304.83±7.6	12.93±0.9	122.80±0.8	0.996
Heat	184.04±5.0	9.78±0.9	124.21±0.9	0.994
5mM H_2SO_4	182.76±2.5	15.88±1.35	124.79±0.4	0.997
Acetone	316.10±14.1	55.06±114.85	124.53±6.7	0.971
PBS	437.20±6.0	24.13±2.1	112.45±0.9	0.997

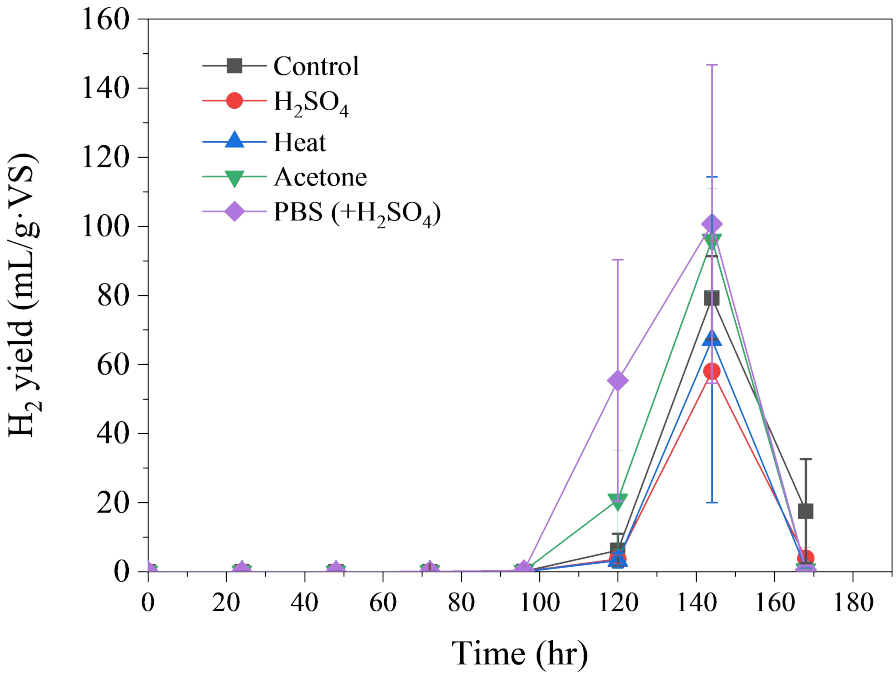


Figure 4-11. Hydrogen yield(mL/g·VS) at different pretreatment methods during operation period (0h-168h).

Chapter Ⅴ. Integrating Anaerobic Digestion and Bioelectrochemical System for Biohydrogen Production (HP-BEAD) in Continuous Stirred Tank Reactor

1. Introduction

The need for energy conversion from the composting and liquefaction methods, which are associated with problems such as decreased farmland availability and excessive fertilizer application, necessitates the adoption of biogasification for livestock manure, which accounts for a significant proportion of domestic organic waste. The government acknowledges this and is establishing measures to expand the utilization of biogas energy. In 2023, the biogasification method utilizing organic waste will be implemented, and the construction of biogasification plants is planned to be expanded. However, the current focus is primarily on methane gas generation within the biogas, but if anaerobic digestion processes, specifically acidogenesis, are utilized, it offers the advantage of simultaneous production of hydrogen gas alongside methane gas from organic waste. This enables biological hydrogen production without greenhouse gas emissions, in contrast to conventional hydrogen production methods that utilize hydrocarbons, which result in greenhouse gas emissions.

Research on continuous biological hydrogen production through successive substrate feeding has been reported, including studies on continuous operation using mixed cultures. However, there is currently no research on continuous hydrogen production using an electrochemical system. Based on the findings of continuous operation studies, the optimal conditions for hydrogen production have been reported as temperatures of 30-36°C, pH of 5.0-6.0, and hydraulic retention time within 12 hours.

In this study, we determined the factors to consider for biological hydrogen production based on previous experiments and evaluated hydrogen production efficiency

in a batch system by introducing a bioelectrochemical system. We aim to compare and evaluate the hydrogen production performance of a continuous substrate (organic waste) feeding bioelectrochemical acidogenesis system with a biological reactor, and propose strategies for achieving high-efficiency hydrogen production.

2. Materials and Methods

2.1 Substrate and Inoculum

The characteristics of the swine wastewater (SW) and food waste leachate (FW) used in this study are presented in Table 5-1.

There are differences in the characteristics of the swine wastewater used in chapter 3 and chapter 4, which is attributed to variations in discharge methods among different sampling site even when collected from the same facility. Therefore, it was difficult to use wastewater with similar characteristics to those previously used.

The SW used in the study had approximately twice the TCOD concentration and T-N concentration, as well as a higher content of solid materials, compared to the previous swine wastewater. To prevent pump clogging during continuous operation, the wastewater was initially sieved using a sieve before use. In preliminary tests, it was determined that the high T-N concentration hindered hydrogen production. Consequently, a 1:1 dilution of distilled water and SW was used. The characteristics of the food waste wastewater fell within the range shown in Table 5-1, and the inoculum sludge used was the same as the one introduced in chapter 3.

Table 5-1. Characteristics of swine wastewater and food wastewater

Parameter	Swine wastewater	Food wastewater
pH	7.26~7.42	4.21~4.63
TCOD (mg/L)	63,700	135,600~144,600
SCOD (mg/L)	39,900	99,500~109,800
TS (mg/L)	5,500	113,500~133,500
VS (mg/L)	12,400~17,500	107,700~120,200
T-N (mg/L)	3,900~4,200	3,200~3,350
NH3-N (mg/L)	2,839	543~644
T-P (mg/L)	1,280~1,400	1,845~2,400

2.2 Reactor Setup and Operations

A 1L bottle was used as the reactor, equipped with a lid containing holes and a silicone seal to prevent air ingress from the external environment. Sampling ports and lines were connected to enable inflow and outflow. The BT100S pump was employed, and based on the results of previous experiments in Chapters 3 and 4, the pH of the influent substrate was maintained in the range of approximately 5.8-6.0. The TCOD/N ratio of the mixed substrate from swine wastewater and food waste wastewater was fixed at approximately 8:2, considering the substrate composition. Due to the variability in the characteristics of the swine wastewater and food waste wastewater, it was not possible to precisely control the TCOD/N ratio, and only the pH range was maintained.

The Hydraulic Retention Time (HRT) was operated in a decreasing manner, with durations of 3 days and 2 days. The influent was prepared daily due to pH variations resulting from the mixing of swine wastewater and food waste wastewater, ensuring a consistent pH level.

Both a biological reactor and a bioelectrochemical reactor were separately operated to

observe the acceleration of hydrogen production under external voltage application. Based on the results of previous experiments, a voltage of 0.3 V was applied.

Graphite felt was used for the electrode (size; 1.5cm x 4cm x 7cm), and pretreatment was performed using PBS and acetone, which were most efficient according to the results of the pretreatment experiment in Chapter 4. The pretreatment methods was after cutting electrode, soak it in acetone for about 10 minutes, dry it 5 5℃ for 1hour, and then soak it a pH 6 of PBS (Na_2HPO_4 and KH_2PO_4) solution for 30 minutes before using experiment. The reactor employed graphite felt electrodes secur ely fixed, and a small magnetic bar was used to achieve a reaction speed of 30 rpm. The operational setup of the reactor is depicted in Figure 5-1 below.

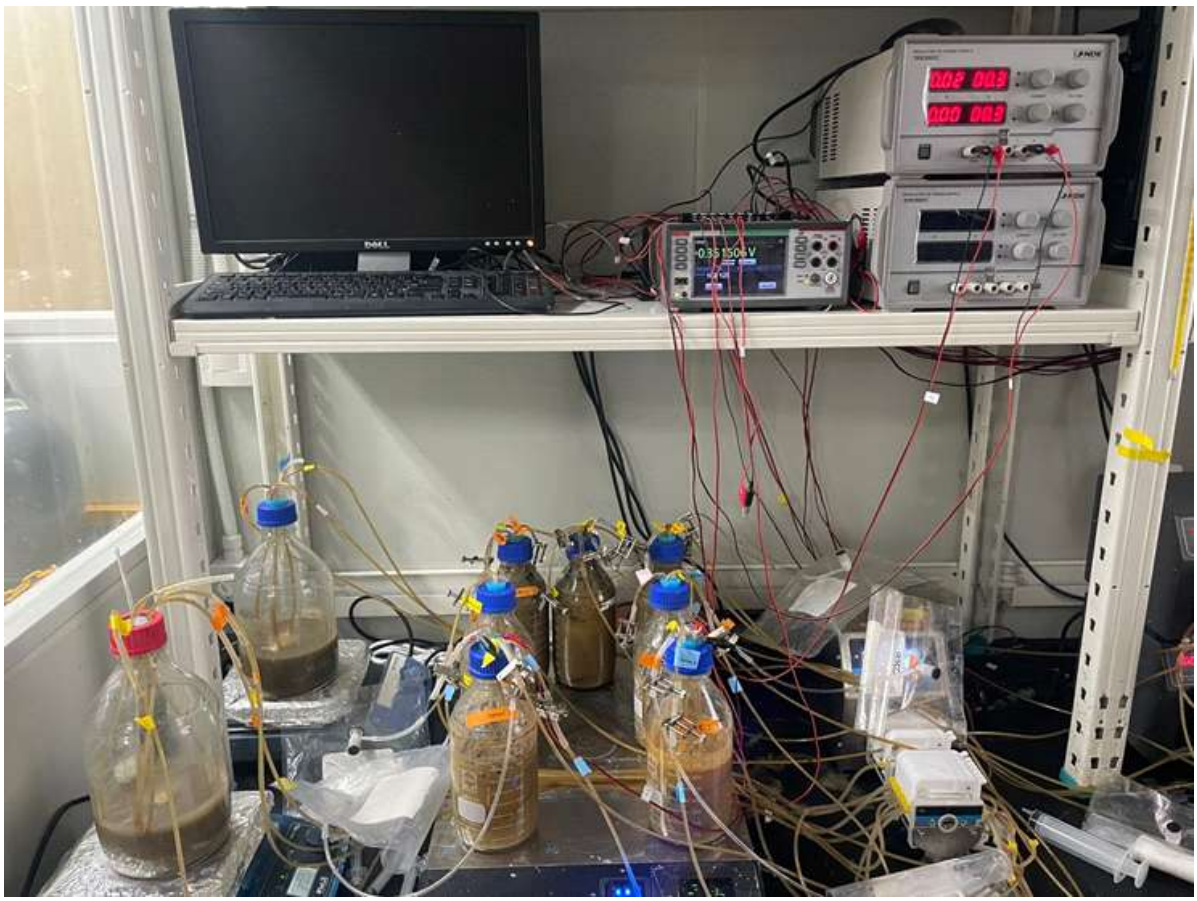


Figure 5-1. Operating anaerobic acid fermenter and hydrogen production bioelectrochemical anaerobic digestion (HP-BEAD) with CSTR.

3. Results and Discussion

The volumetric gas production rates (mL/L-d) of anaerobic digestion (AD) and HP-BEAD operated in a continuous stirred tank reactor (CSTR) configuration are presented in Figure 5-2.

The operation was conducted for 16 days with a hydraulic retention time (HRT) of 3 days, followed by 23 days with an HRT of 2 days. The pH of the influent was maintained within the range of 5.95 to 6.2 based on the optimal pH experiment results from chapter 3.

Observing the pH changes daily, when operated with an influent pH of 6.18, both AD and HP-BEAD exhibited no gas production for the first 5 days and showed a pH of 5.15 at the effluent after one day of operation. On the 4th day, the pH decreased to 4.97 and then increased to 5.64 (AD) and 5.87 (HP-BEAD) on the 7th day when gas production became active. This pattern of pH change was similar to the pH variation observed in sludge heat-treated batch operation for hydrogen production, as shown in Figure 4-3 (a). It was concluded that this was the result of a prolonged lag phase of microbial cell clusters due to the heat shock caused by sludge pretreatment.

The volumetric gas production (mL/L-d) at HRT 3days for AD and HP-BEAD showed that methane production (VMPR) remained below 2.68mL/L-d during the 16-day operation, indicating hydrogen production only after the initial operation. In HP-BEAD with applied voltage, the maximum volumetric hydrogen production rate (VHPR) of 723.75mL/L-d was observed within 6 days, while in AD, the maximum VHPR of 509.52mL/L-d was achieved on the 7th day. This indicated that the induction time of microbial cell clusters was shortened by approximately one day in HP-BEAD with an applied voltage of 0.3V, resulting in a 42.04% increase in VHPR.

After changing the HRT from 3 days to 2 days, HP-BEAD exhibited a VHPR of 165.95mL/L-d starting from the 19th day, while AD did not show hydrogen production until the 25th day and then started producing at a VHPR of 126.48 mL/L-d. During the HRT 2-day operation, the maximum hydrogen production was recorded on the 26th day for AD with a VHPR of 1057.21mL/L-d and on the 27th day for HP-BEAD with

a VHPR of 1724.93mL/L-d, demonstrating a 63.15% higher efficiency in HP-BEAD compared to AD.

Both AD and HP-BEAD exhibited a rapid decline in hydrogen production after reaching the maximum value. Afterwards, AD showed an increasing rate of 549.04 mL/L-d VHPR, while HP-BEAD showed an increasing rate of 743.29mL/L-d VHPR until the 31st day when a sharp decrease in hydrogen production was observed. During this period, methane production remained below 50mL/L-d, indicating the inhibition of methanogenesis while enabling active hydrogen production. HP-BEAD with applied voltage demonstrated approximately 42.04% and 63.15% higher efficiency compared to AD.

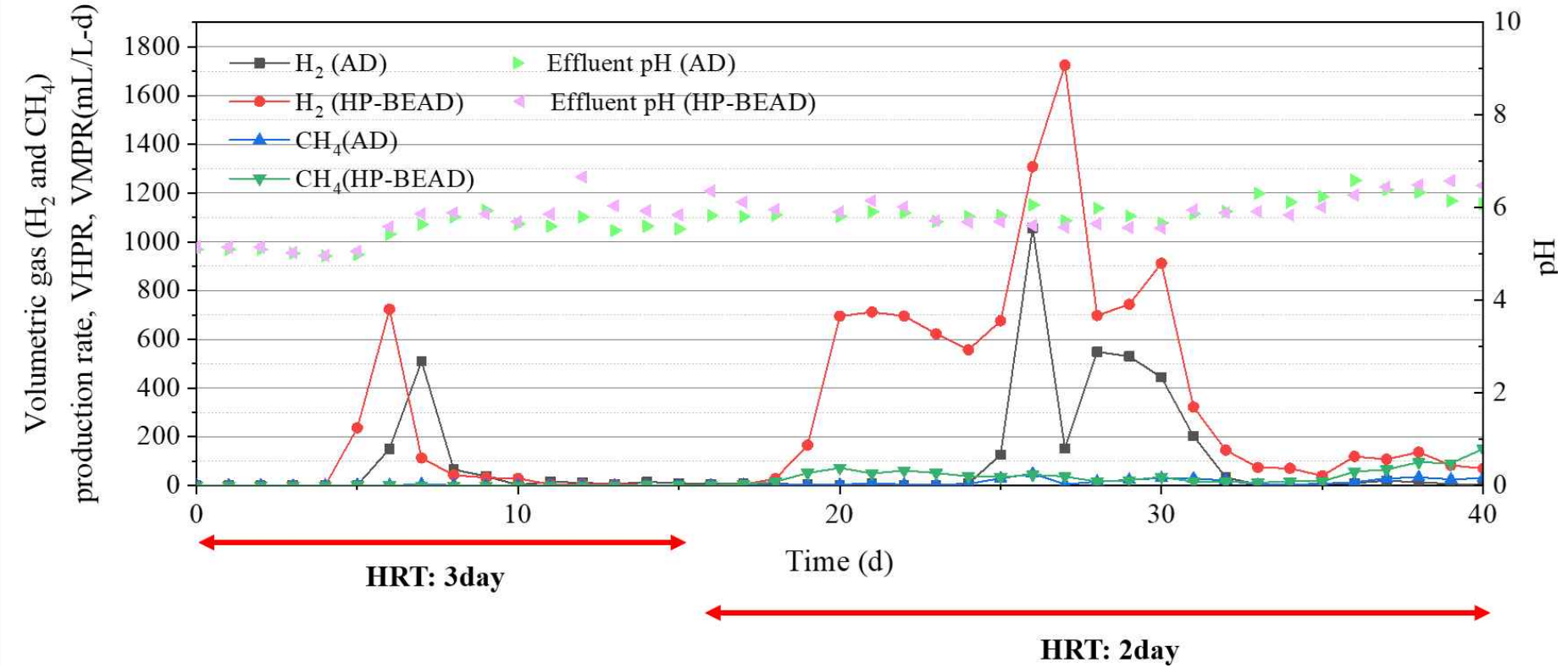


Figure 5-2. Volumetric gas production rate about hydrogen and methane operating anaerobic digestion fermenter (AD) and hydrogen production bioelectrochemical anaerobic digestion (HP-BEAD) with CSTR reactor

Chapter Ⅵ. Conclusion

In this study, firstly the optimal conditions for hydrogen production was explored through a biological process using anaerobic digestion (AD), after that with the bioelectrochemical anaerobic digestion (BEAD) using different ratios of swine wastewater (SW) and food waste leachate (FW) as a substrate for biohydrogen production. Hydrogen production-bioelectrochemical anaerobic digestion (HP-BEAD) was conducted under different voltages, tested control, 0V(only electrode), 0.3V, 0.6V, 0.9V, and 1.2V. The following was discovered:

1. A high hydrogen production rate was observed when SW and FW at a mixture ratio of 7:3 and 5:5, respectively, were inoculated with acid fermenter sludge. Through pH analysis, optimum pHs were recorded. The SW at pH 6.0 exhibited the fastest hydrogen production rate, while pH 5.5 resulted in the highest hydrogen production quantity. However, the hydrogen production yield (mL/g·VS) increased by 37.1% at pH 6.0 compared to pH 5.5, indicating that pH 6.0 was the favorable condition for hydrogen production. In contrast, no gas production was observed in the FW at different pH conditions, and all pH conditions showed a decrease to pH 4.4 within 24 hours, indicating the absence of hydrogen-producing bacteria due to insufficient alkalinity in the FW. The optimum pH of the substrates (SW: FW) at ratios of 7:3 and 5:5 were recorded at 6.1 and 5.8, respectively. Therefore we identified the optimal influent pH range of 5.5-6.10.
2. Utilizing substrate containing appropriate pH for anaerobic acid fermentation is more advantageous for biohydrogen production. Considering the high pH of SW, the addition of FW with a lower pH helped overcome the pH challenge of SW and the relative shortage of carbon source in SW. The optimal COD/N ratio of the substrate is 50-56 for biohydrogen production. Therefore, maintaining an optimal C/N ratio and a pH of 6.0 is essential for the successful production of hydrogen in anaerobic acid fermentation.
3. In the AD-BEAD, the pH variations could also be influenced by heat shock

resulting from the sludge heat shock pretreatment. The pH changes were primarily caused by acidogenic fermentation rather than applied voltage.

4. Cumulative hydrogen production analysis using the modified Gompertz function showed that the 0.3V reactor had the highest cumulative hydrogen production of 695.28±12.9 mL, a 63.4% increase compared to the control and a 78.0% increase compared to the 0V reactor. The lag phase was the shortest at 0.3V and control, while higher applied voltages prolonged the induction period of microbial consortia. Methane gas production was not observed.
5. Among all the pretreatment, the optimal electrode pretreatment method for bioelectrochemical acid fermentation system (HP-BEAD), the PBS (with 5mM H_2SO_4),was best. The control treatment achieved higher cumulative hydrogen production than the 5mM H_2SO_4 pretreatment, indicating the negative effect of H_2SO_4 on microbial activity. Acetone had the highest hydrogen production rate, while PBS had the shortest lagphase. PBS also achieved the highest hydrogen production yield, followed by acetone and the control.
6. The continuous operation of the HP-BEAD system was carried out based on all the optimized conditions for efficient HP-BEAD operation. Continuous operation was conducted with an influent substrate pH range of 5.8-6.0 and a hydraulic retention time (HRT) of 3 days. As a result, the HP-BEAD system achieved a hydrogen production rate of 723.75mL/L-d in 6 days, followed by a subsequent hydrogen production of 509.52mL in the simple acidogenic reactor for 7days. This demonstrated a relatively rapid production in the HP-BEAD system, with a 42.04% increase compared to the anaerobic digestion (AD) reactor after changing the HRT time 2 days, confirming continuous hydrogen production for approximately 15 days.
7. In comparison, the application of the HP-BEAD system showed significantly higher efficiency compared to the simple anaerobic digestion reactor, suggesting that the integration of the bioelectrochemical system into the acidogenic stage of anaerobic digestion processes holds the potential for increased biohydrogen production.
8. In conclusion, even a low applied voltage of 0.3V achieved highly efficient

hydrogen production in HP-BEAD, outperforming the control and 0V reactors. The electrode material and pretreatment method may have affected the microbial consortium. Further research with different bioelectrodes and pretreatment methods is needed to improve our understanding of the hydrogen production process.

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