Thesis for the Degree of Master of Science

A microbially catalyzed anode and cathode microbial electrosynthesis system for efficient metformin removal and volatile fatty acid production

School of Architectural, Civil, Environmental, and Energy Engineering

The Graduate School

Abdul Samee Ali

December 2022

The Graduate School

Kyungpook National University

A microbially catalyzed anode and cathode microbial electrosynthesis system for efficient metformin removal and volatile fatty acid production

Abdul Samee Ali

School of Architectural, Civil, Environmental, and Energy Engineering

The Graduate School

Supervised by Professor Dae Sung Lee

Approved as a qualified thesis of Abdul Samee Ali
for the degree of Master of Science
by the Evaluation Committee

December 2022

Chairman   <u>Prof. Woong Kim</u>   (인)

<u>Prof. Chang min Park</u>   (인)

<u>Prof. Dae Sung Lee</u>   (인)

The Graduate School Council
Kyungpook National University

Abstract:

The removal of different pharmaceuticals and personal care products from surface water is crucial. This study focused on the removal and transformation of metformin (MTF), an emerging contaminant in aqueous solutions using a dual bioanode (BIND) and biocathode (BICD) microbial electrosynthesis system. Successful biodegradation of MTF (91%) was achieved within 120 h with improved bioelectrochemical performance. The current density (-849 mA/m^2) with drug loading at 0.5 ppm was 10.3, 7.6, and 2.4 times higher than that of the control, 0.1 ppm, and 0.3, ppm, respectively. Volatile fatty acids (VFA) production also improved with excellent acetate, propionate, and butyrate production at both BIND and BICD. Liquid chromatography Mass spectrometry (LC-MS) studies indicated improved mineralization with more MTF bioproducts. MTF regulated the microbial flora through enrichment of the electroactive phyla *Proteobacteria* and *Bacteroidetes*. This study provides a new perspective for the use of dual biocatalyzed microbial electrosynthesis systems in bioremediation research.

Acronyms:

1. Metformin (MTF)

2. Diabetes mellitus type 2 (T2DM)

3. Guanyl -urea (GUA)

4. Hybrid Microbial electrosynthesis system (Hybrid MES)

5. Bioanode (BIND)

6. Biocathode (BICD)

7. Volatile fatty acid (VFA)

8. Dimethyl sulfoxide (DMSO)

9. Cyclic voltammetry (CV)

10. Chrono amperometry (CA)

11. Bioelectrio chemical system (BES)

12. Wastewater treatment plants (WWTPs)

Table of content

List of Table

List of Figures

inoculum and the CF samples. Bacterial taxonomic makeup and relative abundance (percent) at the (a) phylum, (b) class, and (c) genus level.

Chapter 1.0

1.0. Introduction:

An increase in the amount of pharmaceutical production has participated tons increase in the concentrations of pharmaceutical presence in the wastewater and surface water[1][2]. Pharmaceutical wastewater pollutants are born during the drug production method (fermentation, chemical synthesis, extraction, and packing)[3][4]. The large pollution pollutants of this chemical synthesis of drugs in the environment are through water excreted by industries [1][5]. Many articles have described industrial drug synthesis and their products are present in systems of wastewater treatment plants (WWTPs) and their removal efficiency is very low [1][6]. Wastewater during the production of pharmaceutical products or in industrial use is a very serious problem due to high production for the aquatic environment [7] many research papers have mentioned the effect of drugs against diabetes in surface and waste water[8][9]. Metformin (MTF) ($C_4H_{11}N_5$) is among the top advised and globally use compounds. Globally, a percentage of the age of the world has been affected by diabetes[10][9]. Now a day's some research has described that MTF is present in very high concentrations in domestic discharge water [9]. Therefore, MTF dis

discharged un-absorbed from the human digestive system in urine and finish up in excreted water and aquatic environments[11]. MTF has maximum amounts in domestic discharge water (64–98 µg/L)[12]. Pharmaceutical and waster coming from industries are treated with very chemical, physio and biological process [2] MTF can be degraded with aerobic bacteria to guanyl urea as a product which is very hard against further biodegradation in different discharge water treatment methods and plants[8][9][13] However, it is a very good idea to remove the MTF aerobically, anaerobically and other industrial pollutants. MTF is a globally spread pharmaceutical drug in domestic and industrial wastewater[14]. MTF is a drug of Diabetes mellitus type 2 (T2DM) and worldwide spreads in Europe waste and domestic water. In wastewater, a large amount of about 60µg/l was detected in 10 others (WWTPs) in Germany [15]. However, in freshwater, the quantity of MTF from 1 to 8µg/l was detected in Belgium and Dutch rivers water. However, because MTF usage is very high, with seventy million individuals using per year and gram quantity per daily advised, the drug is extremely found in domestic and wastewater treatment processes and plants. There are a lot of pollutants in the environment, stability level of biodegradable compounds often increases due to those of the original component and this is the same invariably the case with MTF. T2DM is a globally spread disease, and it affects 9.6% of total adult individuals in the US and two hundred million persons globally [15]. The biguanide MTF is of the

best drugs which are used for the cure of all type's diabetes [15]. MTF usage start in Europe in the 1950s, but in the United States since the 1990s [16]. According to worldwide/wide people absorption qualities in the body, more than 90% of the compound is excreted by urine within 12 hours and is also not absorbed through the feces [17]. MTF prescribed in daily dosage differs from five hundred mg to twenty-five hundred mg, it comes in the list of most prescribed drugs. MTF discovered for per year increase amount of 90.9 tons annually 2004, moving it is the second number most advised drug in Australia [18] .In the United Kingdom, over205.8 tons of MTF were advised annually. New research for MTF in cancer and immuno-modulating therapies move it to globally spread pollutant of environments in coming years[15].

1.1 LITERATURE REVIEW

1.2. Historical perspective of bioelectrochemical system (BES)

BES is a collection of technologies that combine traditional biological methods with modern electrochemical processes for waste remediation, energy generation, or the manufacturing of useful chemical commodities.[19]. All BESs had two electrodes (anode/cathode), which provided a platform for microbial growth and helped improve extracellular electron transfer (EET), which is an important aspect of BES performance.[20–22] Galvani discovered electric

current by twitching a frog's leg in the late 17th century and realized that biological reactions and electric current are inextricably linked[23]. Potter claimed in 1911 that microbes (Escherichia coli) were capable of converting organic substrates into energy. [24]. This was the beginning of the MFC field, which is the most common type of BES. The temporal events of BES discovery are depicted schematically in (Figure 1). Following that, many researchers turned their attention to BESs to learn more about this emerging subject. With time, several ways to improve BES performance have been tried, such as the use of a mediator to reduce direct electron transport. [25]. Nevin and colleagues have proved the production of organic molecules from inorganic carbon, giving rise to the concept of MES, which has given BES a new focus. [26]. These activities provide the framework for BESs to come up with fresh ideas in this field that need to be investigated.
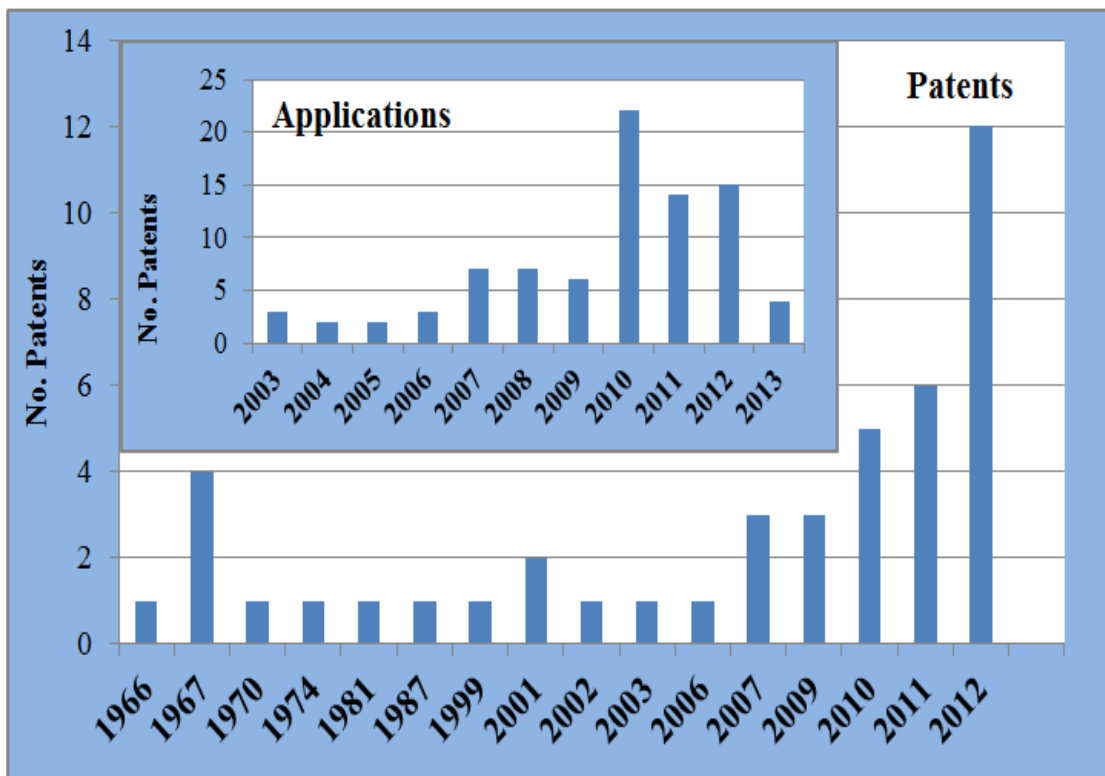
Figure 1 History and patents grants in the discovery of bioelectrochemical systems (BESs) [27].

With the increase of energy shortages and greenhouse gas emissions such as carbon dioxide, the usage of BES has become more important (CO2)[28]. CO2 is the most abundant and cost-effective carbon source, and it may be used to make high-value-added goods like biofuels because of its wide availability. [29]. However, due to its great stability, CO2 reduction is challenging to accomplish. [30]. As a result, the demand for innovative CO2 conversion technology has become increasingly pressing. such as MFC

and MES, may provide an alternative platform for energy and biofuel production in the face of the crisis. MES has demonstrated its ability to reduce Using CO_2/bicarbonate and electrical energy, multicarbon compounds are produced that can be used as valuable organic commodities. [31]. Furthermore, due to their low greenhouse gas emissions, Long-term power generation with MFCs has emerged as a viable clean energy solution.[32].

1.3.   Microbial fuel cell (MFC)

MFC is a long-term power generating platform that includes pollution cleanup and efficient wastewater treatment. [32]. The electrode material (anode) is important in MFC because it acts as both an active location for bacterial activity and a platform for EET. As a result, The long-term viability of MFC power generation depends on the manufacture of anode materials with enhanced properties, such as high conductivity, wide surface areas, increased electrocatalytic activity for EET, and appropriate biocompatibility[33]. Anodes for metals, such as polyaniline/TiO_2 foam anodes for nickel coatings, are used in these situations. [34] and stainless steel-coated graphene [35] MFC power densities were increased by two and eight times, respectively In addition to increasing power output, some transition metal oxides, like RuO_2-coated carbon felt, have demonstrated a 17-fold increase in transient/stationary electron storage [36]. Iron oxide, for example, has demonstrated feasibility as an MFC anode material while being unstable, serves as a non-precious electrocatalyst with mixed valent ions (Fe(II)/Fe(III)). [37]. As a result, more suitable anode materials must be developed to improve MFC performance.

1.4 Microbial electrosynthesis (MES)

The development of effective methods for converting greenhouse gases like carbon dioxide (CO_2) into high-value commodities has huge environmental and economic implications. [28]. MES, which uses CO_2/bicarbonate as the microbial feed to produce usable organic commodities, has recently emerged as a potential BES technology [38]. Microbial electrosynthesis (MES) technology, which is designed to reduce carbon emissions, can thus be used to produce chemicals for large-scale energy storage. [39]. The biocathode, which serves as an electron transfer conductor as well as a site for microbial growth, has a big impact on how well MES works. [40]. The modified cathode has been shown to improve total VFA production. [31,40,41]. Zhang et al reported enhanced acetate production on treating carbon cloth with gold (0.35 g/L) and palladium (0.45 g/L) nanoparticles [31]. Compared to an uncoated graphite cathode, the formation of acetate was enhanced by coating the graphite stick with nickel nanoparticles (0.12 g/L) and nanowire (0.54 g/L). [42]. Improved MES performance and current density were seen by Jourdin et al. after developing a flexible multiwalled carbon nanotube on reticulated vitreous carbon (NanoWeb-RVC). [41]. However, expensive electrode materials, low current densities, and multiple electrosynthesis products are major challenges to this field.

1.5. Opportunities for performance enhancement in BES

Since its discovery, efforts have been undertaken to develop BES so that it may be

employed in practical applications such as wastewater treatment, pollutant remediation, electricity generation, chemical manufacture, and many more. These efforts highlight opportunities and parameters that can be optimized to improve and enhance BES performance, such as anaerobic conditions (feed, temperature, pH), applied potential, microbial culture selection and enrichment, BES reactor design, and electrode (anode/cathode) material and design. [43]. (Figure 2) shows the various BES parameters that can be tweaked to increase system performance. Microbial inoculum selection and electrode material/design are two of the most important elements in maximizing BES performance. Mixed cultures, as opposed to pure strains, are more tolerant to environmental disturbances and produce more biomass at a faster rate. [44]. As a result, it's critical to employ cultures that can consume trash, generate electric current (MFC), and produce certain organics and VFAs (MES). Another significant thing to consider is the electrode material and design, as an adequate electrode is critical for assisting microbe development while facilitating EET. [20–22]. Furthermore, the electrode is critical for selective microbial enrichment and the formation of stable biofilms. [45].
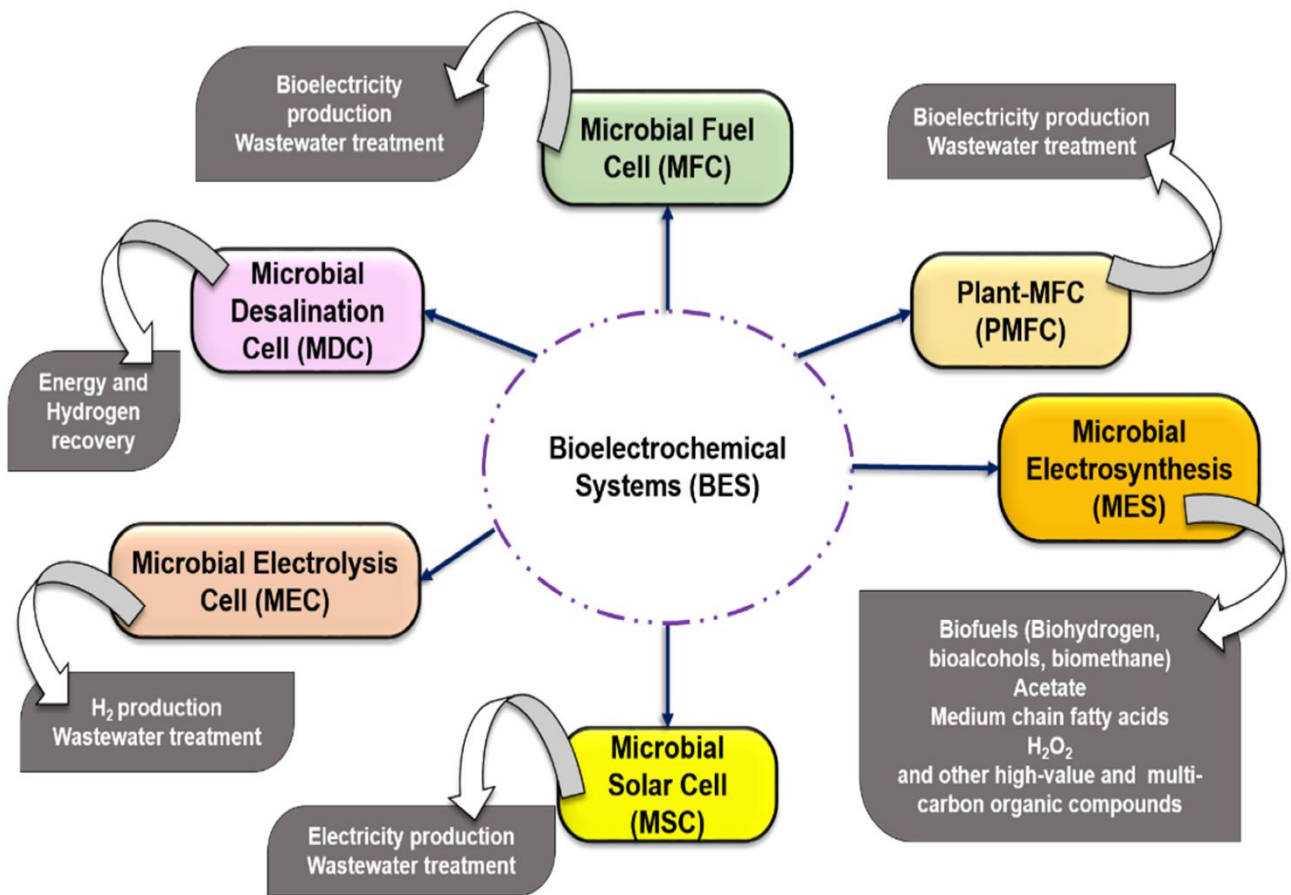
Figure 2. The dynamic environment of BES: potential for scientific research and difficulties with commercial uses[46]**.**

1.6. Electrode modification in a BES

The electrodes of the BES have a big impact on their performance since they supply microbial growth sites as well as act as an electron transfer conductor at the microbe/electrode interface. [40]. (Figure 3) depicts several electrode material designs and their impact on microbial adhesion/growth, with uneven electrode surfaces assisting more microbial growth than flat electrode surfaces. [47]. As a result, several strategies for developing new electrode materials and surface modification techniques to improve BES performance have been proposed. [38,40,41]. Functionalizing the electrode surface to promote electrode-microbe electron transport is one of these methods [38]. Using nanowires or nanoparticles to lower the activation energy of electron transport [42], By using various carbon

materials, such as carbon nanotubes, to coat or fabricate electrodes [41,42]. To assess the efficacy of a proposed electrode material for use in MES or MFC, it must first be coated on a biocompatible, flexible, and conductive substrate. Due to its great mechanical strength, superior conductivity, and increased microbe density, a carbon-based electrode is an ideal choice for supporting material. [48,49]. Carbon felt (CF) has been widely employed among the carbon-based electrode materials accessible because of its high porosity and remarkable flexibility, making it amenable for further surface modifications. [50]. Traditional electrode coating processes, such as mixing, are, however, ineffective. [51,52], adsorption coating [53,54], atomic layer deposition [55], and other composite in-situ growth routes [56] are usually time-consuming and expensive, making them unsuitable for everyday use. As a result, simple approaches for fabricating highly effective electrodes remain critical.
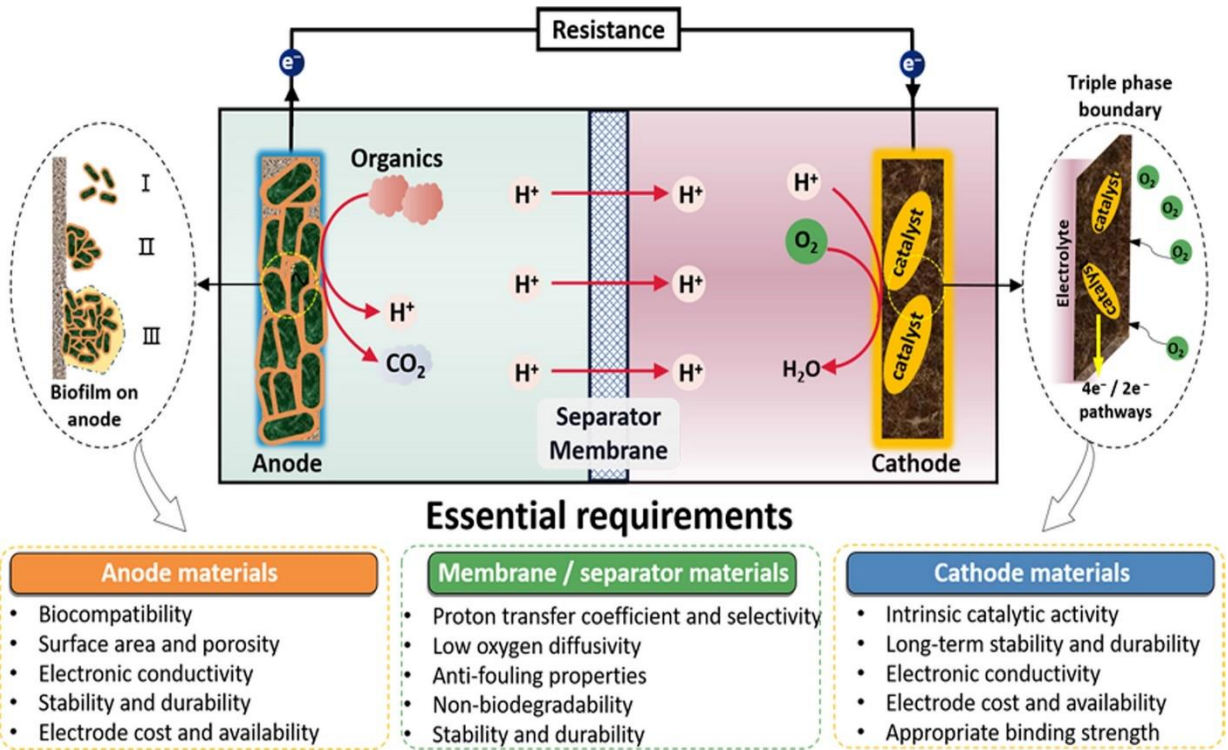
Figure 3. An overview of how electrode characteristics and microbial electrocatalysis are affected by the chemistry and topography of material surfaces in bioelectrochemical systems [57].

1.7.BES main components

The main components of MFC which influenced their performance are briefly described as below [58].

- Anode materials with high electronic conductivity, biocompatibility, chemical stability, high specific surface area, and high porosity function well in BES. Electrodes (anode and cathode). All the materials that are used as anodes may be utilized as cathodes. The most common cathode materials used in BES include carbon, carbon without a platinum catalyst, plain carbon, metals other than platinum, and bio cathodes.

- Catalyst at the electrode: When oxygen, protons, and electrons come together at the catalyst at the cathode, a triphase reaction takes place. For electrons and protons in the various phases of the catalyst to arrive at the same location without a delay, it is necessary for it to be exposed to both air and water.

- Anolytes and catholytes in anodic and cathodic chambers: The biological phenomena of BES are primarily focused on the anodic chamber. BES employs a variety of anolytes that include various exoelectrogens from pure or mixed cultures. Studies on BES also show that various substrates and bio film electrodes are used, and that these two components interact to improve performance, such as current density or power density. Sugar, acetate, lactate, sodium formate, starch, urban wastewater, synthetic wastewater, and other

substances are examples of substrates used in BES. These substrates engage with the right microbes (inoculums) and break down, releasing protons and electrons in the process. Since oxygen is infinitely available, it has been utilized often as an electron acceptor in the majority of BES research.

• Ion (Proton) exchange membrane: The main criteria for a membrane to be utilized in BES are great thermal, chemical, and mechanical stability, strong ionic conductivity, cheap cost, and low degradation. The decision between two conflicting interests is represented by the membrane that separates the anode from the cathode: High stability: Membranes must be strong in a colloidal and nutrient-rich environment. High selectivity: The better the biofuel cell functions, the lower the resistance of the membrane will be.

1.8. Basic operating principle of BES.

Electrons can be produced with the aid of bacteria as bio-catalyst in an anaerobic environment using organic matters in BES [59,60]. It generally has two compartments, anaerobic anode section and aerobic cathode section (Fig. 4). Both sections are separated by an ion exchange membrane (proton exchange membrane) or salt bridge. Organic matters are oxidized in anodic chamber and generate electrons and protons in the course. Electrons are transferred to the anode that passes through an external circuit, generating current. After crossing a PEM or a salt bridge, the protons enter the cathodic chamber where they combine with oxygen to form water [61,62].

By considering glucose as an example of principal substrate, the chemical reactions taking place in different electrodes are discussed below.

(1) Anode: $C_6H_{12}O_6 + 6H_2O \xrightarrow{\text{bio-electricity}} 24e^- + 24H^+ + 6CO_2$

(2) Cathode: $24e^- + 24H^+ + 6O_2 \longrightarrow 6H_2O$

(3) Overall reaction: $C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$
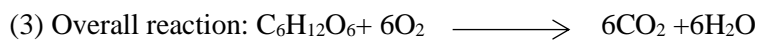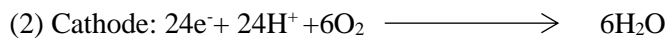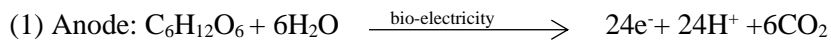
The overall reaction is the breakdown of the substrate to carbon dioxide and water with a simultaneous production of electricity as a by-product.

Waste water
➢ Agricultural
➢ Municipal
➢ Industrial
➢ Diary
➢ Brewery/Food

CO_2

e^-

Anode catalyst

H_2O
$HCO3^-$

Organics

Anode

H^+

H^+ H^+

H^+ H^+

H^+

Cathode

H^+

Cathode catalyst

O_2
H^+
CH_3COO^-
HCO_3^-
NO_3^-

H_2O
H_2
C_2H_5OH
H_2O_2
N_2

Proton exchange membrane
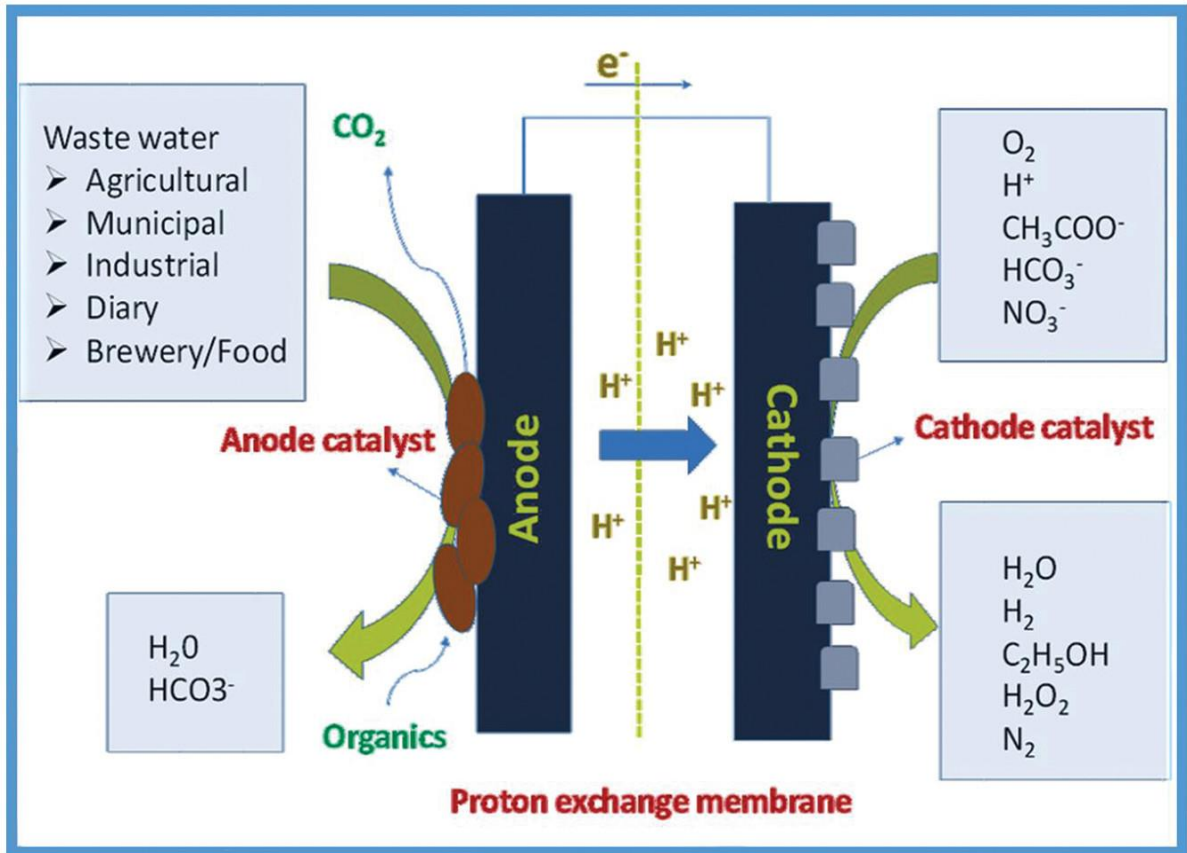
Fig. 4. Basic operating principal schematic illustration for BES[63].

1.9. Factors Effecting BES performance

Biological as well as electrochemical processes effect the generation of electricity in a microbial fuel cell [64]. Parameters important for optimization of electroactive biofilms are illustrated in (Fig 5) and brief discussion on significant factors is as below:

- The anolyte conversion rate; depends on the quantity of bacterial cells, the mixing and mass transfer phenomena in the reactor, the bacterial kinetics, the biomass organic loading rate [65], the efficiency of the proton exchange membrane for transporting protons [66] and the potential over the BES.

- The electrode surface, the electrode's electrochemical properties, its potential, and its kinetics, as well as the BES current and the electron transfer method, all have an impact on the anode's overpotentials. Overpotentials at the cathode; losses occur in the cathode compartment due to overpotentials as in anodes. Even small current densities flow through the electrode surface, these losses need attention. To decrease the activation overpotentials, catalysts need to be added to the electrode, or a suitable mediator is needed to transfer the electrons from the cathode to oxygen. To be sustainable, BES cathodes preferably should be open-air cathodes [67].

- The internal resistance of the BES is influenced by the membrane resistance as well as the

16

resistance of the electrolyte between the electrodes. Anode and cathode should be as near together as feasible for best performance[68]. Additionally, proton migration has a major impact on losses caused by resistance[68]. good mixing might reduce these losses.

- The electrical component of external resistance, which regulates the relationship between electric current and operating voltage, is crucial for the production of electricity. The fuel cell's internal resistance must be equivalent to its exterior resistance in order to produce the most power. Anode biofilm's bacteria composition may be impacted by external resistance as well. [69].Enhancing biofilm development and maximizing BES power output may be achieved by using an external resistance that is lower than or equivalent to the internal resistance. [70,71].

- Conductivity/ionic strength, pH, and temperature. These three environmental variables have a significant influence on the development and physiology of bacterial cells. For the generation of electricity, a high acidity in the cathode and a neutral pH in the anode is preferred. [72]. Temperature influences BES power generation as well as bacterial growth. [73]. Current generation was seen to rise by 80% when the temperature was raised from 30 to 40 $^{\circ}$C.
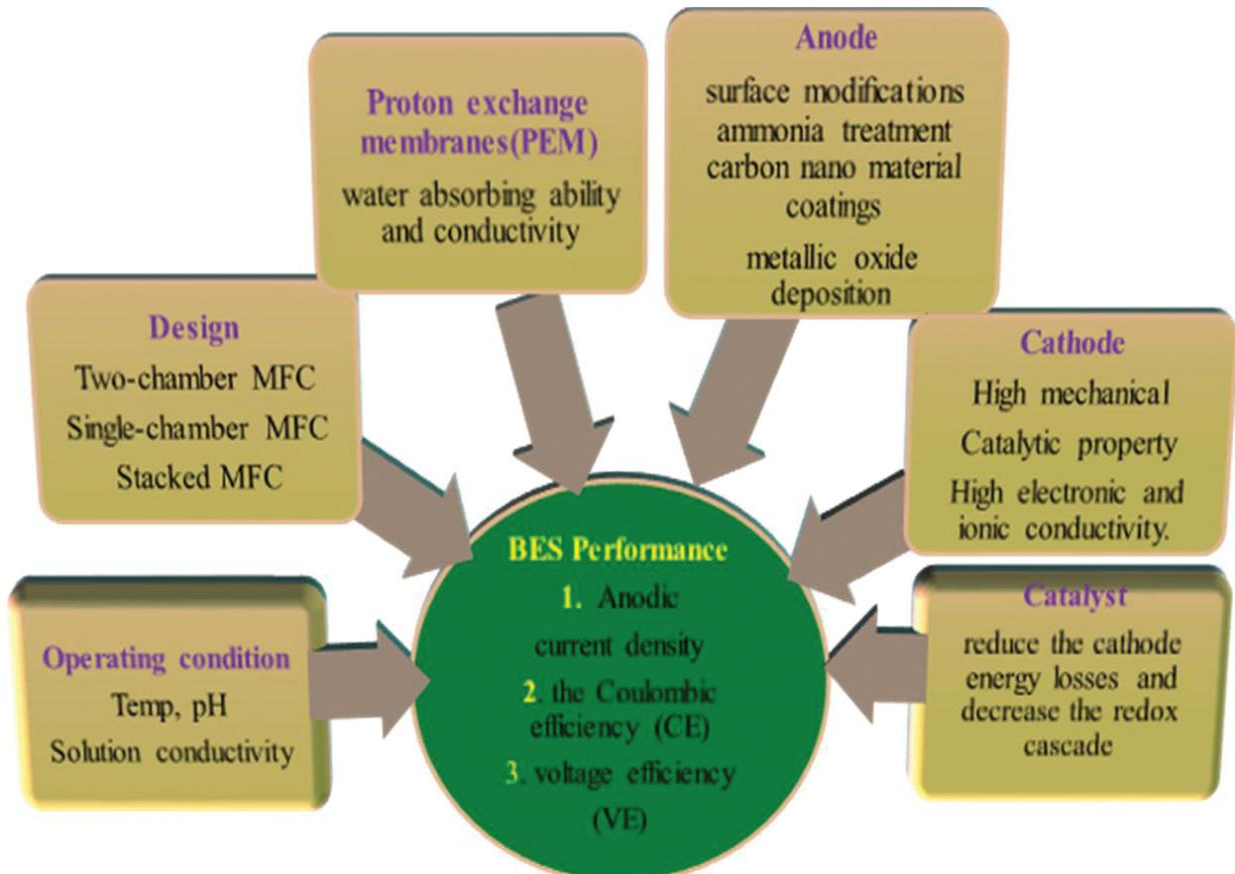
Fig. 5. Important variables for the improvement of electroactive biofilms in bioelectrochemical systems[63].

Chapter 2.0

2.1. Microbial electron transfer mechanisms

There are several potential processes by which electron transfer occurs when microbes interact with conductive solid surfaces. (Fig. 6 and 7).

Mediated electron-transfer (MET)

soluble redox mediators such flavins, quinones, and phenazines, which act as electron carriers for a variety of oxidation-reduction cycles, are excreted by the body during MET. Shewanella is one of the bacteria that depends on these substances for extracellular electron transport. [74], Lactococcus [75], and Pseudomonas [76].

Direct Electron-Transfer (DET)

The cytochromes in the outer membrane are directly contacted by the electrode during DET. These types of electron transfers are modeled by the organisms Shewanella and Geobacter. [77]. Significantly, DET may also incorporate the, as of yet hypothetical, potential contribution of electron-transfer proteins incorporated in the matrix of extracellular polymeric materials (EPS)[78,79].

DET through microbial nanowires

Specifically, conductive pili that have a metal-like conductivity and may transfer electrons straight from the bacterial membrane to the electrode. This electron-transfer method has been demonstrated for several species, although Geobacter and Shewanella are its prototypes. [80,81].
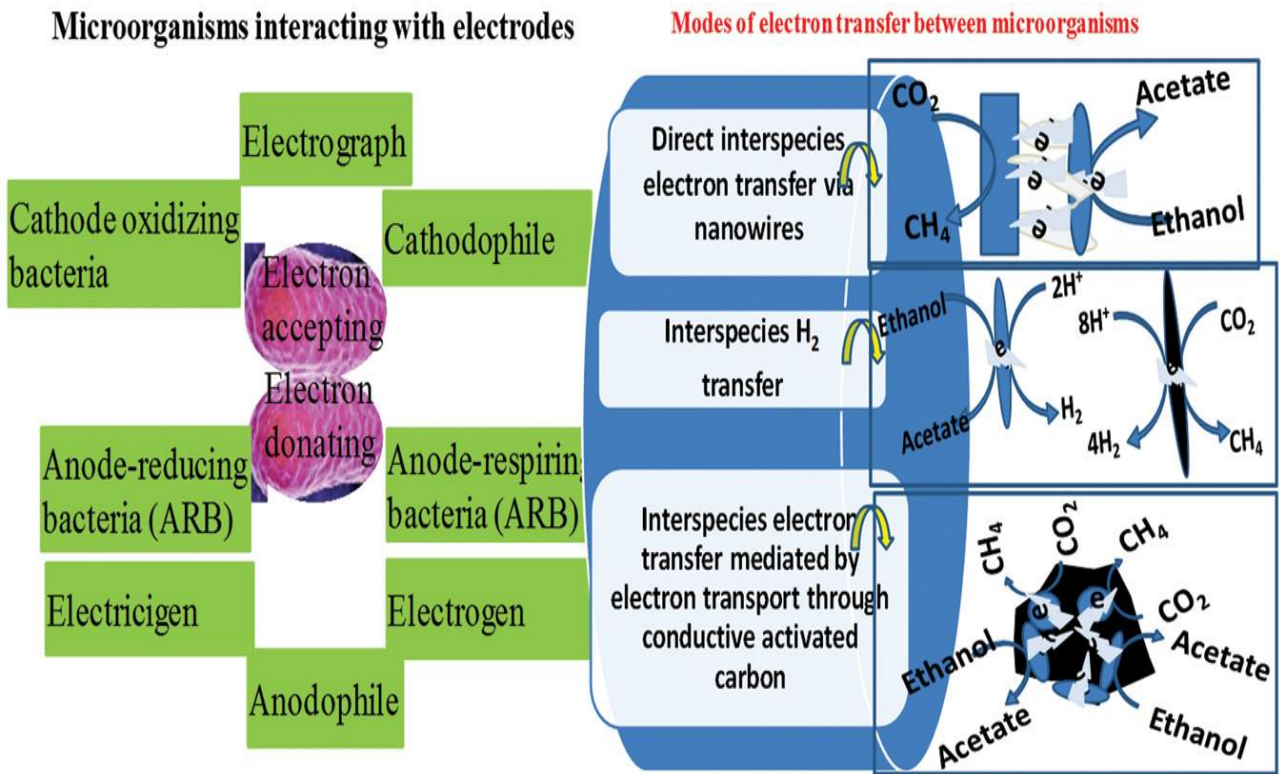
Fig.6. Diagram illustrating the solution-based electrochemical reaction pathways in the presence of a solid biofilm electrode: a)between the biofilm and the electrode directly; or, (b) indirectly, through the electroactive metabolites[63].
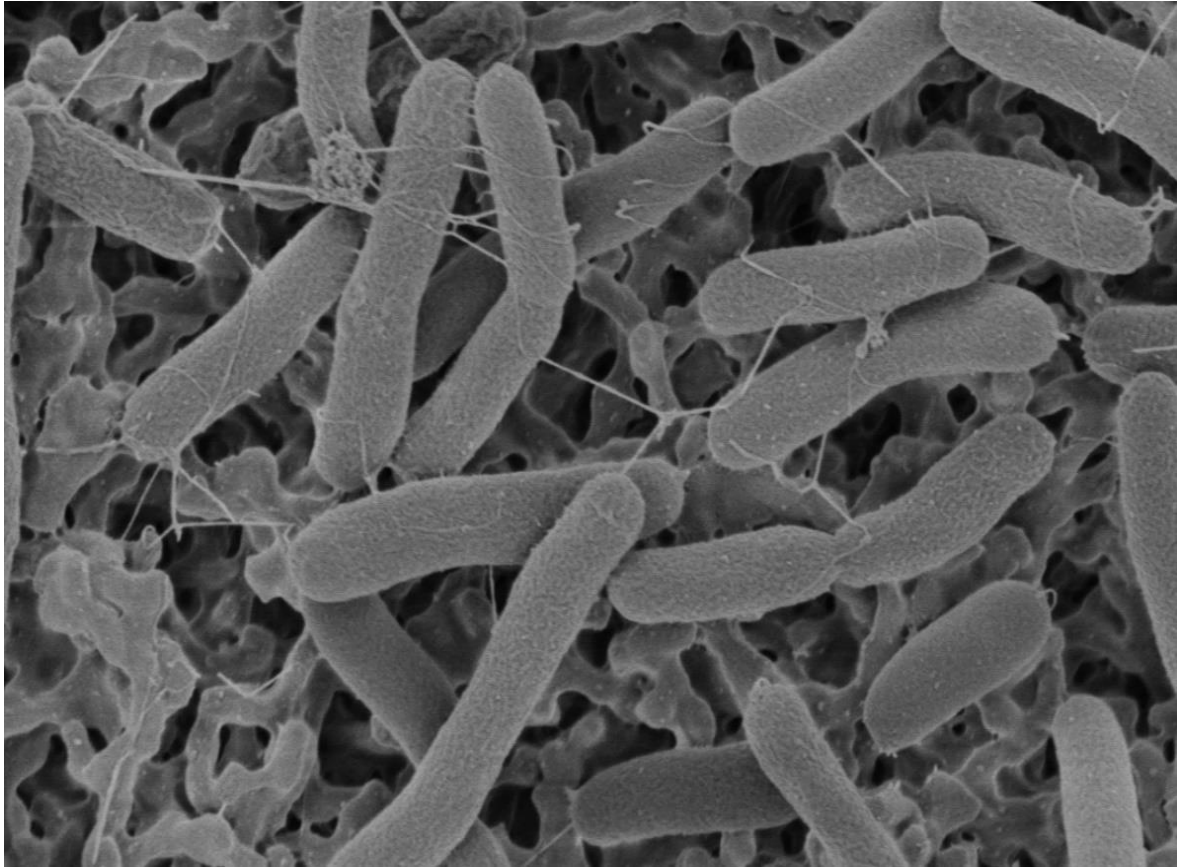
Fig. 7. Image from Scanning Electron Microscope, Shewanella oneidensis MR-1 produced electrically conductive pilus or nanowires [81].

2.2. Combination of Bioanode and biocathode (BIND and BICD):

Bioelectrochemical systems with both BICD and BIND are helpful due to the stability and less expense management attached with the help of microbes as biocatalysts [82]. The

system of BIND and BICD is very excellent with very benefits more than abiotic systems. First of all, the BIND and cathode contain living microbes that adhere to BIND or BICD [83]. This is due to microorganisms are acting as biocatalysts and it is a self-produced system in which food of bacteria and substrate is spontaneously added to the BIND or BICD [84]. However, nonliving electrodes use unreal catalysts which cannot be produced products by themselves and we can't use them for long-term systems. Second, the biocatalysts depend on microbes that are more powerful than others systems and survive on sulfate or another organic dangerous compound in comparison to nonliving catalysts. Thirdly, we can get microorganisms from our natural environments which are low cost than abiotic anode and cathode particularly when expensive metals like platinum are working as catalysts [85][86]. However, it is very novel for environmental techniques like purification of particular domestics and industrial wastewaters whether it is excellent for Bioelectrochemical systems because the microorganisms have the potential to resist both the availability and reaction of chemical compounds. Bioelectrochemical systems (MES hybrid) as mentioned in (Fig 8). are used especially between electricity-production BIND and different products at BICD. To recognize the interconnection, microbiology skills and knowledge is very necessary and BES maintenance is essential for success. Anyhow, BICD and BIND attached in Hybrid MES, electricity production at BIND aided in the production of products at BICD [87]. Double-chamber reactors with a combination of BIND and BICD splinted by a cation-exchange membrane (Nafion). Novel step in the direction of the usage of (Hybrid MES) for the transformation of organic or inorganic substrates

22

into biofuels and other products. attaching a CO_2-reducing BICD with an electron-producing BIND into a BES not only low the importance of outer power input for the production of important bioproducts but also work as an important system for wastewater treatment [87][88].
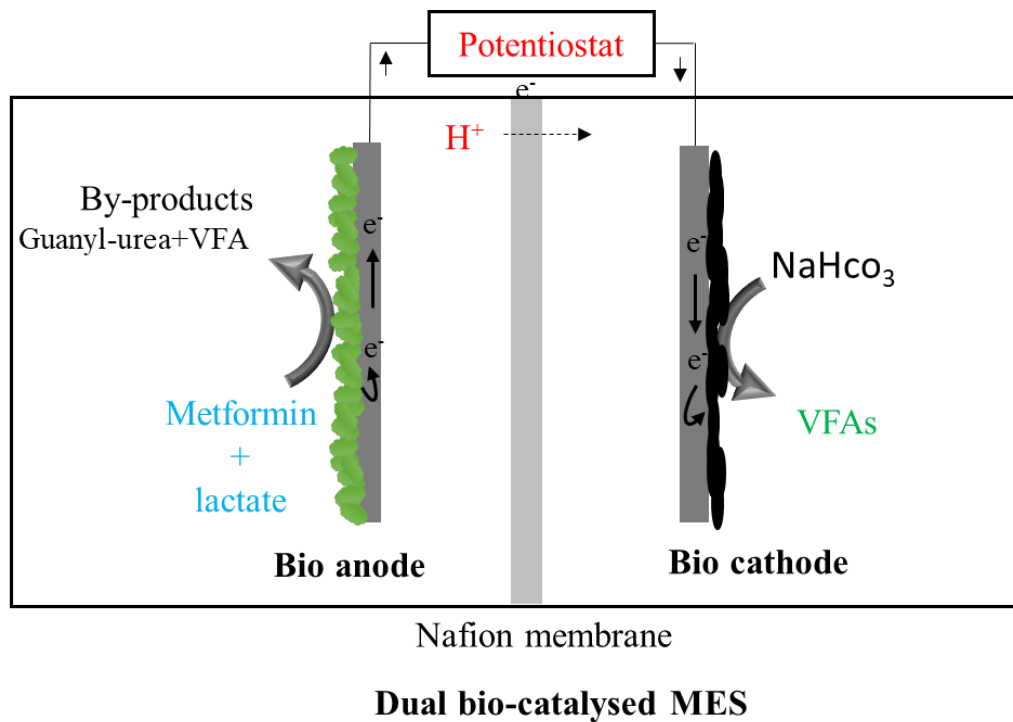
Fig. 8. Schematic of the dual biocatalyzed bioelectrochemical system for microbial electrosynthesis operation and metformin biodegradation.

Materials and methods

2.3. Materials and characterization

Metformin (1,1-Dimethylbiguanide hydrochloride) CAS NO (1115-70-4) product of Alfa Aesar, KH2PO4 Duksan CAS NO (7778-77-0), Methanol (HPLC. grad 98% purity), and H2SO4 purchased for the experimental analysis. Samples were collected on both sides (BICD and BIND) on daily basis after 24hours. After sampling nitrogen was injected into the reactor with the help of the attached nitrogen cylinder. The samples are stored at -20 degrees. After 5-days (120 hours) samples are observed under the below-mentioned method in HPLC UV dad. Metformin biodegradation was observed by UV HPLC Agilent's techniques 1260 infinity (II) diode ray detector HS. Quaternary pump with integrated 4 channel degassing unit, co. Agilent poro-shell C18 column (120EC-C18,4.6×15mm,4micro meter). Methanol (HPLC grade 98% purity) and 0.5mM KH2PO4 are used as a solvent (35:65v/v). The flow rate is 1.7ml/min and the detection wavelength is 235nm. Peak timing is 4.9 min. Run the method for the 15 min[89]. Volatile Fatty acid detection (VFA) microfilters, and examined by operating the isocratic elution mode with a mobile phase of hydro sulfuric acid (4 millimolar) at 60 °C. High-performance liquid chromatography (HPLC) (Agilent 1200, USA) operated with a refractive index detector (G1362A) and anion-exchange column (Aminex HPX-87H; 300 mm × 7.8 mm) was applied to analyze the formation of VFA in the MES systems. This analysis was monitored

at 60 °C using isocratic elution with 4 mM H_2SO_4 as the mobile phase. The transformed products were detected based on retention times compared with available standards. Each sample was analyzed in triplicate, and the average values were used for each VFA.LC/MS analysis were observed by a waters Xevo QTS mass spectrometer coupled to an acuity ultra-performance liquid chromatography. The mass spectrometer was operated using an electrospray (Z spray) ionization source in positive ion and multi reaction monitoring mode. An Agilent Eclipse Plus C18 RBD 1.8 µm, 2.1 × 100 mm analytical column was used for the analysis of metformin. The mobile phase consisted of water and methanol 0.1% formic acid (A) and acetonitrile (B). Others conditions of LC/MS analysis and time are mentioned in table [3][4].

2.4. MES system construction and operation

A double plexiglass reactor of 0.2 Liter volume (BICD/BIND) and five cm × five cm × eight cm size was applied as microbial electrosynthesis system construction. Carbon felt five cm×five cm the BIND/BICD chamber was isolated by a Nafion® 117 proton exchange membrane (DuPont Co.; Wilmington, DE, USA) preprocessed with hydrogen peroxide (Three %) and H2SO4 (0.5 Molar). The two chambers were repeatedly injected with a gas carrier have Nitrogen. before installation, both BIND/BICD construction were autoclaved to maintain system sterilization. The microbial electrosynthesis system working was judged for four rotations (labeled as Cycle one, two, three, and four), each controlling for 120 hours at a

25

stable cathode potential of −800 millivolt (vs silver). Pure nutrient medium with HCO3- as carbon source was injected at the BICD and lactate injected at the BIND to start each cycle. Throughout the microbial growth cycle, sodium bromoethane sulfonate was used to forbid further methanogens' enrichment and CH_4 generation[90]. Whole details according to the MES culture, enrichment, and nutrient media provided in supporting information. A temperature maintainer managed the temperature of the system at 35 ±. 1 °C while a magnetic stirrer (180 rpm) confirm the stability of the biological catholyte as in (Fig 9).

2.5. Growth media and microbial enrichment

2.6. Bioanode and biocathode:

The initial inoculum (anaerobic reactor sludge) was obtained from a domestic wastewater treatment plant (WWTP) in Daegu, South Korea. The foreign particles were removed by sieving the sludge and continuous stirring (180 rpm) to produce a homogeneous liquid phase for the final microbial inoculum. After pretreatment, the sludge (0.2 L) was introduced into a 1-L Erlenmeyer flask and fresh growth media (0.8 L) was added. The microbial culture used was initially enriched and grown for six weeks under anaerobic conditions at 35 ± 1°C and 180 rpm. The flask was equipped with ports to facilitate the introduction of fresh media and the removal of exhausted media.
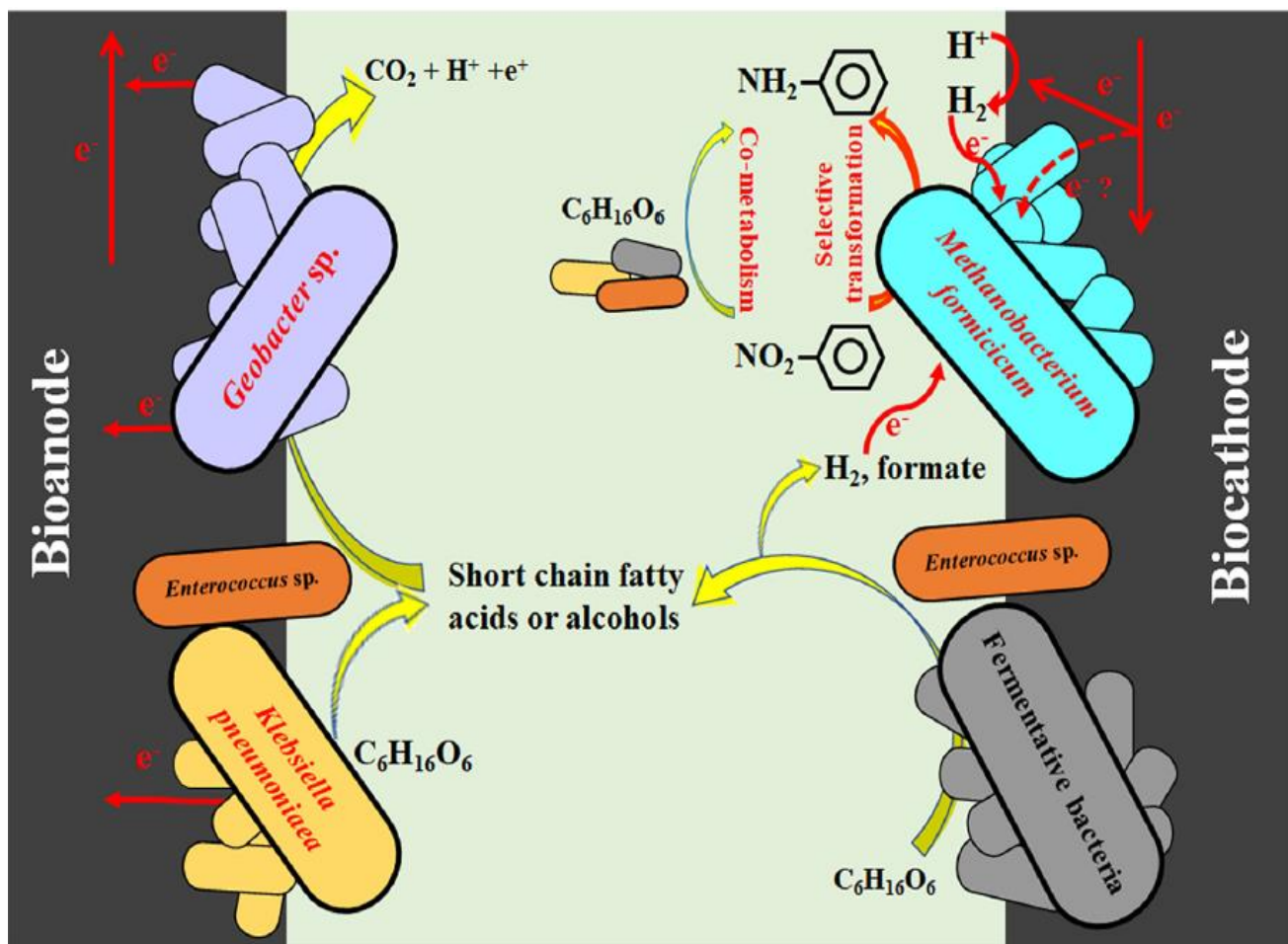
Figure 9. A schematic diagram of MFC and MES assembly and operation[91].

Bioanode (BIND)

The media comprising the solution of the following compound, respectively. Lactate /L (KH_2PO_4 0.5g, NH_4Cl 1.0g, $MgSO_4.7H_2O$ 0.06g, Na_2SO_4 2.0g, Sodium lactate 60% 3.5 ml, sodium citrate 0.3g, $FeSO_4.7H_2O$ 0.1g, Trace mineral 1ml, Glycolic acid O.1 ml and Ascorbic acid O.1 g.as feed. To ensure anaerobic conditions, the medium was subjected to nitrogen gas (100%) purging and autoclaved at 121 °C for 20 min to kill the possible microbes present in the media. After every fifth day, the exhausted media (70%) was decanted and replaced with fresh growth media.

Biocathode (BICD)

The media consisted of the following compounds (g/L): Bicarbonate /L (KH_2PO_4 0.5g, NH_4Cl 1.0g, $MgSO_4.7H_2O$ 0.06g, Na_2SO_4 2.0g , $NaHCO_3$ 3.5g , sodium citrate 0.3g, $FeSO_4.7H_2O$ 0.1g, trace mineral 1ml, Glycolic acid O.1 ml, Ascorbic acid O.1g.The composition of the trace element solution was (g/L): 1.50 $N(CH_2CO_2H)_3$, 3.0 $MgSO_4.7H_2O$, 0.50 $MnSO_4.H_2O$, 1.0 NaCl, 0.10 $FeSO_4.7H_2O$, 0.18 $CoSO_4.7H_2O$,0.10 $CaCl_2.2H_2O$, 0.18 $ZnSO_4.7H_2O$, 0.01 $CuSO_4.5H_2O$, 0.02 $KAl(SO_4)_2.12H_2O$, 0.01 H_3BO_3,0.01 $Na_2MoO_4.2H_2O$, 0.03 $NiCl_2.6H_2O$ 0.03 g with 0.03 mg/L $Na_2SeO_3.5H_2O$ and 0.4 mg/L of $Na_2WO_4.2H_2O$.

2.7 Bioelectrochemical measurements

Electrochemical measurements were taken using a three-electrode system controlled by a multi-channel potentiostat (wizECM-8100 Premium, Korea). For the Hybrid MES system, the BICD was operated at −800 mV versus the Ag/AgCl reference electrode, with the BIND acting as the counter electrode. However, for the MFC system, an anode potential of +200 mV (v/s Ag/AgCl) was operated as a Bioanode (CF) as working electrodes, whereas a cathode served as the counter electrode. Briefly,

28

chronoamperometry (CA), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS) analyses were monitiored.CA was used to study the current-time behavior at a fixed potential (-800 t0 +200 volt) for both systems. The electrode biofilm was analyzed using CV, with the exoelectrogens bacterial activity due to the MTF at BIND examined. The fresh growth media was used before CV analysis (24 h) to ensure equilibrium between the electrodes and the electrolyte. Three consecutive CV cycles run at different scan rates (1–200 mV/s) were used to assess the electrochemical parameters. The EIS analysis was also conducted using a three-electrode setup connected with a potentiostat, which comprised a platinum wire as a counter electrode, a saturated Ag/AgCl electrode as the reference electrode, and the synthesized composite as the working electrode. EIS was carried out in a frequency range of 100 kHz–0.01 Hz. The electrolyte used was a solution mixture of a phosphate buffer (5 mM) and potassium ferricyanide (5 mM), and the analysis was carried out at a direct current potential of +200 mV.

2.8. Volatile fatty acids analysis

The Hybrid MES system was operated for 120 h, with 24 h intervals fixed for sampling and chemical analysis. A 2 mL sample from both BIND and BIND chambers was taken and filtered through a 0.22-micrometer membrane every 24 h. High-performance liquid chromatography (Agilent 1200, USA) equipped with a refractive index detector (G1362A) and anion-exchange column (Aminex HPX-87H; 300 mm × 7.8 mm) was utilized to operate the formation of VFAs in the MES systems. This analysis was monitored at 60°C using isocratic elution with 4 mM H_2SO_4 as the mobile phase. The products were identified based on retention times compared with available standards. Each sample was analyzed in duplicate, and the average values were used.

2.9. Microbial community analysis

Microbial community analysis was carried out at the end of the experiment to evaluate the enriched biofilm as a result of MTF biodegradation. As a pretreatment, the collected samples were centrifuged at 12,000 rpm for 10 min, and the supernatant was discarded. The final sample obtained in the form of a pellet was stored at −80°C before further analysis. A FastDNA SPIN Kit for Soil (MP Biomedicals) was used to extract the genomic deoxyribonucleic acid according to the manufacturer's protocols. The following setup was used for the polymerase chain reaction (PCR): a single cycle at 94°C (3 min); 30 cycles at (a) 94°C (20 s), (b) 56°C (45 s), and (c) 72°C (45 s); and, a single cycle at 72°C (3 min). The PCR products were then purified using a PCR purification kit (Solgent, Korea). A second PCR run was conducted under the same PCR conditions, as mentioned above, except for eight cycles instead of 30 (for a, b, and c). The PCR products were quantified using an immunosorbent assay reader equipped with a Take3 multivolume plate. The Illumina MiSeq platform at Macrogen (Seoul, South Korea) was used for the next-generation high-throughput sequencing of the final composite sample formed by mixing both amplicons (equivalent amounts) of the purified PCR. Data analysis was performed using Qiime2 software https://qiime2.org/, [92], while the adapter sequences were eliminated using the Scythe (v0.994 BETA) and Sickle programs. The generation and removal of chimeras removed the ambiguous base calls and sequences <200 bp. The operational taxonomic units were defined and taxonomically classified using similarity (97%) and divergence (3%) clustering and the Silva v128 database [93], respectively. The Chao1 and

Shannon–Weaver biodiversity indices were calculated for the normalized sequences of the samples to avoid bias as mentioned in table 1.

Table 1 Primer and template details for microbial community analysis.

Primers[a]	Templates[b]
341F (5′-<u>TCGTCGGCAGCGTC-AGATGTGTATAAGAGACAG</u>-CCTACGGGNGGCWGCAG-3′)	i5 (5′-AATGATACGGCGACCACCGAGATCTACAC-[i5]-TCGTCGGCAGCGTC-3′)
805R (5′-<u>GTCTCGTGGGCTCGG-AGATGTGTATAAGAGACAG</u> GACTACHVGGGTATCTAATCC-3′)	i7 (5′-CAAGCAGAAGACGGCATACGAGAT-[i7] GTCTCGTGGGCTCGG-3′)

[a]The underlined sections show the adaptor sequences, with the adaptors targeting the 16S rRNA genes specifically in the V3-V4 hypervariable regions.
[b][i5] and [i7] represent unique barcodes for Illumina MiSeq sequencing.

Chapter 3.0
3.0 Results and discussion
3.1. Metformin biodegradation

Metformin (MTF) biodegradation under different dug loading (0.5, 0.3, and 0.1 ppm) was investigated using the dual bio catalyzed BIND/BICD MES system. As most of the literature suggested oxidation as a potential route for MTF degradation, the drug was introduced into the BIND chamber [94,95]. Fresh growth media was added at the start of experiment and samples were taken out every 24 h to evaluate MTF biodegradation. Exceptional biodegradation performance was observed for all MTF concentrations with a maximum removal of 91% for 0.1 ppm drug within 120 h. On increasing the MTF loading, removal efficiency of 89% and 78% were observed for 0.3 ppm and 0.5 ppm, respectively (Fig 10). These biodegradation efficiencies are quite high as a number of studies have evaluated MTF degradation; however, lower removal efficiencies were reported [96–99]. Trautwein et al. performed a series of biodegradation tests for 28 days and found MTF biodegradation performance ranging from 9.9% to 51.3% [100]. Some advanced treatment processes such as photolysis, photocatalysis and chlorination gave removal efficiencies of 9%, 31% and 60% respectively [98]. In another study, 56% and 63% MTF removal was reported for UV/H_2O_2 and neutral photo-Fenton process, respectively [101]. On the other hand, bioelectrochemical systems operating at poised potential and enriched with mixed microbial communities can target a wide range of waste chemicals including pharmaceutical drugs effectively [102,103]. However, most of these systems have biotic/abiotic catalytic chamber which are expensive and

32

use excessive energy. The use of the dual biocatalyzed MES system with BIND is beneficial as it not only removed unwanted compounds such as MTF but also produce useful products such as VFAs at BICD. Even with the increase in drug concentration by 5 times, only a 13% decrease in biodegradation performance was observed, signifying the capability of the BIND to effectively degrade MTF. The improved biodegradation performance at higher concentration could be attributed to the release of microbial enzymes that assisted the drug removal [104]. Moreover, additional electrons would be generated by the MTF biodegradation at BIND that could assist the bioreduction at BICD [105,106].
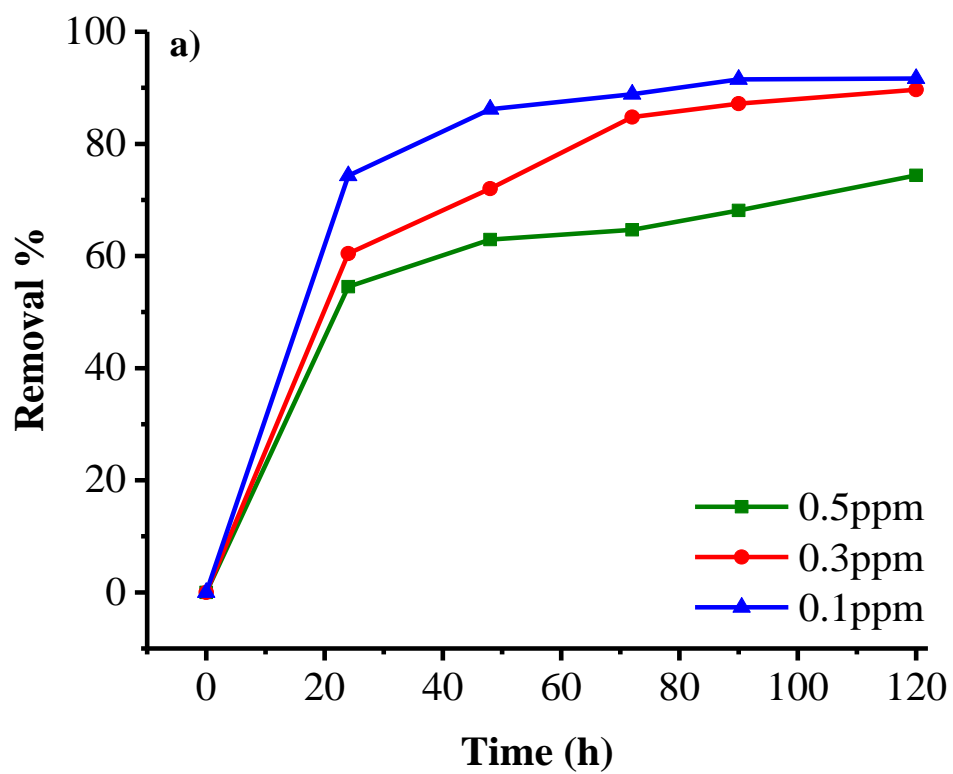
Figure. 10. (a) Metformin biodegradation and microbial electrosynthesis system for the drug loading of 0.1 ppm, 0.3 ppm, and 0.5 ppm

3.2. Current density performance

Current desnity is one of the most significant parameter that depicts the bioelectrochemical system performance and stability. MES dual biocatalyzed system performance was evaluated with MTF drug at different concentrations and compared with MES system without MTF (control). In sumary, both MES systems were poisd at −800 mV vs Ag/AgCl using a multi-channel potentiostat with different drug loadings. On comparing with control, an increase in current density was observed all drug concentrations indicating the role of MTF in improving current output. Moreover, the current density also improved upon changing the drug concentration with -849, -354, -112 and -82 mA/m^2 for 0.5, 0.3, 0.1 ppm and control, respectively (Fig 11). Altunkaynak et al. also observed a positive relation of current output and MTF loading which is in agreement with these results [107]. This could be attributed to the breakdown of MTF by microbial metabolism generating additional electrons that adds up into the system and assisting the bioreduction at BICD [105,106]. This signifies the potential of using dual biocatalyzed MES to process reluctant compounds at bioanode, thereby ensuring environmental remediation and producing useful products at biocathode. Lactate, acetate, propionate, butyrate sulfide, MTF, and BIND electrode were electron givers, while the BIND electrode was an electron acceptor, according to the proposed mechanism. Microbial colonies aided MTF, lactate, acetate, propionate, butyrate, and sulfate reactions, as well as extracellular electron transport to the anode for the current generation. At the top of the BIND biofilm, sulfate was largely decreased. [108]. MTF and lactate biodegradation is acceptable in a large

35

phase or on the anode biofilm, and many other compounds are produced mentioned in fig [15]

[17]and table [7]. So Current density evaluation revealed in (Fig 11).
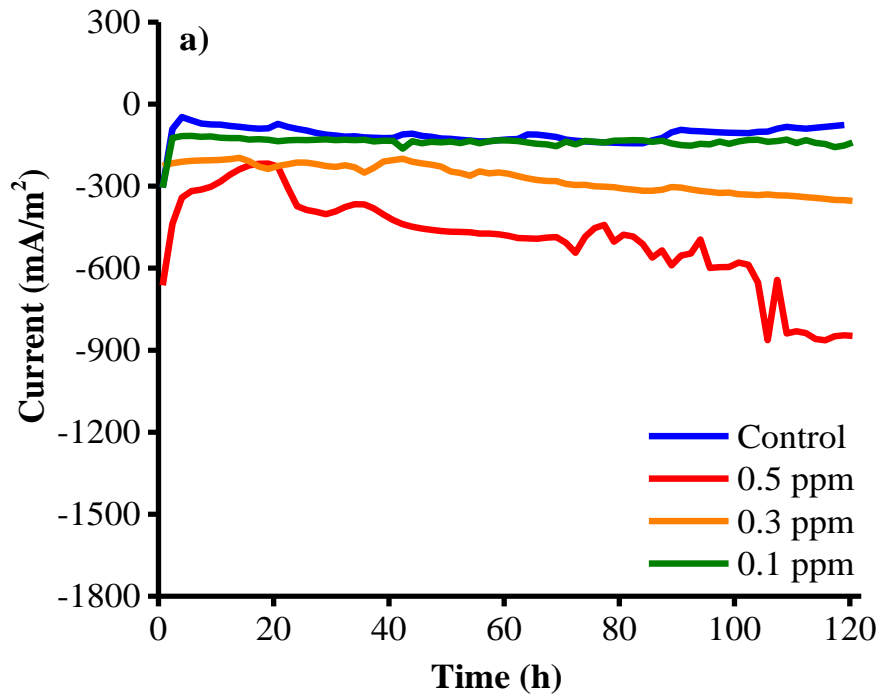
Figure 11. a) Current density performance of dual biocatalyzed microbial electrosynthesis system for the drug loading of 0.1 ppm, 0.3 ppm, and 0.5 ppm

36

3.3 Cyclic voltammetry and First derivative

The biocatalytic activity of the dual bio catalyzed MES system with and without MTF was evaluated at the end of experiment. In summary, the three-electrode system performed the CV analysis in the potential range of −800 to +200 mV at different scan rates (1 - 200 mV/s). It was interesting to see that MES without MTF (Fig 12) showed minimal current as compared to MTF exposed MES systems. Furthermore, with increase in drug loading of 0.5 (Fig 11), 0.3 (Fig 11), 0.2 ppm (Fig 3d), and control, a substantial increase in the CV cathodic current (mA) was found at -1.6, -0.87, -0.79, and -0.32, respectively (Fig 11). These findings were in line with the current density results, indicating that MTF biodegradation had a favorable impact on current production. The improved biocatalytic performance was further confirmed by first derivative analysis. To identify redox peaks involved in the electron intake process, the derivatives of the CVs scanned from positive to negative voltages were employed. [109]. A number of substantial redox peaks were detected for all MES systems (+74, +147, +186, −141 mV, −194 mV, −364 mV, −458 mV, −595 mV, −602 mV, and −743 mV v/s. AgCl) which signified the existence of different mediators assisting the electron transfer (Fig 13b) [110,111]. The -201 mV, -185 mV and +90 mV redox potentials corresponded to OmcA and OmcB species [111,112]. Flavoproteins are responsible for -436 mV and -375 mV, respectively. [113]. R3dox peaks (mV) of +50 [114], +222 [115], −800 [116], −530, and −400 mV [117] has been reported in literature which is close to our observed values. In a MES investigation with a

37

mixed microbial culture, redox potentials of -520 mV, -459 mV, and -261 mV have also been detected [85].Experimental factors, such as electrode material, microbial source, and the presence of free or biofilm-bound mediators, could be related to the differences in redox potential values [118]. Because a mixed microbial culture was employed, the detected electron mediators could not be linked to any specific bacteria. peeks than without MTF.
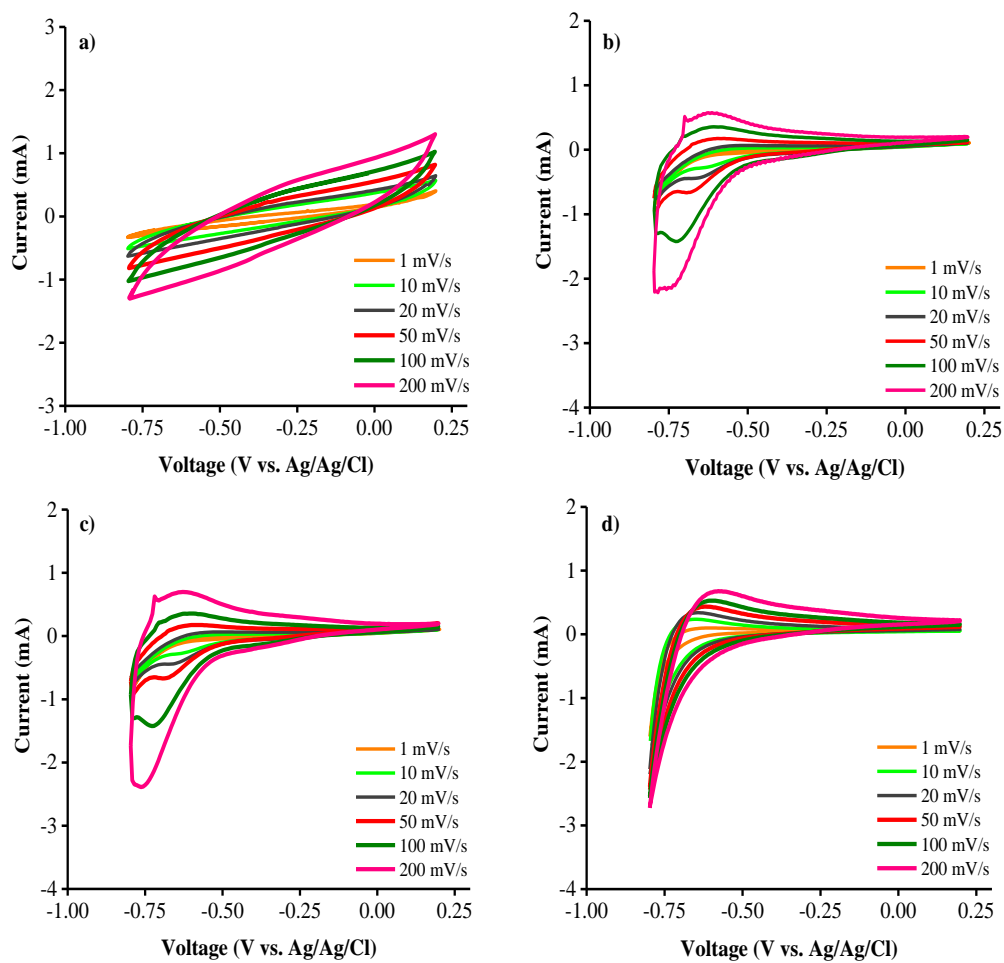
Figure. 12. Cyclic voltammogram profiles recorded at different scan rates (1–200 mV/s) and applied potential (−0.8 to +0.2 V) for (a) control, (b) 0.1 ppm, (c) 0.3 ppm, and (d) 0.5 ppm metformin concentration

39
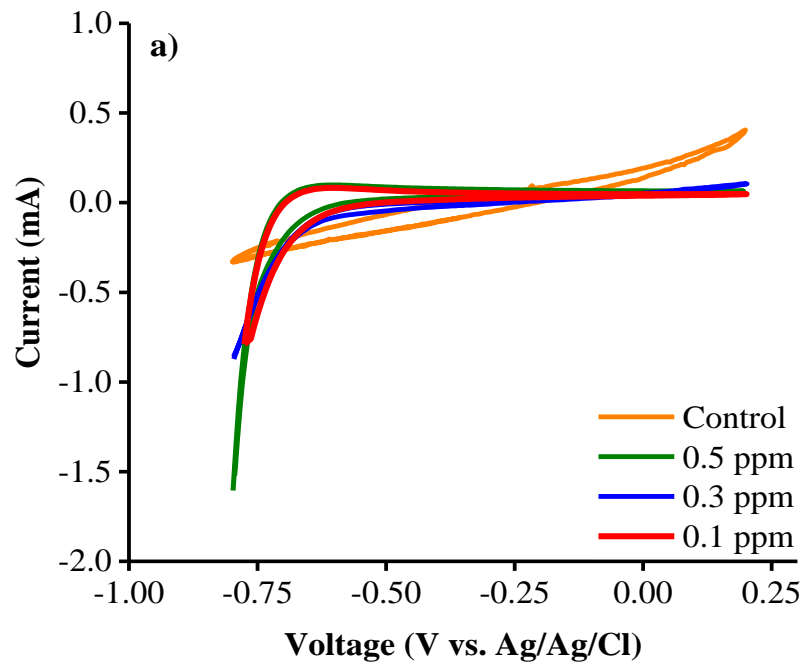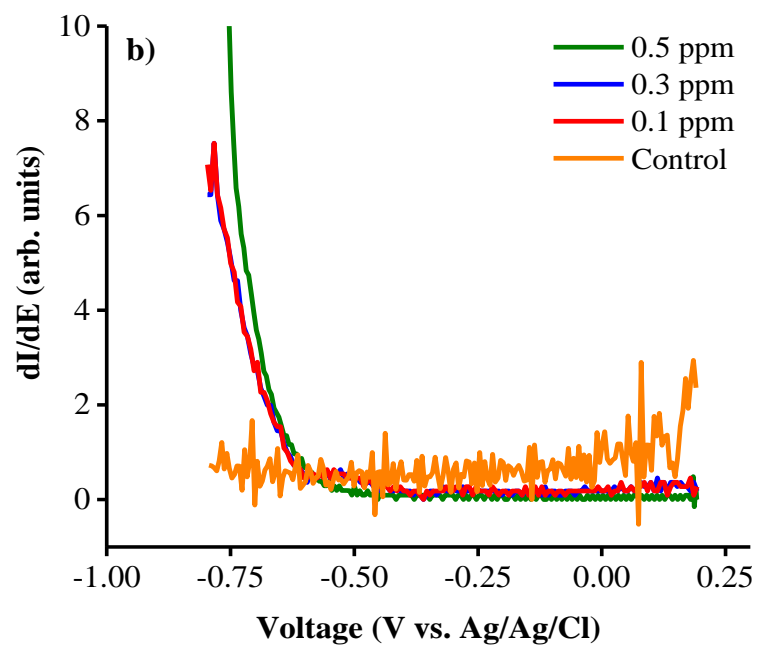
Fig 13. (a) Comparative cyclic voltammogram profiles at 1 mV/s, (b) First derivative deduced from the cyclic voltammogram (from positive to negative voltage).

3.4 Electrocatalytic activity evaluation

the electrocatalytic activity was evaluated using EIS analysis in a typical three-electrode system. The circuit component parameters were evaluated across a large frequency range using an equivalent circuit of R(QR)(QR), and the equivalent circuit model fitting was performed by ZSimpWin software (AMETEK, USA) for plotting the Nyquist curve. EIS evaluation (Fig 14) revealed that 0.5 ppm MES system holds the lowest resistance (68 Ω) as compared to 0.3 ppm (181 Ω), 0.1 ppm (554 Ω) and control (454 Ω). The lower electrode resistance signified the improved electrocatalytic activity which was in accordance with the improved CV and current density results [119]. An increase in the concentration of metformin with different kinds of electrodes material revealed an increase in current density with the lowest EIS value [120]. MTF is observed with lactate solution which is used as a feed of bacteria. Results revealed red straight lines(0.1ppm) in the high-frequency range(0.5ppm), whereas green lines were observed in the lowest frequency range.
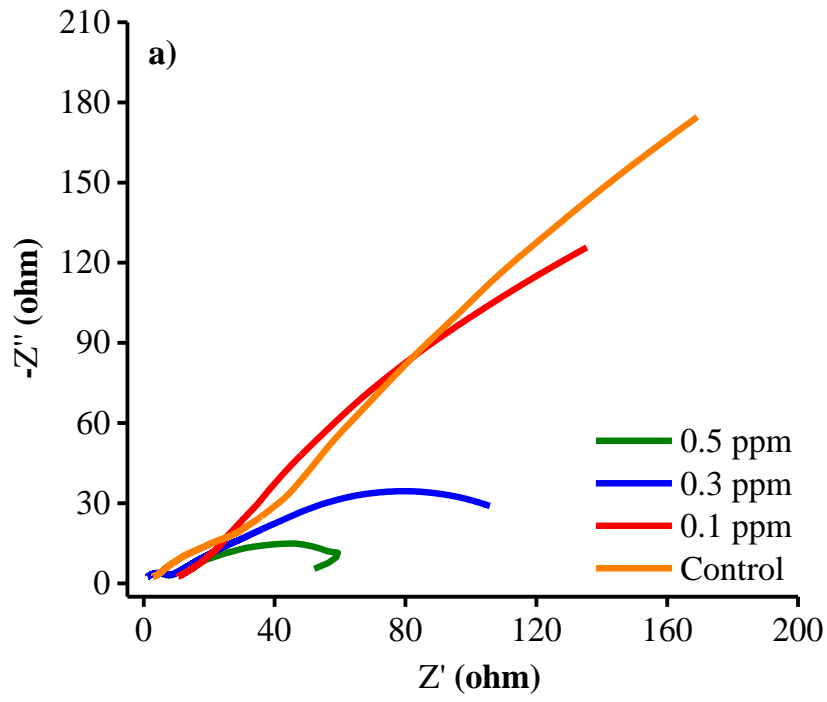
Figure 14. Comparison analysis for EIS results at a) of 0.5,0.3, and 0.1 ppm and control.

3.5. Volatile fatty acid production

The BIND and BICD samples were collected every 24 hours from both dual biocatalyzed MES systems, and the retention periods were compared to available standards to monitor the VFA production. On analyzing the BIND, VFA production of acetic (Fig 15a), propionic (Fig 15b), and butyric acid (Fig 15c), were observed, however, MTF exposed BIND showed improved acetate and propionate production signifying the overall positive effect of MTF on VFA. Moreover, a positive co-relation of VFA and MTF concentration was also observed on exposing system to different drug loadings. On increasing the MTF concentration from 0.1 to 0.3 ppm, the acetate, propionate, and butyrate concentrations (mg/L) increased from 146, 718, 125, to 244, 800, and 204, respectively. The 0.5 ppm loading further improved the VFA generation with a 11.5, 3.3, and 2.6 times higher acetic, propionic, and butyric acid production as compared to 0.1 ppm loading. The selective enrichment of the different fermentative bacteria as a result of MTF exposure in the BIND could explain the improved VFA production. It has been reported that MTF regulated the gut microbial community resulting in an increased concentration of acetate and propionate [121]. In another study, increased fecal propionate and acetate amounts were found for diabetic patients treated with MTF which is in agreement with higher VFAs production as compared to control [122–124]. On observing VFAs production at BICD chamber, the acetate (600, 540, 370 mg/L) (Fig 15d), propionate (9, 4, 3mg/L) (Fig 15e), and butyrate (651, 637, 583 mg/L) (Fig 15f) and table 2, concentration also improved for 0.5, 0.3, and 0.1 ppm loading implying that MTF

biodegradation at BIND has contributed extra electrons for production of chemical commodities overall improving the MES performance.

45
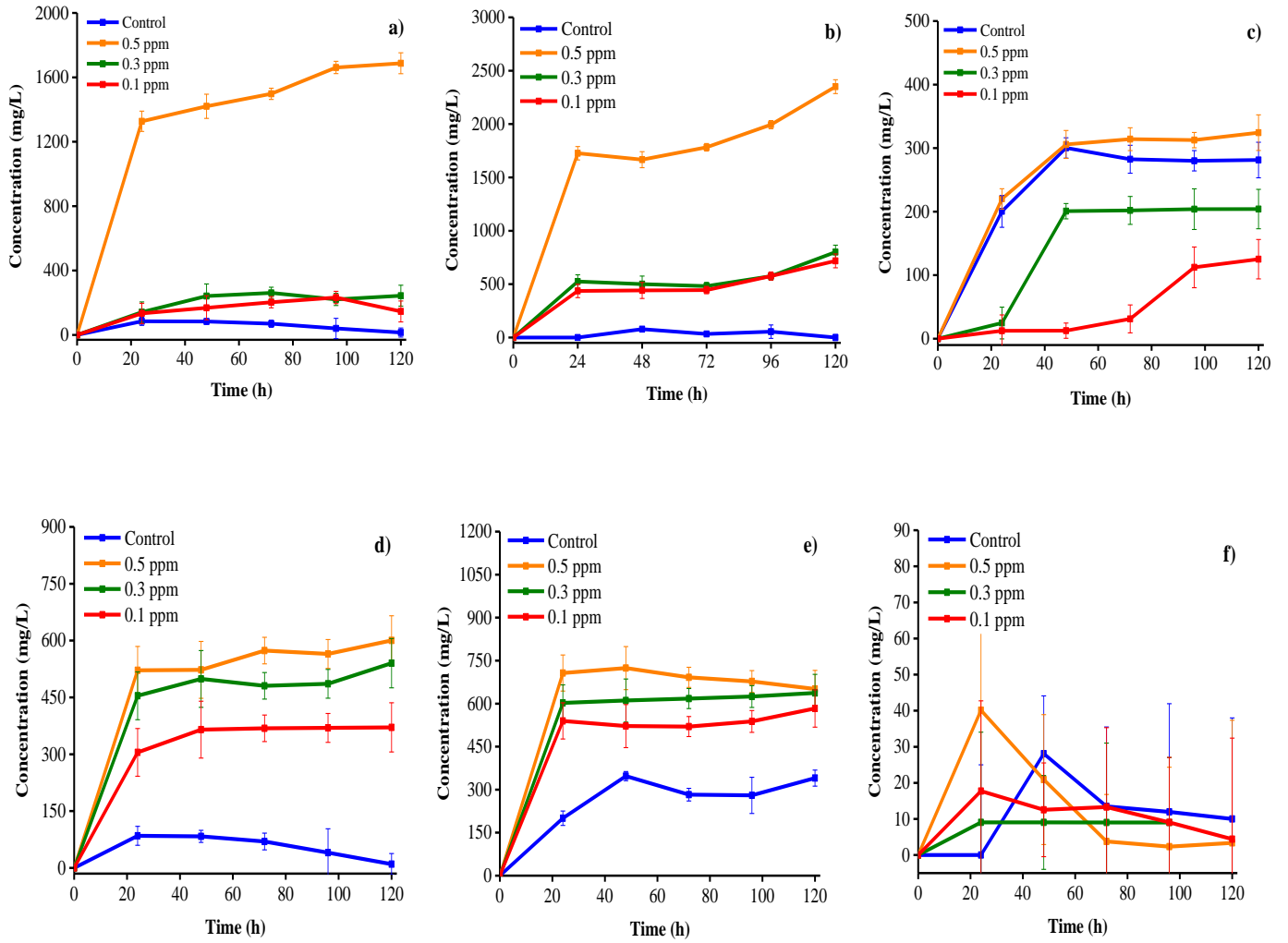
Figure. 15. Accumulated concentration profile of (a) acetic, (b) propionic, and (c) butyric acid using biocatalyzed anode (BIND), and (d) acetic, (e) propionic, and (f) butyric acid using biocatalyzed cathode (BICD) electrode for control, 0.1 ppm, 0.3 ppm, and 0.5 ppm metformin concentration.

	0.5ppm		0.3ppm		0.1ppm	
	BICD	BIND	BICD	BIND	BICD	BIND
Acetic acid	600	1687	540	244	370	146
Butyric acid	651	324	637	204	583	125
Propionic acid	-	2380	-	800	-	718

Table 2. Maximum VFA (acetic acid, butyric acid, and propionic acid) concentration generated by BIND and BICD at a fixed cathode potential of - 0.8 V using dual biocatalyzed MES system

3.6. Metformin biodegradation pathway

The MTF biodegradation along with byproduct formation was analyzed using LC-MS/MS analysis. Moreover, based on the detected m/z value, possible degradation pathways were proposed (Fig 16). The precursor–product transition ion m/z 60.05 (MTF2), m/z 103.7 (MTF3) and 88.1 (MTF8) were used as a qualifier to confirm the degradation of MTF in the samples [125][100]. The ion at m/z 103.4 (MTF3) corresponds to the cleavage of amine from m/z 130.4 (MTF) whereas loss of methylene from MTF give rise to m/z 60.05 (MTF2) [97]. The formation of guanyl-urea m/z 103.4 (MTF3) by two-fold dealkylation of MTF has also been reported and its further oxidative deamination gave formation to m/z 60.05 (MTF2) and 88.7 (MTF4) [100]. The m/z 88.08 (MTF6) originated from m/z 113.08 (MTF5) due to loss of one amine and a further methyl group removal from m/z 88.08 (MTF6) caused the formation of m/z 74.04 (MTF7) [97]. Detected peaks of m/z 88.1 (MTF8) and m/z 60.05 (MTF2) might be equivalent to protonated ions by losing guanidinium ion from m/z 85.08 (MTF1) and m/z 130.4 (MTF), respectively. Such fragmentation mechanisms of [M+H] + ion of MTF was previously reported and are in agreement with our results [97]. In another study, similar byproducts were reported and formed mainly by dimerization, dehydrogenation, demethylation, and peroxidation [126]. All the detected transformation products are in agreement with many past studies [97,100,127,128]. Most of the existing literature reported limited number of byproduct as a result of MTF degradation [100,129,130]. However, a number of biodegradation products

were detected in this study signifying the potential of BIND to successfully removal MTF in (Fig 17) and (Table 5). Moreover, many studies have reported guanyl-urea as a dead-end transformation product of MTF which was further degraded in this study [100,129,130]. The detected m/z values signified that MTF has been successfully biodegraded by dual bio catalyzed MES system.

Table 3. Experimental conditions for LC-MS analysis for MTF bioproduct analysis.

EWATRS, XEVO-TQS micro	Capillary voltage: 1 kV
Source Temperature: 150	De-solvation Temperature: 300
Cone Gas Flow (L/HR): 150	Mass range: 50-150
Flow :0.2mL/min	Column: BEH 2.1(2.7X50mm 1.8um)
Detector : ESI(+)	Cone Voltage: 15V
Mobile phase	0.1% FA: ACN (A: B)

Time (min)	Elluent A	Elluent B
0	90	10
1	90	10
6	0	100
7	0	100
8	90	10
10	90	10

Table 4. Eluent A and B flow rate ratio with time
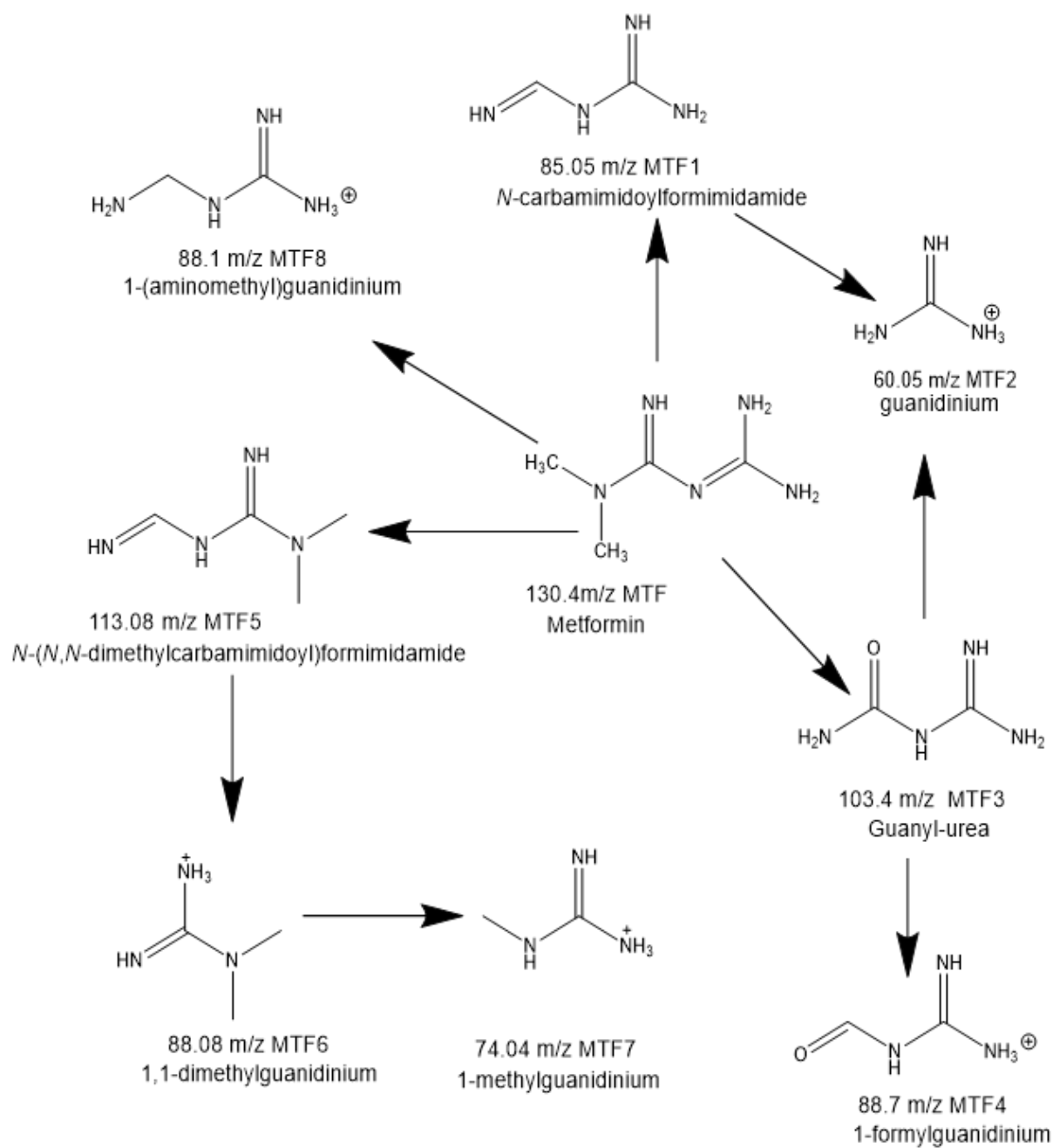
Figure 16. Proposed pathways for metformin biodegradation using the dual biocatalyzed

microbial electrosynthesis system
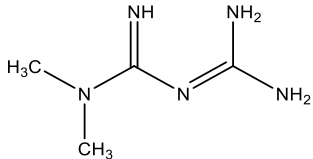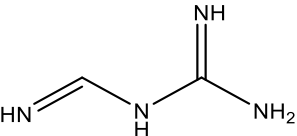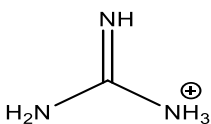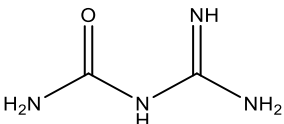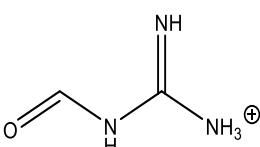
Chemical compound	Precursors ions LC/MS	PRODUCT ION M/Z
METFORMIN	130.4(C4H12N5)	113.0820, 88.0867,85.0508, 71.0605, 60.05,46.0649

Table 5. MTF precursor and product ions values.
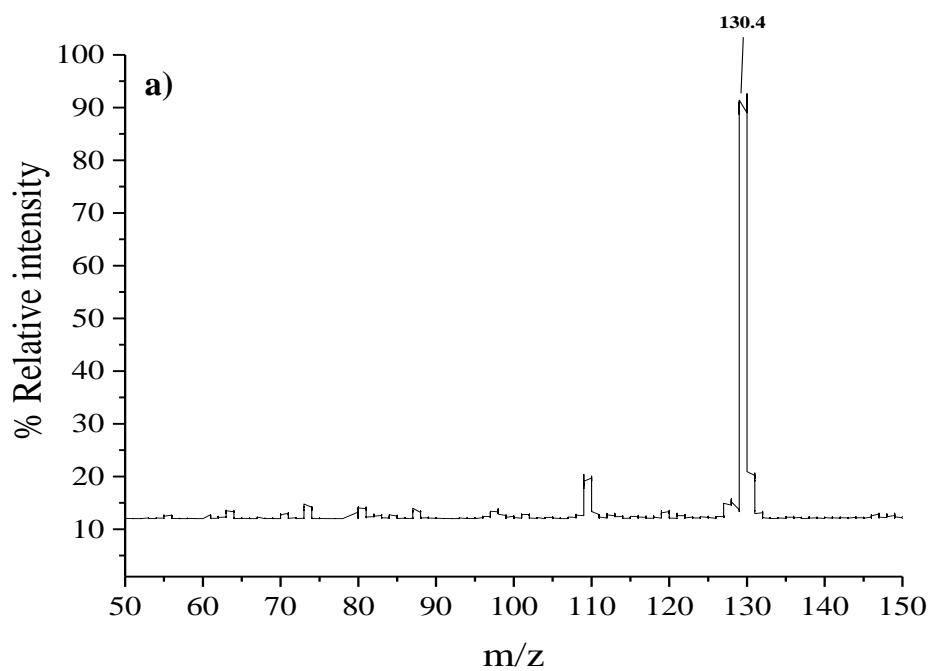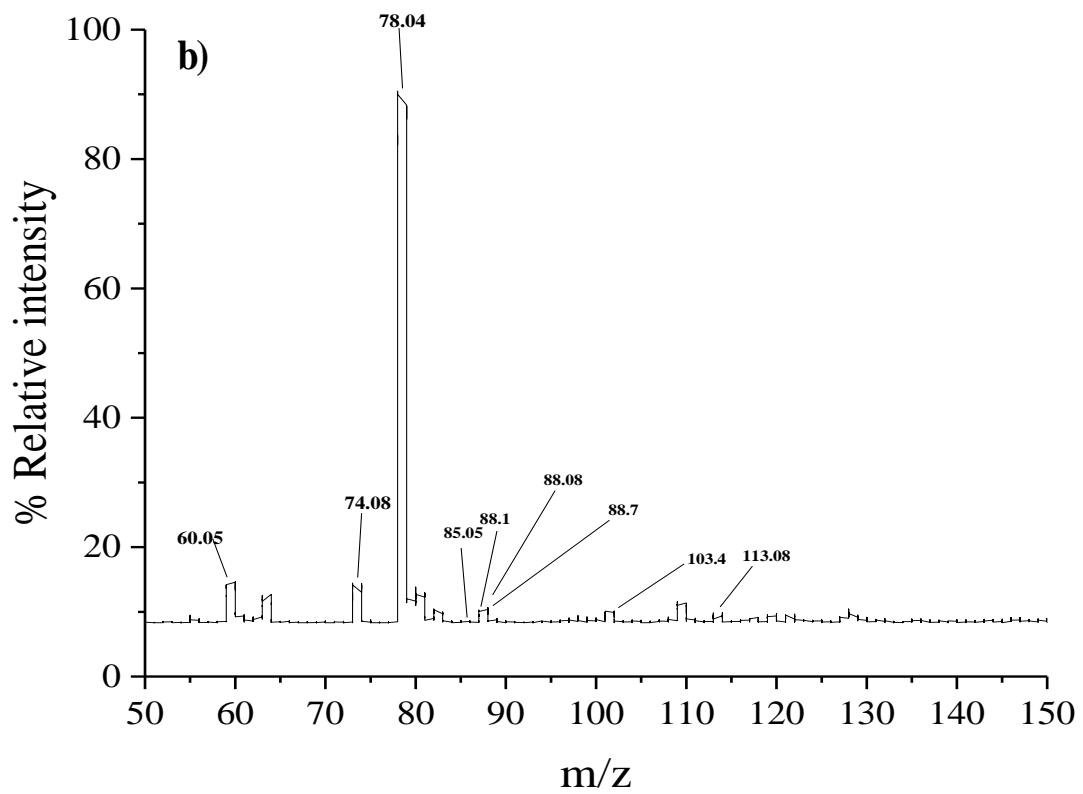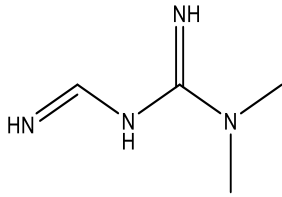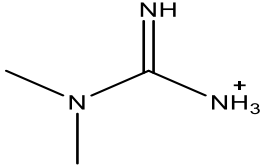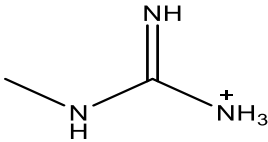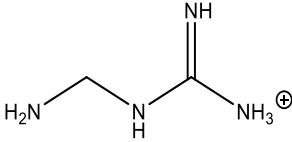
Figure 17. MTF biodegradation at different products m/z a) control b) sample

Precursors ion (m/z)	Chemical Structure	Chemical name	Product symbol
130.4		Metformin	MTF
85.05		N-carbamimidoyl-formimidamide	MTF1
60.05		Guanidinium	MTF2
103.4		Guanyl-urea	MTF3
88.7		1-formylguanidinium	MTF4

53

113.08		N-(N, Ndimethylcarbami midoyl) formimidamide	MTF5
88.08		1,1-dimethylguanidinium	MTF6
74.04		1-methylguanidinium	MTF7
88.1		1-(amino methyl)guanidin ium	MTF8

Table 5. Details of the MTF biodegradation products: m/z value, molecular formula, chemical name and structure

3.7 Microbial community analysis

At the end of experiment, the transformation and enrichment of the original inoculum for both dual biocatalyzed MES systems were assessed. Based on 16S rRNA gene pyrosequencing, operational taxonomic units and enriched microbial communities were recognized and categorized into phylum, class, and genus levels. The BIND chamber was abundantly enriched with acetate and propionate-producing phylum of *Proteobacteria* and *Bacteroidetes* [131,132]. On comparing the relative abundance, *Bacteroidetes* increased from 11% to 27% and *Proteobacteria* from 18% to 22% for dual biocatalyzed MES without and with MTF, respectively (Fig 7a). The MTF promoted microbial growth/adhesion and biofilm selective enrichment, as evidenced by the boost in relative abundance of certain bacteria. It has been reported that members of *Proteobacteria* increased in response to the MTF which is in agreement with above results [133]. An increase in propionate production was also observed which was associated with the proteobacterial groups [134]. In another study, MTF regulated the gut microbial community resulting in an increased concentration of acetate and propionate [121]. On observing class level (Fig 18b), microbial classes such *Bacteroidia* (10 to 17%) and *Deltaproteobacteria* (4 to 9%) increased with belonged to the acetate and propionate producing phylum of *Bacteroidetes* and *Proteobacteria*, respectively. An enhance in fecal propionate amounts have been determined in patients treated with MTF which signifies positive link between MTF and VFA production [122,123]. At the genus level, *Trichococcus*, *Microbacter*,

55

Desulfovibrio and *Petrimonas* contributed to the highest relative abundance (Fig 18c). A positive relationship between MTF and *Microbacter* has been reported [135]. Treatment with metformin resulted in a significant increase in the population percentage of *Desulfovibrio* and has been reported to be an excellent exoelectrongenic bacteria assisting electro transfer [136,137]. *Trichococcus* did not change much (40 to 41%), signifying that *Trichococcus* is resilient to MTF. *Petrimonas* genus has been linked with acetate and propionate production [138]. The abundance of these microbial species is in agreement with the improved VFA and current.

b)

Legend (top to bottom):
- Other
- Unclassified
- *Thermotogae*
- *Synergistetes*
- *Verrucomicrobia*
- *Planctomycetes*
- *Acidobacteria*
- *Ignavibacteriae*
- *Chloroflexi*
- *Actinobacteria*
- *Bacteroidetes*
- *Proteobacteria*
- *Firmicutes*

X-axis: Initial inoculum, Control, MTF

Y-axis: % Relative abundance
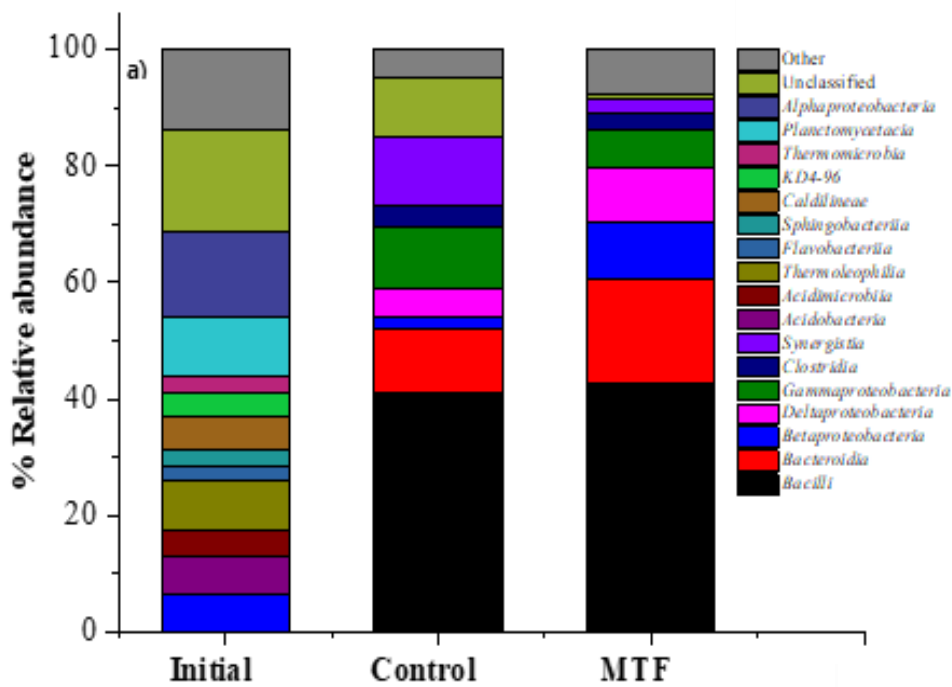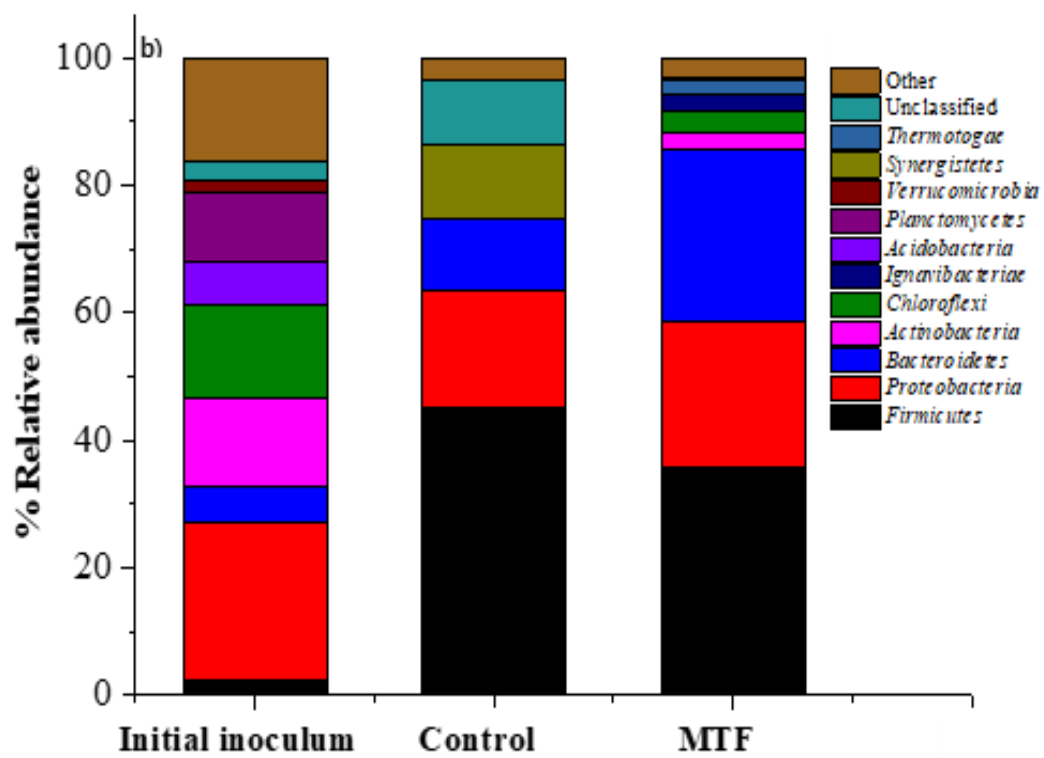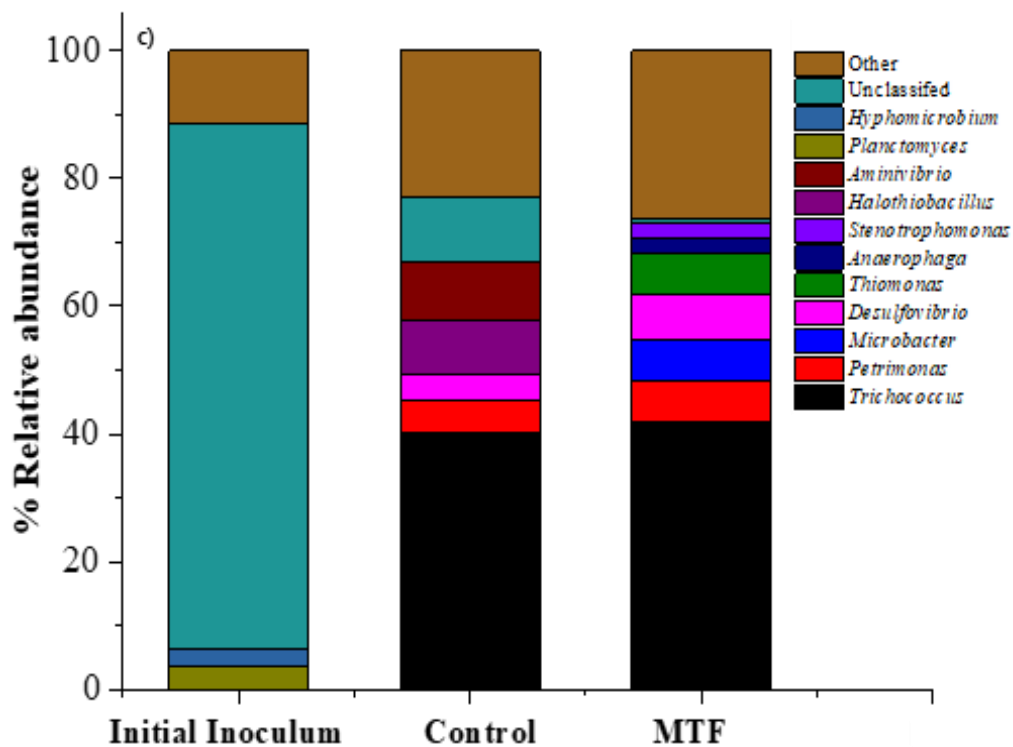
Figure. 18. Bacterial taxonomic composition and relative abundance (%) at the (a) phylum, (b) class, and (c) genus level for the initial inoculum, control, and MTF injected dual biocatalyzed microbial electrosynthesis system

3.8. Conclusion

Successful biodegradation of metformin (91%) was achieved using a dual biocatalyzed anode (BIND) and cathode (BICD) MES system. Different bioelectrochemical tests revealed improved MES performance in terms of both treatment and current density efficiency with 10.3 times higher current density than control (without MTF). MTF loading also improved the VFA production with excellent acetic, butyric, and propionic acid electrosynthesis performance. LC-MS studies supported improved mineralization of MTF with more bioproducts formation. Microbial community analysis revealed enrichment of electroactive and fermentative phylum of *Proteobacteria* and *Bacteroidetes*. These outcomes demonstrated the potential role of dual biocatalyzed BIND and BICD MES system for ensuring environmental remediation and producing useful products.

References:

[1] C. Afonso-Olivares, Z. Sosa-Ferrera, J.J. Santana-Rodríguez, Occurrence and environmental impact of pharmaceutical residues from conventional and natural wastewater treatment plants in Gran Canaria (Spain), Sci. Total Environ. 599–600 (2017) 934–943.

[2] R.M. Briones, A.K. Sarmah, L.P. Padhye, A global perspective on the use, occurrence, fate and effects of anti-diabetic drug metformin in natural and engineered ecosystems, Environ. Pollut. 219 (2016) 1007–1020.

[3] C. Gadipelly, A. Pérez-González, G.D. Yadav, I. Ortiz, R. Ibáñez, V.K. Rathod, K. V. Marathe, Pharmaceutical industry wastewater: Review of the technologies for water treatment and reuse, Ind. Eng. Chem. Res. 53 (2014) 11571–11592.

[4] X. Shi, K.Y. Leong, H.Y. Ng, Anaerobic treatment of pharmaceutical wastewater: A critical review, Bioresour. Technol. 245 (2017) 1238–1244.

[5] W.C. Li, Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil, Environ. Pollut. 187 (2014) 193–201.

[6] E. Fabbri, Pharmaceuticals in the environment: Expected and

unexpected effects on aquatic fauna, Ann. N. Y. Acad. Sci. 1340 (2015) 20–28.

[7] F.F. Al-Qaim, M.P. Abdullah, M.R. Othman, J. Latip, Z. Zakaria, Multi-residue analytical methodology-based liquid chromatography-time-of-flight-mass spectrometry for the analysis of pharmaceutical residues in surface water and effluents from sewage treatment plants and hospitals, J. Chromatogr. A. 1345 (2014) 139–153.

[8] E.N. Evgenidou, I.K. Konstantinou, D.A. Lambropoulou, Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: A review, Sci. Total Environ. 505 (2015) 905–926.

[9] M. Scheurer, A. Michel, H.J. Brauch, W. Ruck, F. Sacher, Occurrence and fate of the antidiabetic drug metformin and its metabolite guanylurea in the environment and during drinking water treatment, Water Res. 46 (2012) 4790-Water Research4802.

[10] M. Markiewicz, C. Jungnickel, S. Stolte, A. Białk-Bielińska, J. Kumirska, W. Mrozik, Ultimate biodegradability and ecotoxicity of orally administered antidiabetic drugs, J. Hazard. Mater. 333 (2017) 154–161.

[11] R.M. Briones, W.Q. Zhuang, A.K. Sarmah, Biodegradation of metformin and guanylurea by aerobic cultures enriched from sludge, Environ. Pollut. 243 (2018) 255–262.

[12] M. Oosterhuis, F. Sacher, T.L. ter Laak, Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional surface water based on sales data, Sci. Total Environ. 442 (2013) 380–388.

[13] W. Mrozik, J. Stefańska, Adsorption and biodegradation of antidiabetic pharmaceuticals in soils, Chemosphere. 95 (2014) 281–288.

[14] G. Muyzer, A.J.M. Stams, The ecology and biotechnology of sulphate-reducing bacteria, Nat. Rev. Microbiol. 6 (2008) 441–454.

[15] M. Scheurer, F. Sacher, H.J. Brauch, Occurrence of the antidiabetic drug metformin in sewage and surface waters in Germany, J. Environ. Monit. 11 (2009) 1608–1613.

[16] M.L. Reitman, E.E. Schadt, Pharmacogenetics of metformin response: A step in the path toward personalized medicine, J. Clin. Invest. 117 (2007) 1226–1229.

[17] D. Caminada, C. Escher, K. Fent, Cytotoxicity of pharmaceuticals found in aquatic systems: Comparison of PLHC-1 and RTG-2 fish cell lines, Aquat. Toxicol. 79 (2006) 114–123.

[18] J.E. Ongerth, S. Khan, Drug residuals: How xenobiotics can affect water supply sources, J. / Am. Water Work. Assoc. 96 (2004) 94–101.

[19] S. Srikanth, M. Kumar, S.K. Puri, Bio-electrochemical system (BES) as an innovative approach for sustainable waste

management in petroleum industry, Bioresour. Technol. 265 (2018) 506–518.

[20] U. Schröder, Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency, Phys. Chem. Chem. Phys. 9 (2007) 2619–2629.

[21] C. Santoro, C. Arbizzani, B. Erable, I. Ieropoulos, Microbial fuel cells: From fundamentals to applications. A review, J. Power Sources. 356 (2017) 225–244.

[22] T. Cai, L. Meng, G. Chen, Y. Xi, N. Jiang, J. Song, S. Zheng, Y. Liu, G. Zhen, M. Huang, Application of advanced anodes in microbial fuel cells for power generation: A review, Chemosphere. 248 (2020).

[23] M. Piccolino, Animal electricity and the birth of electrophysiology: The legacy of Luigi Galvani, Brain Res. Bull. 46 (1998) 381–407.

[24] Electrical effects accompanying the decomposition of organic compounds, Proc. R. Soc. London. Ser. B, Contain. Pap. a Biol. Character. 84 (1911) 260–276.

[25] S.K. Chaudhuri, D.R. Lovley, Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells, Nat. Biotechnol. 21 (2003) 1229–1232.

[26] K.P. Nevin, T.L. Woodard, A.E. Franks, Z.M. Summers, D.R. Lovley, Microbial electrosynthesis: Feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular

organic compounds, MBio. 1 (2010) 1–4.

[27] V.G. Gude, B. Kokabian, V. Gadhamshetty, Beneficial bioelectrochemical systems for energy, water, and biomass production, J. Microb. Biochem. Technol. 5 (2013).

[28] S. Bajracharya, K. Vanbroekhoven, C.J.N. Buisman, Application of gas diffusion biocathode in microbial electrosynthesis from carbon dioxide, Environ. Sci. Pollut. Res. 23 (2016) 22292–22308.

[29] Haixia, T. Song, K. Fei, H. Wang, J. Xie, Microbial electrosynthesis of organic chemicals from -CO2 by Clostridium scatologenes, Bioresour. Bioprocess. 5 (2018) 1–10.

[30] B.R.J. Pearson, M.D. Eisaman, J.W.G. Turner, P.P. Edwards, Z. Jiang, V.L. Kuznetsov, K.A. Littau, L. Marco, S.R.G. Taylor, Energy storage via carbon-neutral fuels made from CO2 , water, and renewable energy, Proc IEEE. 100 (2012) 440–460.

[31] T. Zhang, H. Nie, T.S. Bain, H. Lu, M. Cui, O.L. Snoeyenbos-West, A.E. Franks, K.P. Nevin, T.P. Russell, D.R. Lovley, Improved cathode materials for microbial electrosynthesis, Energy Environ. Sci. 6 (2013) 217–224.

[32] B.E. Logan, K. Rabaey, Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies, Scienc. 339 (2013) 686–690.

[33] Y.Q. Wang, H.X. Huang, B. Li, W.S. Li, Novelly developed three-dimensional carbon scaffold anodes from polyacrylonitrile for

microbial fuel cells, J. Mater. Chem. A. 3 (2015) 5110–5118.

[34] Y. Qiao, S. Bao, C.M. Li, X. Cui, Z. Lu, J. Guo, Nanostructured polyaniline/titanium dioxide composite anode for microbial fuel cells, ACS Nano. 2 (2008) 113–119.

[35] Y. Zhang, G. Mo, X. Li, W. Zhang, J. Zhang, J. Ye, X. Huang, C. Yu, A graphene modified anode to improve the performance of microbial fuel cells, J. Power Sources. 196 (2011) 5402–5407.

[36] Z. Lv, D. Xie, X. Yue, C. Feng, C. Wei, Ruthenium oxide-coated carbon felt electrode: A highly active anode for microbial fuel cell applications, J. Power Sources. 210 (2012) 26–31.

[37] X. Peng, H. Yu, X. Wang, Q. Zhou, S. Zhang, L. Geng, J. Sun, Z. Cai, Enhanced performance and capacitance behavior of anode by rolling Fe3O4 into activated carbon in microbial fuel cells, Bioresour. Technol. 121 (2012) 450–453.

[38] T. Zhang, H. Nie, T.S. Bain, H. Lu, M. Cui, O.L. Snoeyenbos-West, A.E. Franks, K.P. Nevin, T.P. Russell, D.R. Lovley, Improved cathode materials for microbial electrosynthesis, Energy Environ. Sci. 6 (2013) 217–224.

[39] L. Jourdin, M. Winkelhorst, B. Rawls, C.J.N. Buisman, D.P.B.T.B. Strik, Enhanced selectivity to butyrate and caproate above acetate in continuous bioelectrochemical chain elongation from CO2: Steering with CO2 loading rate and hydraulic retention time, Bioresour. Technol. Reports. 7 (2019) 100284.

[40] M. Cui, H. Nie, T. Zhang, D. Lovley, T.P. Russell, Three-dimensional hierarchical metal oxide– carbon electrode materials for highly efficient microbial electrosynthesis, Sustain. Energy Fuels. 1 (2017) 1171–1176.

[41] L. Jourdin, S. Freguia, B.C. Donose, J. Chen, G.G. Wallace, V. Flexer, A novel carbon nanotube modified scaffold as an efficient biocathode material for improved microbial electrosynthesis, J. Mater. Chem. A. 2 (2014) 13093–13102.

[42] H. Nie, T. Zhang, M. Cui, H. Lu, D.R. Lovley, T.P. Russell, Improved cathode for high efficient microbial-catalyzed reduction in microbial electrosynthesis cells, Phys. Chem. Chem. Phys. 15 (2013) 14290–14294.

[43] M. Aghababaie, M. Farhadian, A. Jeihanipour, D. Biria, Effective factors on the performance of microbial fuel cells in wastewater treatment–a review, Environ. Technol. Rev. 4 (2015) 71–89.

[44] Z. Dong, H. Wang, S. Tian, Y. Yang, H. Yuan, Q. Huang, T. shun Song, J. Xie, Fluidized granular activated carbon electrode for efficient microbial electrosynthesis of acetate from carbon dioxide, Bioresour. Technol. 269 (2018) 203–209.

[45] K. Tahir, W. Miran, J. Jang, A. Shahzad, M. Moztahida, B. Kim, D. Sung, A novel MXene-coated biocathode for enhanced microbial electrosynthesis performance, Chem. Eng. J. 381 (2020) 122687.

[46] M. Quraishi, K. Wani, S. Pandit, P.K. Gupta, A.K. Rai, D. Lahiri,

D.A. Jadhav, R.R. Ray, S.P. Jung, V.K. Thakur, R. Prasad, Valorisation of co2 into value-added products via microbial electrosynthesis (Mes) and electro-fermentation technology, Fermentation. 7 (2021).

[47] S.A. Patil, K. Rabaey, K. Guo, A. Pre, Engineering electrodes for microbial electrocatalysis, Curr. Opin. Biotechnol. 33 (2015) 149–156.

[48] V.A. Online, V. Flexer, J. Chen, B.C. Donose, P. Sherrell, G.G. Wallace, J. Keller, The nanostructure of three-dimensional scaffolds enhances the current density of microbial bioelectrochemical systems, Energy Enviornmental Sci. 6 (2013) 1291–1298.

[49] S. Chabi, C. Peng, D. Hu, Y. Zhu, Ideal three-dimensional electrode structures for electrochemical energy storage, Adv. Mater. 26 (2014) 2440–2445.

[50] N. Aryal, F. Ammam, S.A. Patil, D. Pant, An overview of cathode materials for microbial electrosynthesis of chemicals from carbon dioxide, Green Chem. 19 (2017) 5748–5760.

[51] X. Wang, G. Li, Z. Chen, V. Augustyn, X. Ma, G. Wang, B. Dunn, Y. Lu, High-performance uupercapacitors based on nanocomposites of Nb2O5 nanocrystals and carbon nanotubes, Mater. Views. 1 (2011) 1089–1093.

[52] M. Min, K. Machida, J.H. Jang, K. Naoi, Hydrous RuO2/carbon black nanocomposites with 3D porous structure by novel incipient wetness method for supercapacitors, J. Electrochem. Soc. 2 (2006)

334–338.

[53] E. Environ, X. Xie, M. Ye, L. Hu, N. Liu, J.R. Mcdonough, W. Chen, H.N. Alshareef, Carbon nanotube-coated macroporous sponge for microbial fuel cell electrodes, Energy Environ. Sci. (2012) 5265–5270.

[54] X. Xie, L. Hu, M. Pasta, G.F. Wells, D. Kong, C.S. Criddle, Y. Cui, Three-dimensional carbon nanotube-textile anode for high-performance microbial fuel cells, Nano Lett. 11 (2011) 291–296.

[55] T. Chen, Z. Cai, L. Qiu, H. Li, J. Ren, H.P. Huijuan Lin, Zhibin Yang, Xuemei Sun, Synthesis of aligned carbon nanotube composite fibers with high performances by electrochemical deposition, J. Mater. Chem. A. 1 (2013) 2211–2216.

[56] D. Ma, Z. Cao, H. Wang, X. Huang, L. Wanga, X. Zhang, Three-dimensionally ordered macroporous FeF3 and its in situ homogenous polymerization coating for high energy and power density lithium ion batteries, Energy Environ. Sci. 5 (2012) 8538–8542.

[57] Q. Zhu, J. Hu, B. Liu, S. Hu, S. Liang, K. Xiao, J. Yang, H. Hou, Recent Advances on the Development of Functional Materials in Microbial Fuel Cells: From Fundamentals to Challenges and Outlooks, Energy Environ. Mater. 5 (2022) 401–426.

[58] J. Khera, A. Chandra, Microbial Fuel Cells: Recent Trends, Proc. Natl. Acad. Sci. India Sect. A Phys. Sci. 82 (2012) 31–41.

[59] S.E. Oh, B.E. Logan, Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies., Water Res. 39 (2005) 4673–82.

[60] Y. Mohan, S. Manojmuthukumar, D. Das, Electricity generation using microbial fuel cells, Int. J. Hydrogen Energy. 33 (2008) 423–426

[61] V.R. Nimje, C.-Y. Chen, C.-C. Chen, H.-R. Chen, M.-J. Tseng, J.-S. Jean, Y.-F. Chang, Glycerol degradation in single-chamber microbial fuel cells., Bioresour. Technol. 102 (2011) 2629–34.

[62] N. Lu, S. Zhou, L. Zhuang, J. Zhang, J. Ni, Electricity generation from starch processing wastewater using microbial fuel cell technology, Biochem. Eng. J. 43 (2009) 246–251.

[63] A. Das, M. Mohapatra, S. Basu, Recent Development in Cathodic Catalyst towards Performance of Bioelectrochemical Systems, ACS Symp. Ser. 1342 (2020) 1–25.

[64] K. Rabaey, W. Verstraete, Microbial fuel cells: novel biotechnology for energy generation., Trends Biotechnol. 23 (2005) 291–298.

[65] K. Rabaey, G. Lissens, S.D. Siciliano, W. Verstraete, A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency., Biotechnol. Lett. 25 (2003) 1531–5.

[66] J.K. Jang, T.H. Pham, I.S. Chang, K.H. Kang, H. Moon, K.S.

69

Cho, B.H. Kim, Construction and operation of a novel mediator- and membrane-less microbial fuel cell, Process Biochem. 39 (2004) 1007–1012. 9592(03)00203-6.

[67] B. Min, B.E. Logan, Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell., Environ. Sci. Technol. 38 (2004) 5809–14.

[68] H. Liu, R. Ramnarayanan, B.E. Logan, Production of electricity during wastewater treatment using a single chamber microbial fuel cell., Environ. Sci. Technol. 38 (2004) 2281–5.

[69] D.Y. Lyon, F. Buret, T.M. Vogel, J.-M. Monier, Is resistance futile? Changing external resistance does not improve microbial fuel cell performance., Bioelectrochemistry. 78 (2010) 2-7.

[70] P. Aelterman, M. Versichele, M. Marzorati, N. Boon, W. Verstraete, Loading rate and external resistance control the electricity generation of microbial fuel cells with different three-dimensional anodes., Bioresour. Technol. 99 (2008) 8895–902.

[71] S. Oh, B. Min, B.E. Logan, Cathode performance as a factor in electricity generation in microbial fuel cells., Environ. Sci. Technol. 38 (2004) 4900–4.

[72] A.P. Borole, G. Reguera, B. Ringeisen, Z.-W. Wang, Y. Feng, B.H. Kim, Electroactive biofilms: Current status and future research needs, Energy Environ. Sci. 4 (2011) 4813.

[73] S. a Patil, F. Harnisch, B. Kapadnis, U. Schröder, Electroactive

mixed culture biofilms in microbial bioelectrochemical systems: the role of temperature for biofilm formation and performance., Biosens. Bioelectron. 2 6 (2 0 1 0) 8 0 3 – 8 .

[74] E. Marsili, D.B. Baron, I.D. Shikhare, D. Coursolle, J. a Gralnick, D.R. Bond, Shewanella secretes flavins that mediate extracellular electron transfer., Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 3968-73.

[75] S. Freguia, M. Masuda, S. Tsujimura, K. Kano, Lactococcus lactis catalyses electricity generation at microbial fuel cell anodes via excretion of a soluble quinone., Bioelectrochemistry. 76 (2009) 14–8.

[76] T.H. Pham, N. Boon, P. Aelterman, P. Clauwaert, L. De Schamphelaire, L. Vanhaecke, K. De Maeyer, M. Höfte, W. Verstraete, K. Rabaey, Metabolites produced by Pseudomonas sp. enable a Gram-positive bacterium to achieve extracellular electron transfer., Appl. Microbiol. Biotechnol. 77 (2008) 1119-29.

[77] D.R. Lovley, The microbe electric: conversion of organic matter to electricity., Curr. Opin. Biotechnol. 19 (2008) 564–71.

[78] T.S. Magnuson, How the xap locus put electrical "Zap" in Geobacter sulfurreducens biofilms., J. Bacteriol. 193 (2011) 1021–2.

[79] B. Cao, L. Shi, R.N. Brown, Y. Xiong, J.K. Fredrickson, M.F. Romine, M.J. Marshall, M.S. Lipton, H. Beyenal, Extracellular polymeric substances from Shewanella sp. HRCR-1 biofilms: characterization by infrared spectroscopy and proteomics.,

Environ. Microbiol. 13 (2011) 1018–31.

[80] M.Y. El-Naggar, G. Wanger, K.M. Leung, T.D. Yuzvinsky, G. Southam, J. Yang, W.M. Lau, K.H. Nealson, Y. a Gorby, Electrical transport along bacterial nanowires from Shewanella oneidensis MR-1., Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 18127-31.

[81] Y. a Gorby, S. Yanina, J.S. McLean, K.M. Rosso, D. Moyles, A. Dohnalkova, T.J. Beveridge, I.S. Chang, B.H. Kim, K.S. Kim, D.E. Culley, S.B. Reed, M.F. Romine, D. a Saffarini, E. a Hill, L. Shi, D. a Elias, D.W. Kennedy, G. Pinchuk, K. Watanabe, S. Ishii, B. Logan, K.H. Nealson, J.K. Fredrickson, Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms., Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 11358-63.

[82] O. Choi, B.I. Sang, Extracellular electron transfer from cathode to microbes: Application for biofuel production, Biotechnol. Biofuels. 9 (2016) 1-14.

[83] D. Xing, S. Cheng, J.M. Regan, B.E. Logan, Change in microbial communities in acetate- and glucose-fed microbial fuel cells in the presence of light, Biosens. Bioelectron. 25 (2009) 105-111.

[84] H.S. Lee, B.E. Rittmann, Significance of biological hydrogen oxidation in a continuous single-chamber microbial electrolysis cell, Environ. Sci. Technol. 44 (2010) 948–954.

[85] J.A. Modestra, S.V. Mohan, Microbial electrosynthesis of carboxylic acids through CO2 reduction with selectively enriched biocatalyst: Microbial dynamics, J. CO2 Util. 20

(2 0 1 7) 1 9 0 – 1 9 9 . [86] G. Mohanakrishna, J.S. Seelam, K. Vanbroekhoven, D. Pant, An enriched electroactive homoacetogenic biocathode for the microbial electrosynthesis of acetate through carbon dioxide reduction, Faraday Discuss. 183 (2015) 445–462.

[87] A.W. Jeremiasse, H.V.M. Hamelers, C.J.N. Buisman, Microbial electrolysis cell with a microbial biocathode, Bioelectrochemistry. 78 (2010) 39-43.

.

[88] S.S. Lim, E.H. Yu, W.R.W. Daud, B.H. Kim, K. Scott, Bioanode as a limiting factor to biocathode performance in microbial electrolysis cells, Bioresour. Technol. 238 (2017) 313–324.

[89] N.S. Abdelwahab, A. Morsi, Y.M. Ahmed, H.M. Hassan, A.M. Aboulmagd, Ecological HPLC method for analyzing an antidiabetic drug in real rat plasma samples and studying the effects of concurrently administered fenugreek extract on its pharmacokinetics, RSC Adv. 11 (2021) 4740–4750. [90]P. Kosse, M. Lübken, M. Wichern, Selective inhibition of methanogenic archaea in leach bed systems by sodium 2-bromoethanesulfonate, Environ. Technol. Innov. 5 (2016) 199–

[91] J. Hu, G. Liu, H. Li, H. Luo, R. Zhang, Synergistic effect of bioanode and biocathode on nitrobenzene removal: Microbial community structure and functions, Sci. Total Environ. 833 (2022) 155190. [92] E. Bolyen, J.R. Rideout, M.R. Dillon, N.A. Bokulich, C.C. Abnet, A. Gabriel, A. Ghalith, H. Alexander, E.J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J.E. Bisanz, K. Bittinger, A. Brejnrod, C.J. Brislawn, C.T. Brown, J. Benjamin, A. Mauricio, C. Rodríguez, J. Chase, E.K. Cope, R. Da

Silva, P.C. Dorrestein, G.M. Douglas, D.M. Durall, C. Duvallet, C.F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J.M. Gauglitz, D.L. Gibson, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, G.A. Huttley, S. Janssen, A.K. Jarmusch, L. Jiang, D. Benjamin, K. Bin Kang, C.R. Keefe, P. Keim, S.T. Kelley, D. Knights, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B.D. Martin, J.L. Metcalf, S.C. Morgan, T. Jamie, L.F. Nothias, S.B. Orchanian, N. Segata, A.D. Willis, QIIME 2 : Reproducible , interactive , scalable , and extensible microbiome data science, PeerJ Prepr. 6 (2018) e27295v2.

[93] C. Quast, E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, F.O. Glöckner, The SILVA ribosomal RNA gene database project : improved data processing and web-based tools, Nucleic Acids Res. 41 (2013) 590–596.

[94] C.F. Carbuloni, J.E. Savoia, J.S.P. Santos, C.A.A. Pereira, R.G. Marques, V.A.S. Ribeiro, A.M. Ferrari, Degradation of metformin in water by TiO2–ZrO2 photocatalysis, J. Environ. Manage. 262 (2020) 110347.

[95] E. Aseman-Bashiz, H. Sayyaf, Metformin degradation in aqueous solutions by electro-activation of persulfate and hydrogen peroxide using natural and synthetic ferrous ion sources, J. Mol. Liq. 300 (2020) 112285.

[96] A. Gumieniczek, A. Berecka-Rycerz, T. Mroczek, K. Wojtanowski, Determination of chemical stability of two oral antidiabetics, metformin and repaglinide in the solid state and solutions using LC-UV, LC-MS, and FT-IR methods, Molecules.

24 (2019).

[97] M. Luo, L. Zheng, S. Wang, J. Zhu, Z. Tang, P. Gao, Characterization of four major degradation products in metformin by 2D LC-QTOF/MS/MS, J. Pharm. Biomed. Anal. 192 (2021) 113662.

[98] F.J.O. Quintão, J.R.L. Freitas, C. de Fátima Machado, S.F. Aquino, S. de Queiroz Silva, R.J. de Cássia Franco Afonso, Characterization of metformin by-products under photolysis, photocatalysis, ozonation and chlorination by high-performance liquid chromatography coupled to high-resolution mass spectrometry, Rapid Commun. Mass Spectrom. 30 (2016) 2360–2368.

[99] S. Gite, V. Patravale, Validation of RP-HPLC method and stress degradation for the combination of metformin hcl, atorvastatin calcium and glimepiride: application to nanoparticles, J. Chromatogr. Sci. 53 (2015) 1654–1662.

[100] C. Trautwein, K. Kümmerer, Incomplete aerobic degradation of the antidiabetic drug Metformin and identification of the bacterial dead-end transformation product Guanylurea, Chemosphere. 85 (2011) 765–773.

[101] N. De la Cruz, L. Esquius, D. Grandjean, A. Magnet, A. Tungler, L.F. de Alencastro, C. Pulgarín, Degradation of emergent contaminants by UV, UV/H2O2 and neutral photo-Fenton at pilot scale in a domestic wastewater treatment plant, Water Res. 47 (2013) 5836–5845.

[102] K. Tahir, W. Miran, M. Nawaz, J. Jang, A. Shahzad, M.

Moztahida, B. Kim, M. Azam, S.E. Jeong, C.O. Jeon, S.R. Lim, D.S. Lee, Investigating the role of anodic potential in the biodegradation of carbamazepine in bioelectrochemical systems, Sci. Total Environ. 688 (2019) 56–64.

[103] P. Chen, X. Guo, S. Li, F. Li, A review of the bioelectrochemical system as an emerging versatile technology for reduction of antibiotic resistance genes, Environ. Int. 156 (2021) 106689.

[104] O.A.H. Jones, N. Voulvoulis, J.N. Lester, The occurrence and removal of selected pharmaceutical compounds in a sewage treatment works utilising activated sludge treatment, Environ. Pollut. 145 (2007) 738–744.

[105] S. Gadkari, M. Shemfe, J.A. Modestra, S.V. Mohan, J. Sadhukhan, Understanding the interdependence of operating parameters in microbial electrosynthesis: A numerical investigation, Phys. Chem. Chem. Phys. 21 (2019) 10761–10772.

[106] Y. Xiang, G. Liu, R. Zhang, Y. Lu, H. Luo, High-efficient acetate production from carbon dioxide using a bioanode microbial electrosynthesis system with bipolar membrane, Bioresour. Technol. 233 (2017) 227–235.

[107] A.L. Yalçın Altunkaynak , Ömer Yavuz, New voltammetric strategy for determination and electrochemical behaviors of Metformin by pencil graphite electrode, J. Indian Chem. Soc. 97 (2020).

[108] K. Rabaey, K. Van De Sompel, L. Maignien, N. Boon, P.

Aelterman, P. Clauwaert, L. De Schamphelaire, H.T. Pham, J. Vermeulen, M. Verhaege, P. Lens, W. Verstraete, Microbial fuel cells for sulfide removal, Environ. Sci. Technol. 40 (2006) 5218–5224.

[109] E. Science, A.R. Rowe, S. Xu, E. Gardel, A. Bose, P. Girguis, J.P. Amend, M.Y. El-naggar, crossm Cathodes by Methanosarcina barkeri, 10 (2019) 1–12.

[110] H. Liu, T. Song, K. Fei, H. Wang, J. Xie, Microbial electrosynthesis of organic chemicals from CO2 by Clostridium scatologenes ATCC 25775T, Bioresour. Bioprocess. 5 (2018).

[111] H. Richter, K.P. Nevin, H. Jia, D.A. Lowy, D.R. Lovley, L.M. Tender, Cyclic voltammetry of biofilms of wild type and mutant Geobacter sulfurreducens on fuel cell anodes indicates possible roles of OmcB, OmcZ, type IV pili, and protons in extracellular electron transfer, Energy Environ. Sci. 2 (2009) 506–516.

[112] X. Jin, F. Guo, Z. Liu, Y. Liu, H. Liu, Enhancing the electricity generation and nitrate removal of microbial fuel cells with a novel denitrifying exoelectrogenic strain EB-1, Front. Microbiol. 9 (2018) 1–10.

[113] P. Batlle-vilanova, S. Puig, R. Gonzalez-olmos, M. Dolors, J. Colprim, Continuous acetate production through microbial electrosynthesis from CO2 with microbial mixed culture, J Chem Technol Biotechnol. 91 (2015) 921–927.

[114] C.A. Pham, S.J. Jung, N.T. Phung, J. Lee, I.S. Chang, B.H. Kim,

H. Yi, J. Chun, A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to Aeromonas hydrophila, isolated from a microbial fuel cell, FEMS Microbiol. Lett. 223 (2003) 129–134.

[115] V. Kumar, P. Kumar, A. Nandy, P.P. Kundu, Crosslinked inter penetrating network of sulfonated styrene and sulfonated PVdF-co-HFP as electrolytic membrane in a single chamber microbial fuel cell, RSC Adv. 5 (2015) 30758–30767.

[116] H.Y. Yang, Y.X. Wang, C.S. He, Y. Qin, W.Q. Li, W.H. Li, Y. Mu, Redox mediator-modified biocathode enables highly efficient microbial electro-synthesis of methane from carbon dioxide, Appl. Energy. 274 (2020) 115292.

[117] S. Li, Y.E. Song, J. Baek, H.S. Im, M. Sakuntala, M. Kim, C. Park, B. Min, J.R. Kim, Bioelectrosynthetic conversion of CO2 using different redox mediators: Electron and carbon balances in a bioelectrochemical system, Energies. 13 (2020).

[118] A. Carmona, Investigation of electron transfer mechanisms in electrochemically active microbial biofilms, Institute of Environmental and Sustainable Chemistry, Technische Universität Braunschweig, Germany, 2012.

[119] M. Hu, X. Li, J. Xiong, L. Zeng, Y. Huang, Y. Wu, G. Cao, W. Li, Nano-Fe3C@PGC as a novel low-cost anode electrocatalyst for superior performance microbial fuel cells, Biosens. Bioelectron. 142 (2019) 111594.

[120] Y. ALTUNKAYNAK, … Ö.Y.-6 th I.B.O., undefined 2020, New voltammetric strategy for determination and electrochemical behaviors of Metformin by Pencil Graphite Electrode, Icntcconference.Com. 97 (2020). http://icntcconference.com/wp-content/uploads/2020/10/ICTNC-2020-BOOK-OF-ABTRACTS.pdf#page=66.

[121] R. Pryor, P. Norvaisas, G. Marinos, L. Best, L.B. Thingholm, L.M. Quintaneiro, W. De Haes, D. Esser, S. Waschina, C. Lujan, R.L. Smith, T.A. Scott, D. Martinez-Martinez, O. Woodward, K. Bryson, M. Laudes, W. Lieb, R.H. Houtkooper, A. Franke, L. Temmerman, I. Bjedov, H.M. Cochemé, C. Kaleta, F. Cabreiro, Host-microbe-drug-nutrient screen identifies bacterial effectors of metformin therapy, Cell. 178 (2019) 1299-1312.e29.

[122] H. Wu, E. Esteve, V. Tremaroli, M.T. Khan, R. Caesar, L. Mannerås-Holm, M. Ståhlman, L.M. Olsson, M. Serino, M. Planas-Fèlix, G. Xifra, J.M. Mercader, D. Torrents, R. Burcelin, W. Ricart, R. Perkins, J.M. Fernàndez-Real, F. Bäckhed, Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug, Nat. Med. 23 (2017) 850–858.

[123] A. Bravard, C. Gérard, C. Defois, B. Benoit, K. Makki, E. Meugnier, D. Rainteau, J. Rieusset, M. Godet, H. Vidal, Metformin treatment for 8 days impacts multiple intestinal parameters in high-fat high-sucrose fed mice, Sci. Rep. 11 (2021) 1–12.

[124] N.T. Mueller, M.K. Differding, M. Zhang, N.M. Maruthur, S.P.

Juraschek, E.R. Miller, L.J. Appel, H.C. Yeh, Metformin affects gut microbiome composition and function and circulating short-chain fatty acids: A randomized trial, Diabetes Care. 44 (2021) 1462–1471.

[125] K. Chaudhari, J. Wang, Y. Xu, A. Winters, L. Wang, X. Dong, E.Y. Cheng, R. Liu, S.H. Yang, Determination of metformin bio-distribution by LC-MS/MS in mice treated with a clinically relevant paradigm, PLoS One. 15 (2 0 2 0) 1 – 2 0 .

[126] F. Collin, H. Khoury, D. Bonnefont-Rousselot, P. Thérond, A. Legrand, D. Jore, M. Gardès-Albert, Liquid chromatographic/electrospray ionization mass spectrometric identification of the oxidation end-products of metformin in aqueous solutions, J. Mass Spectrom. 39 (2004) 890–902.

[127] S. Tisler, C. Zwiener, Aerobic and anaerobic formation and biodegradation of guanyl urea and other transformation products of metformin, Water Res. 149 (2 0 1 9) 1 3 0 – 1 3 5 .

[128] B.A.J. Poursat, R.J.M. van Spanning, M. Braster, R. Helmus, P. de Voogt, J.R. Parsons, Biodegradation of metformin and its transformation product, guanylurea, by natural and exposed microbial communities, Ecotoxicol. Environ. Saf. 182 (2019) 109414.

[129] E. Haiba, L. Nei, K. Herodes, M. Ivask, M. Lillenberg, On the degradation of metformin and carbamazepine residues in sewage sludge compost, Agron. Res. 16 (2018) 696–707.

[130] C. Trautwein, J.D. Berset, H. Wolschke, K. Kümmerer,

Occurrence of the antidiabetic drug Metformin and its ultimate transformation product Guanylurea in several compartments of the aquatic cycle, Environ. Int. 70 (2014) 203–212.

[131] R. El Hage, E. Hernandez-Sanabria, M. Calatayud Arroyo, R. Props, T. Van De Wiele, Propionate-producing consortium restores antibiotic-induced dysbiosis in a dynamic in vitro model of the human intestinal microbial ecosystem, Front. Microbiol. 10 (2019) 1–17.

[132] N. Reichardt, S.H. Duncan, P. Young, A. Belenguer, C. McWilliam Leitch, K.P. Scott, H.J. Flint, P. Louis, Phylogenetic distribution of three pathways for propionate production within the human gut microbiota, ISME J. 8 (2014) 1323–1335.

[133] L. Silamiķele, I. Silamiķelis, M. Ustinova, Z. Kalniņa, I. Elbere, R. Petrovska, I. Kalniņa, J. Kloviņš, Metformin Strongly Affects Gut Microbiome Composition in High-Fat Diet-Induced Type 2 Diabetes Mouse Model of Both Sexes, Front. Endocrinol. (Lausanne). 12 (2021).

[134] H.S. Ejtahed, R.Y. Tito, S.D. Siadat, S. Hasani-Ranjbar, Z. Hoseini-Tavassol, L. Rymenans, K. Verbeke, A.R. Soroush, J. Raes, B. Larijani, Metformin induces weight loss associated with gut microbiota alteration in non-diabetic obese women: A randomized double-blind clinical trial, Eur. J. Endocrinol. 180 (2019) 165–176.

[135] C. Desouza, M. Keebler, D.B. McNamara, V. Fonseca, Drugs affecting homocysteine metabolism: Impact on cardiovascular

risk, Drugs. 62 (2002) 605–616.

[136] J. Kuka, M. Videja, M. Makrecka-Kuka, J. Liepins, S. Grinberga, E. Sevostjanovs, K. Vilks, E. Liepinsh, M. Dambrova, Metformin decreases bacterial trimethylamine production and trimethylamine N-oxide levels in db/db mice, Sci. Rep. 10 (2020) 1–9.

[137] C.S. Kang, N. Eaktasang, D.Y. Kwon, H.S. Kim, Enhanced current production by Desulfovibrio desulfuricans biofilm in a mediator-less microbial fuel cell, Bioresour. Technol. 165 (2014) 27–30.

[138] S. Qin, S. Wainaina, H. Liu, A.M. Soufiani, A. Pandey, Z. Zhang, M.K. Awasthi, M.J. Taherzadeh, Microbial dynamics during anaerobic digestion of sewage sludge combined with food waste at high organic loading rates in immersed membrane bioreactors, Fuel. 303 (2021) 121276.

효율적인 Metformin 제거 및 volatile fatty acid 생산을 위한 미생물 촉매 양극 및 음극 미생물 전기합성 시스템

압둘 사메 알리

경북대학교 대학원 건설환경에너지공학부 환경에너지공학전공

(지도교수 이대성)

(초 록)

지표수에서 다양한 의약품과 퍼스널 케어 제품을 제거하는 것이 중요합니다. 이 연구는 이중 bioanode (BIND) 및 biocathode (BICD) 미생물 전기합성 시스템을 사용하여 수용액에서 새롭게 부상하는 오염물질인 Metformin(MTF)의 제거 및 변형에 중점을 둡니다. MTF 의 성공적인 생분해(91%)는 개선된 생전기화학적 성능으로 120 시간 이내에 달성되었습니다. 약물 로딩은 대조군 0.1ppm 및 0.3ppm 에 비해 10.3, 7.6, 2.4 배 더 높은 0.5ppm 에 대해 전류 밀도($-849mA/m^2$)를 증가시켰습니다. VFA(volatile fatty acid) 생산은 또한 BIND 및 BICD 에서 우수한 acetate, propionate 및 butyrate 생산으로 개선되었습니다. LC-MS(liquid chromatography mass spectrometry) 연구는 더 많은 MTF 바이오 제품으로 개선된 광물화를 의미했습니다. MTF 는 Proteobacteria 및 Bacteroidetes 의 전기 활성 문의 농축으로 미생물 군집을 조절했습니다. 이 작업은 생물 정화 연구에서 이중 생물 촉매 미생물 전기 합성 시스템의 적용에 대한 새로운 관점을 엽니다.