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August 2023

Master's Thesis

**Assessment of Modified Cathode Electrode
for Bioelectrochemical Hydrogen Production
in Anaerobic Digestion**

Graduate School of Chosun University

Department of Environmental Engineering

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Assessment of Modified Cathode Electrode for Bioelectrochemical Hydrogen Production in Anaerobic Digestion

생물전기화학적 수소생산을 위한 혐기성 소화조
내에서의 변형 캐소드 전극의 적용가능성 평가

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Assessment of Modified Cathode Electrode for Bioelectrochemical Hydrogen Production in Anaerobic Digestion

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초록

생물전기화학적 수소생산을 위한 혐기성 소화조 내에서의 변형 캐소드 전극의 적용가능성 평가

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세계적인 기후변화에 대한 걱정과 화석연료의 고갈 문제는 신·재생에너지에 대한 관심을 갖게 하였다. 수소는 청정한 에너지이며 높은 에너지 함량을 갖고 있어 산업계에 미래 잠재에너지원으로 활용하기 위한 여러 노력을 하고 있다. 수소는 수증기 개질, 전기분해와 같은 공정의 물리화학적인 방법과 암발효와 광발효와 같은 생물학적인 방법으로 생산이 가능하다. 이 중 바이오매스를 활용한 바이오수소는 탄소중립과 연계한 친환경에너지로서 각광을 받고 있다. 바이오매스 중 하나인 유기성폐자원의 혐기성 소화를 통한 바이오가스 생산 기술은 에너지 측면에서 메탄가스를 생산하기 위한 공정으로 오래전부터 활용되어 왔으나 바이오수소의 관심으로 산발효를 통한 수소의 이용가능성에 대한 연구가 관심을 받고 있다. 본 연구는 가축분뇨와 같은 유기성폐자원에서부터 기존 혐기성 소화 공정에 생물전기화학 기술을 융합하여 고효율의 수소를 생산을 하고자 한다. 특히, 생물전기화학 기술의 핵심재료인 캐소드 전극과 변형 캐소드 전극의 적용가능성을 확인하기 위해 각 전극의 전기화학적, 생물전기화학적 특성을 파악하고자 하였다.

먼저, 효율적이고 경제적인 생물전기화학적 바이오수소 생산하기 위해 캐소드(cathode) 전극으로서 연구적으로 많이 사용하고 있고 경제적인 측면에서 흑연펠트(graphite felt), 흑연판(graphite plate), 니켈판(nickel plate), 스테인리스판(stainless steel plate) 전극을 선별하였으며 순환전류법을 이용한 전기화학적 성능을 확인하여 각 전극의 환원반응에 문제 없음을 확인하였다. 또한, 이러한 전극들을 생물전기화학적 반응조에 설치하고 전극을 삽입하지 않은 생물학적 반응기를 추가하여 회분식 반응으로 수소생산을 평가하였다. 그 결과, 흑연 펠트 전극은 8일 운전 동안 생물학적 반응기에서 가장 많은 수소 생산을 보였다. 수소 수율 또한 흑연 펠트 27.2 ± 2.0 mL/g VS로 전극을 삽입하지 않은 생물학적 반응기(10.5 ± 3.0

mL/g VS)와 다른 캐소드 전극을 삽입한 반응기(흑연판 12.7±9.0 mL/g VS, 니켈 판 3.9±0.0, mL/g VS, 스테인리스판 1.4±0.0 mL/g VS)보다 높았음을 확인하였다.

같은 실험구성에 0.3 V의 외부전압을 인가한 생물전기화학적 반응을 진행한 결과 흑연판과 스테인리스판 순으로 수소 수율이 높게 나왔으며 이러한 수소생산측면과 전극의 가격을 고려하여 흑연펠트전극과 스테인리스판을 생물전기화학적 수소생산반응을 위한 캐소드 전극으로 선정하였다.

전극의 비표면적과 전기화학적 오버포텐셜을 고려하여 수소생산 효율을 향상 시키기 위해 흑연펠트와 스테인리스판 전극에 carbon nanotube (CNT) 및 CNT+TiO₂로 딥 코팅하여 수소생산 성능을 비교해보았다. 그 결과 딥 코팅을 하지 않은 대조군(흑연펠트와 스테인리스판) 대비 CNT를 코팅한 전극을 사용한 반응기에서 우수한 수소생산과 수소생산수율을 보였으나 전극에 CNT+TiO₂ 코팅한 전극에서는 수소생산이 상대적으로 감소하거나 발생하지 않은 것을 확인하였다. 따라서 본 연구에서는 스테인리스판 혹은 흑연펠트에 CNT를 코팅한 전극이 수소생산 측면에서 유리하다고 판단할 수 있었다.

추가적으로 효율적인 수소생산을 위한 생물전기화학 반응기 내 전극의 위치 선정 방법을 알기 위해 애노드와 캐소드 사이의 전극 간격(1cm, 2cm, 4cm)에 따른 최대 수소생산성을 확인한 결과 전극 간격이 가까울수록 보다 높은 수소생산을 확인할 수 있었으며, 이는 저항에 관련된 것이라 추정한다.

본 연구에서는 생물전기화학적 고효율의 수소생산을 위해 기존 혐기성 소화 공정 내 생물전기화학적 융합 기술 적용시 변형캐소드의 특성평가에 따른 전극 선별과 전극거리의 중요성을 확인할 수 있었다.

1. Introduction

1.1 Background

Emissions from the burning of fossil fuels degrade the quality of the atmosphere all around the world. The byproducts of carbon have a remarkable impact on global warming. Hence the need for hydrogen generation has risen noticeably in recent years. The assembly of hydrogen from natural gas is inexpensive but it is associated with a variety of greenhouse gas emissions (Sazali et al., 2020). As a sustainable energy carrier hydrogen is considered a viable solution for lowering global warming through limiting the utilization of fossil fuels. Due to the fact that hydrogen is carbon-free, it can be considered a green fuel because, when it burns, only pure water is produced. In South Korea, for instance, the hydrogen economy could bring in over 420,000 employment and \$32.8 billion in income per year by 2040. Steam reforming, electrolysis of H₂O, pyrolysis, gasification, and other types of hydrogen-producing methods are energy-demanding and need high temperatures. To address this the biological technique is being explored for sustainable development and waste reduction, which employs microorganisms to make hydrogen from renewable resources such as biomass (Kapdan, I.K et al., 2006). Biomass energy sources include but are not limited to plants, livestock, sewage sludge, municipal solid wastes, and forestry residues. Considering the fact that it can be obtained in solid, liquid, or gaseous form, it is the greatest versatile renewable energy source. Different paths may be taken to get the most energy from biomass feedstock.

Anaerobic digestion (AD) of biodegradable biological materials produces biogas. Biogas production with AD has the benefit of being more environmentally friendly. The fundamental advantage of this technology is that it converts trash into energy in the form of biogas (50-60% CH₄ and 40-50% CO₂), whereas be change into the energy. AD has received increased attention in recent decades as a sustainable way to manage organic wastes since anaerobes use organic wastes as carbon sources and create methane and carbon dioxide as end products. BES working principles similar to

microbial electrolytic cells (MECs), which was applied external voltage and produce hydrogen on the cathode electrode, another one microbial fuel cells (MFCs), that developed for treating the wastewater and during the operation produce electricity (Logan et al., 2006, Logan et al., 2008). MECs are similar to MFCs, with the exception of the type of energy produced and the inclusion of external voltage. Electrochemically active microorganisms linked to MECs' anodes decompose organic material into electrons protons and carbon dioxide. These bacteria produce electrons which are then transmitted to the cathode, where hydrogen is produced. The cathode electrode is the most important components of a bioelectrochemical system since it is directly connected to hydrogen production. The most expensive part of the reactor is always the anode and cathode electrode materials, which accounts for 47% of the overall cost (Rozendal et al., 2008). Many research is now working on the utilization of low price cathode material to improve cathode catalytic achievement by lowering the hydrogen overpotential. The pace and content of biogas generation are impacted by variations in the electrochemical method characteristics of the electrode material which has an impact on process effectiveness. The platinum (Pt) and an associated coating compounds have been chosen as the noble metal for the cathode electrode in the manufacture of hydrogen despite their expensive cost. To swiftly offer effective massive transfer a multiple dimension scaffold and large specific surface area structures like two or three-dimensional (2D or 3D), had been designed. Carbon-based brushes and cloth were often utilized as materials for electrodes in early BEAD investigations. Carbon-based electrodes have a large specific surface area are highly electrochemically efficient and are bio-compatible. Lately, in BEAD, metal electrodes, notably stainless steel (SS) electrodes, have been employed. The key benefit of stainless steel is its inexpensive cost as well as its excellent durability and strength. The cost of stainless-steel much lower than carbon based felt, cloth and paper. In recent investigations, four distinct types of stainless steel (SS) alloys were mostly employed for cathodes: SS316, SS420, SSA286 and SS304. According to another research conducted on SS304 mesh, an applied voltage 0.5 V had shown a low cycle good energy efficiency and a time to reduce hydrogen loss. Nickel and steel and carbon materials, which are carbon brush and carbon cloth, with high reaction potential but

less catalytic capacity are preferred for methane composite (Wenzong et al., 2019). Given how different electrode materials and microorganisms interact, it is important to choose effective cathode materials. A better cathode material and catalyst are needed to get a high hydrogen yield. The main goals of the research are always to improve efficiency and decrease costs. In recent research, various cathode electrode materials include stainless steel, nickel, graphite, and carbon-based electrodes showed good efficiency. There has been a surge in the production of synthetic nanomaterials, driven by the anticipation that unexpected capabilities may emerge from their nanoscale structural peculiarities. Over the last two decades, there has been a surge in the production of synthetic nanomaterials, driven by the anticipation that unexpected capabilities may emerge from their nanoscale structural peculiarities. The dimensions of these nanomaterials can be used to classify them: Nanotubes and nanowires are one-dimensional, whereas nano clays and graphene are two-dimensional, and spherical and cubical nanoparticles are three-dimensional. CNTs have caught attention of mainstream researchers on account of their exceptional properties and are regarded considered the best promising materials for nanoelectronics, composite materials, energy storage devices and the medical sector, nano sensor applications, biological applications, smart materials, water and air purification systems, and a variety of other uses. A large number of conductive polymers and nanomaterials (CNTs) placed on electrodes with redox-active treatments to modify the surface of the material can be effective in reducing the overpotential of the reaction and promoting microbial adhesion and electron transfer. The relatively richest content of electroactive nitroaromatic reducing agents was found to be 15% more hydrophilic modifiers than hydrophobic groups, indicating that hydrophobic electrodes improved the adhesion of functional microorganisms (Xie, et al., 2021). Carbon nanotubes (CNTs) and titanium dioxide (TiO₂) coupling have gained a lot of interest in the literature as an approach to improve photo induced catalysis for environmental and sustainable energy utilization like solar energy conversion, photo and electro catalysis, greenhouse gases photoreduction, hydrogen the theory of evolution dye affected solar power cells and devices for sensing Coupling carbon nanotubes with TiO₂ at the level of the nanoscale may help in achieving the separation of electron hole energies developed during illumination.

Furthermore, CNTs can offer TiO₂ physical encirclement and significant promoting surface areas, which causes quicker measured oxidation rates of reaction. The type of organic feedstock to be utilized is another important consideration during biohydrogen generation in bioelectrochemical anaerobic digestion systems.

The aim of the research was focused on looking into utilizing four different cathode electrodes in a bioelectrochemical anaerobic digestion system to enhance the production of hydrogen from swine effluent. In this bioelectrochemical anaerobic digestion system utilizing swine wastewater, we investigated hydrogen generation using four different cathode electrodes to determine if we could select an optimum cathode electrode to improve satisfactory hydrogen production.

1.2 The objectives of research

In recent years bioelectrochemical anaerobic digestion systems is utilizing for wastewater treatment. The most crucial component of a bioelectrochemical anaerobic digestion system is the cathode since it is directly connected to the creation of hydrogen and methane (A. Kadier et al., 2016). That is why, the most expensive part of the reactor is always the anode and cathode electrode materials, which accounts for 47% of the overall cost (Rozendal et al., 2008). However, many studies are working on the application of affordable electrodes of cathode material and higher cathode catalytic effecting for hydrogen production. Therefore, by using graphite felt (GF), stainless steel plate (SS 304), graphite plate (Gp), and nickel plate (Ni p), this thesis aims to choose an affordable and highly conductible cathode electrode for the bioelectrochemical system. It also evaluates the biological and bioelectrochemical (applied voltage 0.3 V) reaction performance of the various embedded cathode electrode in a hydrogen-producing, operated at mesophilic temperature, and compared it. In addition, CNT and CNT/TiO₂ dip-coated cathode electrodes have been prepared and investigated in order to improve hydrogen production

2. Literature review

2.1 Hydrogen

The gas hydrogen is colorless, tasteless, odorless, light, and non-toxic (A.Kadlier et al.,2020). Bioelectrochemical treatment of wastewater had been a steady developing study area since the discovery of direct electron transfer by electrochemically active microorganisms on electrodes around the turn of the previous century MFCs for the generation of energy and bio catalyzed electrolysis for the generation of hydrogen are the bioelectrochemical treatment methods that have received the most research to date (Liu et al., Logan et al., 2005). However, many research hurdles remain before bioelectrochemical wastewater treatment can be deemed a mature wastewater treatment method.

The biological generation of hydrogen is mostly dependent on bacteria and algae. Hans Gaffron who made the discovery that algae may alternate between producing O₂ and H₂ in 1939. The microorganisms carried out the procedure at room temperature, pressure and needing less energy. Hydrogenase and nitrogenase, for example, are essential bio-catalysts in these processes. In Mohan et al., 2011 the hydrogen may be generated from a range of fundamental sources, including water, biomass and fossil fuels. There are various significant sustainable energy sources it may be produced from flowing water, earth's heat, wind, biomass, sun, and for biological hydrogen generation from microbes. Many microorganisms, including blue-green algae, which use light energy to split water for hydrogen creation, and cyanobacteria, which usually use carbohydrates to store energy from photosynthesis to manufacture hydrogen from water, are known to do so under particular circumstances. Various organic-based starch industry waste, industrial waste biodiesel, lignocellulosic materials including wood and its products, food, home trash, and others can be used to produce hydrogen gas from sustainable biomass resources. The term "low-carbon hydrogen" is widely used to describe blue hydrogen despite the fact that the steam reforming method does not completely stop the creation of greenhouse gases. There are other forms of hydrogen. Natural gas, which is transformed into gaseous steam through a process called steam

reforming, is the main source of blue hydrogen. Carbon dioxide is a byproduct even if the final product is hydrogen. By employing steam methane reformation, grey hydrogen is made from natural gas, or methane, without capturing the resulting greenhouse gases. Blue hydrogen and grey hydrogen are comparable in many aspects, however grey hydrogen does not use carbon capture and storage. By electrolyzing water with a clean energy produced in excess from renewable power sources like wind or solar power which was green hydrogen is created. With no carbon dioxide produced electrolysis use an electrochemical mechanism to break down water into its component parts hydrogen and oxygen. Due to the high cost of generation green hydrogen only makes up a small part of total hydrogen production at the moment. Green hydrogen will become more affordable as it becomes easier to obtain much as wind energy has. One of the goals that governments throughout the world have set for 2050 is to the reduce the carbon the earth. One way to do this is to produce green hydrogen by carbonizing the manufacturing of an element like hydrogen which today contributes more than 2% of global CO₂ emissions.

Biohydrogen

Dark fermentation photo fermentation and direct and indirect methods are the most extensively used researched and created techniques for creating biohydrogen from organic waste. Biohydrogen is presently regarded as the best biofuel since it can meet energy demands and can be created from renewable resources such as biological splitting of water molecules by biomass conversion and solar thermal splitting of water. There is a sort of biofuel called biohydrogen. Biohydrogen is a form of biofuel that's comparable to bioethanol, biodiesel and bio-oil. Both biological and chemical methods can be used to produce hydrogen. Therefore, biohydrogen refer to a process of producing hydrogen biologically through microorganisms in a bioreactor. As shown Fig 2-1. Schematic diagram of biohydrogen production process. All of these processes are related to many process parameters, each of which has its own significance. Each process has its own set of parameters and operating factors, as well as advantages and disadvantages, which are detailed further below.

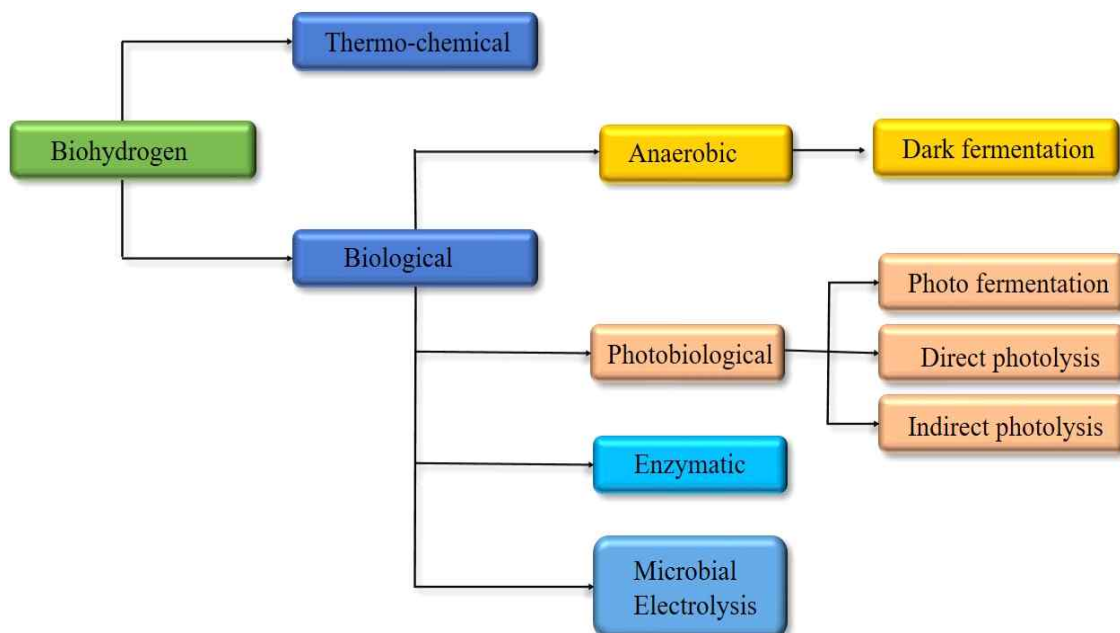


Figure 2-1. Biohydrogen production process modified from (Rahul et al., 2023)

2.1.2 Biohydrogen production method

Dark fermentation

Dark fermentation is a well-known biohydrogen generation technology that allows microorganisms to create H₂ in an absence of light anaerobic environment. The anaerobic pyruvate metabolism increases hydrogen generation, while ferredoxin oxide reductase and formate lyase promote pyruvate breakdown. The optimal bio-hydrogen generation, key variables such as pH, substrate, temperature, nutrition feed, and so on must be preserved. In particular, pH impacts hydrogenase enzyme activity.

Variable amounts of H₂ are produced by glucose, isomers of hexoses, or polymers in the form of starch or cellulose depending on the fermentation route and end product (Equation 2-1 to 2-3)

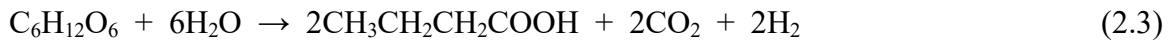
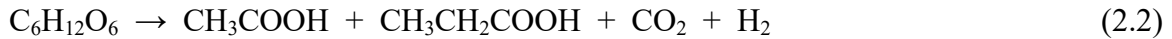
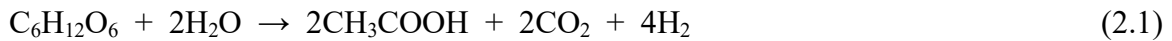
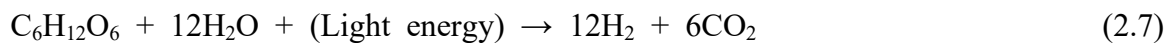
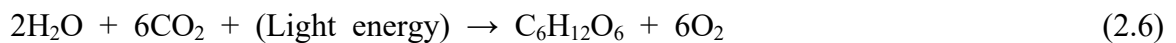


Photo fermentation

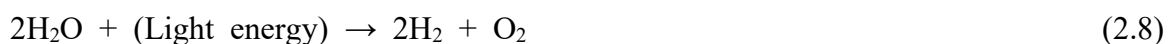
With the aid of light-dependent, sulfur and non-sulfur purple bacteria, photo fermentation breaks down organic acids to create H₂. When immobilized in or on a solid matrix, photoheterotrophic bacteria often create more hydrogen than when they are free to move about. In nitrogen-deficient environments, purple non-sulfur bacteria combine light energy and reduced molecules (organic acids) to produce molecular H₂ that is catalyzed by nitrogenase. the process by which glucose and acetic acid are converted into H₂ by photo fermentation. By oxidizing organic acids including acetic acid, propionic acid, butyric acid, lactic acid, and malic acid, photo fermentative bacteria produce H₂ and CO₂. According to (Equation 2-4 to 2-5)



Bio photolysis, known as water splitting photosynthesis, is the progress by which H_2 may be created using just sunlight and water by oxygenic photosynthetic microorganisms such as cyanobacteria and green microalgae. There are two methods for producing H_2 via bio photolysis: (a) direct bio photolysis and (b) indirect bio photolysis. Indirect photolysis process cyanobacteria are an immense and varied genus of photoautotrophic microbes that developed and diversified early in world history. Cyanobacteria may undertake oxygenic photosynthesis and include photosynthetic pigments such as chlorophyll A, and phycobiliproteins. They are a morphologically varied category that includes species that are unicellular, filamentous, and colonial. Cyanobacteria's dietary needs are straightforward: air nitrogen and oxygen, water, mineral salts, and light (Hansel et al., 1998). Through photosynthesis, cyanobacteria it may also create and develop H_2 via the processes: shown (equation 2-6 and 2-7)



Direct bio photolysis: The photosynthetic generation from a biological mechanism that converts sunlight into usable, stored chemical energy involves the broad reaction outlined below. It occurs when hydrogen is extracted from water. ((Equation 2-8)



2.2 Anaerobic digestion

2.2.1 Principles of anaerobic digestion

Anaerobic digestion is a method that uses anaerobic conditions to breakdown and stabilize organic materials while producing methane as a byproduct. By liquefaction or hydrolysis, polymeric organic substances may polysaccharides, proteins, and lipids are transfer to monosaccharides, amino acids, glycerol, and fatty acids in anaerobic digestion tanks. Acid-producing bacteria utilize the transformed material to create lower fatty acids equally acetic acid, alcohol, carbon dioxide, hydrogen, ammonia, as well as sulfur compounds. Acetic methanogens and hydrogenotrophic methanogens are now converting acetic acid and hydrogen into methane Sludge, garbage, sewage, and waste from a swine manure feedstock are a few examples of substrates with high quantities of organic matter that are frequently used in the biochemical process of anaerobic digestion. Anaerobic digestion consists of the following four basic processes. (Fig. 2-2.)

(a) Hydrolysis

The first stage is carried out by hydrolytic bacteria, and maybe also by fungi, which convert long-chain polymers such as lipids, polysaccharides, proteins are to tiny monomers of glycerol, amino acids, LCFA, sugars (Deng, L et al., 2014). Hydrolysis is the term for the procedure of severing chains with the aid of hydrolyzing enzymes. Bacteria may easily access simple sugars and monomers that are produced during high molecular weight polymeric parts disintegration. These processes create acetate, hydrogen, and certain volatile fatty acids. VFAs must first be catabolized into tiny molecules that the bacteria can utilize since they cannot be used directly by microorganisms.

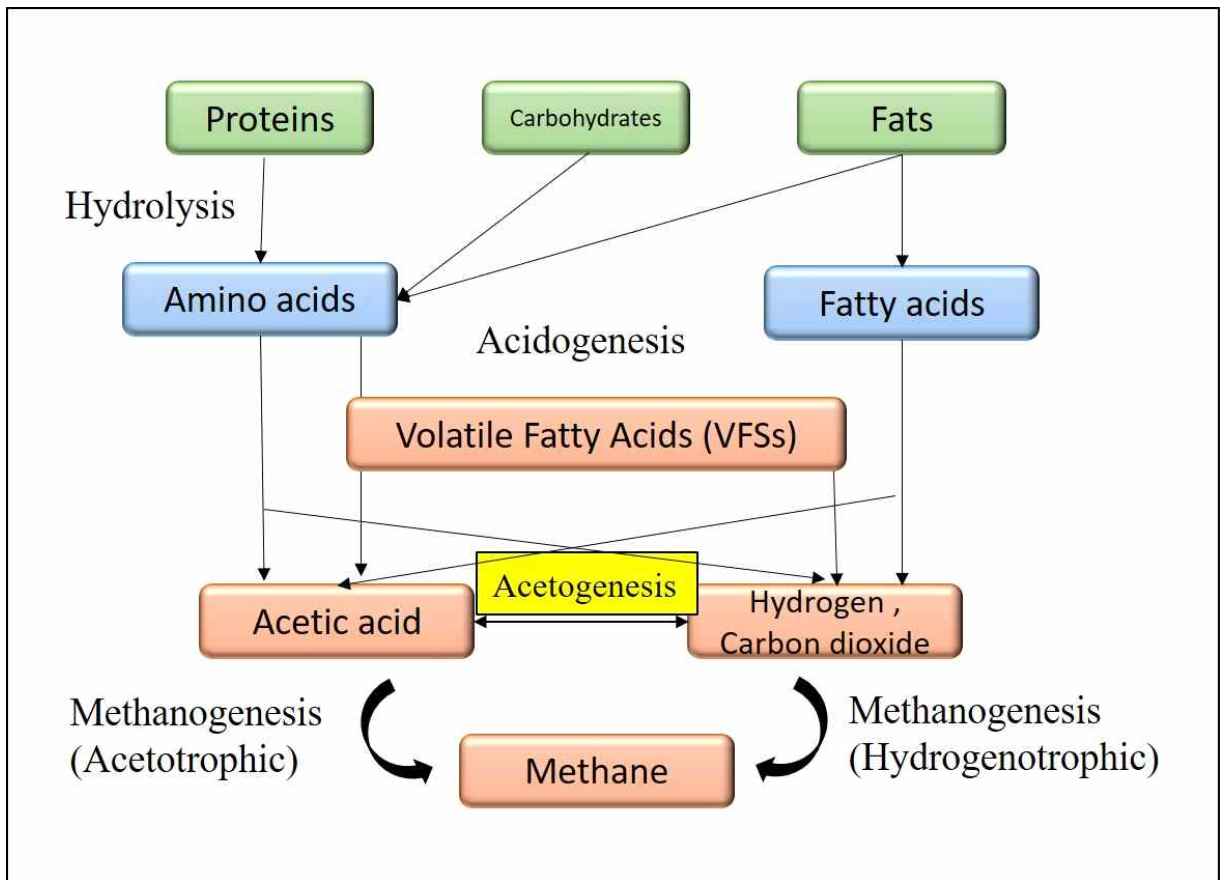
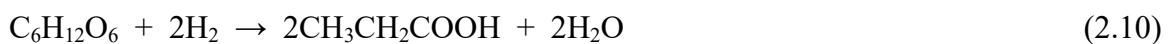


Figure 2-2. Schematic mechanism of anaerobic digestion

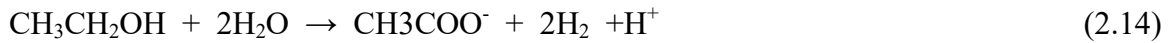
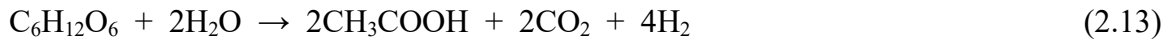
(b) Acidogenesis

Acidogenesis is the fermentation step in which acidogenic bacteria destroy hydrolysis alcohols, aldehydes, volatile fatty acids (VFAs), and other compounds (soluble organic monomers of sugars and amino acids) and acetate along with H₂ and CO₂ (Sambusiti, 2013). The chemical equations linked with the acidogenic process (2-9, 2-10 and 2-11)



(c) Acetogenesis

Acetogenesis, a process that results in the production of acetic acid, uses acetogens. As a result of this process, the main byproducts are carbon dioxide and hydrogen. Additionally effectively using the amino acids and sugars produced during hydrolysis, acetogenic bacteria also oxidizes pyruvate. Acetate is produced from the anaerobic breakdown process's intermediate product, pyruvate. Acetogens carry out a number of tasks under thermodynamic conditions. (Kim et al. 2007) According to (Ostrem & Themelis, 2004; Strauber et al., 2012; Zheng et al., 2015), propionate, glucose, and ethanol are converted to acetate during the third stage of anaerobic fermentation.



(d) Methanogenesis

This is the process that generates methane, the end result of anaerobic digestion. Methane-producing bacteria produce methane gas by using lower fatty acids, hydrogen, and carbon dioxide. The methane production reaction is broken down into two parts: an acetic acid-based methane production reaction (Equation 2.15), which utilizes acetic acid, a lower fatty acid, and a hydrogen-based methane production reaction (Equation 2.16), which converts carbon dioxide to methane using hydrogen. Between 6.5 and 8, a pH-sensitive process that is taking place occurs. In this process the intermediate product from previous stages is used to make methane, carbon dioxide, and hydrogen.



2.2.2 Microorganism involved in the anaerobic digestion

There are many different microbial species involved in anaerobic digestion but acid bacteria and methane-producing germs are the most common. Anaerobic bacteria involved in the hydrolysis reaction vary according to the breakdown ingredient and are resistant to environmental changes. Acid-producing bacteria are not much impacted by pH, organic acid concentration or temperature however methane-producing bacteria are (Yoo, et al., 2003)

Table 2-1 Products and substrate capital microorganisms in MFC (Feng et al, 2004)

Anaerobic bacteria	Substrate	Products
Hydrolysis bacteria	Complex organic matter	Monomer
Acetogens	Short chain fatty acids	Acetic acid
Methanogens	Acetic acid, methanol, CO ₂ , and formic acid	Methane
Denitrifying bacteria	Oxidized nitrogen	N ₂ , NH ₃ , N ₂ O
Sulfate-reducing bacteria	Sulfate	H ₂ S
Homoacetogens	formic acid, propionic acid compounds	Acetic acid

2.2.3 Factors affecting anaerobic digestion process

The most crucial thing to consider while operating an anaerobic digestion tank is pH parameter. The pH level for methane generating bacteria to grow to an anaerobic digestion tank is between 6.5 and 8.0. Acid-producing bacteria are active even at low pH, but methane-producing bacteria are significantly less active when the pH is decreased to 6.5 or below. Alkalinity with buffering capacity may grow as a result of VFAs or ammonia produced by a reaction in the fire extinguisher tank and high alkalinity does benefit pH stability.

The thermostability of intracellular proteins determines the stability of a variety of microbial species against changes in temperature (Dohanyos and Zabranska, 2001). The anaerobic digestion process is a low-temperature metabolizing method (15-25°C) depending on the operating temperature.

The organic loading rate (OLR) is the amount of biologically degradable organic matter that enters the anaerobic digester per unit volume (Song et al., 2016). The reactor design, wastewater properties, settleability, and biomass activity are the criteria linked with the highest potential OLR (Zaher et al. 2007). If the organic material loading rate is low, an association reaction may develop as a result of insufficient substrate, reducing the quantity of methane gas generated in an anaerobic digestion tank. Furthermore, if the organic material load rate is high, VFA formed by acid fermentation might build, lowering pH and alkalinity and worsening anaerobic digestion tank stability.

VFA concentration is an important factor in the anaerobic digestive system constitute two of them. Excess volatile fatty acids created through acid fermentation can rapidly decrease pH and alkalinity, inhibiting the activity of methane-producing bacteria. The high concentration of volatile fatty acids could suggest a problem with the reduction reaction during the anaerobic digestion process's production of methane. Toxic chemicals that impact the anaerobic digestion process include ammonia, salt, heavy metals, and others. In the case of metal ions, sulfide and sulfate are generated within the fire extinguisher tank and precipitate. However, ammonia is a very poisonous chemical that inhibits methane-producing microorganisms.

One of the factors that has a direct impact on the effectiveness of anaerobic digestion is inflow waste. The compounds that can be digested in anaerobic digestion are restricted to some organic substances, while those that cannot be decomposed by anaerobic microbes accumulate inside the digestive tract, affecting its function. As a result, the amount of methane gas created might mostly base on how much of the incoming rubbish is made up of biodegradable organic material. Two-phase anaerobic digestion (AD) of municipal wastewater separates the hydrolytic-acidogenic phase from the methanogenic phase, compared to conventional single-phase treatment. This leads to optimization of the corresponding microbial communities, increased stability, shorter residence times, and higher methane production rates. There has been much research on increasing methane production yield utilizing two-phase operation, but just a few studies on hydrogen generation using acid fermentation tanks have been performed.

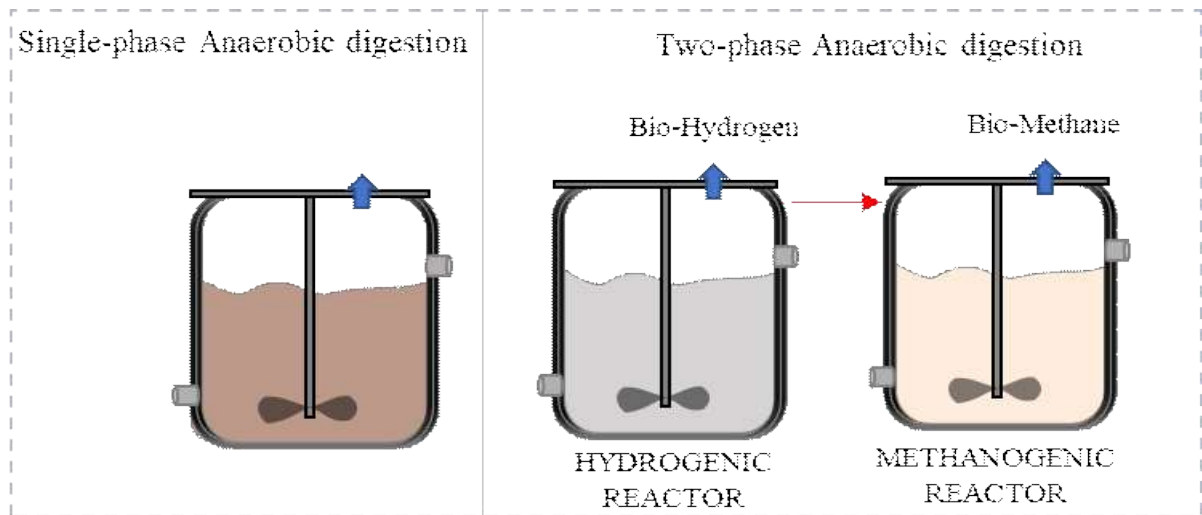


Figure. 2-3. Anaerobic co-digestion schematic design

2.2.4 Advantage and disadvantage of anaerobic digestion

In organic waste treatment anaerobic digestion is restricted by the slow rate at which it progresses. Due to the slow rate of anaerobic digestion long hydraulic retention times must be maintained the result in high operating costs for anaerobic digestion. Similarly the low rate of anaerobic digestion also causes problems such as longer start-up time and longer recovery time after disturbance. Currently many studies focus on get the better of the slow rate of anaerobic digestion. All researchers try to optimize running conditions as well as temperature, pH, organic loading rate, and time of residence the pre-treat organics including ultrasonics, microwaves, high temperature, high pressure, and oxidant treatment to speed up the anaerobic digestion reaction rate. Although the above methods can be used to improve anaerobic digestion rates, they cannot completely solve the problem. The most important thing about anaerobic digestion is that the bacteria that produce methane grow slowly have a low pH, alkalinity, and external environmental conditions, such as decreased activity when exposed to outside air, as well as a slow growth rate. Due to its sensitivity it is difficult to operate and requires a considerable amount of processing time because of the operating conditions.

2.3 Bioelectrochemical system

2.3.1 Working principles of bioelectrochemical system

A bioelectrochemical system for producing H_2 from a large area of substrates utilizing microbial electrolysis cells is a novel technology that has gained traction in the latest years. MEC technology is also known as electro fermentation or bio catalyzed electrolysis cells (Chandrasekhar et al., 2015). BES is a hybrid electrochemical device in which at least one of the electrode processes anodic or cathodic is carried out by a microorganism and biocatalyst, often under strictly anaerobic circumstances (Rabaey et al., 2007), (Enamala et al., 2020). The application of an external voltage is a new concept in anaerobic digestion. Wastewater treatment may be used to generate energy and value-added compounds via bioelectrochemical processes. Solid electrode materials and metals in general participate in the extracellular electron transport chain and function as electron acceptors for microorganisms that are electrochemically active in the BES system.

2.3.2 Microbial fuel cell (MFC)

Microbes in MFCs use catalytic breakdown of organic substrates to transfer chemical energy to electrical energy. Organics are oxidized in the anode compartment by certain bacteria, resulting in the generation of electrons and protons. MFCs that use mixed cultures now reach power densities that are significantly higher than those that use pure cultures. MFCs are produced using a variety of materials and in an ever-increasing range of combinations. The operating conditions for these systems vary according to the temperature, pH, electron acceptor, electrode surface area, reactor size, and running time. Electrode material is important parameter in MFC. The main variables influencing the performance of the biocathode are its properties and configuration (Wei et al., 2011). In addition to serving as a conductor, bio-electrodes also serve as carriers of

bacteria. Electrode materials with unique surface characteristics, such as high surface roughness, strong biocompatibility, and effective electron transport between bacteria and electrode surface, are necessary for high biocatalytic activity. In the realm of MFC research, electrode surface modification has gained increased attention as a way to enhance bacterial adherence and electron transmission. The electrode material for air cathodes with a catalyst is composed of a base substance, a catalyst, a binder, and a waterproof coating. Each component has a unique set of functional and material characteristics. The base material often simply functions as a supporting material and a current collector. Both mechanical strength and excellent conductivity are required. There are no specific prerequisites for bacterial adhesion, though. For air cathodes, a catalyst is helpful but not necessary. If necessary, a binder is used to secure the catalyst to the substrate's surface, and a hydrophobic coating is routinely added to the cathode to stop water loss. Activated carbon, among other highly specialized materials, has been used to reduce the price of air cathodes. The most versatile electrode material is carbon, which may be found in a number of shapes including glassy carbon, fibrous material (felt, cloth, paper, fibers, foam), compact graphite plates, rods, or granules. The most basic materials for anode electrodes are graphite plates or rods since they are fairly inexpensive, manageable, and have a predetermined surface area. Graphite felt electrodes have a much bigger surface area which is 3D type. Furthermore, carbon nanotube (CNT) anode modification has shown highly promising features for usage as electrode materials because of their enormous specific surface area, remarkably high conductivity, and mechanical flexibility. Low corrosion, strong electric conductivity, high porosity, and a large specific surface area are all characteristics of cathode electrodes.

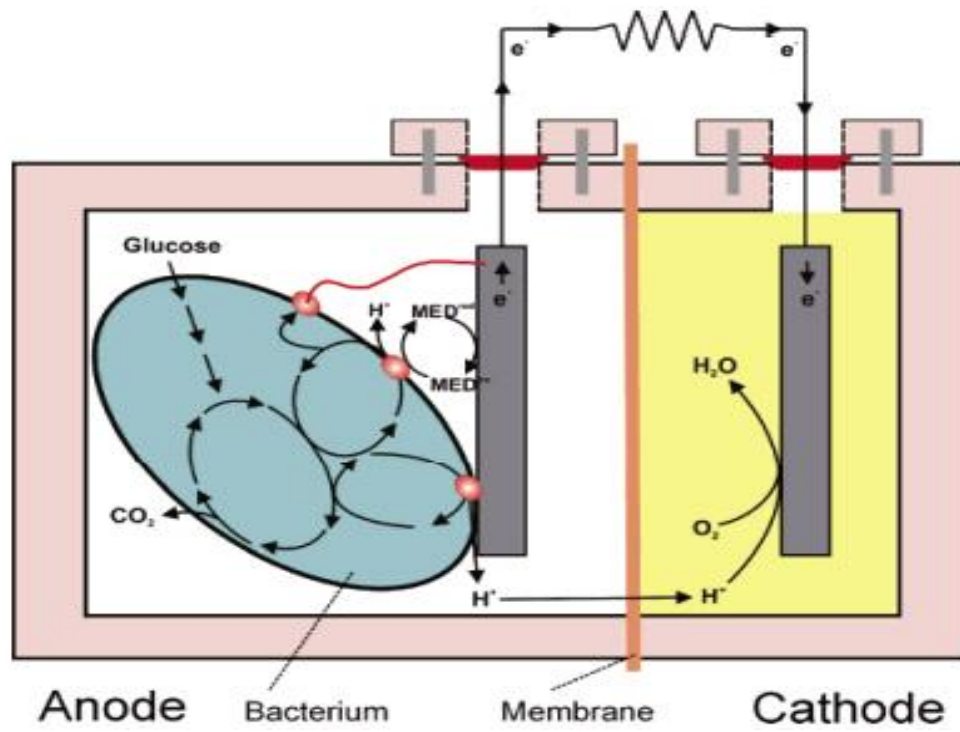
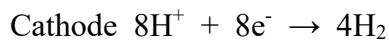
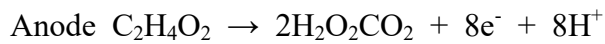


Figure .2-4. Schematic diagram of MFC (H. Bird et al)

2.3.3 Microbial electrolysis cell (MEC)

A MEC is an electrolytic reactor which produces hydrogen in the cathode electrode via a microbial anode catalyst and uses electrical power. Electrons traveling to an anode through electron shuttle move or electron transfer via conductive materials and biofilm (Kumar et al., 2017). Earlier experiment was focused on the two chambers MEC system which need a membrane for the separate the two electrode or using membrane less MEC system. An external electric circuit transports the anode's produced electrons to the cathode, where they are used for oxygen reduction. Acetate was oxidized at the anode when hydrogen generation took place at the cathode, with half reactions happening at both the anode and cathode.



Acetic acid, butyric acid, lactic acid, glucose, cellulose, and other types of wastewaters are just a few of the diverse substrates that may be used to make hydrogen in MECs (Lu et al., 2009). MECs are bioelectrochemical systems that use electricity to power the cell's bioelectrochemical operations. These systems are susceptible to changes in the operational environment. The performance of the anode, which may be transferred from MFCs, MECs, and MEC-ADs, is crucial to the performance of bio-electrochemical systems. According to research, the performance of the overall system may be constrained by the action at the anode.(Lim et al., 2017).

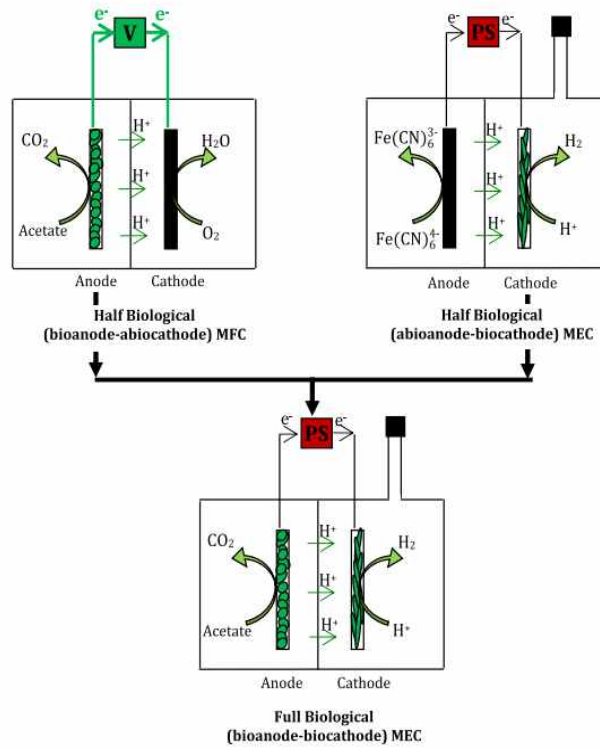


Figure 2-5. Design of half biological MFC and half biological MEC, full biological MEC (Taherreh et al., 2019)

2.3.4 Challenges of a bioelectrochemical system

The application of materials that are cheap plays an essential role in releasing BES technology. Excellent performance BESs for the reclamation of resources require catalysis that can promote cathode reactions at minimum overpotential. Developing economically feasible larger surface area materials for electrodes that have superior conductivity, alongside innovations related to BES designs or architecture, could substantially improve the productivity of the process and save prices.

2.4 Bioelectrochemical anaerobic digestion (BEAD)

2.4.1 Fundamental of bioelectrochemical system

The microorganism connected to electrodes accelerates the decrease in oxidation processes in the bioelectrochemical system. One instance is the coupling of a bioelectrochemical system to anaerobic digestion, which aids in enhancing the productivity of waste remediation and the formation of biogas from biomass. Combining AD with BES is a working electro-fermentation (EF) technique for boosting energy transformation productivity (substrate to substance) and reliability of operations when using conventional AD. By inserting electrodes into the micro environment, the AD production rate and process performance might be increased. The process can be accelerated by additionally connecting the electrodes outside when applying a voltage throughout both. Considering the microorganism mechanism and the microbial composition on the electrode surface aids in enhancing particular goods output after AD combination with BEAD. The voltage that is supplied to the BES system for microbial synthesis supports the acceleration of the reactions rather than actively participating in the response as a donor of electrons.

2.4.2 Factor affecting bioelectrochemical anaerobic digestion

(a) Electrode

The anode and cathode electrodes are important part of the bioelectrochemical system. Higher conductivity, an attraction for bacteria to form, a material that is permeable with a sizable area of specific surface for organisms to attach an anode, chemical compounds and living equilibrium and strength, the electrode's structure needed to be simply constructed with economical assemblies, no getting stuck in for microbial proliferation, which is simply scaling up for the bioelectrochemical anaerobic digestion. The bioelectrochemical cathode uses the electrons passed on from the anode to convert carbon dioxide into methane, acting in principle as an electron acceptor. The cathode's catalyst is crucial in accelerating the rate whereby methane is generated from the carbon dioxide for better effectiveness. Therefore, an important consideration for establishing the process rates is the electrochemical composition of the bioelectrochemical cathode. To confirm that the reduction mechanism occurs flawlessly the bioelectrochemical cathode must have a high specific surface area. By decreasing carbon dioxide, a good catalyst on the cathode can improve methane generation efficiency. In the past, materials made from carbon have typically satisfied the anode and cathode standards established for bioelectrochemical anodes and cathodes. As bioelectrochemical electrodes, materials with names like carbon paper, carbon cloth, graphite rod graphite granule, fraphite felt and graphite fiber brush, stainless steel, carbon nanotube are commonly used Table 2-2. For the purpose of high-rate bioelectrochemical biogas generation, a more effective and enduring bioelectrochemical electrode material is still needed.

Table 2-2 Mostly used electrode materials in MFCs

Materials	Advantages	Disadvantages
Carbon paper	High conductivity	Brittle, low specific surface area, expensive
Carbon cloth	High conductivity, flexible, high specific surface area	Expensive
Graphite felt	High conductivity, high porosity, large specific surface area, flexible	Low strength
Stainless steel	High conductivity, low cost	
Graphite rod	High conductivity, defined surface area	Low specific surface area, expensive
Reticulated vitreous carbon	High conductivity, high porosity, large specific surface area,	Brittle
Graphite granules bed	High conductivity, high porosity, high surface area	High contact resistance
Graphite fiber brush	high porosity, large specific surface area, flexible	Expensive

(b) Applied voltage

The conceivable variance across anode and cathode within a bioelectrochemical anaerobic digester corresponds to one of a number of key variables for its optimum performance. The organic matter begins to oxidize by electrically active bacteria which attach to the anode's surface to produce the protons, molecules of carbon dioxide, and ions. By providing a modest voltage via a direct current (DC) supply, electrons are transported to the cathode, wherein carbon dioxide and proton are turned into biogas. Based on a previous study (Wang et al., 2009), the greatest amount of hydrogen gas came into existence while the voltage being applied occupied a range of 0.5-0.9 V, Table 2-3. But the highest amount of methane gas formed when the supplied voltage

was quite using the range of 0.3-0.6 V in a MEC. Nevertheless, the voltage that was utilized with bioelectrochemical anaerobic digestion has caught less consideration and remains unresolved.

Table 2-3 Performance of different cathode materials and cathode catalysts used in MEC

Cathode materials and catalyst	Cathode potential (V)	Hydrogen yield (%)
Stainless steel A286	0.9	80±2
Stainless steel 304	0.9	77±1
Ni 625	0.9	67±9
Ni 625+ NiO _x	0.6	76±5
Ni 210 (60 mg Ni in 267μL Nafionon carbon cloth)	0.6	92±0
MoS ₂ on carbon cloth	-	92±3
SS A286+NiO _x	0.6	76±2

(c) Others

The biomass gas subsequent generation rate in a bioelectrochemical reactor was demonstrated to be more than thirty percent at a pH level of 5.8 when compared to 7.0 pH in a year-published study (Hu et al., 2008). The hydrogen manufacturing in bioelectrochemical anaerobic digestion at 10°C was 5.3-6.6 fold higher than in the control digestion (without the voltage being applied and electrodes) at 10°C and almost clearly the same as the yield in a mesophilic condition (35°C) (Liu et al., 2016). However, it can be challenging to say definitively whether bioelectrochemical anaerobic digestion cannot be impacted by its surrounding environment. The successful result of bioelectrochemical anaerobic digestion can be affected by impacting characteristics

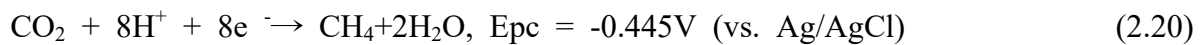
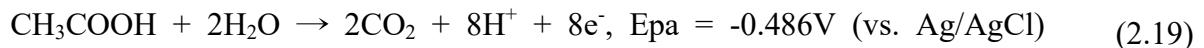
involving biological material, organic loading rate, and temperatures.

Table 2-4 Cathode material types and structure in other study (Wenzong et al., 2019)

Material	Base material	Base materials Structure	Catalyst
SS brush	SS	brush	-
Nickel sheet	Nickel	Mesh	-
Carbon cloth+Platinum	Carbon	Fiber	Platinum
Graphite	Carbon	3D	Microorganism
Graphite felt	Carbon	3D	Microorganisms
SS sheet	SS	Mesh	-
Nickel foam	Nickel	Mesh	-
Carbon cloth+MoS ₂	Carbon	Fiber	MoS ₂
Titanium sheet	Titanium	Mesh	
Steel mesh	Steel	Stacked mesh	-
Carbon felt+CNTs	Carbon	Porous	Microorganism
Titanium+Platinum	Titanium	Mesh	Platinum
Carbon cloth+graphene	Carbon	Fiber	Microorganism
Carbon paper +Pd Nanoparticle	Carbon	Fiber	Pd
GFF+Fe	Carbon	Fiber	Microorganism

2.4.3 The performance of BEAD for biogas production

BEAD generates biogas by direct interaction of electrons with electrodes. Anaerobic digestion could be coupled utilizing bioelectrochemical technologies via an anode and a cathode inside a currently operational conventional anaerobic digester while preserving a small potential distinction across both of them (Song et al., 2016). A couple of expressions reveal one of the methane-generating processes of bioelectrochemical anaerobic digestion: On the anode surface, organic matter is oxidized producing the elements protons, electrons, and carbon dioxide; the electrons travel through the anode to the cathode within an additional circuit employing an applied voltage; and the formed oxidized substances are then broken down on the surface of the cathode in order to produce biogas. Equations 2.19 and 2.20, respectively explain the reactions that happen on the anode and cathode surfaces.



3. Methodology

3.1 Substrate and inoculum

Substrate

Swine wastewater (SW) and food wastewater (FW) substrates have been obtained at the Nonsan Gyeryong Livestock Cooperative Natural Circulation Agricultural Center in South Korea. The samples of swine wastewater and food wastewater were divided into 1L bottles and stored at -20°C until use. The pH, ammonia nitrogen (NH₃), total nitrogen (T-N), chemical oxygen demand (COD), total solids (TS), soluble chemical oxygen demand (SCOD), and volatile solid (VS) characteristics of raw swine manure and food waste are shown in Table 3-1

The sludge used was anaerobic acid sludge generated in Nonsan, South Korea, via a two-phased anaerobic digestion process. Following a sludge sample, the sludge is put in the big bottle reactor and purged with high-purity nitrogen gas to establish an anaerobic environment. The features of sludge are presented in Table 3-1

Table 3.1 Characteristic of raw substrates and acid fermentation sludge

Parameter	Swine wastewater	Food wastewater	Sludge
pH	7.26~7.42	4.21~4.63	5.56
TCOD(mg/L)	28,000~39,100	135,600~144,600	49,600
SCOD(mg/L)	13,300~19,000	99,500~109,800	43,700
TS(mg/L)	19,300~25,400	113,500~119,400	1.9%
VS(mg/L)	12,400~17,500	107,700~120,200	1.16%
T-N (mg/L)	3,900~4,200	3,200~3,350	3,200
NH ₃ -N(mg/L)	2,723~3,173	543~644	2,434
T-P(mg/L)	1,280~1,400	1,845~2,400	1,210

3.2 Preparation of electrode

Electrode fabrication

The electrode materials utilized in this research, such as graphite felt (GF), graphite plate (G plate), stainless steel plate 304 (SS plate), and nickel plate 99.999% (Ni plate) purchased from (Nara Cell tech., Seoul, South Korea). Graphite felt was selected for the anode electrode for the whole reactor and design for a rectangle and size was 1 cm × 2 cm × 3.5 cm (width × length × height). In addition, anode electrodes of 0.2 cm × 2 cm × 3.5 cm (width × length × height) were fabricated for another experiment. Stainless steel 304, nickel, and graphite plates were chosen for cathode electrode. The cathode electrodes were trimmed the same shapes as an anode electrode then small holes were lasered over each plate electrodes to connect to the titanium wires. All cathode electrodes size was 0.2 cm × 2 cm × 3.5 cm (width × length × height) for fit into the reactor. Before starting the experiment, all new electrode materials were pretreated to remove their contaminant that feasibly harmful to growth the microbial to inoculate. First, carbon-based graphite felt electrode materials were rinsed deionized water and prepare the solution of 5 mM H₂SO₄ and soaked for each electrode then swilled the deionized water and stored in soaked water for 24 hours. Rather for the metal-based plate electrodes were wash with deionized water and soaked with acetone 15 min then properly wash with deionized water again, all electrodes were soaked in sludge to provide an environment for rapid growth of microorganisms. Stainless steel 304, nickel, and graphite plates electrode after the storage and connected to titanium wire using an epoxy until is glued from titanium wire. As seen Fig. 3-1. Photographs of preparation of electrode process.

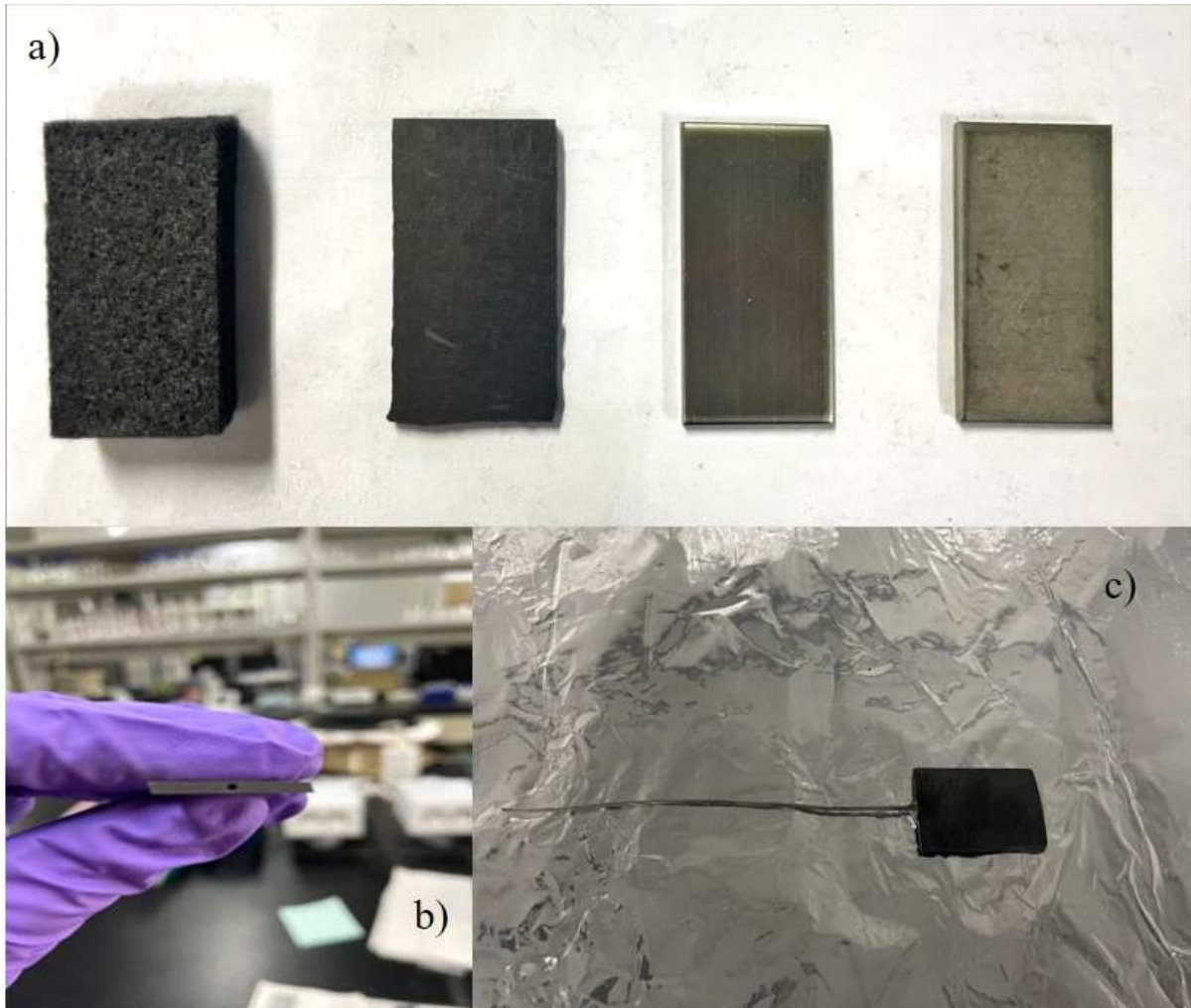


Figure 3-1. a) left to right graphite felt (GF), graphite plate (G plate), stainless steel plate (SS plate) and nickel plate (Ni plate) electrode design for a rectangle, b) process of a making a hole bottom of electrode for connecting to titanium wire, c) after connected to titanium wire

3.3 Reactor setup and operation

As shown Figure 3-3. The lab-scale bio-electrochemical reactor was prepared total a volume of 250-mL glass bottles (Duran) covered a plastic cap (G45). Anode and cathode electrodes were similarly spaced apart, at a distance of 4 cm, on the reactor's side. A gas sampling hole was drilled into the cap for biogas analysis and a water sampling hole was installed. Acid fermentation sludge was used as inoculum and the substrates were in a ratio of 8:2 (swine wastewater and food wastewater). The both electrodes were entirely glued to titanium wire using epoxy sealant. The applied voltage to the bioelectrochemical reactor was linked through copper wire electrodes from an adjustable DC power supply. For the loop current, an external resistance of 10 ohm was attached to measure and record the current across this resistance and recorded every 5 min using computer. Each reactor was filled with 200 mL of swine wastewater/food wastewater mixture and 50 mL of inoculum, then purged with nitrogen for 5 minutes to eliminate oxygen. Keithley power supplies with 0.3 V voltage were used to power the electrodes. All reactors were put in a mesophilic temperature-controlled chamber at 30. Each reactor was also duplicated. The daily gas output was measured utilizing the gas sampling needle throughout the operation. In the Table 3-2 is shows bio-electrochemical operation condition in this study.

In the following experiment, graphite felt and stainless steel plate electrodes were changed with CNT and CNT+TiO₂ dip coatings to enhance their surface area and hence improve hydrogen generation in biological reactors. The operating settings of modified cathode electrodes are shown in Table.3-3. Coating chemicals included isopropyl alcohol for the CNT, TiO₂ 1 g, ETOH 60 g, PEG 5 g, KH570 2 g, and TEOS 2 g for the I of CNT+TiO₂.

Table 3-2 Operational conditions of bio-electrochemical reactors

Parameter		Reactors		
		AD	BEAD	BEAD
Inoculum		Sludge (acid fermentation) 50 mL		
Substrates	SW	Mixing ratios of 8:2 (SW:FW) 150 mL		
	FW			
Voltage (V)		-	-	0.3
Working volume (mL)		200		
Electrodes materials and size	Anode	GF 1 cm 3.5 cm 2 cm		
	Cathode	GF, G palte, SS plate and Ni plate 0.2 cm 3.5 cm 2 cm		
Electrode distance		4 cm		
Operation type		Batch		
HRT		8 days		
Temperature		30°C		

Table 3-3 Operational conditions of modified with CNT and CNT+TiO₂ coated biological reactor

Parameter		Reactors					
		GF	GF+CNT	GF+CNT/TiO ₂	SS	SS+CNT	SS+CNT/TiO ₂
Inoculum		Sludge (acid fermentation) 50 mL					
Substrates		Mixing ratio of 8:2 (SW:FW) 150 mL					
Electrode materials	Anode	Graphite felt (GF)					
	Cathode	Graphite felt (GF) and stainless steel plate (SS plate)					
Surface modified	Anode	-					
	Cathode	carbon nanotube (CNT) and carbon nanotube titanium dioxide (CNT+TiO ₂)					
Electrode distance		4 cm					
Operation type		Batch					
HRT		18 days					
Temperature		30°C					

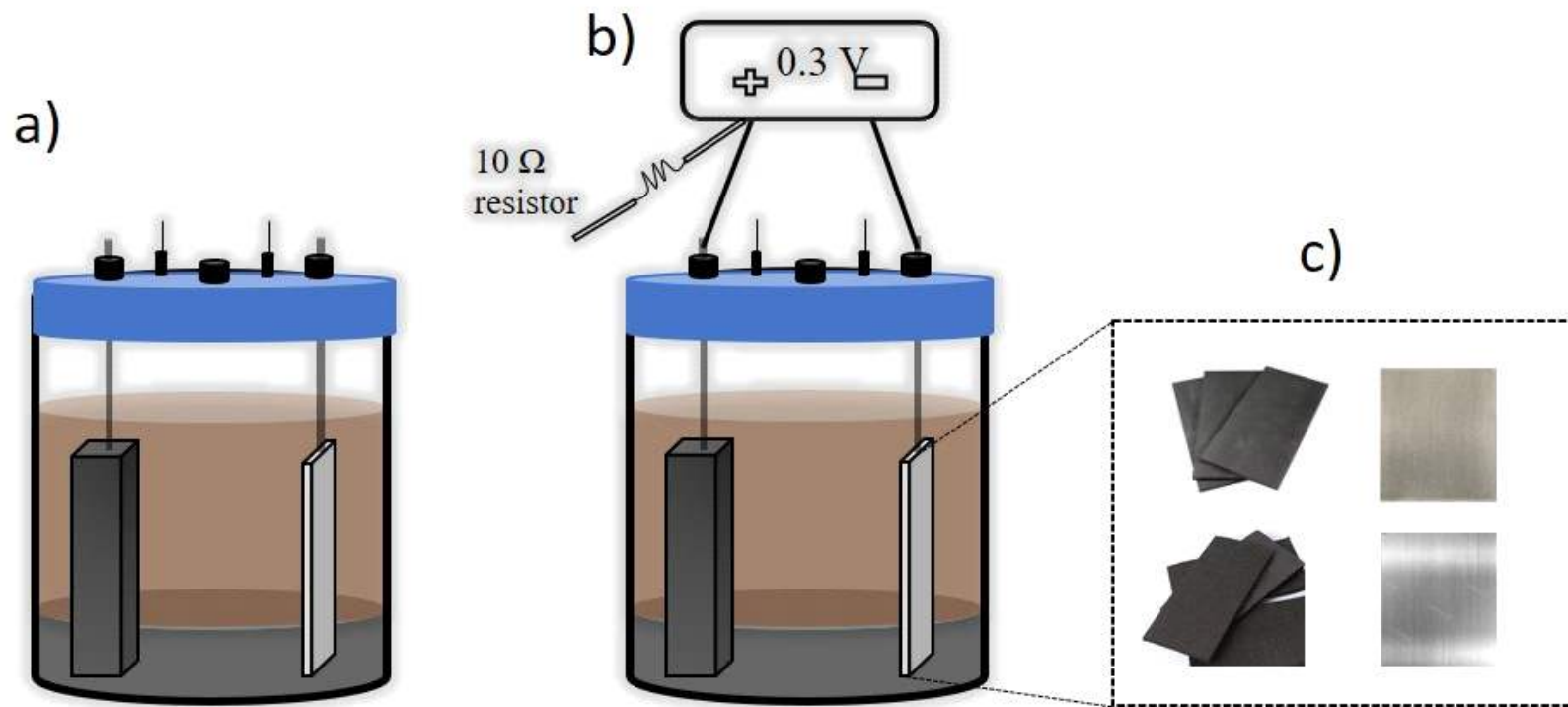


Figure 3-2. Experimental setup. a) Biological reactor b) bio-electrochemical reactor with applied voltage of 0.3 V and c) electrode materials used in this study

3.4 Analysis

Determination of biogas production was measured using gas chromatography-GC (Young in Chromass, YL-6500 system, South Korea) equipped with a thermal conductivity detector (GC-TCD); the column was Mol Sieve 13X (3FT X 1/8IN X 2.1 MM) and a flame-ionization detector (GC-FID/Methanizer) column were PORAPAK N (10FT x 1/8IN x 2.1 MM). The operating temperature of the GC oven was maintained at 40°C for 8 min and the injection temperature was 150°C. 0.1 mL of sample was injected using 0.5 mL Pressure-Lok® Precision Analytical syringe(LA, USA). Nitrogen was used as the carrier gas for the GC.

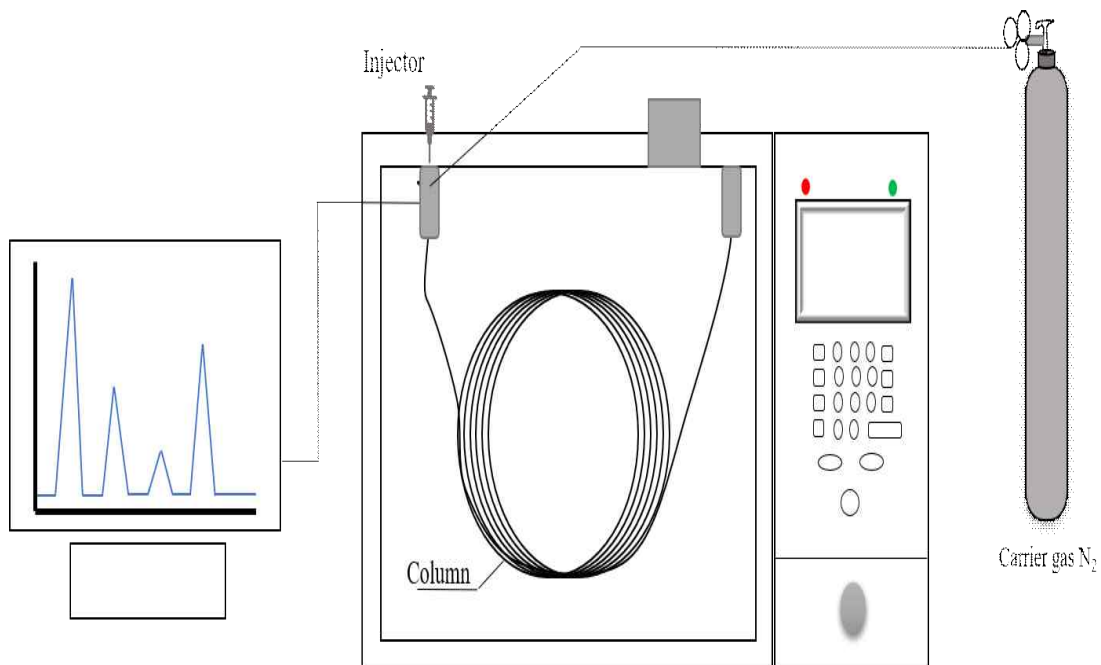


Figure 3-3. Gas chromatography used in this study

3.4.1 Water analysis

Using assay kits from the Hach Company, Inc. (Colorado, USA), samples' total nitrogen (T-N), total phosphorus (T-P), NH₃N, total solids (TS), total chemical oxygen demand (TCOD), and soluble chemical oxygen demand (SCOD) were calculated. The materials were digested using a heater (HACH DR 200), and the results were then analyzed using a UV Spectrometer (HACH DR 5000). A Metrohm 912 Meter was used to measure the pH at both the beginning and end. Total solid (TS) using (JEIO TECH FO-600M) drying oven at 105°C for 2 h, and put into the desiccator until cooled down and Volatile solid (VS) was measured using the (LAB 24 DY-6062-6 South, Korea) heat oven temperature maintained 550°C for 30 min. Each sample was diluted by 10 times for analyzed TS and VS.

3.4.2 Electrochemical analysis

Using swine wastewater as a substrate, the impact of an external supplement voltage of 0.3 V on biogas production was examined. Keithley, a DC power source, was used to supplement the required external voltage. The cathode and anode electrodes were connected to the negative and positive leads of the power supply with an external resistance of 10. At 5-minute intervals, the voltage across the external resistance was measured with a digital multimeter linked to a computer. With a multi-potentiated, three-electrode configuration, all electrochemical studies CV were carried out. The working, counter, and reference electrodes were connected to the cathode, anode, and Ag/AgCl electrodes, respectively. In a three-electrode configuration, cyclic voltammetry (CV) was carried out with a potential range of -0.9 V to 0.9 V (against Ag/AgCl) with scan rates of 100, 50, 20, 10, and 2 mV/s. The potential and current of the redox peaks were calculated using the acquired CV curve and Origin.

3.4 Scanning electron microscope

The surface analysis of the electrodes was performed using a scanning electron microscope (SEM equipment from Jeol, Japan). SEM analysis was carried out in the graphite felt and stainless steel plate 304 electrodes surface are modified with carbon nanotube (CNT) and carbon nanotube titanium dioxide (CNT+TiO₂) using dip coating methods. Checking these two-electrode materials surface area fully covered.

4. Results and discussion

4.1 Evaluation of different cathode electrodes for improvement of bio-electrochemical H₂ production

4.1.1 Effect of embedded electrodes for biological H₂ production

a) Biogas production

The biological reactors which were embedded with four different cathode electrodes and a control reactor without electrode batch mode operated for about 8 days. As shown in Fig. 4-1 on the initial day no hydrogen was detected in all reactors however from the second day hydrogen production was slowly realized by all the electrodes and control except the SS plate. But while other electrodes hydrogen producing capacity increased steadily to 96 h, a very high surge in hydrogen production was observed with graphite felt (GF) than other between electrodes 72 h and 96 h. For most of the electrodes and control after 96 h, a gradual decreased in hydrogen production was observed until no detection was found. For Ni plate and SS plate a little hydrogen production was observed at 144 h, but the production was not sustained. The specific hydrogen production of 236.9 ± 37.91 mL/L-d for graphite felt (GF) and 69.91 ± 27.11 mL/L-d for control, 59.53 ± 50.31 mL/L-d for graphite plate (G plate) at 96 hours, which was about 29.61%, 74.8% higher than the control and G plate, respectively. In this result, both electrode materials used graphite felt, producing more hydrogen than other cathode electrode materials. At the top of Fig. 4-1. pH changes are shown in all reactors. The initial pH was around 6.3~6.4; when pH was 6.0, hydrogen was obtained from all reactors.

Evaluation of biological reactions due to hydrogen generation using four different cathode electrodes from swine wastewater is shown in Fig. 4-2. In the short-time batch tests, hydrogen yields were 27.18 ± 2.41 , 10.54 ± 3.02 , 1.43 ± 0 and 3.92 ± 0 (mL/g VS) for

graphite felt (GF), graphite plate (G plate), stainless steel 304 plate (SS plate) and nickel plate (Ni plate), respectively, while the control was 12.72 ± 9.22 mL/g VS, respectively. The highest hydrogen yields of 27.18 ± 2.41 mL/g VS for graphite felt (GF) reactor, was approximately 53.2% and 61.2% higher than the graphite plate (G plate) and control reactor, respectively. The lowest hydrogen yields in the stainless-steel plate and nickel plate.

In Fig 4-2. Cumulative hydrogen production obtained from the biological reactor with different cathode electrodes and control anaerobic reactor in 8 days operation. The pH change over time in the biological reactor at the top of Figure 4-1, the initial pH of 6.27 was maintained in the reactor except for the graphite felt-stainless steel plate (GF:SS plate) for 24 hours, and then began to decrease rapidly to 6.04-6.13 at 48 hours.

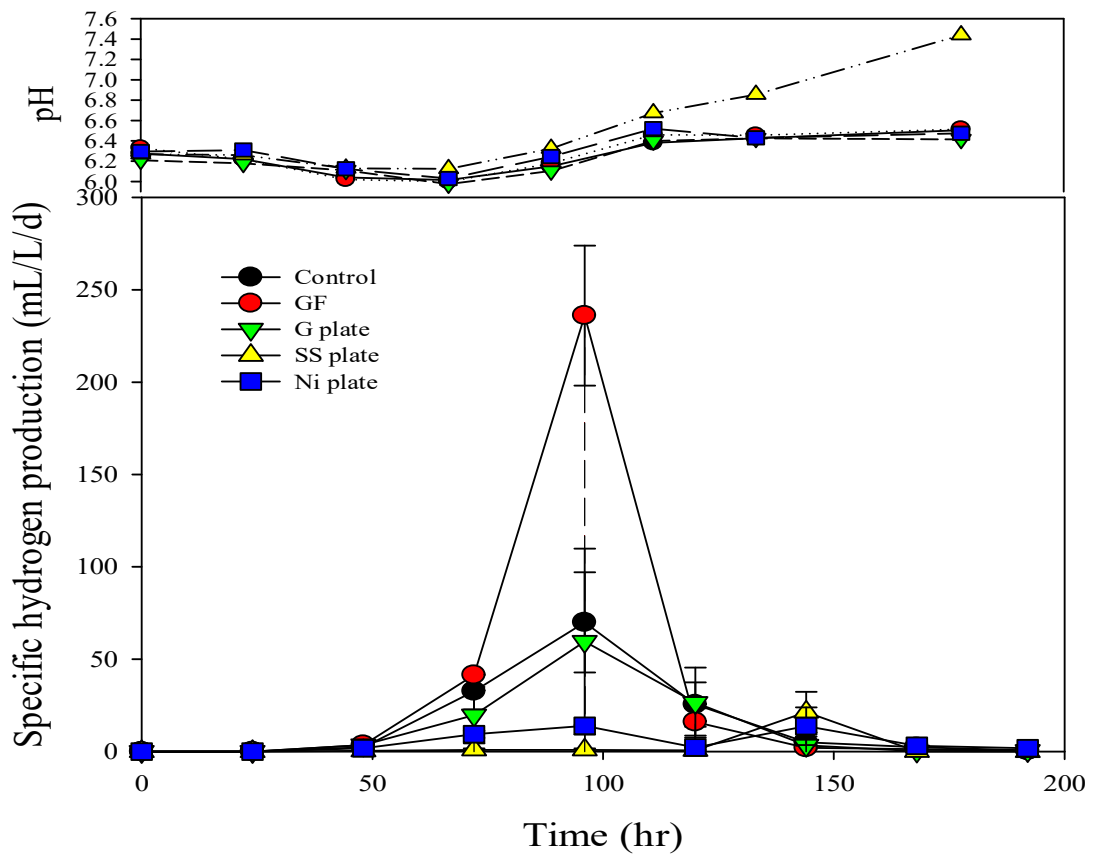
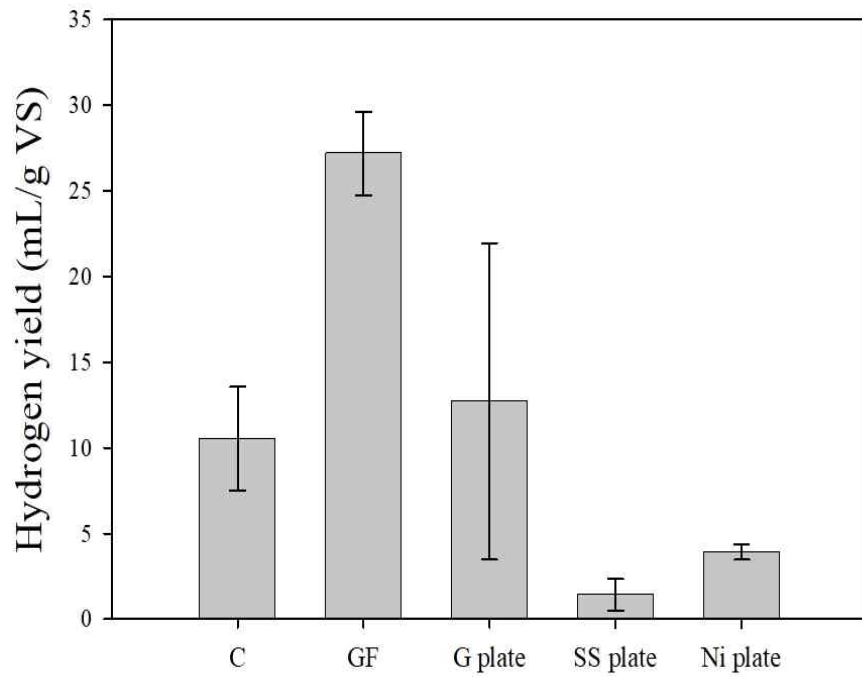


Figure 4-1. Specific hydrogen production (mL/L/d) with pH changes in biological reactor during 192 hr (8 days)

a)



b)

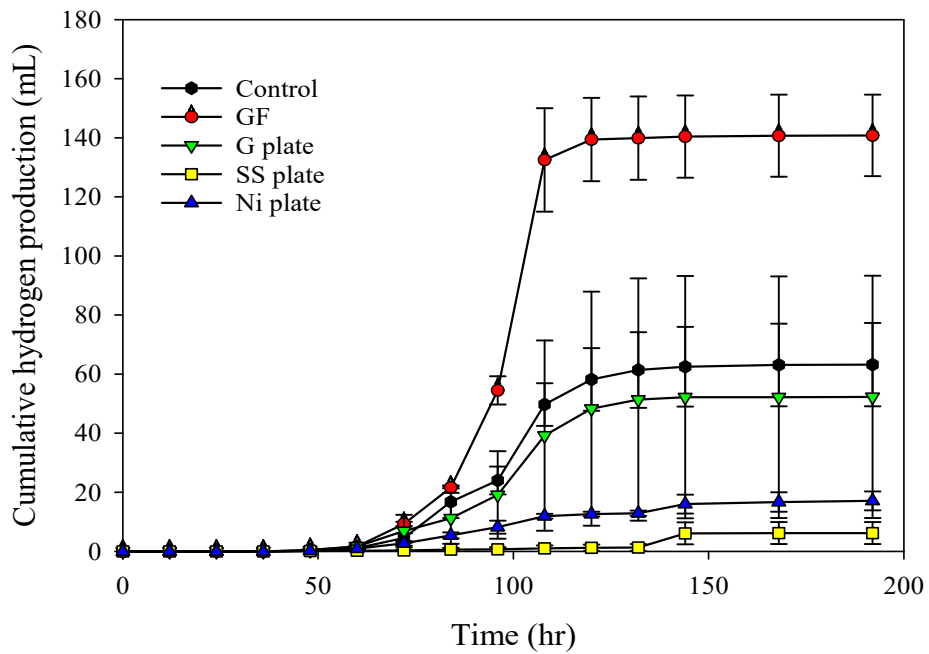


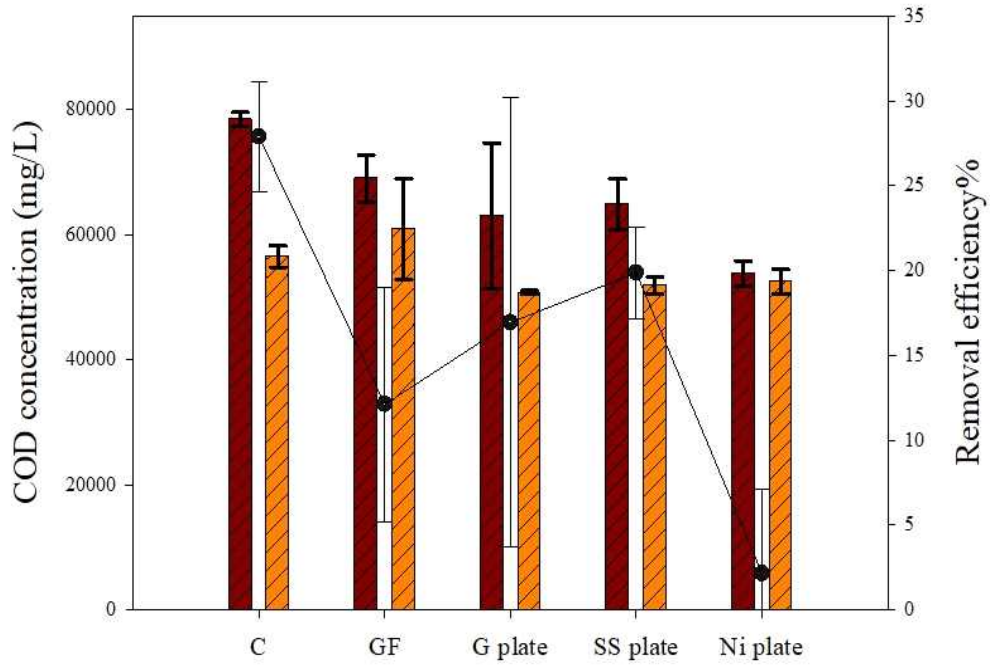
Figure 4-2. a) Hydrogen production yield (mL/g VS) and b) cumulative hydrogen production (mL) in biological reactor compared to without electrode (control) and different electrodes

b) Evaluation of water compounds

The initial and final chemical oxygen demand (COD) and removal efficiency% from all the reactors are shown in Fig 4-3a) The initial (COD) concentration at the beginning of the experiment was 78,450 mg/L for the control reactor. For the different electrodes, initial COD concentration was 69,000, 63,000, 64,900 and 53,800 mg/L for GF, G plate, SS plate and Ni plate reactors. After the experiment, final CODs were 56,550 mg/L (27.86% removal, control), 60,900 mg/L (12% removal, GF), 50,775 mg/L (16.95% removal, G plate), 51,900 mg/L (19.8% removal, SS plate) and 52250 mg/L (2.14% removal, Ni plate).

As shown Fig 4-3b) TS and VS removal efficiency in the biological reaction was confirmed in the order of graphite felt, nickel felt, control and stainless steel plate were checked. TS removal efficiency of $38.82 \pm 2\%$, $56.80 \pm 14.42\%$, $39.6 \pm 3\%$, $34.3 \pm 4\%$ and $45.8 \pm 5.3\%$ for control, GF, G plate, SS plate and Ni plate, respectively. The highest TS removal efficiency from organic solids in graphite felt could be from 3D structure of graphite felt, due to the higher surface area, which probably facilitated high reactions than other electrodes. A similar result was observed with the VS removal efficiency. The lowest VS removal efficiency was observed with graphite plate ($41.35 \pm 6\%$), $41.6 \pm 1.5\%$ for stainless steel plate, $44.5 \pm 6\%$ for control, $49.6 \pm 6\%$ for nickel plate while the highest VS removal efficacy was recorded by the graphite felt ($63.1 \pm 14\%$).

a)



b)

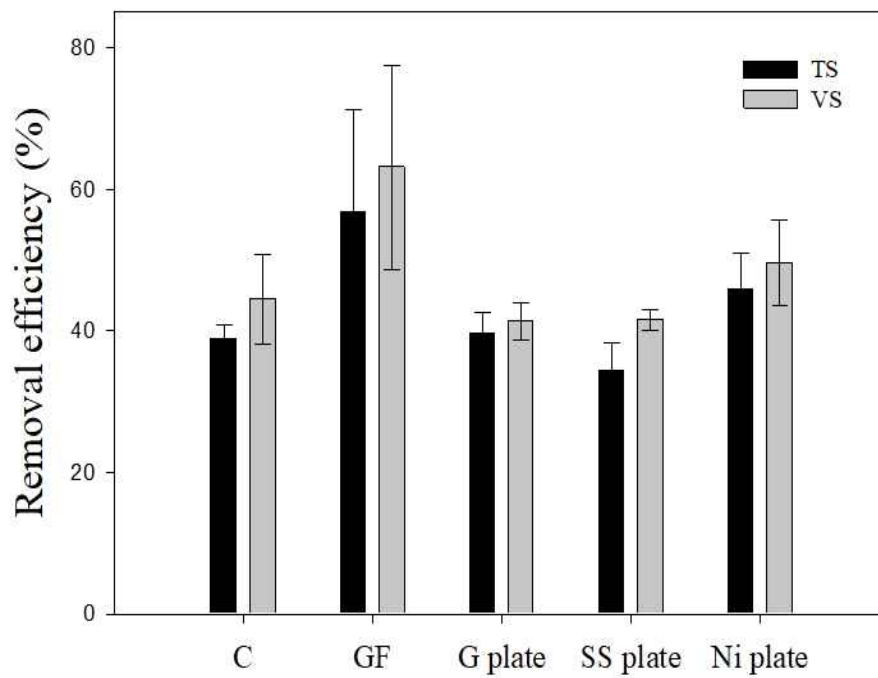


Figure 4-3. a) Total chemical oxygen demand (TCOD), and b) TS and VS initial and final concentration and removal efficiency in biological reactor using different electrodes

4.1.2 Performance of bioelectrochemical H₂ production

a) Biogas production

The hydrogen production according to the voltage during the bio-electrochemical experiment period is shown Fig.4-4. Other previous studies, summarized that the optimal voltage for hydrogen production is approximately in the range of 0.3 V to 1.2 V. This experiment, a voltage of 0.3 V was applied to improve hydrogen production from swine wastewater using biological reactors, which were embedded with different cathode electrodes. The highest hydrogen specific production was observed at 96 h with all different electrode. The highest hydrogen specific production of 127.6±15.33 mL/L-d was observed in the reactor embedded with the graphite plate, followed by 107.9±10.2 mL/L -d for the stainless steel plate reactor, 71±58 and 11.8±5 mL/l-d for nickel plate for graphite plate reactors, respectively. Hydrogen production was detected faster than biological reactors in all electrodes with applied voltage. The higher specific hydrogen production detected with the graphite plate was 15.4%, 44.3% and 90.7% higher than stainless steel plate, nickel plate and graphite felt, respectively. Hydrogen production with the all plate (stainless steel plate and graphite plate and nickel plate) cathode electrodes increased with applied voltage. In an electrochemical system, it is considered with a voltage of 0.3, the activity of microorganisms involved in the removal of organic matter and hydrogen production increased at the oxidation and reduction electrodes in the reaction tank. The initial pH was around 6.2~6.5; however, the highest hydrogen production detected during the operation was at pH 6.0.

Fig 4-5a) shows hydrogen yield from an applied voltage of 0.3 V at different cathode electrodes. In biological reaction, graphite felt detected high hydrogen production, but bioelectrochemical reaction which applied a supplement voltage of 0.3 V, showed low hydrogen production. Furthermore, graphite plate gave a hydrogen yield of 14.4 mL/g VS and 10.5 mL/g VS for stainless steel which represents a higher hydrogen yield of 39.5% and 17.1% higher than nickel plate, respectively, in the

bioelectrochemical anaerobic digestion system.

The electric potential on the polarized electrodes drives the DIET in the bioelectrochemical reactor, which increases hydrogen generation more than the biological reactor by enriching the electrode surface. The cumulative hydrogen production was 101.6 ± 1.5 mL for the graphite plate, 92.6 ± 5.8 mL for the stainless steel plate, 58.7 ± 4 mL for the nickel plate and 14.5 ± 3 mL for graphite felt, respectively. However, the stainless steel plate produced hydrogen production faster than the graphite plate in the first 3 days. (Fig 4-5b).

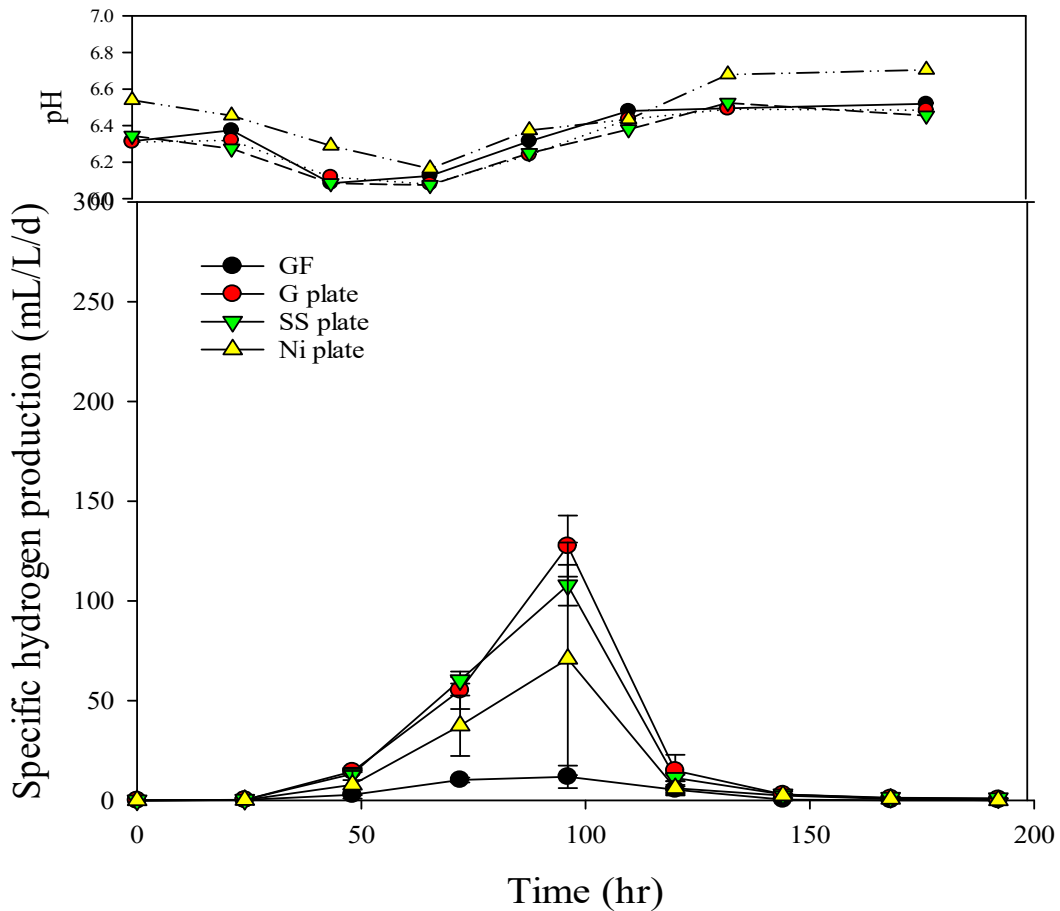


Figure 4-4. Specific hydrogen production rate mL/L-d and pH change in bioelectrochemical reaction during 8 days operation

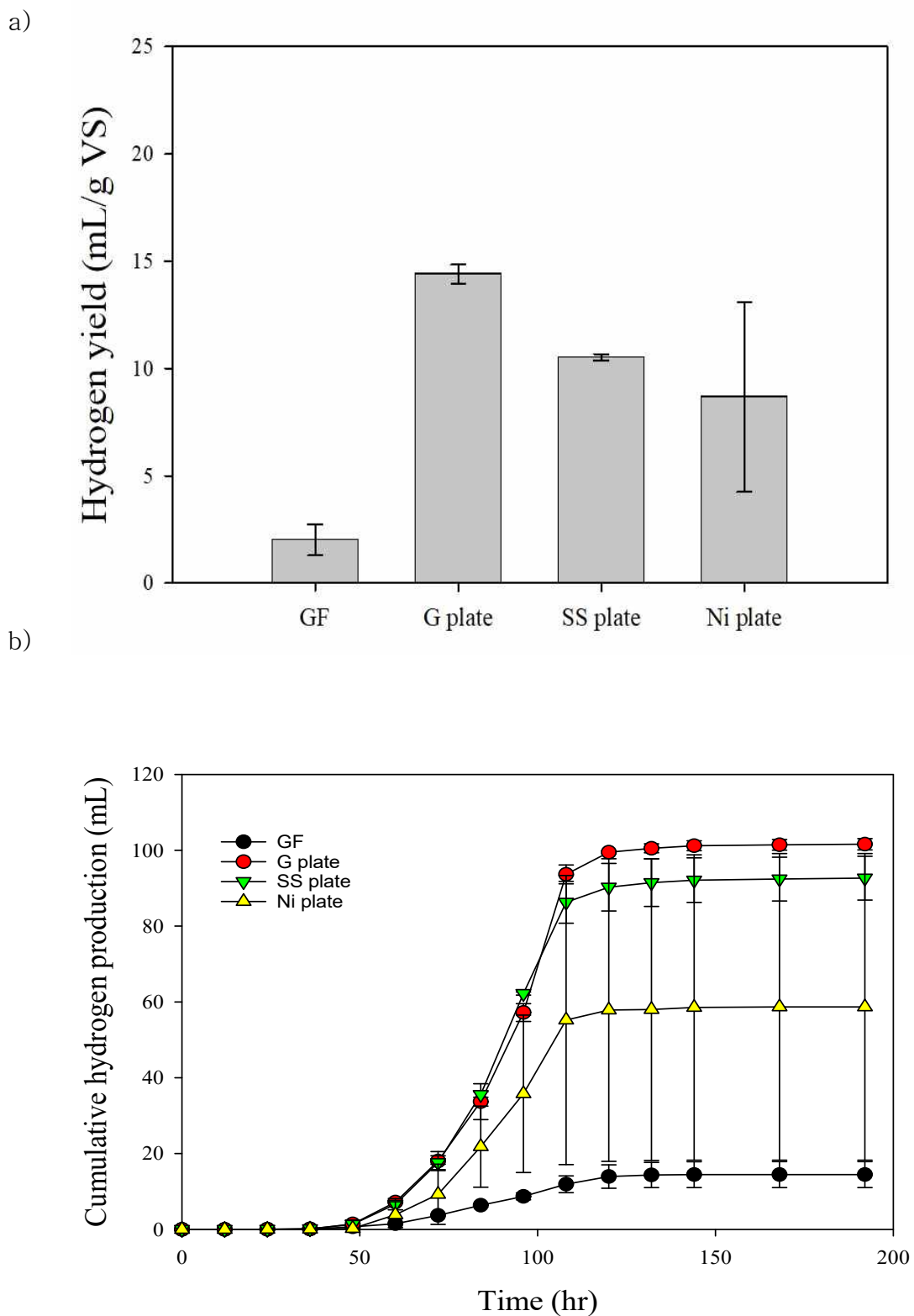


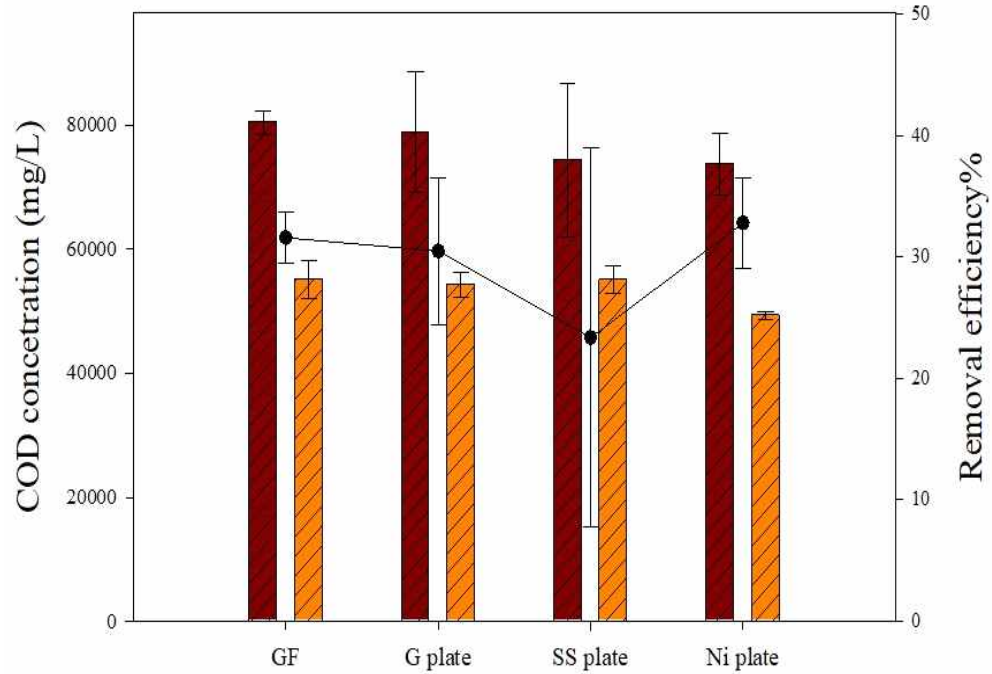
Figure 4-5.a) Hydrogen production yield (mL.g VS) and b) cumulative hydrogen production at an applied voltage of 0.3 V

b) Evaluation of water compunds

The bioelectrochemical reactor COD initial and final concentration and removal efficiency results are shown in (Fig. 4–6. a) The performance of the bioelectrochemical reactor with an applied voltage of 0.3 V was faster and had a higher COD concentration than the biological reactor. The initial TCOD concentration was 83,650 with graphite felt electrode but decreased to 55,125 mg/L after 8 days of operation. Other electrode materials, such as graphite plate, stainless steel plate, and nickel plate, were 78,900, 74,400, and 73,700 mg/L, respectively. The organic matter removal efficiency was 32.7% for nickel plate, 31.5% for graphite felt, 30.4% for graphite felt, and 23.3% for stainless steel plate in the bioelectrochemocal reactor.(Fig 4-6)

Figure 4-6.b) shows TS and VS removal efficiency with applied voltage of 0.3 V at different cathode electrodes. TS removal efficiency end of the experiment showed $49.5\pm 1\%$, $54.0\pm 3\%$, and $50.8\pm 6\%$ for graphite felt, graphite plate, and nickel plate respectively, while the stainless steel plate had the highest TS removal efficiency of $64.0\pm 4\%$ a similar trend was observed for the VS removal. The highest VS removal efficiency was $66.7\pm 4\%$ for stainless steel plate, followed by $57.9\pm 1\%$ for graphite felt, $52.8\pm 1\%$ for graphite plate and the least was $52.4\pm 8\%$ for nickel plate.

a)



b)

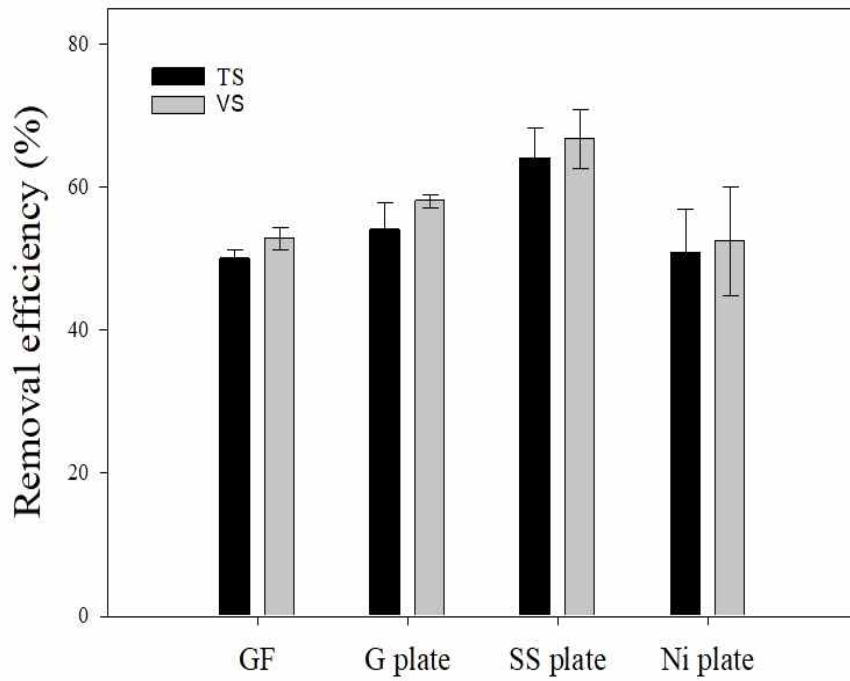


Figure 4-6.a) COD initial and final concentration and removal efficiency and b) TS and VS removal efficiency at different electrodes in bioelectrochemical reactor

4.2 Electrochemical characteristics of different cathode electrodes

4.2.1 Cyclic voltammetry analysis

Electrochemical characterization of the graphite felt and stainless steel plate was measured to examine the catalytic behavior of the bare electrode and coated with CNT and CNT+TiO₂. Fig. 4-7 a shows the cyclic voltammetry (CV) analysis of a bare graphite felt electrodes redox activities in substrate as mixing ratio of swine wastewater and food wastewater and inoculum source was acid fermentation sludge used in solution.

The CV investigation of bare electrodes and their redox activities in solution as a mixing ratio of swine wastewater, food wastewater, and sludge utilized in the solution is depicted in Fig. 4-7a) and b). The CV measurement was performed throughout a potential range of -0.4 V to 0.4 V and at various scan rates of 2 mV/s, 10 mV/s, 20 mV/s, 50 mV/s, and 100 mV/s. The bare graphite felt electrode at 2 mV/s scan rate produced a greater peak than the other scan rates (Fig.4-7a). b) At different scan rates, the bare stainless steel plate electrode produced a varied result. However the modified CNT coating peak was greater than the CNT+TiO₂ coated graphite felt peak in Fig.4-8a), indicating that the redox reaction of modified CNT graphite felt was improved. However, in Fig.4-9 a), the CNT coated stainless steel plate has a lower peak than the naked stainless steel plate. However, the modified CNT+TiO₂ stainless steel plate had a wider range of reduction peaks than the others (Fig.4-9b). .

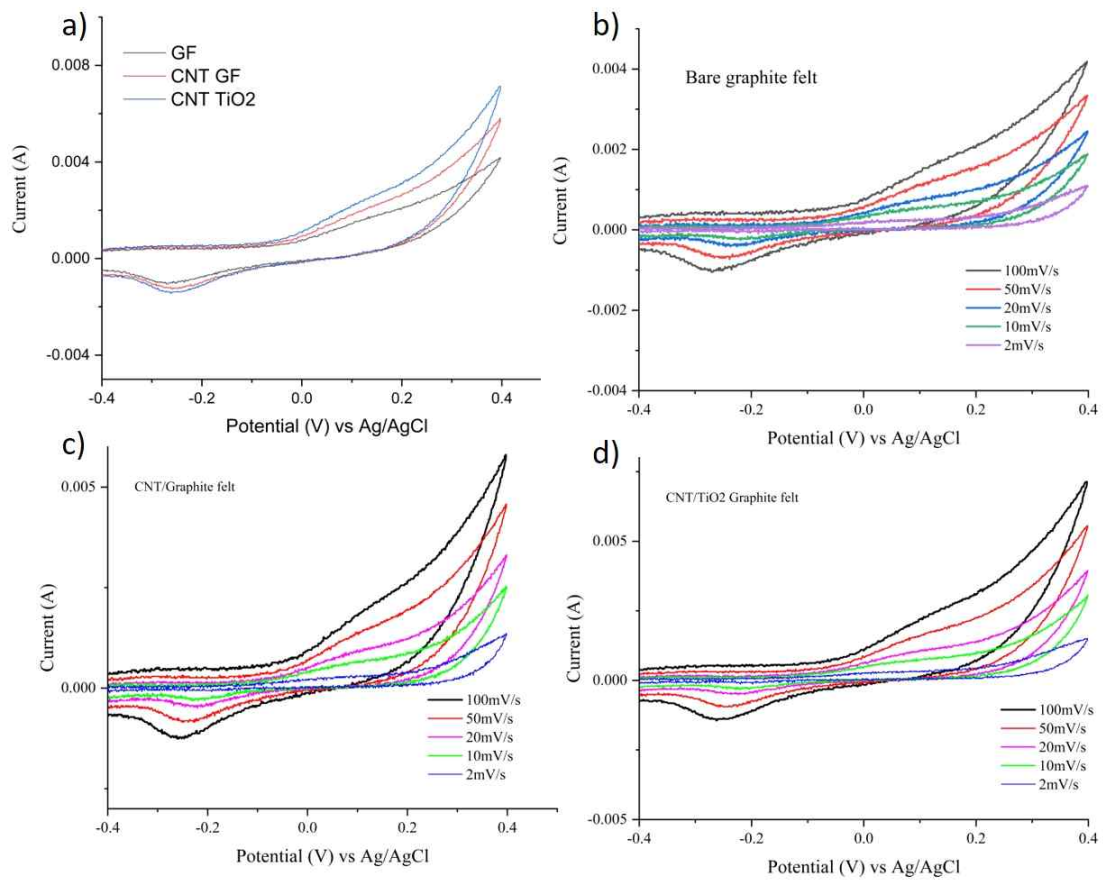


Figure 4-7. Cyclic voltammetry (CV) analysis of a) bare GF, CNT coated GF, and CNT+TiO₂ at 100 mV/s b) bare GF, c) CNT coated and d) CNT+TiO₂ coated at different scan rates

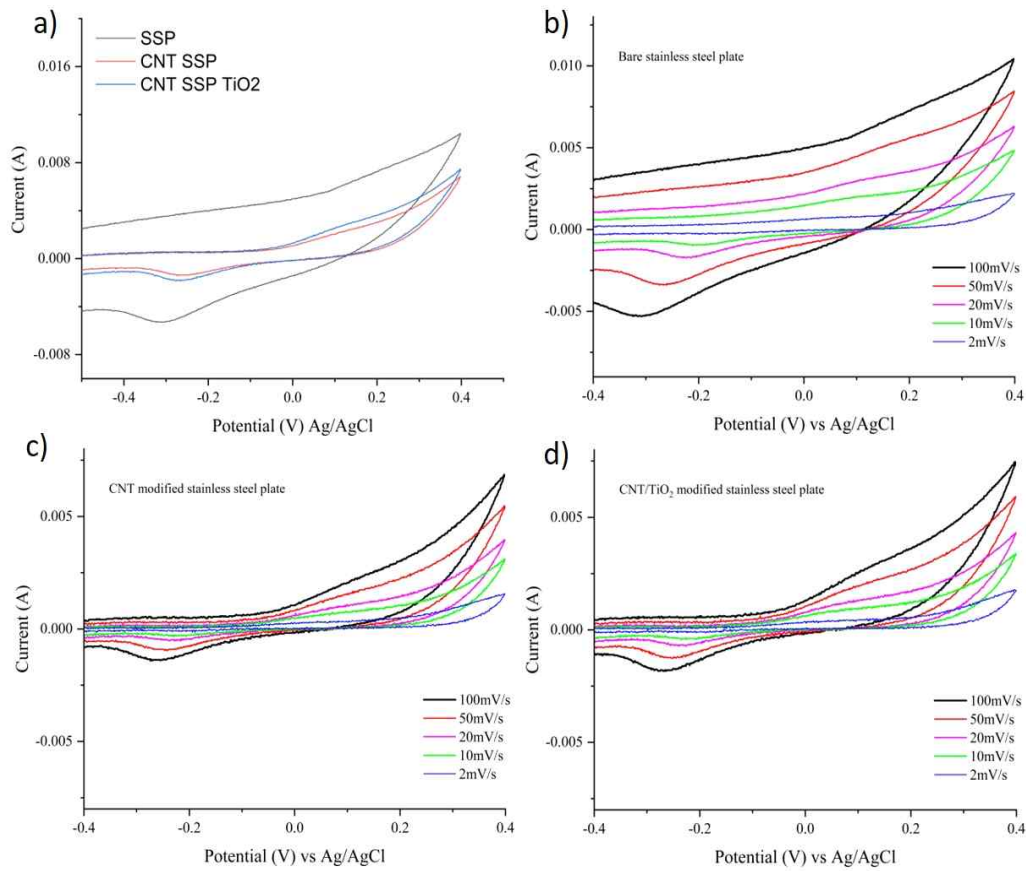


Figure 4-8. Cyclic voltammetry (CV) analysis of a) bare SSP, CNT and CNT+TiO₂ coated at 100 mV/s b) bare SSP, c) CNT coated and d) CNT+TiO₂ coated at different scan rates

4.2.2 SEM analysis

SEM was used to evaluate the cathode surface appearance. Stainless steel plate 304 and graphite felt electrodes performed best during hydrogen production in a bio-electrochemical reactor. Graphite felt and stainless steel plate electrodes will be used in the next experiment to increase their performance for hydrogen generation in bioelectrochemical anaerobic digestion. As shown in Fig 4-13. a-b, bare graphite felt electrode coated with 1 wt% carbon nanotube (CNT) and 1 wt% carbon nanotube titanium dioxide (CNT+TiO₂) totally covered by CNT and (CNT+TiO₂). Figures 4-10. d-f depict a stainless steel plate coated with nanoparticles. The cathode electrode is coated with CNT and CNT+TiO₂ nanoparticle size, which increases the bare electrode surface area and microorganism attachment in the cathode.

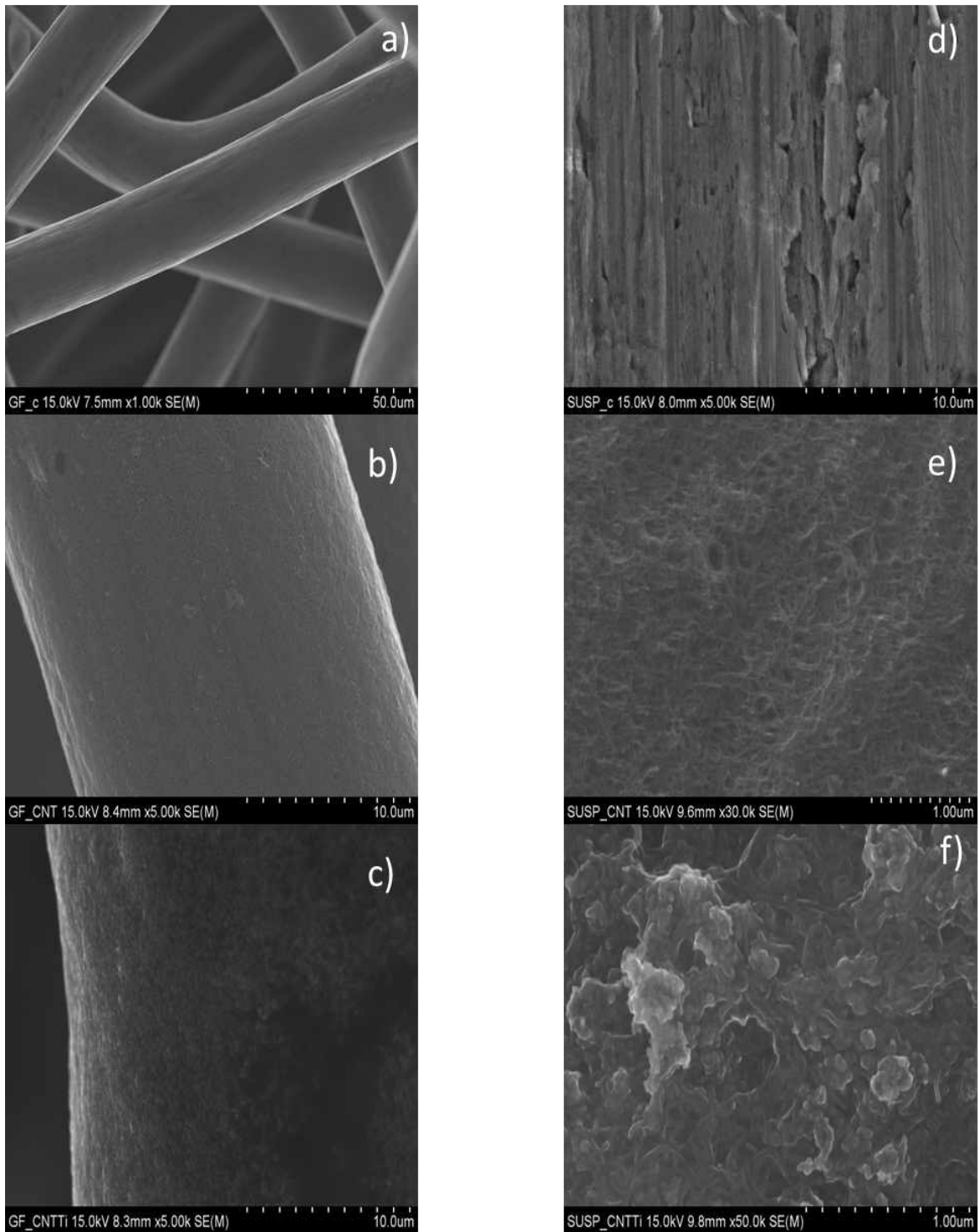


Figure 4-9. SEM images of a) bare graphite felt (GF) b) modified CNT graphite felt (GF+CNT) c) modified CNT+TiO₂ (GF+CNT/TiO₂) d) bare stainless steel plate (SS plate) e) modified CNT stainless steel plate (SS+CNT) f) modified CNT+TiO₂ stainless steel plate (SS+CNT/TiO₂)

4.3 Effect of CNT and CNT+TiO₂ coating on selected cathode electrodes

4.3.1 Effect of embedded modified electrodes for biological H₂ production

a) Biogas production

From above the result shows that graphite felt had high hydrogen production in biological reactors. Also, with applied voltage of 0.3, the bioelectrochemical reactor shows high hydrogen production from graphite plate and stainless plate electrodes. The rate of hydrogen production in the bioelectrochemical anaerobic digestion system reactor produced hydrogen for three to four days, but this experiment's result showed no hydrogen was discovered until 8 days (216 hours). After 8 days of operation, hydrogen production rapidly increased in the CNT coated stainless steel, and graphite felt. (Fig. 4-9) Also, bare graphite felt electrode shown higher hydrogen production in bare stainless steel plate electrode. The highest hydrogen generation was recorded in SS+CNT and GF+CNT (538.8 mL and 219.2 mL, respectively), and the pH was 5.9 and 5.5.(Fig 4-9). CNT+TiO₂ coated graphite felt electrode slowly produced hydrogen during the operation. But stainless steel plate no hydrogen was detected until the end of the experiment.

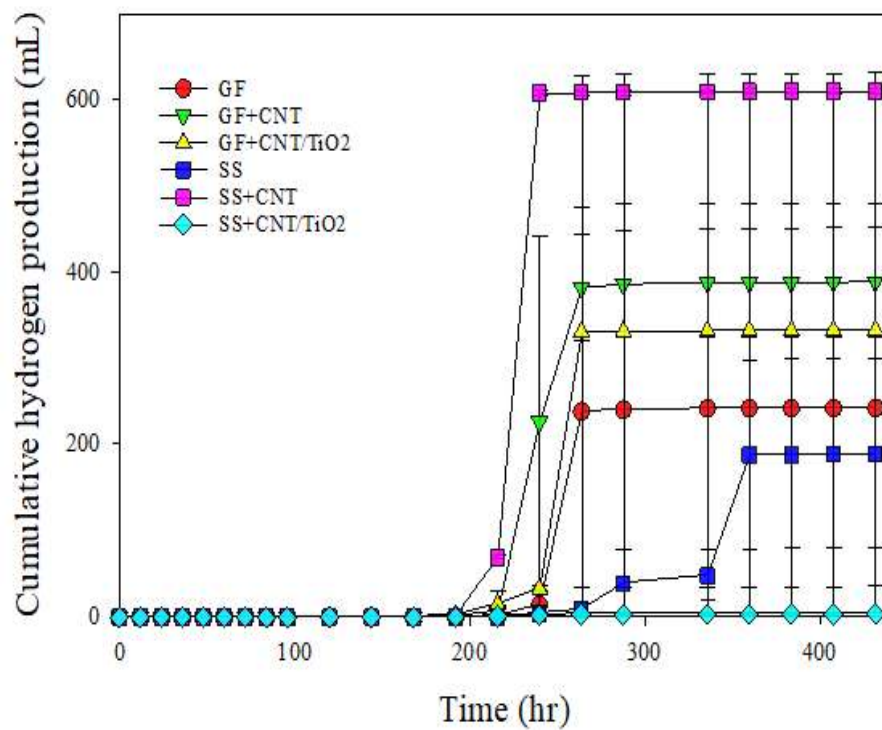
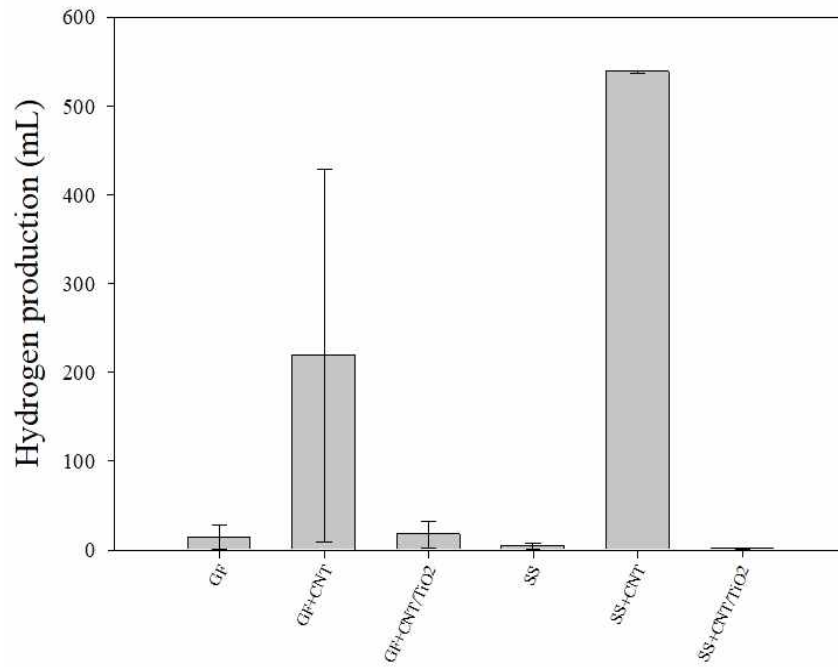


Figure 4-10. Cumulative hydrogen production (mL) bare electrode and CNT and CNT+TiO₂ coated electrode

a)



b)

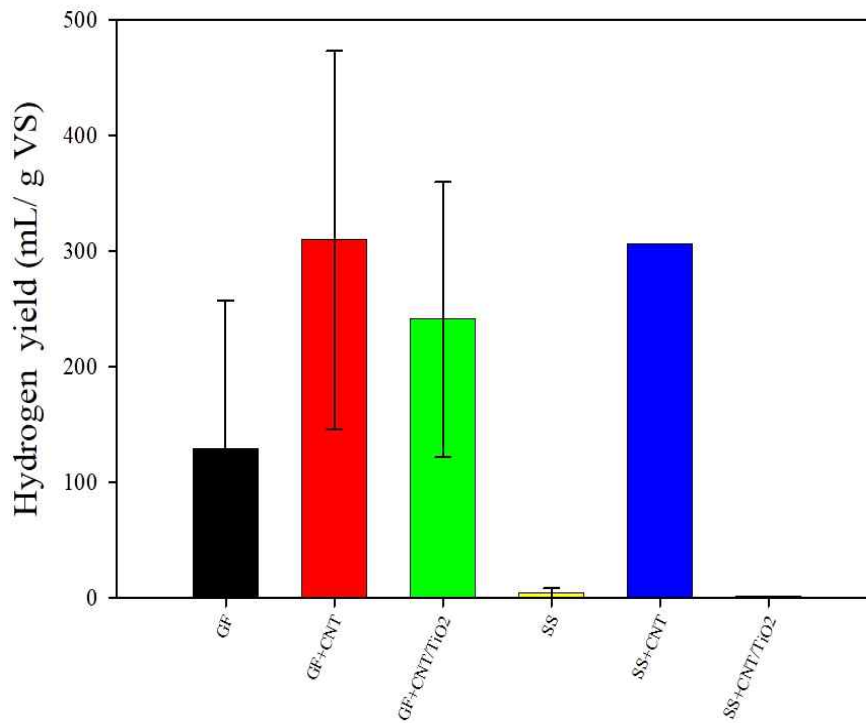


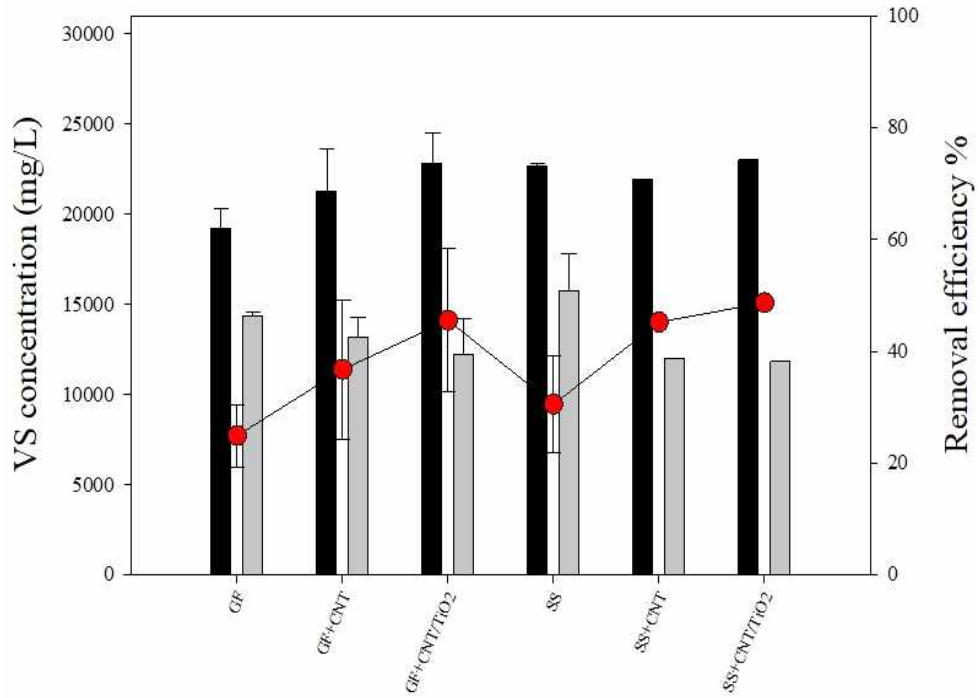
Figure 4-11.a) Hydrogen production (mL) and b) hydrogen yield (mL/g VS) of bare graphite felt and stainless steel compared to CNT and CNT+TiO₂ coated electrodes

b) Evaluation of water compounds

The modified cathode biological reactor COD initial and final concentration and removal efficiency results are shown in Fig. 4-11.b The performance of the modified cathode electrode biological reactor COD concentration was lower than the bioelectrochemical reactor. The initial COD concentration was 35,250 with bare graphite felt electrode, 37,350 mg/L for bare stainless steel electrode but there was no reduction showed in each reactor end of the experiment. Other modified electrodes, such as GF+CNT, SS+CNT, GF+CNT/TiO₂, and SS+CNT/TiO₂ were 39,900, 43,000, 41,350, and 41,400 mg/L, respectively. The highest organic matter removal efficiency was showed 20.3% for SS+CNT/TiO₂, following by 18.1% for SS+CNT, 16.6% for GF+CNT/TiO₂, 12.6% for bare GF, 10.7% for GF+CNT, respectively, however bare stainless steel plate electrode showed lowest organic removal efficiency.

Figure 4-11.a shows VS concentration and removal efficiency from bare and modified cathode electrodes in the biological reactors. The initial VS concentration was around 19,000~23,000 mg/L in all reactor. VS removal efficiency end of the experiment similar result showed 48.6%, 45.5%, and 45.2% for SS+CNT/TiO₂, GF+CNT/TiO₂, and SS+CNT respectively, while bare graphite felt had the lowest VS removal efficiency of 24.9% observed expect other. The highest VS removal efficiency was 48.69% for SS+CNT/TiO₂ which was no hydrogen detected whole operation period.

a)



b)

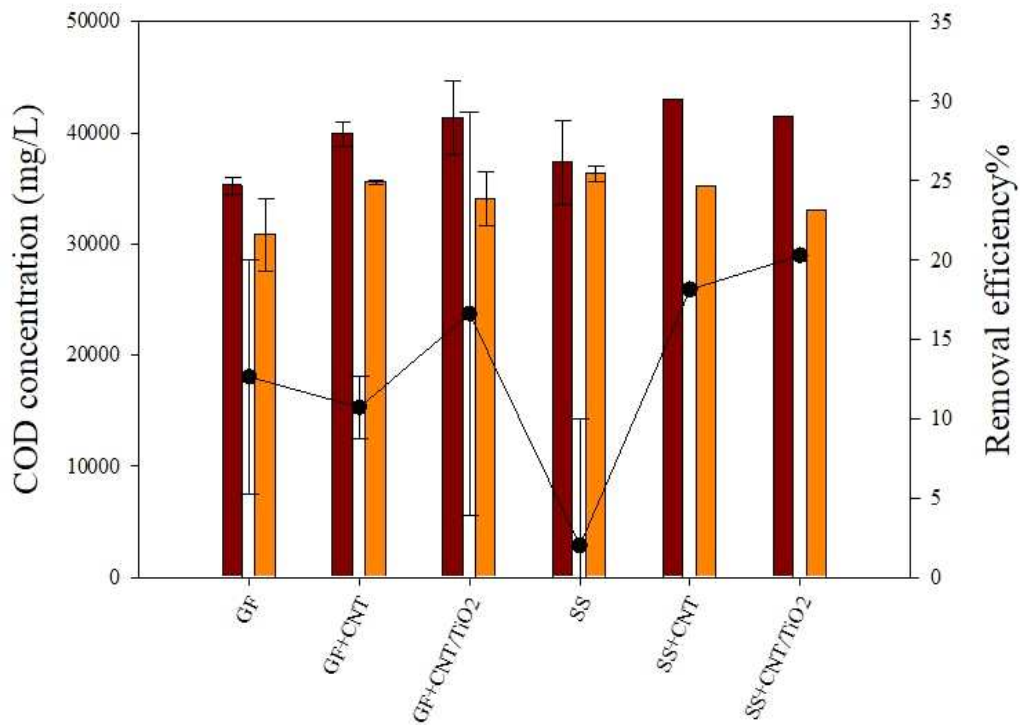


Figure 4-12.a) VS and b) COD concentration and removal efficiency in electrodes coated with CNT and CNT+TiO₂ compared to bare electrodes

4.4 Hydrogen generation according to electrode distance

4.4.1 Biogas production

When the electrode spacing was reduced from 4 cm to 1 cm during the biological process, hydrogen production increased. The greatest increases in hydrogen production occurred at 1 cm and 2 cm. The maximum hydrogen production was 13.06 mL for electrode distances of 1 cm, while the minimum hydrogen production was 2.73 mL for electrode distances of 4 cm after 24 hours. The hydrogen production was 8.1 mL when two electrodes were separated by 2 cm. The 4 cm generated very little hydrogen. This was most likely due to a lack of surface area on the anode for bacteria. Furthermore, an electrode distance of 1 cm demonstrated hydrogen production. When an applied voltage was provided, the electrode separation was 2 cm and 1 cm in earlier studies. However, comparable findings were obtained in this investigation when no voltage was provided to the electrode. Fig. 4-14 a) depicts the hydrogen yield of electrode spacings of 1.12 cm for 4 cm, 2.57 mL/g VS for 2 cm, and 4.37 mL/g VS for electrode spacings of 1 cm for 4 cm, respectively. According to the TS and VS study, the VS removal rate was 40.37% higher at the electrode distance of 2 cm, an increase of (48.04%), and the TS removal rate was 2 cm higher in the order of 2 cm, 1 cm, and 4 cm, an increase of 40.2% in comparison to 4 cm.

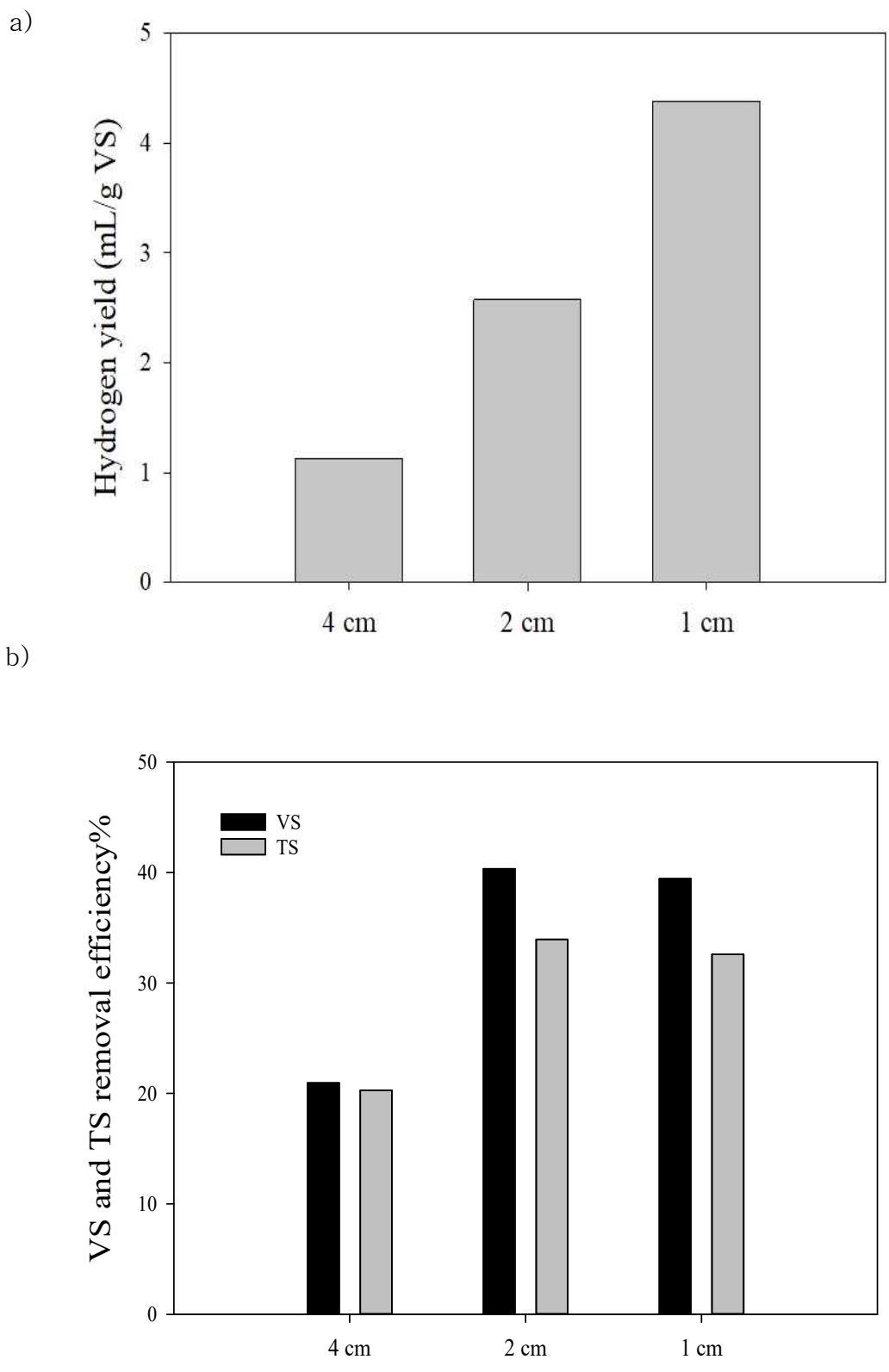


Figure 4-13.a) Hydrogen production yield (mL/g VS) and b) VS and TS removal efficiency at different distance

5. Conclusions

This study investigated the bio-electrochemical biohydrogen production from swine wastewater in bioelectrochemical anaerobic digestion systems using four different cathode electrodes namely: graphite felt, graphite plate, nickel plate and stainless steel plate. The research concludes that:

1. Graphite felt electrode has the best capacity for hydrogen production in a biological system than other electrodes used. In contrast graphite felt capacity for enhancing hydrogen production in a bioelectrochemical system is very poor.

2. In the bioelectrochemical reactor, with an applied voltage of 0.3 V, the stainless steel plate has the best capacity to enhance hydrogen production among other electrode used. The finding of this experiment add to understanding of the effect of applied voltage on the electrode on hydrogen production.

3. The assessment of two cathode electrode surface areas that have been changed with CNT and CNT+TiO₂ dip coatings to be larger than the original, in order to increase hydrogen production in the biological reactor was performed. The results revealed that no hydrogen was obtained from each reactor throughout the 8-day period, however at 216 h, minimal hydrogen was obtained from the SS+CNT and GF+CNT reactors and rapidly grew compared to the other reactors. At 240 h, the bare stainless steel plate and graphite felt electrode materials treated with carbon nanotube (CNT) produced the most hydrogen, 538.8 and 219.2 mL, respectively. Across the way, CNT+TiO₂ coated stainless steel plate electrode no hydrogen obtained during the operation while organic removal efficiency showed higher than the other reactor.

4. Reducing the anode and cathode electrode distance could increase hydrogen production, the highest hydrogen yield was 4.37 mL/g VS for 1 cm.

5. Despite recent breakthroughs in research towards electrode materials developments and improvements, current BEAD technology still has more room for improvement in order to be effectively utilized. Therefore more developments in this sector are still required.

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