ENHANCEMENT OF BIOGAS PRODUCTION FROM CATTLE MANURE USING A COMBINED MICROBIAL ELECTROLYSIS CELL AND ANAEROBIC DIGESTER

KOMBİNE MİKROBİYAL ELEKTROLİZ HÜCRESİ VE ANAEROBİK ÇÜRÜTÜCÜ KULLANILARAK BÜYÜKBAŞ HAYVANSAL GÜBRESİNDEN BİYOGAZ ÜRETİMİNİN ARTTIRILMASI

KENAN DALKILIÇ

PROF. DR. AYŞENUR UĞURLU
Supervisor

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ABSTRACT

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Kenan DALKILIÇ

Doctor of Philosophy, Department of Environmental Engineer

Supervisor: Prof. Dr. Ayşenur UĞURLU

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It is assumed that fossil fuel sources which accounts for 80 % of the total energy production in the world, will be depleted in the near future. Because of the depletion of fossil fuel sources and environmental concerns, the attentions have been canalized to renewable energy resources. Being one the most utilized renewable energy resource in the world, biomass is also used extensively in anaerobic digestion processes for methane production. Recently a new technology called Microbial Electrolysis Cell (MEC) was introduced as an alternative and sustainable approach to harvest hydrogen, methane and other valuable chemicals from the organic materials and to treat waste and wastewater simultaneously. The studies conducted on MECs revealed that the newly technology is superior to conventional anaerobic digestion (AD) in terms of methane production and organic removal efficiency. Therefore, combined/integrated MEC+AD systems have been introduced to overcome the limitations of anaerobic digestion such as unstable process, insufficient treatment, low rate methane production, etc.

So far, organic materials used as substrate in MECs included synthetic wastewater, acetate, waste activated sludge, leachate, food waste, pig slurry and other wastes. And yet the operating conditions such as hydraulic retention times (HRT) and organic loading rates (OLR) were not chosen in the range of that would force the limits of the reactors. Thereby, cattle manure which to our knowledge have not been applied to MEC reactors before was chosen as the substrate in this study. Also, because it was stated in many studies that MEC technology was superior to conventional AD technology, it was thought that combining MEC and AD could overcome the challenges of conventional AD technology and enhance the treatment and methane production performances. As a result the main objective of this thesis was determined as to enhance methane production from cattle manure in a combined MEC+AD reactor operated at different conditions. Firstly Biochemical Methane Potential (BMP) of cattle manure was investigated in MEC+AD and control reactors to determine the differences in terms of methane production, degradation efficiency and treatment time. BMP tests were conducted at the start-up period and at the acclimatized conditions of the reactors. Then MEC+AD and control reactors were operated on semi-continuous mode by feeding with manure at fixed content (3 % VS, 4.15 % TS, 30 g VS/L) and HRTs from 6 days to 1 day in descending order. The feeding corresponded to OLRs from 5 to 30 g VS/L/d. After that, the reactors were operated at fixed HRT of 2 days by feeding with manure at content of 4.5 % VS and 6 % VS corresponding to OLR of 22.5 and 30 g VS/L/d respectively. Meanwhile MEC+AD reactors operated at different HRTs and OLRs were supplied with external voltages of 0.3, 0.6 and 1.0 V as well. Biogas productions, methane yields, organic removal rates and current productions of the reactors were observed during the entire study.

The results showed that biogas productions increased consistently in MEC+AD reactors from the lowest OLR of 5 g VS/L/d to highest OLR of 30 g VS/L/d. Biogas productions in MEC+AD reactors changed between 1.23 L/L/d (HRT:6 day, OLR:5 g VS/L/d, 0.3 V) and 5.11 L/L/d (HRT:2 day, OLR:30 g VS/L/d, 1.0 V) depending on HRT and OLR. Methane yields of the MEC+AD reactors changed between 0.09 and 0.24 L CH_4/g VS, decreasing by the increase in OLR. The highest methane yield of 0.24 L CH_4/g VS was obtained at OLR and HRT of 5 g VS/L/d and 6 days respectively in MEC+AD with supplied voltage of 0.6 V. Methane content of the biogas produced from MEC+AD reactors were in the range of 75-80 % at all operational conditions. The methane content of biogas was totally independent of the input voltage and the applied HRTs and OLRs

in this study. Input voltages of 0.6 and 1.0 V were significantly effective on biogas productions at OLRs of as high as 20-30 g VSL/d. During the entire study biogas productions and methane yields of MEC+AD reactors were superior to control reactors at all HRTs (6, 4, 3 days). Energy assessments of the reactors showed that $(MEC+AD)_{0.3V}$ reactor exhibited the highest energy efficiency according to the energy input and energy output. The energy content of methane obtained from $(MEC+AD)_{0.3V}$ reactor were 200 folds of the energy supplied to the reactor. Highest COD, TS and VS removal efficiencies obtained in MEC+AD reactors were observed at HRT of 6 days and OLR of 5 g VS/L/d. The highest removal efficiencies were between 41.4 and 44.9 % for COD, 26.1 and 29.5 % for TS and 34.3 and 37.7 % for VS respectively. Current productions in MEC+AD reactors were strictly depended on the supplied voltage amount. Highest and lowest current productions were generally in the range of 4 - 6 mA/L and 1 - 2.5 mA/L at supplied voltages of 1.0 and 0.3 V respectively.

Keywords: Microbial electrolysis cell, anaerobic digestion, combined MEC+AD, methane production, cattle manure, short hydraulic retention time

ÖZET

KOMBİNE MİKROBİYAL ELEKTROLİZ HÜCRESİ VE ANAEROBİK ÇÜRÜTÜCÜ KULLANILARAK BÜYÜKBAŞ HAYVANSAL GÜBRESİNDEN BİYOGAZ ÜRETİMİNİN ARTTIRILMASI

Kenan DALKILÇ

Doktora, Çevre Mühendisliği Bölümü

Tezdanışmanı: Prof. Dr. Ayşenur UĞURLU

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Dünyadaki toplam enerji üretiminin % 80`ine karşılık gelen fosil yakıt kaynaklarının yakın gelecekte tükeneceği düşünülmektedir. Fosil yakıt kaynaklarının tükenecek olmasından ve çevresel kaygılardan dolayı, dikkatler yenilenebilir enerji kaynaklarına kanalize olmuştur. Dünyanın en çok kullanılan yenilenebilir enerji kaynaklarından birisi olan biyomas, ayrıca metan üretimi için anaerobik çürütme proseslerinde yaygın şekilde kullanılmaktadır. Son dönemlerde, Mikrobiyal Elektroliz Hücresi (MEH) olarak adlandırılan yeni bir teknoloji, organik materyallerden hidrojen, metan ve diğer değerli kimyasalların üretimi ve aynı zamanda atık ve atıksuların arıtımı için alternatif ve sürdürülebilir bir yaklaşım olarak öne sürülmüştür. MEH ile yapılan çalışmalarda, yeni teknolojinin metan üretimi ve organik madde giderim verimliliği konularında geleneksel anaerobik çürütmeye (AÇ) göre daha iyi olduğu ortaya konulmuştur. Bu sebeple, anaerobik çürütmenin istikrarsız süreç, yetersiz arıtma, düşük oranlı metan üretimi vb.

sınırlandırıcı özelliklerinin üstesinden gelebilmek için kombine/entegre MEH+AÇ sistemler öne sürülmüştür.

Bugüne kadar MEH`lerde substrat olarak kullanılan organik materyaller, sentetik atıksu, asetat, atık aktif çamur, sızıntı suyu, gıda atıkları, domuz çiftliği atıksuları ve diğer atıklardan oluşmaktaydı. Bununla birlikte, hidrolik bekleme süresi (HBS) ve organik yükleme oranı (OYO) gibi işletme koşulları, reaktörlerin sınırlarını zorlayacak aralıklarda seçilmemiştir. Böylece, bilgimize göre daha önce MEH`lere substrat olarak uygulanmamış olan büyükbaş hayvansal gübre bu çalışmada substrat olarak seçilmiştir. Ayrıca, birçok çalışma sonucunda, MEH teknolojisinin geleneksel AÇ teknolojisine göre daha iyi olduğu ileri sürüldüğünden dolayı, MEH ile AÇ`nin birleştirilerek, geleneksel AÇ teknolojisinin sıkıntılarının üstesinden gelinebileceği ve arıtma ve metan üretme performanslarının arttırılabileceği düşünülmüştür. Sonuç olarak, bu çalışmanın ana amacı kombine mikrobiyal elektroliz hücresi ve anaerobik çürütücüde büyükbaş hayvansal gübreden farklı işletme koşullarında biyogaz üretimin arttırılması olarak belirlenmiştir. İlk önce, MEH+AÇ ve kontrol reaktörlerinde, metan üretimi, organik madde parçalama verimi ve arıtma süresi konularındaki farklılıkları belirlemek için büyükbaş hayvansal gübrenin Biyokimyasal Metan Potensiyeli (BMP) araştırılmıştır. BMP testleri, çalışmanın başlangıcında ve reaktörlerin alışmış olduğu koşullarda gerçekleştirilmiştir. Sonrasında, MEH+AÇ ve kontrol reaktörleri yarı-sürekli çalışma modunda, HBS`si 6 günden 1 güne azaltılarak ve içeriği sabit olan gübre (% 3 UKM, %4,15 TKM) ile beslenerek işletilmiştir. Besleme 5 ila 30 g UKM/L/gün OYO denk gelmiştir. Bundan sonra reaktörler, OYO 22,5 ve 30 g UKM/L/güne karşılık gelecek şekilde 2 günlük sabit HBS`de % 4,5 UKM ve % 6 TKM içeriği sahip gübre ile beslenerek işletilmiştir. Bu esnada farklı işletme koşullarında çalıştırılan MEH+AÇ reaktörleri, 0,3, 0,6 ve 1,0 V`luk enerji ile de desteklenmiştir. Bütün çalışma boyunca, reaktörlerin biyogaz üretimleri, spesifik metan üretimleri, organik uzaklaştırma oranları ve akım üretimleri gözlemlenmiştir.

Sonuçlar göstermiştir ki, MEH+AÇ reaktörlerinin biyogaz üretimleri, en düşük OYO olan 5 g UKM/L/gün`den en yüksek OYO olan 30 g UKM/L/gün`e kadar devamlı olarak yükselmiştir. MEH+AÇ`nin biyogaz üretimleri, HBS ve OYO`na bağlı olarak 1,23 L/L/gün (HBS: 6 gün, OYO: 5 g UKM/L/gün, 0,3 V) ile 5,11 L/L/gün (HBS: 2 gün, OYO: 30 g UKM/L/gün, 1,0 V) arasında değişmiştir. MEH+AÇ`nin spesifik metan üretimleri

OYO`nun artması ile azalarak, 0,09 ve 0,24 L $CH_4$/g UKM arasında değişmiştir. 0,24 L $CH_4$/ g UKM olan en yüksek spesifik metan üretim oranı, 0,6 V voltaj uygulanan MEH+AÇ`de 6 günlük HBS ve 5 g UKM/L/gün OYO`nda elde edilmiştir. Reaktörlerde üretilen biyogazın metan oranı, bütün işletme koşullarında % 75-80 arasında olmuştur. Bu çalışmada, biyogazdaki metan oranı, uygulanan voltajdan, HBS ve OYO`dan tamamen bağımsız sonuçlanmıştır. 0,6 ve 1,0 V olarak uygulanan voltajlar, 20-30 g UKM/L/gün gibi yüksek OYO`nda gerçekleşen biyogaz üretimleri üzerinde belirgin şekilde etkili olmuştur. Bütün çalışma boyunca, MEH+AÇ reaktörlerinin, biyogaz üretimleri ve spesifik metan üretimleri her bir HBS`nde (6, 4, 3 gün) kontrol reaktörlerinden daha üstün olmuştur. Reaktörlerin enerji değerlendirmeleri göstermiştir ki, $(MEH+AÇ)_{0.3V}$ reaktörü, reaktörlere verilen enerjiye ve alınan enerjye göre, en yüksek enerji verimliliğini sergilemiştir. $(MEH+AÇ)_{0.3V}$ reaktöründen elde edilen metanın enerji içeriği, reaktörlere sağlanan enerji içeriğinin 200 katı kadar olmuştur. MEH+AÇ reaktörlerinde en yüksek KOİ, TKM ve UKM giderim verimleri, 6 günlük HBS ve 5 g UKM/L/gün`lük OYO`nda elde edilmiştir. En yüksek giderim verimleri, sırasıyla KOİ için % 41,4 ile % 44,9, UKM için % 26,1 ile % 29,5 ve UKM için % 34.3 ile % 37,7 arasında gerçekleşmiştir. MEH+AÇ reaktörlerindeki akım üretimleri, uygulanan voltaj miktarlarına kesin olarak bağımlı olmuştur. En yüksek ve en düşük akım üretimleri, sırasıyla 1,0 V ve 0,3 V güç uygulanan reaktörlerde genel olarak 4-6 mA/L ve 1-2,5 mA/L aralığında gerçekleşmiştir.

Anahtar Kelimeler: Mikrobiyal elektroliz hücresi, anaerobic çürütme, kombine MEC+AD, metan üretimi, büyükbaş hayvan gübresi, kısa hidrolik besleme süresi

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SYMBOLS AND ABBREVIATIONS

Symbols

CO2	Carbon dioxide
H2	Hydrogen gas
CH4	Methane gas
CFC	Chlorofluoro carbon
HCF	Hydrofluoro carbon
SF6	Sulfur hexafluoride
t_R	Alıkonma zamanı

Abbreviations

AD	Anaerobic digestion
BES	Bioelectrochemical system
COD	Biochemical oxygen demand
EU	European Union
HRT	Hydraulic retention time
MEC	Microbial electrolysis cell
MEC+AD	Combined microbial electrolysis cell and anaerobic digestion
MFC	Microbial fuel cell
Mtoe	Million tonnes of oil equivalent
OLR	Organic loading rate,
TS	Total solids
VS	Volatile solids
SEM	Taramalı Elektron Mikroskobu

1. INTRODUCTION

Presently, life standarts, technological improvements and changes in habits increase the energy consumption per capita consistently. According to World Bank data, petroleum equvalent energy consumption per capita increased from 1.33 to 1.92 tonnes petroleum/year-capita from 1971 to 2015 [1]. In reference to United Nations` World Population Prospects report [2], world population should have reached to 7.7 billion in 2019. When it is compared to the population of 1973 which was 2.5 billion, it can be understood that energy consumption has increased enormously for the last 50 years [3].

It is assumed that energy produced from fossil derived fuels which accounts for 80 % of total energy production in the world today, will be extinct in 60 to 120 years [1, 4]. International political crises aroused from petroleum prices in 1970s, depletion of fossil-derived sources and the idea of reduction of dependence to these sources triggered the search for alternative energy sources [5]. In the last 30 years, there have been discussions on the international platforms that fossil-derived energy resources have been depleted, and the use of these resources put pressure on the environment and climate change [5, 6]. The process, which started with the Kyoto Protocol in 1997, obliges the parties to protocol to reduce the emission of gases (CO_2, CH_4, NO_2, and industrial gases such as CO_2, CH_4, NO_2 and SF_6, HFC and CFC) in energy production and consumption at certain times and at certain rates. At this point, it is understood that some of the targets have been achieved in a short time by investing in renewable energy resources in the countries which are the parties to the Kyoto protocol, for example in the developed countries of European Union (EU) [7]. From 1990 to 2017, the EU increased the total energy produced from renewable sources more than 3 times to 230 Mtoe (million tonnes of oil equivalent). In addition, the part of the energy derived from renewable sources in the total amount of energy consumed within the EU has been increased from 4 % to 14 % in 30 years [7].

Renewable energy can be described as the energy that can be obtained from the natural resources such as solar, wind, water (hydroelectric), geothermal and from self-renewing resources such as biomass [6, 8]. It is stated that renewable energy sector is the fastest growing energy production sector in 2017 and this will continue until 2040 [5]. Renewable energy is shown as a resource that can be used to meet the demand which is growing day by day. The renewable energy sector also has become one of the most

dynamic sectors that can contribute to the world economy and is seen as a tool to eliminate the threat of global climate change [8].

Nearly 94 % of the energy derived from renewable energy sources consists of hydroelectric, solar and wind energy. [6]. However, if biomass resources that provide basic needs such as heating and incineration are also considered as renewable source, it can be seen that 80 % of the energy obtained from renewable energy sources is supplied from biomass [9, 10]. Biomass is defined as the living or recently living raw biological materials such as plants and animal materials. It is stated that a potentially renewable biomass is the material that can be grown equally or less than the used one [11]. Biomass differs from other renewable sources with the possibility of being used as fuel (biodiesel, bioethanol). It is pointed out that it is the only carbon-based renewable energy source to replace the fossil fuels regarding to its storable, transportable and convertible features [9, 12].

The energy obtained from the biomass is called bioenergy and it can be in the forms of power, heat, and solid, liquid, and gas fuels. Bioenergy can be generated by using a wide range of plant and agricultural crops, food and animal wastes which are all composed of cellulose, hemicellulose, starch, protein, and lipids [13]. These wastes are used as resources to produce bioenergy by using various biological and physicochemical methods. These methods are mostly anaerobic digestion, fermentation, pyrolysis, esterification, gasification, incineration, landfill and also bioelectrochemical systems. Bioelectrochemical systems have recently been started to be researched with the known names of microbial fuel cells and microbial electrolysis cells [13].

Bioelectrochemical systems (BES) or microbial electrochemical systems are promising alternative bioenergy production technology that can convert chemical energy in biodegradable organics into direct energy. Direct energy derived from organics can be in the form of electricity, hydrogen, methane gases and other value-added products derived from all kinds of organic materials. In addition to energy production, waste/wastewater treatment and bio-remediation can be applied as well [14]. They are mainly comprised of microbial fuel cell (MFC), microbial electrolysis cell (MEC) and other bioelectrochemical remediation systems. These systems include conductive electrodes such as metals and carbonecous materials. Beside to the electrodes, waste/wastewater or biodegradable organic materials and bacteria are the main components of the BESs. BES

processes may operate in one or multiple reactors linked to each other electrically and/or physically by membranes or salt bridges. The electrodes in BESs are called anode and cathode. Microbial break down of organic matters take place in anode chamber and generally electron reduction reaction occurs in the cathode chamber. Both electrodes are connected via an external circuit and electrons flow from high redox potential to low redox potential by this external circuit [14, 15].

Being one of the most studied BES for the last ten years, Microbial Electrolysis Cell (MEC) is a new emerging method for harvesting energy and other valuable goods from organic materials and simultaneously treating waste and wastewater [15]. The difference between MFCs and MECs is the operating modes of the systems which is galvanic in MFCs and electrolytic in MECs [16]. In MFCs electricity is produced by the flow of electrons as a result of spontaneous redox reaction that arises from the degredation of organic material. On the other hand, in MECs, non-spontaneous redox reactions such as hydrogen, methane, ethanol, and hdrogen peroxide formation occur through the application of electrical energy which is theoretically between 0.2–0.8 V [17]. Seen as a candidate of future waste biorefinery plants, MECs use microbes which are called as exoelectrogenics to convert organic materials in the medium of an anode into electrons, protons and CO_2. Afterwards, the electrons are transfered to cathode via an external circuit while protons move from anode to cathode through a selective membrane or salt bridge. At the cathode protons are reduced by the electrons and they form valuable products such as H_2, CH_4, ethanol, etc. by applying a small voltage. This voltage initiate process to overcome the thermodynamic barrier because microbial electrolysis is an endothermic reaction (positive free Gibbs energy) [17].

Conventional anaerobic digestion (AD) have been successfully applied to various organic material including waste streams to produce biogas for a long time now. It has some significant limitations such as destabilization of the process, insufficient degradation of the substrates, low rate biogas production and treatment efficiency and long hydraulic retention times (HRT). Recently, combined MEC and AD systems are being studied bench scale to tackle these limitations. As it is a new method of interest, there is a lack and an opportunity to make a statement on how efficiently a combined MEC and AD system would produce biogas from animal manures and treat the waste stream. In this context, it was aimed to enhance the biogas production from cattle manure using a

combined MEC+AD system in this thesis. To observe the results and to obtain the optimum performance criterias, cattle manure was fed to the system at different operation conditions. For example, in this study MEC+AD systems were operated under HRTs of 1 to 6 days which in AD systems only, HRT is mostly higher than 10 days. Since power application is needed to trigger the biogas production in MECs, various electrical power, 0.3, 0.6 and 1.0 V, were supplied to the system to observe the effects of the different voltages. Other than application different HRTs and voltages to the system, different organic loading rates (OLR) between 5-30 g VS/L/day were implemented to see the effect of organic load on MEC+AD systems.

Following the introduction, a detailed literaure review was given about MECs and MEC+AD systems, the mechanism and driving force of MECs, effects of operational conditions on MEC performances. Substrates used in MEC studies were also mentioned. Biogas and methane production rates of other MECs were presented to make comparisons with the results obtained in this study. Subsequently, in the material and methods chapter, construction of the combined MEC+AD system was explained in detail. Construction of electrodes and external circuit, maintainance of power supply units and the operational methods of the study were expressed clearly. Analytical methods conducted to monitor the related parameters in MEC+AD system was also given in detial. Then, the results of the study were evaluated and discussed. Biogas production rate and the treatment efficiency of the system are evaluated according to the operational conditions such as HRT, OLR, and supplied voltage. Percentage of methane in the biogas was also evaluated. Optimum operational conditions of the MEC+AD systems were clarified according to biogas production rate and treatment efficiency. An assesment of energy balance of the process were performed to see the feasibility of the MEC+AD system.

2. LITERATURE REVIEW

2.1. Backround and Definition of Microbial Electrolysis Cell

It was first found out by M.C. Potter [18] in 1911, that electrons are revealed in consequence of degreadation of organic materials by microorganisms. Afterwards, in 1931 B. Cohen was able to produce 35 V at a current of 2 mA with a stacked biological fuel cell. And NASA had utilized this phonomena to supply electricity from organic wastes for small devices which may be useful during the long space flights in 1960s. However as early as these findings were, invention and rapid evolvement of photo voltaic systems delayed the enhancement of biological fuel cells [19]. Consequently for the last 20 years these biological fuel cells have been on the focus due to the fast depletion of the fossil fuel sources which cause global climate change and environmental problems.

MFCs and MECs are the most studied alternative renewable technologies among all bioelectrochemical systems. In MFCs, chemical energy stored in organic matters, can be turned into electricity by microorganisms at suitable conditions. MECs on the other hand perform the same process with the help of a little power supply and harvest hydrogen, methane, ethanol and other valuable products. MEC which is a potentially alternative renewable energy technology was first found out in 2005 by two different groups in Penn State University and Wageningen University [16, 17, 19, 20]. At the early stages of the MEC technology, the name of the technology was entitled as “electrochemically assisted hydrogen generation” and “biocatalyzed electrolysis” or “electrohydrogenesis”. Finally the name “microbial electrolysis cells (MECs)” was accepted by the scientists to emphasize the process in general terms and to specify the technology in terms of products [20, 21]. In MECs, electrochemically active bacteria named as electrogens, exoelectrogenic or anodophilic bacteria break down organic matter and as a result CO_2, electrons and protons are generated. Exoelectrogens then send the electrons to the anode and the protons are left to the anode solution. The electrons transferred to anode travel through a conductive wire to an other electrode, cathode. Meanwhile protons move from high concentration gradient to low concentration gradient through a selective membrane if the anode and the cathode chambers are separated. Cathode chamber must not contain electron donors such as O_2, NO_3^-, or SO_4^- if the target is to produce H_2 and CH_4. Since the microbial electrloysis is an endothermic reaction (positive Gibbs free energy), electron flow is needed to produce H_2 and CH_4 by reducing the protons that are coming

from anode. Therefore a small voltage between 0.2-0.8 V is needed to be supplied to the cathode for this process. Finally electrons are combined with available protons (free/not bounded) in cathode medium to generate H_2, CH_4, C_2H_5OH or other goods [16, 17, 19-22]. Figure 2.1 represents the schematics of a single and two chamber MEC.

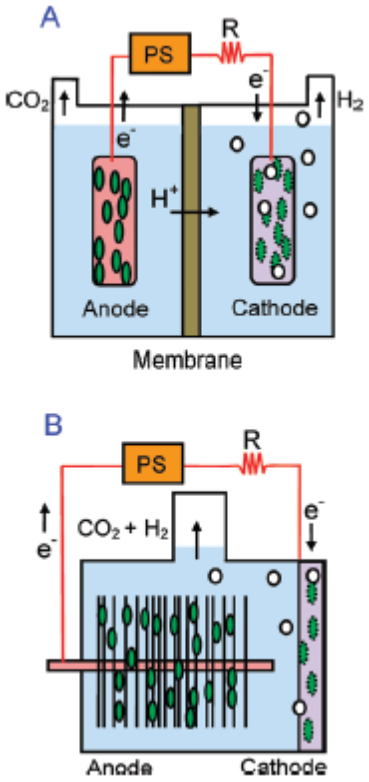

Figure 2.1.Schematics of (A) two chamber MEC with membrane separation and (B) single chamber membraneless MEC [22].

2.2. Working Principles and Dynamics of MECs

Chemotrophic organisms provide the energy they need for living and reproducing by transferring electrons from a low redox potential molecule that is an electron donor, to a high redox potential molecule which is an electron acceptor [19]. These processes are called oxidation-reduction processes. The maximal work done by the oxidation and the reduction processes can be determined in terms of the Gibbs free energy [19, 23]. Gibbs free energy defines the energy of a system and it determines whether the reactions are favorable or not regarding the entalphy and entropy. Entalphy and entropy are the two driving force of a particular reaction and these two driving forces determine the spontaneity of that reaction. Therefore, Gibbs free energy is a function of entrophy (S) and enthalpy (H) as it is shown in Equation 2.1. Entropy is the energy in a system that is available for doing work; it is the tendency in nature for systems to proceed toward a state

of greater disorder or randomness. When matter is converted from solid to liquid, liquid to gas phases, entropy increases. On the other hand entalphy is the the sum of the internal energy and the product of the pressure and volume of a thermodynamic system. It is usually expressed as the change in enthalpy, for a process between initial and final states. For example when a process occurs at constant pressure, the heat either released or absorbed is equal to the change in enthalpy. In this context Gibbs free energy is:

$$\Delta G = \Delta H - T\Delta S \qquad \text{Equation 2.1}$$

Here ΔG, ΔH, T and ΔS stand for Gibbs free energy, enthalphy, temperature and entropy of the system respectively. If ΔH<0 and the ΔS>0, then both the enthalphy and the entropy are favorable and ΔG<0, and the reaction is spontaneous. If ΔH>0 and ΔS<0, then both the enthalphy and the entropy are not favorable and ΔG>0, and the reaction is non-spontaneous. But in the case of one of entropy and entalphy is not favorable then the Gibbs free energy of that system must be calculated to find out whether the reaction is spontaneous or not. If a general redox reaction is $\nu_A A+\nu_B B \rightarrow \nu_C C+\nu_D D$, then Gibbs free energy can be calculated using the molar concentrations of the reactants,

$$\Delta G_r = \Delta G_r{}^o + RT \ln\left(\frac{[C]^{\nu_C}[D]^{\nu_D}}{[A]^{\nu_A}[B]^{\nu_B}}\right) \qquad \text{Equation 2.2}$$

In Equation 2.2 ΔG^o_r stands for the Gibbs free energy at standart conditions (at 1 bar pressure, 298.15 K temperatue and 1 M concentrations of the reactants), ΔG_r stands for the Gibbs free energy at a certain condition, T is the absolute temperature (K), and R is the univesal gas constant (8.3145 /mol.K). Regarding a bioelectrochemical conversion, electromotive force ($E_{emf,}$ in volts) can be used to assess the reaction`s Gibbs free energy:

$$-\Delta G_r = Q.E_{emf} = n.F.E_{emf} \qquad \text{Equation 2.3}$$

Q stands for the charge transferred during the reaction. It is stated in coulumns (C), and it is the product of electrons (n, mol) interchanged in the reaction. F stands for the Faraday`s constant ($F=9.64853x10^4$ C/mol). For standart conditions Equation 2.2 can be rearranged as

$$-\Delta G^o_r = n.F.E^o_{emf} \qquad \text{Equation 2.4}$$

When Equations 2.2, 2.3 and 2.4 are combined, an electromotive force for a certain oxidation and reduction reaction at given terms is formed. Equation 2.5 is the familiar equation with the common name of Nernst Equation. If the results of this equation, E_{emf}, positive then the redox reaction can proceed by itself (spontaneous), if it is negative then the reaction needs a trigger (nonspontaneous).

$$E_{emf} = {E_{emf}}^{o} - \frac{RT}{nF}\ln\left(\frac{[C]^{\nu_C}[D]^{\nu_D}}{[A]^{\nu_A}[B]^{\nu_B}}\right) \qquad \text{Equation 2.5}$$

In the case of an MEC, E_{emf} is mostly negative. For example under standart biological conditions, the Gibbs free energy of acetate oxidation to H_2 is [22]:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3 + H^+ + 4H_2 \quad (\Delta G^o{}_r = +104.6 \text{ kJ/mol}) \qquad \text{Equation 2.6}$$

Acetate cannot be converted into H_2 because Gibbs free energy of this reaction is positive. In order to overcome this thermodynamical barrier to generate H_2, an additional energy is needed for this system to make it happen. To initiate the bioelectrochemical formation of H_2 and indirectly H_2 to CH_4, higher than minimum voltage of ΔG_r/ n.F is needed to be supplied to pass the equilibrium point.

$$E_{emf} = E_{eq} = -\Delta Gr/n.F = -104.6x10^3/8x96485 = -0.14 \text{ V} \qquad \text{Equation 2.7}$$

In general, voltage supply to the bioelectrochemical systems should be higher than the result found in Equation 2.7. The voltage supply should be higher than 0.2 V because of the limitations in the system such as ohmic losses, mass transfer limitations, resistance of the membrane or electrolyte, bacterial usage and etc. [19, 22].

2.2.1. Electron Transfer Pathways in MECs

It is known that oxidation and reduction potential difference between major electron donor and the final electron acceptor specify the net energy gain of chemotrophic microorganisms. And this energy is stored through generation of adenosine triphosphate (ATP) molecules. The basic respiration process is the transfer of electrons from a low redox potential electron donor to a final electron acceptor at high redox potential [19, 24]. In BES systems, exoelectrogens on the electrodes transfer the electrons that are derivated from substrates to anode in order to maintain the energy gain process. This is called extracellular electron transfer (EET) which is illustrated schematically in Figure 2.2 and

it is carried out by two methods according to the characteristics of the microorganisms. One of them is the direct electron transfer (DET) and the other one is the mediated electron transfer (MET). In direct electron transfer pathway, microorganisms that are bounded to the electrode surface with membrane-bound redox enzymes (cytochromes) or microorganisms that can generate extracellular solid pili (nanowire bridge) transfer electrons to the electrode directly without the need of any external or internal soluble redox shuttles [19, 24, 25].

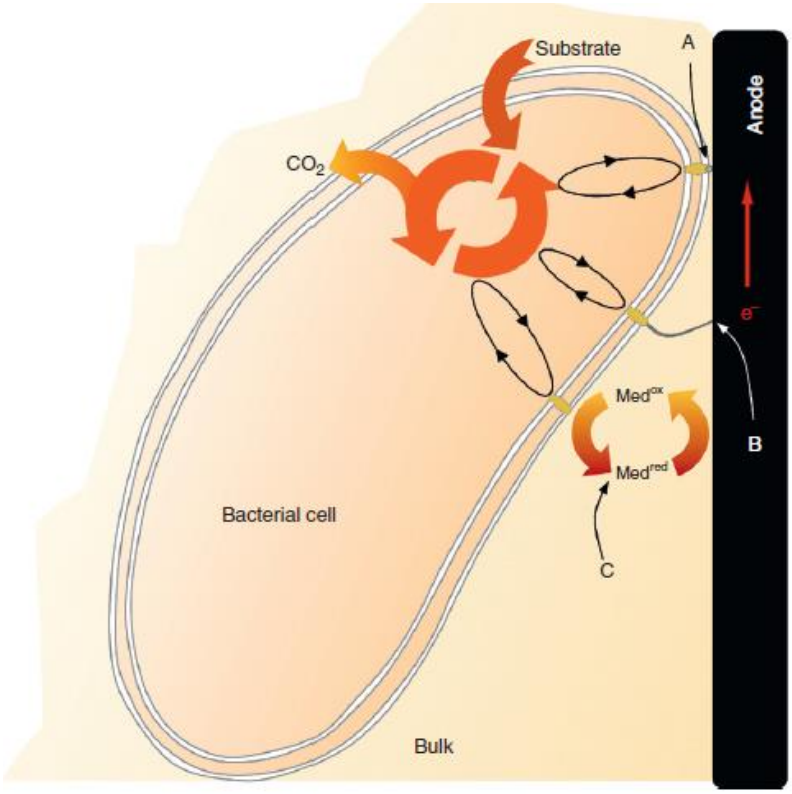

Figure 2.2 Schematic presentation of electron transfer from microorganisms to electrode through: (A)membrane-bound proteins, (B)electrically conductive pilus, (C)redox mediators [19].

In mediated electron transfer, there are redox shuttles such as organic and inorganic soluble compounds that enable the electron transfer by accepting (getting oxidized) the electrons first from the cell/cell membrane and leaving them to electrode by being reduced. These redox shuttles can be oxidized and reduced back and forth at the inside and outside of the cell repeatedly. In some cases redox mediators operate between cell membrane and electrode only if they do not have the ability to penetrate through the cell membrane. Redox mediators that are used in mediated electron transfer can be added externally (exogenous) to the BES or they can be produced by the bacteria itself (endogenous). Meadiators added externally to the system must be resistant to biological

degradation and should have fast kinetics of oxidation at an electrode. It is important for the mediators to diffuse through bacterial membranes easily and to be nontoxic against the microorganisms. Exogenous mediators for example, neutral red, thionin, phenazines, phenothiazines, phenoxazines and various metals (Fe^{3+}, Mn^{4+}) are used to promote EET. Self-secreted mediators by the bacteria are riboflavins, phenazines and quinones [15, 19, 21, 24, 25].

Basic respiration that provides energy is the electron transfer from a low redox potential molecule such as nicotinamide adenine dinucleotide (NAD^+ and NADH: oxidized and reduced back and forth) to the final electron receiver such as O_2 or H_2O at a high redox potential. This respiration being an oxidation process involves tricarboxylic acid (TCA) cycle. Before the TCA cycle, organic substrates are degraded into monomers and finally to pruvate, acetyl-CoA or glycolysis to enter the TCA cyle. NADH, nicotinamide adenine dinucleotide phosphate (NADPH), and flavin adenine dinucleotide ($FADH_2$) are the primary electron donors (reduced molecules) which are produced through the TCA cycle for the electron transport chain. The oxidation and reduction of NADH, NADPH and $FADH_2$ is performed by the electron carriers related with the membrane (a part of membrane structure), including flavoproteins, iron–sulfur proteins, quinone pool, and a series of cytochromes. Energy is released when the electrons are transferred from an electron donor to the next electron acceptor. This process may go on couple of times, there by an electron transport chain can be occured. The energy released during the electron transport chain is gained by the cell to synthesize ATP. Figure 2.3 explains how the energy is gained by electron transport chain.

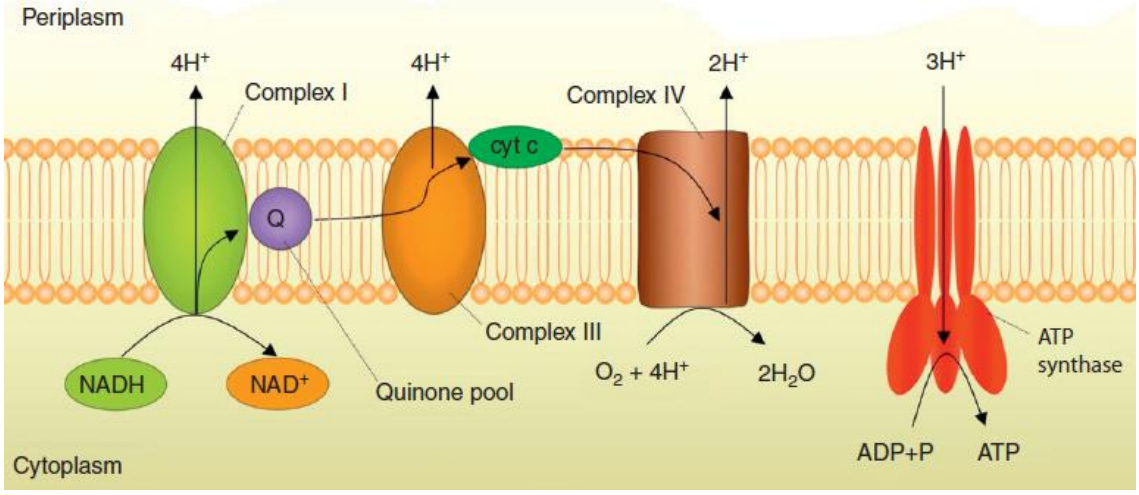

Figure 2.3 A model of electron transport series of respirating organism [19].

Hydrogen atoms in molecules such as NADH, NADPH, $FADH_2$ are cut off from the electrons during the electron transfer. The electrons are sent to the next carrier, simultaneously the protons are ejaculated from the cell. Thus, due to the pH gradient across the cell membrane, a proton motive force is generated that drives ATP synthesis through a process called phosphorylation. An enzyme called proton translocating ATP-synthase benefits the potential unleashed by the protons as they turn back to the cytoplasm [19, 24-26].

2.2.2. Methane Formation in MECs

Methane (CH_4) is formed in two ways in nature. One way is the abiogenic chemical reaction of carbon dioxide or carbon monoxide with hydrogen in extreme conditions and the other one is the biogenic way which is a biochemical reaction that includes organic material, microbes and suitable anaerobic environment [27]. Abiogenic methane formation can be in two ways, natural thermal splitting of kerogen that is in sedimentary rocks and catalytic formation of methane from carbondioxide and hydrogen (Sabatier Process) under high temperature and pressure using catalysts [27, 28].

Biogenic methane formation is a more common and known way that microbes in anaerobic and suitable environment (temperature, pressure) use organic materials and produce methane as an end product. This process is called anaerobic digesiton. Over a hundred years, anaerobic digestion process has been used to produce methane through engineered reactors. In these engineered technological reactors different kinds of organic materials and anaerobic bacteria meet to produce methane. Temperature adjustment and stirring of the medium are applied to improve the methane production.

Bioelectrochemically methane production was first reported in 1999 [29]. It is stated that beside the known pathway of acetoclastic methanogenesis, methane can also be produced through the conversion of carbon dioxide using electrons as electron donor. This process occur with the help of mediators that transfer electrons from cathode to methanogens [28, 29]. Further studies revealed that in hydrogen producing BESs, hydrogenotrophic methanogens were consuming the hydrogen produced by microbial electrolysis. This was shown by Clauwaert et al. [30] by producing hydrogen in an abiotic cathode and feeding hydrogen to a separate anaerobic digestor. Bioelectrochemically methane production were first entitled as “electromethanogenesis” by Cheng et al. [31] to refer an alternative

methanogenic pathway. In electromethanogenesis, supplied electrical current to a system were used to reduce CO_2 by a single Archeon (*Methanobacterium palustre*) and produce methane. Equation 2.8 shows the reduction of CO_2 by the electrons catalyzed by methanogen bacteria [27, 31].

$$CO_2 + 8H^+ + 8e^- \leftrightarrow CH_4 + 2H_2O \qquad \text{Equation 2.8}$$

Many studies carried out to clarify the pathways of methane production in BESs revealed that only one of the two pathways take place in MECs. However, Villano et al. [32] suggested that methane generation in MECs can be in two ways. Firstly, methanogens can accept electrons directly from electrode and produce methane by reducing the CO_2 with electrons (Equation 2.8). The second one is the pathway that hydrogentrophic methanogens produce H_2 by using the electrons coming through electrode first (Equation 2.9) and then H_2 is used to produce CH_4 by methanogens (Equation 2.10) [27, 32]. Figure 2.4 represents the proposed methane generation pathways in a MEC that take place in cathode chamber.

$$2H^+ + 2e^- \leftrightarrow H_2 \qquad \text{Equation 2.9}$$

$$CO_2 + 4H_2 \leftrightarrow CH_4 + 2H_2O \qquad \text{Equation 2.10}$$

Although the ratios of the methane production through direct electron capturing or hydrogen reduction by hdrogenotrophic methanogens is not determined exactly, set cathode potential is an important force to drive the pathway of methane generation. It is stated that at a set negative cathode potentials such as -750 to -900 mV, methane generation through hydrogenotrophic methanogens is much more favorable compared to methane generation through direct electron accepting [32].

EET to anode can be fulfilled in couple of ways as it is mentioned earlier: 1) directly transfer through direct contant of microorganisms with the electrode surface or contact to electrodes by nanowires that microorganism produce, 2) indirect electron transfer through redox mediators. These electron transfer pathways are also valid for the electron uptake from cathode electrode. The deatails of these electron uptake are made clear in this section. Electrons captured from cathode, are used to reduce CO_2 or H_2 to generate CH_4. There is also another proposed pathway beside the direct CO_2 and H_2 reduction to methane. This pathway is the production of fumerate and acetate by specific

microorganisms such as *Geobacter sulfurreducens*, acetogenic bacteria *Sporomusa sphaeroides*, *Clostridium aceticum*, and *Moorella thermoacetica*which lack of hydrogenase enzymes. The latter stage is the methane production by methanogens through the reduction of fumerate, acetate and formate [33]. It is also stated that electron uptake with the reduction of fumerate, formate and acetate to methane is promoted by microorganisms which have c-type cytochrome enzymes.

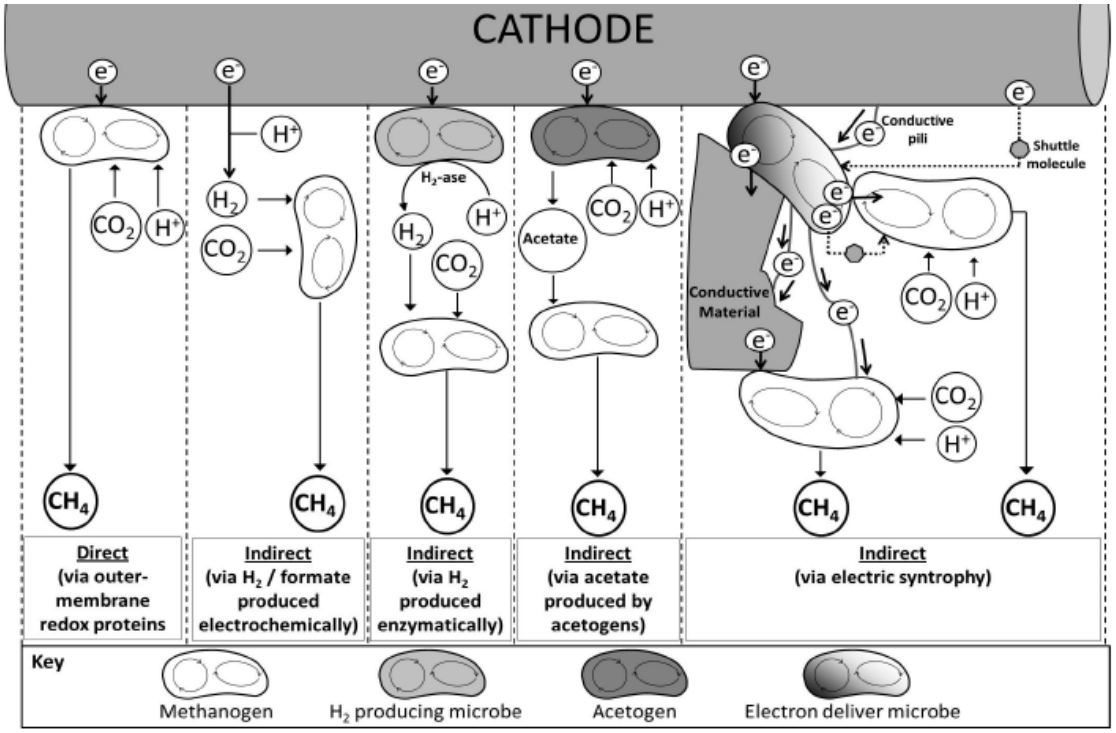

Figure 2.4. Proposed electron transfer pathways that results in methane generation in cathode chamber or around cathode electrode [26].

2.3. Components of Microbial Electrolysis Cell

An ordinary MEC consist of an anode and a cathode chamber with the electrodes in these chambers. Membranes are also one of the main components of the MECs if the MEC configuration is two chambered. The external electrical circuit in a MEC system is indispensable because electrons occured in anode is transferred to the cathode and additional power can be supplied through this external circuit. Also power supply unit is the other essential component of the MEC system. In the following sections, components of an MEC system are explained in detail.

2.3.1. Anode

Anode is the chamber that includes an electrode (anode) material in it and that microbial degredation of organic substrates take place. Exoelectrogens use the organic substrates for energy production and for the metabolic activities. Then they release the electrons to the environment, such as electrode or electrolyte. Electrodes in anode should have high ionic and electronic conductivity. Since anodes carry out the electron transfer to cathode and energy supply to electrolyte, anode material should be highly conductive regarding the energy efficiency. Anode material should be stable biologically, chemically and phisically. It is important for the anode materials to be durable againts biological degredation and extreme pHs. They should not be corrupted at highly ionic concentrations and electrical potentials. Also these materials should be affordable and accessible due to economical reasons [16, 21].

Among the materials used for electrodes, carbon originated materials provide many of the necessities remarked earlier. This is the reason , why carbon originated materials have been used as electrode materials more than others. It is easy and cost effective to obtain in nature or can be formed by various technics including carbonization and pyrolysis. The cost of carbon-based materials may change from couple of dollars to one thousand dollar per square meter owing to its structure [21]. A great amount of common carbon originated materials employed in laboratory studies are graphite fibre/felt, carbon cloth/felt/paper, carbon mesh, graphite plate, granular activated carbon (GAC), graphite granules and graphite brushes [19, 21]. Figure 2.5 presents examples of carbon-based materials which are used for electrodes. All these carbon based materials have their own specific features. For example, graphites are very conductive and stable materials. By making a brush type of anode from graphite, a high surface area for microorganisms can be created. GAC is very cheap and has a high surface area also. However it has limitations such as distance to conductive external wire or electron collector. Granule particles can be distant to eachother which lead to low conductivity and poor electrical contact.

There are various pretreatment methods for electrode materials. These methods are acidic or basic cleaning of the electrode surface, ammonia (NH_3) or heat treatment (450 ^oC for 30 min.), electrochemical oxidation/reduction and surfactant treatment. Pretreatment of the electrodes is used to enhance the anode perfromance due to their electron transfer capacity, surface area improvement, and compatibility to biofilm formation. Different

materials other than carbonoceous materials, such as metals (titanium and steel, iron, nickel) have also been experimented as anodes in BESs. These metals has a very high conductivity and stability however they are usually poor regarding to their surface area which is not appropriate for biofilm development [16, 21].

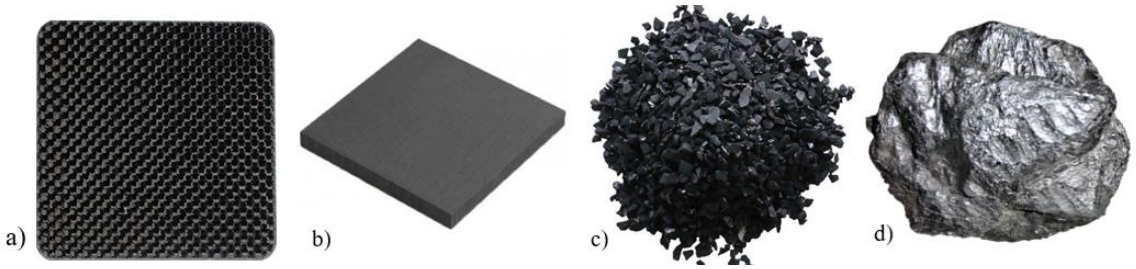

Figure 2.5. Carbon based electrode materials a)Carbon fiber, b)graphite plate, c)Granular activated carbon, d)Graphite mineral

Carbon nanotubes are the other alternative anode materials regarding their extraordinary electrical, mechanical, stabile and conductive features with great specific surface area. On the other hand they impose some serious disadvanteges such as bacterial toxicity as well. It is essential to modify them before using in large-scale applications [16, 21].

2.3.2. Cathode

Cathode is the other electrode in MEC systems. In MEC systems, anode and cathode electrodes are connected to each other with an external circuit to maintain the electron transfer and extra voltage supply [15, 17]. Valuable end products such as H_2, CH_4, ethanol, hydrogen peroxide and etc. are generated in cathode chambers. Cathode chamber must be free of electron acceptors such as oxygen, nitrate or phosphate in order to perform H_2 and CH_4 generation [20]. Carbon-based materials that are accessible and economical can be used as cathode electrode/material as they are used for anodes. However if the main target is H_2 production with a plain carbon-based cathode, it is difficult to overcome the slow evolution reaction of H_2 due to high overpotential of electrode. To come through this limitation and accelerate H_2 formation, catalysts are being used on carbon-based electrodes as reaction accelerator. Platinum and palladium are known as the most used metals so far due to their stableness and fine catalytic features. However environmental and economical concerns set back usage of platinum. On the other hand, metals such as nickel, cobaltmolybdenum, stainless steel, their alloys have been testified to be appropriate cathode materials as well. They are easily reachable, cheap, stable and they have low overpotentials [16, 21]. Carbon nanotubes and graphene are growing into more

preferable materials for anode and they have also been applied victoriously on the surface of cathode materials as an alternative to expensive metals. Nickel alloys with iron, molybdenum and cobalt have been investigated and discovered as good alternative cathode materials [16].

2.3.2.1. Biocathode

Cathodes has a crucial effect in MECs since the products are generated in cathode. It is desired for a process to be economical and feasible. Cathode materials along with the metal catalysts comprise almost half of the cost of a BES system [34]. At the early stages of the researches, MECs had comprised of abiotic cathodes. Those cathodes had included metal and metal alloys which were expensive and environmentally harmful. Afterwards, Rozendal et al. [35] for the first time found out that randomly collected mixed culture that include electrochemically active microorganisms could produce hydrogen as a biocatalyzer/biocathode. Further more, Jeremiasse et al. [36] conducted a research in a MEC, in which both the anode and cathode included bacteria as catalyzer. They stated that cobalt recovery was achieved in MEC with generation of methane and acetate as side products as well. Recently, investigations have focused on metabolic processes occur in cathode, looking for alternatives to abiotic cathodes. Usage of microorganisms as cathode catalysts has some significant advantages over chemical catalysts mentioned in the earlier section. Microbial catalysts are economical, self-generating, environmental friendly and resistant to certain levels of impurities such as sulphur [20, 34]. Biocathodes are very suitable for large scale applications. In a biocathode MEC, microorganisms are able to use the surface of an electrode (cathode) as an electron source to motivate the combination of electrons and protons to perform hydrogen and methane production. Microorganisms on the cathode electrodes form biofilm and bulk sludge so that they can reach out to electrons coming from anode.

It is stated in many studies that MECs with a biocathode configuration can generate much more H_2 gas and provide a higher substrate conversion rate compared to conventional processes such as dark fermentation and photo-fermentation. Also the content of the gas produced in MEC is much purer in comparison with the gas produced by other methods [16, 21, 33, 37]. Gas purification methods are expensive and they contribute to a respected part of the total cost of hydrogen production systems. Water hydrolysis is another conventional technology that is used to produce H_2 gas. But the energy input of water

electrolysis per liter of H_2 gas varies from 5 to 50 folds of energy input that is needed for MEC systems [21].

2.3.3. Membrane and Separator

Membranes are used to separate the chambers in which different processes take place in MECs. At the anode chamber, organic matters are degraded by microorganisms at certain conditions. At the cathode chamber electrons that are derived from the degredation of substrates and supplied from the external power addition, are reacted with protons and other chemicals [22, 33]. These electrons are used to generate H_2, CH_4, ethanol, methanol, hydrogen peroxide and etc. through biological or chemical reduction of electrons [14, 15, 19]. By using membranes and seperators, it is aimed to prevent the interference of the processes that occur in both electrodes [22]. With the application of a separator BES configuration would be divided into two chambers: called anode and cathode chambers. However it is optional to use a membrane in BES systems, there are some important advantages that membranes can provide to the system. It is possible to enhance the percentage H_2 and CH_4 in the generated gas and to avoid consumption of the produced gas (especially H_2) by the anode bacteria. Membranes can retard the diffusion of produced liquid-phase electrofuels toward the anode chamber and following reconsumption of the fuel by the exoelectrogens in the biofilm occured on bioanode. Membranes also maintain stability of the ionic and physical conditions of both chambers, in order the reactions pursue. Also a membrane existence ensures to dispose the danger of short-circuiting [16, 21, 22, 33].

The first and the most used membranes in MEC studies are the cation exchange membranes (CEM) such as Nafion 117 or Fumesap FKE. CEMs are very suitable for the proton transfer from anode to cathode, however they also lead to pH gradient accros the membrane. High pH in cathode and low pH in anode occur due to the accumulation of molecules such as Na^+, K^+, NH_4^+, and Ca^{2+} at the anode and the consumption of the protons (H^+) at the cathode [22]. Other types of membranes used in MECs are anion exchange membranes (AEM) such as AMI-7001 and Selemion AMV, bipolar membranes (BPM) and microporous membranes [16, 21, 22, 33]. It is found out that in the case of using an AEM instead of CEM, internal resistance decreased and as a result hydrogen production rate increased with the help of the phosphate anions that were on the membrane that carry protons accross the membrane. Although membranes are suitable

and serviceable for MECs, there are some disadvantages that they pose. One of them comes to mind is the cost of the membrane which is very high compared to other parts of the MECs. Also they cause voltage losses and an internal resistance for protons to pass through and reach the cathode. Thus a pH gradient may occur accross the membrane. Reduction in pH at anode and increment in pH at cathode may affect the microorganism performance and deteriorate the reactions at both chambers. They also hinder the mass transportation due to membrane fouling [21, 33].

2.3.4. Membraneless MEC

It is mentioned in the paragraph above that membrane is an optional material for MEC technology. So, MECs could also be built free of membranes and as a result they could be cost-effective in terms of construction, operation and competiveveness in large scales. Call and Logan [38] had developed the first membraneless hydrogen producing MEC. They stated that H_2 production of 3.12 m^3H_2/m^3 per day (292 A/m^3) was achieved at an input voltage of 0.8 V. Their motive was that oxygen had not been produced at the anode, therefore a membrane would have not been needed to distinguish the gas generated at the cathode. And if hydrogen generation rate was sufficiently rapid, transformation of hydrogen to methane by methane-producing bacteria in the anode chamber could be ignored due to the low solubility of hydrogen in water [33, 38]. Membraneless MEC configuration could result in lower internal resistance and less complex MEC designs. It would be easier for protons to reach to the cathode and production of H_2 would be favorable due to reduced internal resistance.

Besides all these practical advantages of membraneless MECs, hydrogen produced at the cathode can go through different operations and conversions that have a remarkable effect on the efficiency of the reactors. Hydrogen produced in the cathode can be oxidised repeatedly on the anode that cause an increase on hydrogen recycling phenomenon, which artificially enhances the current in terms of supplied power.

This phenomenon causes the energy efficiency of the system decline. Also microorganisms other than exoelectrogens may use hydrogen to produce acetate or hydrogenetrophic methanogens can benefit from hydrogen and acetate for methane production which cause a mixed content in off gas`s. Finally competition between

exoelectrogens and other microorganisms would end up with the decrease of MEC performance if those migrooranisms are not restrained [16, 33].

The first reason that MECs were developped was to promote hydrogen production. Although it is an efficient method to generate H_2 in MECs, mass and ionic transportation resistance caused by membranes and the need for a cost effective configuration prior to industrialization were still the important obstacles to overcome [33]. For these challenges membrane free MECs came into consideration. Although, H_2 generation has increased due to the lack of membrane originated resistance, another problem showed up, methanization. H_2 produced by the exoelectrogens on the cathode, can be used by hydrogenetrophic methanogens and also direct reduction of CO_2 via methanogens with electrons coming through cathode lead to methanization in MECs [29-32]. Methanization process reduce H_2 generation and electrical efficiency. It causes a competition between exoelectrogens and methanogens over the substrates. Numerous methods has been offered to eliminate undesired methane production in hydrogen production MECs. These are addition of chemical inhibitors like 2-bromoethanesulfonate, 2-chloroethane sulfonate and chloroform into the enviroment; control of pH; exposure of bioelectrodes to air at certain periods; employing lower hydrogen retention time by continuous nitrogen sparging; using double chamber configuration with a membrane; control of temperature and voltage (higher voltages) [21, 33, 39]. Even though many methods were used to prevent methane generation in MECs in order to promote H_2 production, it was inevitable to cut methane generation down to zero. Nevertheless, when applying these methods to the system to prevent methane generation, chemical and physical conditions of MEC medium may become unfavorable for many of the microorganism consortia including exoelectrogens. This can cause hydrogen production to decrease and lead the system to become inefficient and unfeasible [16, 20, 33, 39]. Therefore researchers argue that encouraging methane generation in MECs instead of avoiding could have some significant benefits compared to hydrogen production [16]. Among these advantages, it can be taken into account that methane is easier to handle and store compared to hydrogen. Also organic matter degredation and biogas generation are independent from each other in MECs which allows superior biogas production and methane rate in biogas. In MECs methane generation can proceed at ambient temperature, not requiring heating process and thus energy can be saved. Inhibitor compounds such as ammonia do not necessarily prevent methane generation because in MECs methanogens can acccept

electrons from cathode and produce methane. Also MECs can process at low substrate concentrations unlike anaerobic digestion [16, 20, 21, 40]. Combined microbial electrolysis cell and anaerobic digestion process (MEC+AD) is also a membraneless configuration where anode and cathode electrodes work in harmony in an anerobic single cell. Combined MEC+AD can exploit synergies between the electrodes and can enhance biogas production. It can also assist to lighten some of the limitations of AD. An MEC replaced inside of an anaerobic digester can provide stability of AD by speeding up VFA consumption during overloading and start-up stages where the metabolic activity of methanogens is relatively low. Moreover, MECs can help healing of processes that have gone through a troublesome failure by keeping biomass that is attached to electrodes and help to keep the biomass inside the system [16].

This thesis statement focuses on combined MEC+AD process, so, further information and discussion on MEC+AD processes can be found in section 2.5.1 and in Chapters 3 and 4.

2.3.5. Power Supply

MEC systems are composed of anode, cathode, optionally membrane, microorganisms, external electrical circuit and power supplier [21]. The difference between MFC and MEC, as it is mentioned at the earlier sections is that MFC system aims to produce electricity, and MEC system aims to produce H_2, CH_4 and other value-added products with the help of external power supply [15, 16, 20, 33]. Equation 2.9 is the chemical reaction that explains how hydrogen production occurs at the cathode of the MECs. Equation 2.11 is an example of how this reaction chain starts. In anode these electrons are derivated by the degredation of substrate (in this case acetate) by microorganisms [21, 33]:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8\,e^- \qquad \text{Equation 2.11}$$

In the absence of oxygen and other electron acceptors, electrons that are coming from anode to cathode, are combined with the protons in the electrolyte and form H_2 by the catalytic reaction of microorganism [16, 17, 33]. For H_2 production take place in MECs, fixed electrical potential in the cathode should be minimum -0.414 V vs Normal Hydrogen Electrode (NHE) at standart biological conditions (pH=7, T=25^{o}C, P_{H2}=1 atm) is needed at cathode [21, 33]. Some part of this potential come from anode due to the substrate degredation and resulting electron flow to cathode. Theoretical potential of

anode (E_{an}) for acetate (CH_3COO^-) break down under standard conditions can be determined according to Nernst Equation presented in Equation 2.12 [21, 33].

$$E_{an} = E_{an}^{o} - \frac{RT}{8F} \ln \frac{[CH3OO^-]}{[HCO_3^-]^2[H^+]^9} \quad \text{Equation 2.12}$$

Here, E_{an} and E_{an}^{o} are the potentials of anode at the specified time and at standart conditions respectively. Standart E_{an}^{o} for acetate is 0.187 V, Faraday`s constant (F) is $9.65x10^4$ C/mol, universal gas constant (R) is 8.31 J/mol/K.

$$E_{an} = 0.187 - \frac{8.31x298.15}{8x9.65x10^4} * \ln\left(\frac{[0.0169]}{[0.005]^2[10^{-7}]^9}\right) = -0.300\ V, \text{ Equation 2.13}$$

On the other hand theoretical cathode potential for hydrogen evolution reaction ($2H^+ + 2e^- \rightarrow H_2(g)$) that is aimed to be occured at cathode at standart conditions is [21, 33],

$$E_{cat} = E_{cat}^{o} - \frac{RT}{8F} \ln \frac{p_{H_2}}{[H^+]^2} = 0 - \frac{8.31x298.15}{8x9.65x10^4} * \ln\left(\frac{1}{[10^{-7}]^2}\right) =$$

$$-0.414\ V \quad \text{Equation 2.14}$$

E_{cat}^{o} is the standart electrode potential for hydrogen evolution (0 V) and P_{H2} is the partial pressure of hydrogen gas (1 atm). Finally the equilibrium potential for acetate degredation at usual process terms at both anode and cathode electrode is,

$$E_{eq} = E_{cat} - E_{an} = (-0.414) - (-0.300) = -0.114\ V, \text{ Equation 2.15}$$

Since result of the Equation 2.15 is negative, it indicates that H_2 cannot be generated spontaneously by acetate degredation or by most of the organic substrates. For this reaction to become favorable and produce H_2, an additional input voltage of at least 0.114 V or more has to be supplied to the system [15, 21, 33]. Because the additional voltage is calculated neglecting the losses derived from limitations such as ohmic and activation losses, mass transport limitations and heat generation, the actual voltage that is needed to be supplied to the system should be higher. Previous MEC studies have showed that regarding the MEC configuration and specific features, voltage supply varying between 0.2-0.8 V is convenient to achieve a reputable current and hydrogen generation in MEC [17, 19, 20]. Higher voltage applications such as 1.3 V to 2.3 V are used for water electrolysis which are much more than voltages used for MECs [17, 20]. In MEC studies,

if the aim is to generate hdrogen gas, generally cathode potential is set to values between 0.4-1.0 V, but in the case of methane production, set cathode potential may be as low as 0.2 -0.3 V [21].

2.4. Microorganisms in Microbial Electrolysis Cell

All bioelectrochemical systems such as MFCs and MECs include microorganisms in the process. Microorganisms commence the process mostly by decompising the organic pollutants/substrates in the electrolyte and catalyze the transfer of electrons from organic material to conductive electrode. For example in MFC and MEC, at anode chamber organic materials are used by the microorganisms for their metabolic activities hence current production as well as H_2 and CH_4 production begins [15, 16, 19]. These microorganisms are called electro-active microorganisms, electrogens or exoelectrogens in general. Exoelectrogens are the microorganisms in anode chamber (electrode) of an MFC/MEC that can transfer the electrons derived from the substrate to the electrodes by one of the extracellular electron transfer mechanisms [21, 41]. These exoelectrogens can be found in various environment such as domestic wastewater, ocean and marine sediments, anaerobic sewage sludge and even in earth soil.

2.4.1. Anodic Microorganisms

The information on genetic groups of exoelectrogens that are obtained from BES studies so far includes: α-Proteobacteria (*Rhodopseudomonas, Ochrobactrum*), β-Proteobacteria (*Rhodoferax*), γ-Proteobacteria (*Citrobacter, Shewanella, Enterobacter, Aeromonas*), δ-Proteobacteria (*Geobacter, Geopsychrobacter, Desulfobulbus*), Epsilonproteobacteria (*Arcobacter*), Firmicutes (*Clostridium and Thermincola*), Acidobacteria (*Geothrix*) [21, 25, 41]. Exoelectrogens that are specified in numerous MFC studies, are mostly anode respiring bacteria that can send electrons to anode by themselves (directly) or by mediators. Table 2.1 presents the anodic and cathodic microorganisms with thier electron transfer pathway. Bacteria for examle *Shewanella, Rhodoferax,* and *Geobacter* that can reduce metals, are mostly in the group of the microorganism that can send electrons directly to anode at final stage which are similar to the microbes that use solid mineral oxides as final electron acceptor [42]. Microorganisms can transfer electrons to anode in four different ways: 1) direct contanct with electrode, 2) contact with electrode by self constructed pili (nano wires), 3) using self secreted mediators such as cytochromes and 4) using externally added mediators such as neutral red, thionin, sulphate, methylene blue,

pyocyanin and phenazine-1-carboxamide and ferric chelate complex [41, 42]. Biofilm (e.g. *Shewanella putrefaciens, Rhodoferax ferrireducens, Geobacter sulfurreducens*) attached to the electrode surface can transfer electrons by outer membrane enzymes called cytochromes while some other consortia (e.g. *Shewanella oneidensis, G. sulfurreducens*) that is far from electrodes build pilus like nanowires that have width of several nanometers to transfer electrons to anode [15, 19, 41-43]. It is also stated that a part of filamentous bacteria exist in nature can transfer electrons by using their conductive filaments that have a diameter of as much as 200 nM and length of 15 mm [43]. Some other exoelectrogens (e.g. *Pseudomonas aeruginosa, S. putrefaciens, S. oneidensis, G. sulfurreducens, Clostridium butyricum*) secrete self-made enzymes such as phenazine, riboflavin and phenazine-1-carboxamide which can stimulate electron transfer for other bacterial strains also in mixed cultures.The mechanism of electron transfer of these self-made enzymes is similar to mechanism of the electron transfer of the exogenous electrochemical redox mediators. They can increase the rate of electron transfer and this leads to an increase in power density by transferring thousands of electrons with one enzyme that is used more than once [43].

It is reported that mixed cultures can produce much more current compared to pure cultures due to their various syntrophic species which all have specified tasks that contribute to the higher rate electron transfer than pure cultures [25, 42, 43]. In a mixed culture, fermentative bacteria work with the electrogens in a harmony. The most appropriate electron transfer method amongst the others is selecting mixed culture for the process due to the operational and environmental conditions [15, 42, 43]. Fermentative and anaerobic bacteria that produce hydrogen and sulfur species by oxidation respectively, contribute to the electron transfer mechanism by carrying electrons from nonelectrogenic communities to exoelectrogens [15]. Therefore species such as *Proteus vulgaris, E. coli, P. aeruginosa and Desulfovibrio desulfuricans* can produce H_2 and sulfur species as interspecies electron acceptors. Electron exchange between specific bacteria recommend that a nonelectrogenic bacteria in a co-operative biofilm medium may support current generation if the electrons of that bacteria are taken up by the electrogenic species through interspecies electron transfer. This interspecies electron trasfer is actualized through production of primary metobolites such as H_2, formate and lactate [15, 43].

2.4.2. Cathodic Microorganisms

In BES studies the first configurations included mostly a biotic anode with an abiotic cathode MFC. After microorganisms were found to be cheap and effective catalyzers, biocathode applications started in BES studies. In MEC studies, precious metal catalyzers such as platinum, paladium and their negative effects on microorganisms and the process led the way to the biological cathodes. Due to the advantages of biological cathodes such as being environmental friendly, cheap, affordable, accesible and effective, they became very common in MEC studies. There are two kinds of biological cathodes in BES studies, aerobic and anaerobic. Since the subject of this thesis statement is about MECs and CH_4 production, anerobic cathodes will be explained in detail.

Table 2.1. Microorganisms that participate in MEC systems in anode and cathode [25, 27, 37, 42, 43].

Anodic biofilm	DET/MET	**Cathodic biofilm**	DET/MET
Actinobacillus succinogenes	MET	*Geobacter sulfurreducens*	DET
Aeromonas hydrophlia	DET	*Acidithiobacillus ferrooxidans*	DET
Pseudomonas aeruginosa	DET	*Shewanella putrefaciens*	DET
Clostridium beijerinckii	MET	*Desulfovibrio vulgaris*	DET
Clostridium butyricum	MET	*Clostridium beijerinckii*	MET
Desulfovibrio desulfuricans	MET	*Pseudomonas spp*	MET
Erwinia dissolven	MET	*Shewanella oneidensis*	MET
Escherichia coli	MET	*Acinetobacter calcoaceticus*	MET
Geobacter metallireducens	DET	*Rhodopseudomonas palustris*	DET
Geobacter sulfurreducens	DET	*Hydrogenophilic methanogenic c.*	DET
Gluconobacter oxydans	DET	*Desulfovibrionaceae*	DET
Klebsiella pneumoniae	MET	*Euryarcheota*	MET
Lactobacillus plantarum	MET	*Methanobacterium sp.*	DET
Proteus mirabilis	MET	*Methanosarcina mazei*	DET
Pseudomonas aeruginosa	MET	*Methanobacterium bryantii*	DET
Rhodoferax ferrireducens	DET	*Methanobacteriales*	MET
Shewanella oneidensis	DET	*Methanobrevibacter ruminantium*	MET
Shewanella putrefaciens	DET	*Methaomicrobium mobile*	MET
Streptococcus lactis	MET	*Methanobacteriaceae*	MET

(DET: direct electron transfer; MET: mediated electron transfer)

Biocatalysts that are used in MECs should have the ability to overcome the thermodynamic limitations for production of H_2, CH_4 and other chemicals with the help of external power. Electrode surface should be utilised as an electron donor by these pure

or mixed culture biocatalysts [34]. In most of the MEC studies, anode as well as cathode compartments were inoculated with the MFC/MEC`s anode electrode or anode electrolyte. Therefore similar to anodic cultures, cathodic cultures include the same microorganisms such as *Geobacter sp., Shewanella sp., Pseudomonas sp., Clostridium sp.* and *Rhodoferax sp., Desulfovibrio sp*., *Bacteroidetes* and *Firmicutes* phyla [34, 37]. Also in cathode, mixed cultures present higher performance compared to pure cultures. Mixed cultures show syntrophic relationships during the process. Self–secreted metabolites such as flavins and phenazines produced by *S. Oneidensis* and *Pseudomonas sp*. respectively, can be utilised by other microorganisms several times to transfer the electrons [43]. Generally, BES process with the specific characteristics of various microorganisms as biocatalysts. Symbiotic relationship of interspecious microorganisms for substrate breakdown and electron production leads to a complementary relationship between microorganisms that can widen the utilizable organic material types. This symbiotic relationship can enhance biodegradation of substrates and improve bioelectrochemical cell efficiency. Moreover, employing mixed cultures in BES can ensure robustness and consistance in BESs against the unexpected problems occur in the process such as fluctuations of temperature and organic loading rate, etc. For these reasons, selection of mixed cultures has been seen an advantage for efficient process in BES [34, 37, 43].

It is known that some microorganisms have the ability to catalyze the hydrogen and methane production with electrons derived from electrodes. Methane is produced either by hydrogenotrophic methanogens that reduce CO_2 and H_2 to form CH_4, or by acetoclastic methanogens that reduce acetate [37] Recently, methane production is highly contemporary in MEC studies because methane is easier and cheaper to produce, and it can be stored and transported effectively compared to hydrogen. In MECs, methane production reactions are mediated by biocathodes communites such as *Desulfovibrionaceae* and the phylum *Euryarcheota*. It is reported that *Desulfovibrionaceae* species have a connection between direct electron transfer or hydrogen transfer from electrode surface to methanogenic consortia [27]. Also in mixed culture at biocathodes, hydrogenotrophic methanogens are found to be the dominant species. However, homoacetogens that reduce H_2 and CO_2 to acetate are also found in biocathodes [27, 37]. Most cultures that are found in methane producing biocathodes are the multiple methanogenic phylotypes such as *Methanobacterium sp., Methanobacterium*

bryantii and *Methanosarcina mazei* and hydrogenetrophic methanogens such as *Methanobacterium or Methanobrevibacter* [27].

2.5. Configuration Types of Microbial Electrolysis Cell

Configuration of the MEC systems are important regarding the optimization, process performance and production of H_2 and CH_4. The first MEC design had two chambers and it was spared with a proton exchange membrane (PEM) [44]. In time, configurations used in MFC studies have also been used in MECs studies such as two chamber, single chamber, AD+MEC single chamber, H type reactors, tubular and plate type reactors, rectangular or cube type reactors, cylindrical and disc type reactors. The most significant thing in MECs systems is that cathode of the MEC is needed to be anaerobic for H_2 and CH_4 production. So far in lab-scale studies, it was aimed for the MEC reactors to be optimized in terms of cost effective treatment and bioenergy production. Fort this purpose, several MEC configurations have been developed and applied with various types of materials as anode and cathode electrode materials and membranes [21].

2.5.1. Two Chamber Microbial Electrolysis Cell

Two chamber MEC systems consist of an anode and a cathode chamber that are disparted from each other with a selective membrane. Only selective ions can travel through the membrane therefore chemical and physical conditions such as pH, alkalinity, biology of the electrolytes of the chambers can be different in a two chamber MEC. However membranes impose an internal resistance which may decrease the rate of biogas production of the MEC [16, 21, 45, 46]. It is stated in a study that 86 % of the total internal resistance of a two chamber MEC was caused by a Nafion membrane [21]. Membranes can cause pH gradients at the opposite sides of the membrane which can cause performance and voltage losses. A unit of pH change can lead to 0.06 V loss [21]. The actual reason of a membrane usage is to avoid the diffusion of H_2 from cathode to anode and transfer of bactearia from anode to cathode which can provoke H_2 consumption by the bacteria. A membrane can also maintain a high purity of the produced gas in cathode by avoiding mixture of possible off gases such as CO_2, CH_4 that may arise from anode chamber activities [21, 45, 46]. For given examples, when using a two chamber MEC with a membrane, it is reviewed that H_2 production from 0.01 to 6.3 $m^3/m^3/d$ was recorded [46]. Although there are several advantages that two chamber MECs offer, there are also some drawbacks such as, complexity, difficulty at operations and scale up problems,

voltage losses and manufactoring cost of the membranes. On this context Figure 2.1 was given to show the single and two chamber MEC configurations. It is also possible to treat different chemical solutions or different waste streams in a two chamber MEC using the chambers for different waste streams at the same time and recovering value-added chemicals. Qin et al. [47] achieved a more energy efficient way to recover ammonia from the catholyte solution compared the energy intensive aeration method.

2.5.2. Single Chamber Microbial Electrlysis Cell

The solubility of H_2 in water is between 0–1.5 mg/L at 25°C and 1 atm P_{H2}. If H_2 production rates are at the foreseen level, it is most probable H_2 will not be converted to CH_4 by the MEC system. Since MEC needs to be totally anaerobic, removing the membrane that separates the anode and cathode chambers will not negatively effect the H_2 production [21]. This presumption and the negative impacts of membrane to MECs such as pH gradient accross the membrane, internal resistance for mass and ion transfer, complexity and building cost of the two chamber system have led the studies to single chamber MECs [16, 21]. In single chamber MECs, both electrodes are placed in the same cell without a membrane and they share the same electrolyte. Single chamber MECs are clearly low cost and easy to operate and installation. They exhibit lower ohmic losses and concentration overpotential due to the lower internal resistances compared to two chamber MECs. It is simple to construct single chamber MECs as well. The problems posed by two chamber systems e.g. fouling,clogging and biodegradation of the membranes are avoided by single chamber MECs. Also constructional cost of the MEC can be reduced [33, 46]. In single chamber MEC one type of electrolyte or liquid usually wastewater is used for treatment beside to H_2 or CH_4 production [16].

The constructional and operational advantages of single chamber MECs made way for various single chamber designs. These reactors can be sorted as combined AD+MEC, continuously upflow MEC in which cathode electrode is at the top of the cell, sequencing MEC reactors and microbial fluidised (GAC) electrode electrolysis cell in which GAC is used to enhance the anode biomass [16, 21, 45]. Most of the reactors used in MEC studies are cylindrical (tubular) because of the well mixed characteristic. Some reactors are constructed in a way that a tubular anode can embrace a cathode or vice versa and electrolyte flow through one of them or between them. Planar reactors are also popular when plate like electrodes are used. Single chamber MEC can also be designed in a way

that consist of multiple anode and cathodes in a line [16, 46]. The first pilot scale MEC was a continuous single chamber MEC reactor equipped with multiple electrodes and had a volme of 1 m^3. The highest gas generation rate was 0.19 m^3 biogas/m^3/d and the gas content was 86 % CH_4 in that study [48]. In a combined single chamber AD+MEC average biogas production per day was 0.59 L/L/d when it was used in batch mode for 23 days [49]. In the same study average biogas production of anaerobic control reactor was only 0.34 L/L/d over 23 days. A very common used anaerobic sequencing batch reactor with 20 L volume, was employed as a combined AD+MEC at HRT of 20 days by feeding with high concentrated food waste [50]. At the final steady state of this process, the daily methane production rate was 17 L CH_4/d and methane yield of the system was 0.34 L-CH_4/g-$COD_{rem.}$.

2.5.3. Other Types of Microbial Electrolysis Cells

Bioelectrochemical system related studies opened a new era for water/wastewater/waste treatment along with other value added chemicals/sources production. So far laboratory scale studies aimed to integrate MECs with MFCs, dark fermentation, anaerobic digestion, desalination cells and bio-photoelectrochemical cells.

In MEC-MFC coupled systems, the external power supply for H_2/CH_4 production in the MEC is provided by a separate MFC. So an extra energy consumption may not be needed by the coupled MEC-MFC system if the energy provided by the MFC is stable and continuous. An MFC-MEC coupled system can also be linked to a dark fermentation reactor in which complex biodegredable organic substrates can be first converted into simple monomers. Afterwards these substrates can be fed to the MFC-MEC system for power and gas generation respectively [21, 33]. Solar cells can also be applied for power supply to MECs.

Microbial desalination cell is an MFC induced salinity removal cell from seawater that aims to decrease the salinity via the electrical current produced by anode and oxygen reduction in cathode. However the electrical input of the MFC may vary due to the biological process occurs in anode that cause unstable salt removal. Hence, a low and stable voltage application can further increase the salt removal from seawater with an additional H_2 production in cathode chamber of an MEC. There must be a third chamber between anode and cathode where the seawater is placed and desalinated. Anode and the

other chamber consists of seawater are spared by an anion exchange membrane which allows negative ions (Cl) pass through to the anode. Cathode and the seawater chambers are separated by an cation exchange membrane which transfer cations (Na^+) to the cathode chamber. Beside the desalination system in Microbial Electrodialysis Cell (MEDC), H_2 can be produced in cathode [33]. Figure 2.6 presents a MEDC for better understanding.

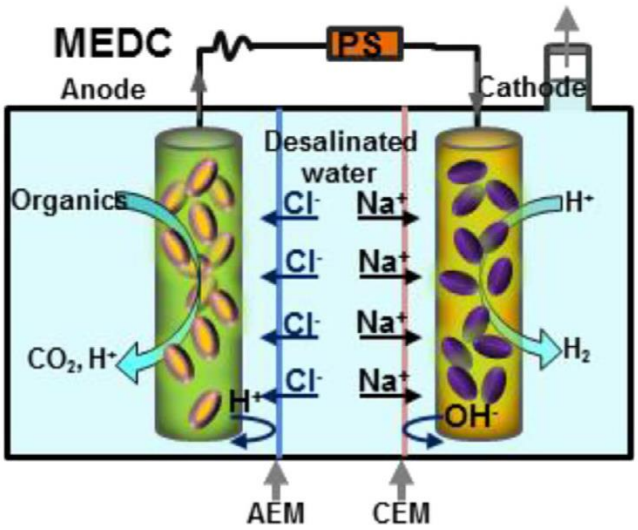

Figure 2.6. Microbial electrodialysis cell [33]

2.6. Substrates Used in Microbial Electrolysis Cell

Hydrogen and methane can be produced in MECs using various kinds of substrates including sodium acetate, volatile fatty acids, glucose, cellulose, and different types of wastes originates from agricultural and industrial processes. These wastes/wastewaters can be sorrted as domestic wastewater, swine wastewater, winery wastewater, dairy and chicken wastes, wastewater treatment plant sludges, food industry wastewater and etc. Because MECs can treat a wide range of wastes/wastewater, they can be an opportunity for biogas production and value-added chemicals production along with the waste/wastewater treatment.

Early MEC studies were conducted using readily usable substrates for exoelectrogens such as acetate, glucose and volatile fatty acids which are known as fermentation end products. For example, in the studies conducted by Liu et al. [44] and Rozendal et al. [51] acetate was used as substrate. Acetate is an end product of fermentation however with an extra power supply, it can be converted into H_2 by bacteria [44, 51]. Liu et al. [51] accomplished specific H_2 production of 2.9 mol H_2/mol acetate with a 0.25 V power

supply. Rozendal et al. [51] presented H_2 production of approximately 0.02 m^3 $H_2/m^3/d$ with an power supply of 0.5 V using acetate as the substrate. The best H_2 production rate observed so far is the 50 m^3 H_2/m^3 MEC/d with an voltage supplementation of 1.0 V using acetate as substrate in a two chamber MEC in which cathode was a nickel foam that had a high surface area [52]. Other than acetate, non-fermentable carbonaceous matters such as glucose and glycerol were also applied as substrates in MECs. Glucose was used as substrate in a single cell MEC that was employed under temperature of 4^{o}C. It was reported that the H_2 yield of approximately 6 mol H_2/mol glucose, and at H_2 production rates of 0.25-0.37 m^3 $H_2/m^3/d$ were achieved in that study [53].

It was stated earlier that since CH_4 production is inevitable in MECs [16], some researchers investigated the CH_4 production in MECs by using different configurations and substrates and by changing operational conditions. Recently in a combined MEC+AD, mixed food waste at highest OLR of 10 kg COD/m^3/d was used to produce CH_4 over a 20 days HRT. The maximum methane yield in this study was 0.36 m^3/kg $COD_{rem.}$when fed with OLR of 6-10 kg COD/m^3/d. MEC+AD was operated at applied voltage of 0.3 V and 35^{o}C [54]. MEC was also used as the second stage of a two stage process that includes dark fermentation as the first stage. In this study recalcitrant lignocellulosic materials were converted into H_2 and then to CH_4. At fermentation stage 1.67 mol H_2/mol glucose at a rate of 0.25 L H_2/L/d was produced with a lignocellulosic effluent [50]. Xiao et al. [55] used aeration tank sludge as feed for MEC in their study. The sludge was first pretreated thermally and alkaline. The pretreatment was made by increasing the pH of the sludge with NaOH addition and then keeping the sludge at 175^{o}C for 30 mins. The methane generation from pretreated sludge increased between 20-80 % when external voltage of 0.6-1.8 V was applied compared to control reactor.

Heidrich et al. [56] treated raw domestic wastewater with a 100 L MEC for 12 months period at ambient temperature changing between 1-22^{o}C. They noted that 100 L of MEC produced hydrogen at the rate of 0.6 L/day and achieved electrical input recovery of 49 %. Also they remarked inconsistent and low level COD removal rates due to the design issues and poor pumping system. Another different type of influent was used by Gao et al. [57] in combined anerobic digestion and MEC for methane production and COD removal. It is reported in this study that municipal solid waste (MSW) incineration leachate was used as feed for MEC+AD for treatment and methane production. It was

found out that COD removal efficiencies and methane productions increased 8.7 % and 44.3 % respectively in MEC+AD compared to control anaerobic reactors. Also MEC+AD can recover more rapidly from rancidness caused by high organic loading rates. Sewage sludge was used as the carbon source in a combined anaerobic digestion and MEC by Guo et al. [58]. It was reported that in the MEC+AD where Ti/Ru based electrodes were used, hydrogen production was 1.7-5.2 fold of the control reactor`s hydrogen production and the methane production was 11.4-13.6 fold of the control reactor`s methane generation at additional power of 1.4 and 1.8 V and at temperaure of 37 ^{o}C. It is clear that various kinds of wastes and wastewater were used as substrate in MECs or MEC+AD reactors for biogas production and treatment. A parallel work conducted by Feng et al. [59] revealed a cumulative methane yield of 0.17 m^3/kg VSS at the end of a 20 days batch study conducted at 35^{o}C and with a voltage supplementation of 0.3 V in a combined MEC+AD with iron and graphite electrodes. Total solid content as high as 10-12 % was used in that study. An UASB and an MEC are combined to enhance methane production and COD removal from glucose based synthetic waste stream at 35^{o}C and HRT of 6 hours wit a given voltage of 1.0 V. The result was combined UASB+MEC could still operate at stable performance (COD removal rate: >70%) at a high organic loading rate of 28 g COD/L/d at short hydraulic retention time of 6 hours. The methane productions were 248.5 mL/h and 51.3 mL/h at combined UASB+MEC and UASB only respectively [60].

In the studies that conducted with lab scale small reactors (<100 mL), most used substrate was the acetate (sodium acetate). The best H_2 production rates, 6.3–50 m^3/m^3/d are obtained from these studies in which voltage of 1-1.5 V were applied [61]. According to Lu and Ren [61], H_2 production rates decrease from non-fermentable substrates (fermentation products) to fermentable products due to the structure change from simple substrates to more complex substrates. Acetate, glucose, organic acids, alcohols and monosaccharide or disaccharide are suitable feed for exoelectrogens to degrade easily. However recalcitrant and polymeric substrates, protein based substrates, wastewater sludges and domestic wastewater lead to very low H_2 production rates such as 0.05–0.54 m3/m^3/d in MECs [61]. Hydrogen production rates in MECs decrease when the lab-scale reactors turn into pilot-scale reactors in which mostly complex substrates such as industrial wastes, food processing wastewater, domestic wastewater and sludges are used as substrates. Several stuies conducted with larger reactors having volume from 100 L to1000 L, reported H_2 production rates that were lower than 0.02 m3/m^3/d [61].

The only study that used dairy wastewater as substrate in MECs reported very little current and H_2 production. However in the same study good performance of H_2 production and current generation were obtained with potato wastewater [62]. Biogas production rate and biogas yield, current generation and VS/COD removal of an MEC systems do not depend only to the substrate type. The results also depend on the anode and cathode materials, microorganism types, operational conditions (pH, temperature, OLR, HRT), applied voltages and reactor configuration such as batch, continuous feed, upflow reactors or two or single chamber MECs.

2.7.Anaerobic Digestion Process

Anaerobic digestion is a multiple phase process that includes the interrelationships of several microbial consortia and interdependency of these microbial consortia to each other [63]. In anaerobic digestion process, various kinds of microorganisms work in harmony to reproduce and harvest energy for metabolic activities by degrading organic substances at the absence of oxygen. As a result of these activities, biogas is produced at the end of the anaerobic process. Biogas is a valuable gas which generally composed of 50-70 % methane. Table 2.2 presents some important characteristics of biogas.

It is accepted that anaerobic process take place in four distinct phases respectively; hydrolysis, acidogenesis, acetonegenesis and methanogenesis [63,64]. Figure 2.7 represents a short summary of the four phases of anaerobic digestion. In anaerobic digestion, each phase is conducted by different type of microbial consortia at specific environmental conditions. These microbial consortia work in a harmony. End product of a substrate can be a new substrate for the next consortia in the food chain. Some bacteria can not degrade substrates in the absence of the other symbiotic bacteria [65].

Table 2.2 Important features of biogas [65].

Content	%55-70 Methane (CH_4), 30-45 Carbondioxide (CO_2), % 1-3 Other Gases (H_2S,H_2O,N_2,CO)
Energy content	6.0-6.5 kWh/m^3
Fuel equivalent	0.60-0.65 L oil/m^3 biogas
Explosion limits	If the volume of the methane in the air is 6-12%
Ignition temperature	650-750 ^oC (at 55-70% methane)
Criticalpressure	75-89 bar
Critical temperature	-82.5 ^oC
Density	1.2 kg/m^3
Molar mass	16.043 kg/kmol (STP :0^oC, 1 bar)

2.7.1. Hydrolysis

Hydrolysis phase can be explained as the degredation and depolymerization of the complex polymeric organic substances into soluble monomers by certain fermentation microorganisms [63]. In hydrolysis phase, complex compounds such as proteins, carbohydrates and fats are degraded into monomers as in soluble amino acids, monosaccharides, sugars, glycerol and short chain fatty acids. Degredation of complex compounds into monomers provided by the enzymes such as hydrolase, cellulase, amylase, protease that are secreted extracellularly by facultative and some other anaerobic bacteria. For example cellulose is converted into lignin and hemicellulose, carbohydrate is converted into glucose and pentose, protein is converted into polypeptide and amino acids and fat is converted into alcohols, fatty acids and hydrogen. Hydrolysis of the carbohydrates can take several hours where as hydrolysis of fats and proteins can take several days [65]. Facultative bacteria such as *Streptococci* and *Enterobactriaceae* and bactericides along with the *Clostridia* involve in hydrolysis process [64]. It is evaluated that hydrolysis phase can be the restrictive phase for the whole anaerobic digestion process because it determines the HRT of the process. HRT, OLR, pH, temperature, the type of organic substance are the main parameters that specifies the hydrolysis rate of the process [63].

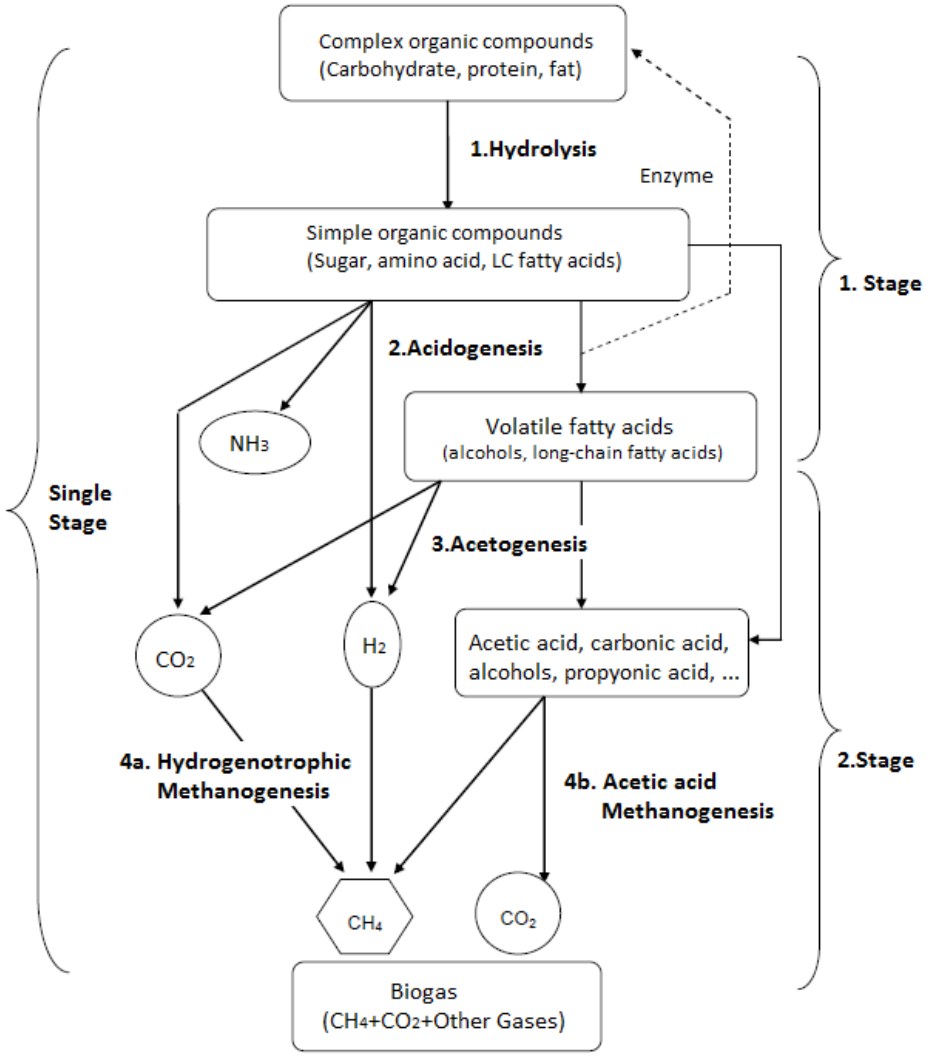

Figure 2.7. Anaerobic digestion and its phases [63, 65, 66].

2.7.2. Acidogenesis

Acidogenesis phase, also known as fermentation, occurs in an environment that is free of an inorganic electron receiver (Oxygen, sulphate or nitrate). Organic materials that were converted into monomers such as amino acids, monosaccharides, glycerol and long chain fatty acids in hydrolysis phase, are broken into one to five carbonaceous short chain fatty acids by various facultative bacteria. Volatile fatty acids (butyric acid, propionic acid, acetic acid) and other common intermediate products such as acetate, alcohols, hydrogen, carbondioxide and etc. are formed at the end of the acidogenesis phase [65]. Some of the bacteria types that participate in acidogenesis phase are *Clostridia*, *Lactobacillus*, *Micrococcus*, *Pseudomonas*, *Desulfuromonas*, and *Streptococcus*. Hydrogen ions that are

produced internally by bacteria can affect the end products of process such that the increase of the partial pressure of hydrogen leads to a decrease in acetate production [65].

2.7.3. Acetogenesis

Acetogenesis means the formation of acetate which can be produced by the reduction of CO_2 and organic acids. The products of acidogenesis phase are used as substrate by the bacteria of acetogenesis phase. In this phase, homoacetogenetic microorganisms use hydrogen and carbondioxide as electron acceptors to produce acetic acid and to harvest energy:

$$2CO_2 + 4H_2 \leftrightarrow CH_3COOH + 2H_2O \qquad \text{Equation 2.16}$$

Homoacetogenic bacteria can compete with methanogenic bacteria over hydrogen, methanol and formic acid. On the other hand some type of acetogenic bacteria produce hydrogen as a side product when they aim to produce acetate from acidogenesis products such as long chain fatty acids (propionic acid, butyric acid) and alcohols (ethanol). However such acetogenic bacteria can obtain the energy required to survive and reproduce at only low hydrogen concentrations. Accumulation of hydrogen in the environment can inhibit metabolic activities of these bacteria. Therefore hydrogen is needed to be at low concentrations for hydrogen producers and acetogenic bacteria. Obligatory, acetogenic and methanogenic microorganisms live in a symbiotic relationship. Since hydrogentrophic methanogens can survive at high hydrogen concentrations and use hydrogen to produce methane, they maintain low hydrogen concentrations in the environment which is suitable for acetogenic bacteria for existence. At this circumstances hydrogen, carbon dioxide and acetate are intensely produced by acetogenic bacteria. However if the H_2 concentration is high in the environment, butyric, carbonic, valeric and propionic acids are also formed which are not useful for methanogenic bacteria to produce methane because only carbondioxide, acetate and hydrogen are used by methanogens to produce methane [63-65].

It is stated that approximately 30 % of the total biogas in anaerobic digestion is formed by the reduction of CO_2 with hydrogen gas. This phenomena is illustrated in Figure 2.8 that H_2 is formed by acetogenic bacteria as a result of metabolic activity and following it is used by methanogens as a electron donor to oxidize CO_2 and produce CH_4. Conversion of fatty acids and alcohols to acetate is a energy waste process for methanogens. Therefore

it is good to have acetogens in the same habitat with the methanogens because acetogens use fatty acids and alcohols as substrate and they form CO_2, H_2 and acetic acid which are used by methanogens for energy and living [65, 66].

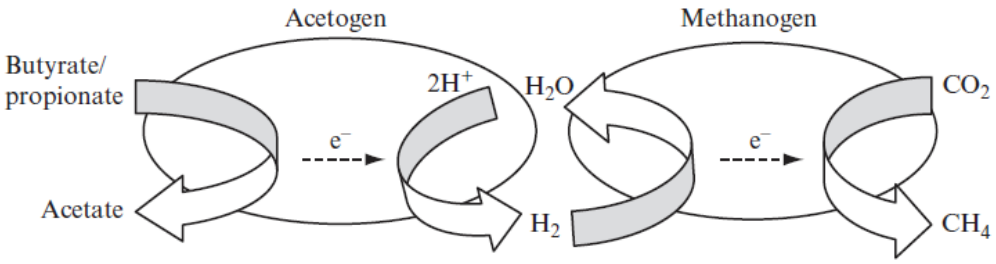

Figure 2.8. H_2 transfer between acetogens and methanogens [63].

In anaerobic process, complex organic molecules are degraded by a variety of fermentation bacteria into compounds (short/long chain fatty acids) such lactate, ethanol, propionate and butyrate. Then in acetogenesis phase, acetogens oxidize hydrolytic products further to hydrogen, formate and acetate. Meanwhile a some homoacetogenic bacteria (homoacetogens) make use of CO_2/H_2 as feed to form acetic acid. At the end, products such as acetic acid, formic acid, and CO_2/H_2 that were originated at acidification phase, converted into methane by methanogens. These anaerobic oxidation reaction series that are implemented by acetogenic bacteria, result with a positive Gibbs free energy change ($\Delta G°$). Because of this, acetogenic bacteria can only perform and produce hydrogen, formate and acetate when they are utilized by the methanogens [66, 67].

Acetogenic bacteria creates acetate which can be utilized directly by aceticlastic methanogens (*Methanosarcina spp*. and *Methanosaeta spp*.) or can be oxidized by syntrophic bacteria (syntrophic acetate oxidizers) and hydrogenotrophic methanogens. CO_2 that yield as an metabolism product of acetotrophic methanogenensis, and H_2 are utilized by hydrogentrophic methanogens to produce methane [69].

2.7.4. Methanogenesis

The last phase of the anaerobic process is the methanogenesis phase. In this phase, acetate, CO_2, H_2, alcohols, formic acid and one carbon methyl compounds are used as substrate by methanogen bacteria and CH_4 is formed at the end of this process. Metabolism of methanogens are different than most of the livings. They obtain their energy not with the known way of substrate level phosphorylation, but obtaine from probably by a proton

motive force. Methanogens can be found in the strict anaerobic environment that is free of electron acceptors such as O_2, NO_3, Fe^{+3} and SO_4^2. Methanogenic activities are exothermic reactions which occur effectively at certain temperatures such as mesophilic and thermophilic. It is not possible for every kind of methanogen to catabolize any kind of organic materials. Therefore methanogens are separated into three groups according to their feeding substrates: Acetotrophic (acetoclastic or acetic acid methanogens) methanogens turn acetate into CH_4 and CO_2; hydrogenotrophic methanogens utilize CO_2 and H_2 and form CH_4; methylotrophic methanogens convert one carbon methyl compouns such as methanol, methylamine, methyl capto propionate, dimethyl sulfide into CH_4 [63].

Acetotrophic (acetoclastic) methanogens, CH_3COO;

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \qquad \text{Equation 2.17}$$

Hydrogenotrophic methanogens, CO_2, HCOO, CO;

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad \text{Equation 2.18}$$

Methylotrophic methanogens, CH_3OH, CH_3NH_3, $(CH_3)_2NH_2^+$, $(CH_3)_3NH^+$, CH_3SH, $(CH_3)_2S$;

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \qquad \text{Equation 2.19}$$

In methanogenesis phase roughly 70 % of the methane is generated by the conversion of acetate and 27-30 % of the methane is produced by the conversion of CO_2 and H_2. When the production of methane is disturbed, acidification occurs in the environment. Also when acetogens interact with the bacteria that produce hydrogen sulfide but not methanogens, methanogens get effected negatively and they can be exposed to toxic effect of hydrogen sulfide hence the process failure take place. Therefore it is important for methanogens to stay in contact with hydrogen producers [63].

Methanogens have an interesting metabolism that has enzymes and co-enzymes. They are the largest and most diverse group in the Archea. Their unique metabolism and genetics differ from the other two domains of life, the Bacteria and Eukaryota. An important characteristic of methanogens is that there is no methanogen kind that harvest energy from the substrate level phosphorylation. However required energy (ATP) is obtained by the driving force of the proton translocation. Most abundant methanogen groups are

Methanosarcinales, Methanomicrobiales, Methanobacteriales, Methanococcales, Methanocellales, Methanotrix, Methanospirillum, and *Methanosaeta* [67].

The process of conversion of acetate into methane is a limiting stage for anaerobic process because methanogens show a slow growth rate. They are very sensitive to saturated oxygen in the habitat even in the range of 0.01-0.08 mg/L concentration. Methanogens are ususally effective at the pH range of 6.5-7.6. Mesophilic and thermophilic temperatures are the best operational temperature for the methanogens [68]. Optimum operational conditions in anaerobic process are given in Table 2.3.

Table 2.3.General specifications of anaerobic digesiton phases [65].

Parameter	**Hydrolysis/Acidogenesis**	**Methanogenesis**
Temperature	25-35 ^{O}C	Mesophilic:32-42^{O}C Thermophilic : 50-58 ^{O}C
pH	5.2-6.3	6.7-7.5
C/N	10-45	20-30
Total solids content	<%40 total solids	<%30 total solids
Redox potential	+400 to -300 mV	< -250 mV
Required C/N/P/S rate	500:15:5:3	600:15:5:3
Trace metals	-	Nickel, Cobalt, Molibdenum, Selenium

Acetotrophic (acetoclastic) and hydrogenotrophic methanogenesis pathways are the two types of methane formation ways in anaerobic digestion. In natüre, hydrogenotrophic methanogenesis is mainly found in marine environments and on the other hand acetate acetoclastic methanogenesis is more dominant in freshwater environments. Acetoclastic and hydrogenotrophic methanogens utilize the hydrolysis products such as acetate and hydrogen and form methane. They use special kind of enzymes in methane formation in which trace elements such as molybdenum (Mo), selenium (Se), nickel (Ni), iron (Fe) and cobalt (Co) are utilized as co-factors of the enzymes. If those elements are not present sufficiently in the medium, restriction of formate and acetate oxidation can result in acid accumulation and severe drop in pH which ultimately cause failure of methane formation [69]. It is also suggested that acetoclastic methanogenis are more sensitive to long chain fatty acids than the hydrogenotrophic methanogens. This is attributed to the composition of the cell membrane, which is different for the two species. However, acetoclastic methanogens were found to be more robust to hign total ammonia nitrogen concentrations [69].

It is stated by many researchers that three and more carbonaceous volatile fatty acids should not be accumulated in the medium for a healthy process. They can not be utilized directly as substrate by the methanogens, so they need to be oxidized into more readily substrate such as acetic acid, hydrogen and formate for methanogens before the methanogenesis phase. Acetic acid is generally formed by acidification of mono substrates, however it can also be transformed by homoacetogens through hydrogen and carbon dioxide reduction. If conversion of acetic acid by homoacetogens can be increased technically, more direct substrate would be available for acetotrophic methanogens for methane generation in the methanogenesis stage. Homoacetogens can reduce a part of CO_2 in the medium to VFAs according to the Wood–Ljungdahl pathway for ATP synthesis. When homoacetogens utilize H_2 and CO_2 for their metabolic activities, carbon enters the Wood–Ljungdahl pathway during CO_2 reduction. Hydrogen is utilized as the electron supplier for the reduction of CO_2 in this reaction.

In anaerobic process, methanogenesis phase is the electron transfer phenomenon from electron supplier to electron receiver. Therefore, enhancement of electron transfer tends to increase methane generation. In hydrogentrophic methanogenessis, H_2 is the primary element than can transfer electrons from organic acid oxidizers to hydrogenotrophic methanogens. Thereby, hydrogenotrophic methanogenesis conducted by some type of methanogens such as *Methanobacteriales*, *Methanococcales* is an important absorber of electrons anaerobic medium. This electron sink is performed through interspecies electron transfer. Hydrogenotrophic methanogens sustain low concentrations of dissolved hydrogen by collateral interaction with hydrogen producers (acetogens). As a result they maintain a favorable environment for hydrogen producing acetogens. Most part of the methane production that originates from hydrogen, occurs in flocks or biofilms. This ebanles direct and fast transfer of hydrogen from the hydrogen producing microorganism to the hydrogen consuming methanogens easily.

2.8. Operational Parameters Affecting Combined MEC and AD

Operational parameters in biological and chemical processes are very determinant and effective for the outcomes of the processes. All living organisms need a stable and reliable environment to carry out metabolic activities otherwise they get stressful and their activities can get interrupted. In wastewater treatment or in anerobic digesiton, operational parameters such as temperature, pH, OLR, HRT, alkalinity and substrate type

are important. Cathode potential or applied voltage are also efficient in bioelectrochemical processes. These parameters determine the rate, performance and outcomes of the combined MEC+AD process in this case.

2.8.1. Temperature

All living organisms need an optimum temperature range to fulfill the essential activities. Anaerobic digestion can be materialized in three temperature ranges which are psychrophilic (10-20^{o}C or <20 ^{o}C), mesophilic (30-40^{o}C or 30-45^{o}C) and thermophilic (50-60^{o}C or 50-70^{o}C) [66, 69]. As it is accepted that actvities of microorganisms enhance with a raise in temperature up to a point. Figure 2.9 illustrates methanogenic activity in anaerobic process at different temperature ranges.

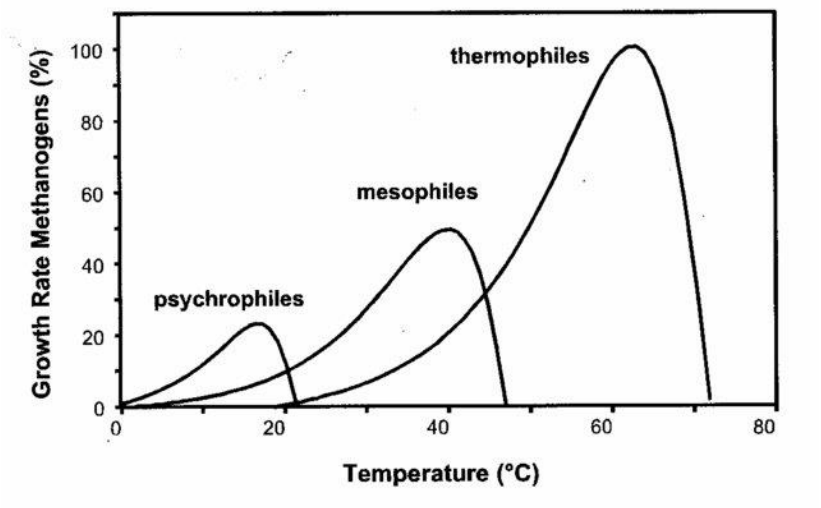

Figure 2.9. Growth and activity rate of methanogens at different temperature ranges [70].

Most of the anerobic processes are operated under mesophilic conditions since mesophilic processes are more stable and resistant to pH, temperature and OLR fluctiations compared to thermophilic ones. However thermophilic reactors are also common due to their accelerated reaction rates, increased gas production and higher rates of pathogen removal compared to mesophilic and psychrophilic reactors. High temperatures also make substrate more readily soluble in water and easy for utilization by methanogens. These facts indicate thermophilic processes are much faster which result in small reactor volumes compared the lower temperatured reactors. But it is worth to note that mesophilic and especially psychrophilic anaerobic digestion processes are much more cost effective compared to thermophilic process because of the required energy amount to heat the process [69, 70, 71]. In psychrophilic reactors, energy is only needed for mixing. Process

temperature is mostly the ambient temperature. Operating temperature in an anaerobic process also specifies the distribution of the active and dominant methanogen archaea. Thermophilic microbiome is more sensitive to pH and temperature fluctiations that may cause a decrease in biogas production [21, 69, 72]. Beside this, at high temperatures, free ammonia accumulation occurs in the process which can lead to process inhibition. At thermophilic conditions it is also likely for hydrogen partial pressure to be higher compared to lower temperatured processes which in turn can lead to acetotrophic methanogens get affected negatively and fail the syntrophic relationship between hydrogen producers and hydrogen scavengers [72].

It is remarked temperature is a critical parameter that affects the activity of exoelectrogenic bacteria. As a result, change in activities can lead to performance alteration of an MEC such as current density, biogas generation, and organic removal rate [71]. Current density of electrochemically active microorganisms at anode can decrease at long term at mesophilic condition (35^{o}C) even though it seems to increase at the beginning of the process. Kyazze et al. [73] indicated an optimum temperature of 30^{o}C (56.5 mL H_2/d, current density 1.69 A/m^2–anode) when working with a two chamber MEC fed with acetate at the temperatures of between 18.5 ^{o}C and 49.4 ^{o}C. In their study, it was found out that the current generation and biogas (H_2, CH_4) production reduced at the temperatures of under 25^{o}C and above 40^{o}C due to the lower activity of exoelectrogens [73]. Another research shared similar results in which highest current density and COD removal rates obtained at the temperature of 31^{o}C in a single chamber MEC [74]. Ahn et al. [71] conducted batch tests with a 2.5 L single chamber MEC fed with sewage sludge to investigate the effect of temperatures of 30, 35 and 40^{o}C on methane generation and organic matter removal at applied voltage of 0.3 V. They reported methane generation, methane yield and COD removal efficiency of 1.11±0.07 m^3 CH_4/m^3 and 104.2±11.2 L CH_4/kg $COD_{rem.}$ 39 % respectively at 35^{o}C. Liu et al. [75] studied the effect lower temperatures (10^{o}C) in a combined bioelectrochemical anaerobic system (BES-AD) and revealed a CH_4 yield of 31 mg CH4-COD/g VSS at a cathode potential of -0.9 V. According to this work, biogas production in the combined system was 5.3-6.6 times higher than that of AD reactor only at 10^{o}C. They also claimed that CH_4 production rate accomplished in the integrated BES-AD reactor at 10^{o}C was almost same with the methane production obtained AD reactor at 30^{o}C [75]. Yang et al. [76] used $NaHCO_3$ as CO_2 source in a synthetic waste and studied the conversion of CO_2 into CH_4 at six

different temperatures between 15 and 70 ^{o}C. They reported that the highest CH_4 generation was at 50^{o}C (2.06 ± 0.13 mmol CH_4/h) and 35^{o}C respectively in a two chamber MEC at fixed cathode potential of -0.9 V. A pilot scale study was conducted with a single chamber continuous flow MEC of 1000 L that included 144 pairs of electrodes with 24 modules. Pilot scale MEC was fed with winery wastewater. After 60 days of exoelectrogenic biofilm enrichment period, MEC was ready to operate at applied voltage of 0.9 V at 31 ^{o}C. With this MEC, SCOD removal rate of 62±20 % was accomplished at a HRT of 1 day and biogas production reached to highest level of 0.19 L/L/d with a CH_4 percentage of 86±6 % [77].

It seems like there are different opinions on optimum temperature ranges in MEC studies. Different results were obtained at MEC studies that focused on biogas production and treatment efficiency at various temperatures. However it is clear that mesophilic and thermophilic temperatures are much more appropriate when the target is methane production but not hydrogen production or current generation.

2.8.2. pH

pH is a measure of H^+ ion concentration in a mild environment or in water that is related to the acidity or alkalinity of the water. pH influences the growth and metabolism of the microorganisms. In anaerobic digestion, pH is so important for the anaerobic microorganisms especially methanogens, that an undesired pH level can inhibit and deterioate the process. Methanogens should be kept in a stable environment because of their sensitive nature against to pH variations [77]. However different optimum pH ranges are remarked for methanogens, it can be concluded that methane production takes place effectively at pH ranges of 6.5–8 independent from substrate type [66, 77]. Methane production can be heavily limited when pH declines below 6.0 or increases above 8.5.

pH increases as a result of ammonia accumulation which arises from intensive breakdown of protein or nitrogenous content. When ammonia concentration escalate pH, ionized ammonia (ammonium-NH_4^+) become free ammonia and this situation exhibits toxic effect on methanogens [78]. On the other hand, pH decreases due to the accumulation of the organic acids or fatty acids (VFA) that comes from carbohydrate degredation. If the alkalinity of the solution is high enough, then pH fluctiations that deteriorates the process stability will not be the subject. Most animal manure has high alkalinity that can stabilize

the pH. VFA concentration over 2 g/L can inhibit methanogen activity [64, 66]. If the pH level decrease as low as to 6.6 and further, methanogen activity gets harmed significantly. After a threshold pH level of 6.2 and lower, methanogens can be affected toxically. At this point, VFA production by acid bacteria continues and this leads to decrase of pH to the 4.5-5.0 levels.

Hydrogenotrophic methanogens stay in contact with hydrogen producing acetogens so that they can benefit from hydrogen produced. In this way hydrogenotrophic methanogens that produce methane by using hydrogen and carbondioxide, maintain the hydrogen concentration stay low and prevent pH decrease [63].

In bioelectrochemical (BES) systems, exoelectrogens are the main bacteria type that transfer electrons derived from organic substances to an electrode and finaly to an external circuit. There have been several efforts to research the effects of pH on exoelectrogens which in some cases have indicated contradictory results. The contradictory results may have been arised from the differences in reactor design such as membrane used reactors or membrane free single chamber BESs and air cathode MFCs and different substrates and catholytes used. It was reported that even though there are conflicting ideas on most suitable anolyte pH level for electron transfer, general view is that neutral pH levels are favorable among exoelectrogens [79]. As a result of the study performed by Min et al. [79], it was found out that anolyte pH ranging from 6.3 to 7.6 was optimal for higher power output and COD removal for the treatment of food waste leachate in MFCs. A research about the yield and decay coefficients of exoelectrogenic bacteria in BES systems, pointed out that at mildly acidic pH level of 5, exoelectrogenic growth is seriously inhibited [80]. It was also found out that exoelectrogens presented similar growth coefficients to anerobic microorganisms which ranged between 0.1 to 0.3 gVSS/g COD at pH ranges of 7-9 [80]. In a two chamber MEC in which an abiotic cathode chamber full of saline solution was used. The highest specific hydrogen production was reported as 3.3 mol H_2/mole per acetate. Volumetric hydrogen generation was 2.2 m^3 $H_2/m^3/d$ and anolyte pH was 9 in the begining of the study [81]. It can be understood that exoelectrogens can perform at a wide range of pH levels but neutral pH range can be used for both exoelectrogens and anaeobic consortia.

2.8.3. Organic Loading Rate and Hydraulic Retention Time

Organic loading rate (OLR) is the quantity of a biologically degredable substrate that is fed to the digestor/reactor during a period of time. It is a function of flowrate, substrate concentration and reactor volume and it is expressed in kg VSS or COD per volume of reactor per day, (kg COD/m^3/d) [82]. Equation 2.20 explains the relationship of OLR between substrate concentration (S_o), flowrate (Q) and reactor volume (V). On the contrary, hydraulic retention time (HRT) is the measured time (hours, days) that feedstock (fed waste, substrate) stays in a digester and it is the contact period of substrate and microorganisms. It is related to digestor capacity and calculated by dividing digester volume by the flowrate.

$$OLR = \frac{S_o}{HRT} = S_o \frac{Q}{V}\ ,\ HRT = \frac{V}{Q} \qquad \text{Equaiton 2.20}$$

OLR and HRT are operational factors that affect the reactor`s performance in terms of biogas production especially in continous mode. Both of them must be optimized for a given reactor, substrate type and temperature range [21]. It can be expected that at thermophilic process, the digestor can overcome higher OLR or lower HRT conditions compared to mesophilic process because the microbial activity is higher at thermophilic conditions [69, 70]. Substrate type is also significant for determining the OLR and HRT of the system. Hardly biodegradable organic matters such as lignocellulosic biomass which are composed of wheat straw, sugarcane bagasse, corn stalks, rye straw, rice straw, and barley straw as well as various types of organic fraction of these crops take time to be utilized [83, 84].

Conventional anaerobic reactor types include fast processing reactors such as upflow anaerobic sludge blanket (UASB) reactors, filters, and anaerobic fluidized bed reactors. The other conventional reactors are anaerobic sequencing batch reactors and continuously stirred tank reactors (CSTR) which all of them operate on totally different working principles. In anaerobic digestion, up to a point, incrementation of OLR results in enhancement of biogas, however at some point, increasing OLR leads to a reduction in biogas production and deterioration in process due to some unfavorable situations such as VFA and ammonia accumulation or pH decrease or increase [85]. Wide range of OLRs can be applied to the anaerobic digestors. For instance, Rico et al. [86] studied methane production with a laboratory scale UASB reactor. Cheese whey and dairy manure liquid

were treated together at 35ºC. They reported that a stable operation was achieved under the highest organic load of 28.7 kg COD/m^3/d at hydraulic retention time of 1.3 days. Organic removal and methane generation rates were 95 % and 9.5 m^3 CH_4/m^3/d in the study. Similar OLR values and results were reported by Torkian et al. [87] when using slaughter house effluent as substrate in a UASB. They stated that OLR values less than 30 kg COD/m^3/d was enough for a stable process at HRTs of as low as 2-7 h. After a threshold value of OLR, reduction in removal efficiency and methane content were observed owing to a combined effect of high OLR and low HRT. Liu et al. [88] used a pilot scale CSTR to produce biogas from co-digestion of municipal solid biomass waste with waste activated sludge. The maximum methane generation in that study was 2.94 m^3/m^3/d at OLR of 8.0 kg VS/m^3/d and HRT of 15 days. It was reported that the operation was highly under at risk with the organic loading rate of 8.0 kg VS/m^3/d due to the reduction in organic degredation, pH level and methane percentage in biogas [88]. Olive mill solid residues were subjected to biogas production in a CSTR at different OLRs and HRTs by Rincon et al. [89]. They reported similar operational conditions such as OLR of 9.2 VS/m^3/d and HRT of 17 days that process was at the most efficient operational condition. However decreasing HRT to 15 days which in turn increased OLR to 11.0 VS/m^3/d, led the way to the destabilization and process failure.

As it is clear, change in OLR and HRT can influence process outputs significantly and furthermore OLR also changes by alteration of HRT when used the same feed. The optimization of the process parameters such as OLR and HRT in this case, is crucial because if the digestor has a high OLR and a short HRT, system failure may possibly occur due to VFA accumulation, pH decrease or ammonia inhibition.

In this work, cattle manure was used as feedstock in a combined MEC+AD system at different OLRs and HRTs. In this context following paragraph gives a brief information about several studies that are conducted by using cattle manure as feedstock which investigated the OLR and the HRT effects in anaerobic processes.

In a study, food waste and cattle manure fed together (ratio of 1:2 weight basis of VS content) to a 16 L CSTR in purpose of decreasing the HRT of the reactor at 37ºC [89]. In that study, different HRTs from 25 to 4 days were tried to evaluate the methane production. Highest production was achieved as 1.48 L/L/d with an HRT of 5 days which corresponded to an OLR of 12 kg VS/m^3/d. However maximum methane yields (236-257

mL/g VS) were attained at HRT of 15 days (4 kg VS/m^3/d). Methane yields decreased at lower HRTs than 15 days and at HRT of 4 days (15 kg VS/m^3/d) process was inclined to deterioration. Similarly, Zhang et al. [90] also studied co-digestion of food waste and cattle manure in a 0.8 L semi continuous reactor at mesophilic temperature. In their work, specific methane productions obtained from digestion of food waste were increased by 53% and 55% corresponding to 388 and 317 mL/g VS respectively when the mixture of cattle manure and food waste at a ratio of 1:2 fed to the reactors at OLRs of 8 and 10 g VS/L/d respectively. Furthermore, it is reported in that study, maximum methane yield of mono digestion of cattle manure was 113 mL/g VS when the OLR was 3 g VS/L/d [90]. Li et al. [91] carried out a study in which a semi continuous stirred 30 L reactor was operated at 37ºC co-digesting rice straw and cattle manure at different mixture ratios and OLRs. Steady and effective process was obtained with a biogas yield of 383.5 L/kg VS and volumetric biogas production rate of 2.30 L/L/d at an OLR of 6 kg VS/m^3/d. However anaerobic co-digestion treatment was seriously restricted by VFA accumulation when the OLR was increased to 12 kg VS/m^3/d. A large scale plug flow anaerobic reactor with working volume of $38.5x10^3$ m^3 operated for a long period of time by Dong et al. [92]. Cattle manure with total solids content of 7-10 % was treated to produce biogas at HRT of 25 days under 37–40 °C with this reactor. They reported an average monthly biogas production of 7.45×10^4 m^3 and daily biogas production rate of 1.07 m^3/m^3/d with methane content of 56.4 % at steady state. Moreover, biogas production yield and the substrate removal efficiency were 0.39 m^3/kg VS and 59 % respectively in this work. Varol and Ugurlu [93] developed a hybrid reactor that is horizontal and operated as a plug flow reactor. It had four sequental compartments for four consecutive stages of anaerobic digestion. They compared a CSTR with hybrid reactor using dairy (cattle) manure as substrate at different OLRs between 1.1-5.4 g VS/L/d. The biogas productions and methane yields obtained from the hybrid reactor (0.45-1.73 L/L/d and 440-320 mL/g VS respectively) were superior to CSTR results at every trial. Also it was stated that co-digestion of manure with maize sludge increased the methane yield about 1.2 folds.

It can be concluded that cattle manure is a complex type of substrate when used in anaerobic digestion as a sole substrate as Tufaner and Avsar implied [94]. Therefore anaerobic treatment of cattle manure together with carbon rich sources leads to resolving imbalances such as VFA accumulation and poor alkalinity and it improves the biogas production with synergistic effect [94].

2.8.4. Applied Potential or Set Electrode Potential

The reason of the application of an external potential to MECs and the mechanism of this application are explained in Section 2.3.4 in details. When the purpose of a study is to produce hydrogen in a MEC a specific amount of external power must be applied to the system because the potential of the cathode must be minumum 0.42 V (vs Normal Hydrogen Electrode) [21]. When used most known organic matters, anode potential can be as high as -0.3 V and therefore a voltage between 0.2-1.0 V can be applied to the MEC [21, 33]. Apllied power of 1.1 V and higher than this is not prefered due to the excess energy application and occurence of water electrolysis [21]. Also voltage amount has essential influence on development and variation of microorganisms, biogas production, and organic removal rates [21].

There are several studies focused on the effects of different voltage levels applied to the MECs. These effects can be indicated as methane or hydrogen production amount, VS or COD reduction, current generation and microbial species distruibution. Choi et al. [95] studied effects of external voltages of 0.5, 0.7, 1 and 1.5 V on a bench scale single cell CSTR MEC fed with glucose concentration of 2 g/L and operated at the temperature of 35 ^{o}C. In batch tests, the MEC that was applied with 1.0 V of power, produced higher methane than the other MECs that were supplied with different external voltages. Methane yield was also superior than theoretical methane yield which is 350 mL/g COD at known standart conditions and the control reactor result (317 mL/g COD). Maximum specific methane production and the current density were 408 mL/g COD and 19 A/m^3 respectively at input voltage of 1.0 V. It was reported that at a certain voltage application, the reactions occur at the cathode were affected considerably. As a result of this methane generation and organic removal efficiency were affected significantly. The oxidation and reduction reactions on the electrodes were attributed to the essential effects of the electrons transported between the electrodes, which ultimately caused enhancement of electrogenic microbial activities and other bioelectrochemical activities [95]. Another study was carried out by Ding et al. [96] in which a two chamber MEC. Different substrates, sodium acetate and saccharose, at 2-3 g COD/L concentration were used in anode and cathode chambers respectively, at HRT of 3 days and 35^{o}C in batch mode. Five different voltages from 0.4 to 2.0 V were applied to the MEC to assess the effects of the different voltages on MEC performance. They stated that maximum organic removal rate and specific methane production were achieved at voltage supplementation of 0.8 V. Both

indicators decreased when the supplied voltage was higher than 0.8 V. It was reported that lactic dehydrogenase (LDH) which is a kind of glycolytic enzyme mainly found in cytoplasm, was found in high concentrations in solution at applied voltages higer than 0.8 V. In normal circumstances, LDH is not found in solution because of cell membrane protection. It is claimed in the paper that higher voltages of 1.0 and 2.0 V led to the cell membrane destruction and performance decrease. Also it was shown that ATP generation decreased due to the destruction of cell membrane in MECs that was supplied with voltages higher than 0.8 V. Cell membrane destruction led to decline of the growth and metabolism activity and following to the decrease of COD removal efficiency and methane yield [96].

Setting the electrode potential as well as voltage application to the MECs is also another way to supply energy to the system. Zeppilli et al. [97] studied biogas upgrading by CO_2 reduction to CH_4 in a cathode of a two chamber MEC in which syntetic media was used at both chamber. In this work, an artificial biogas (v/v: 30% CO_2, 70% N_2) was upgraded by the biofilm on the cathode electrode via current utilization to reduce CO_2 to CH_4. At set cathode potential of -0.65 V (vs. Standart Hydrogen Electrode, SHE), complete energy recovery was obtained by CH_4 generation. On the other hand at set cathode potential of -1.0 V inhibition was observed on the biofilm at cathode. In a study, comparison of the hydrogen and methane production rates and overall energy recoveries of MECs were made. The MECs were conducted batchwise with input voltage of 0.6 V and fixed anode potentials of -0.4, -0.2, 0 and 0.2 V [98]. In all experiments, the MEC with fixedanode potential of -0.2 V performed better results than the other MECs in terms of energy input per reactor volume (3 kWh/m^3) and H_2 generation (2.3 kWh/m^3 H_2) and overall energy recovery (58±6 %). The MEC that was applied with voltage of 0.6 V during the study, had the second best performances although the energy given to this system (1.7 kWh/m^3) was almost half of the energy (3 kWh/m^3) that was given to the MEC at set anode potential of -0.2 V. A study related to bioelectrochemical anaerobic digestion of acetate in a fed-batch single cell MEC revealed the highest specific methane production as 0.351 L CH4·/g at different voltage supplementations [99]. In the same study, organic removal (SCOD) of 83.6% were reported at acetate concentration of 2 g/L and power application of 1.0 V. It was stated that methane production decreased at given voltage of 0.5 V due insufficient potential force and also inhibition occured at given potentials over 1.0 V due to high-voltage. In respect of voltage application, highest current generation of 19.5 mA

was achieved at supplied voltage of 1.0 V. It was suggested that high methane generation was highly relevant with high current flow [99]. Another paper related to the study published by Flores et al. [99] which focused on the the distribution of microbiome at supplied voltages of 0.5, 1.0, and 1.5 V [100]. It is observed that the bioelectrodes with power supplementation of 1.0 V had higher numbers of unique operational taxonomic units compared to those at power applications of 0.5 and 1.5 V. It is pointed out statistically that there was distinction at the biocathode biofilm at power supplementation of 1.0 V due to the diversity and number in the microbial distribution compared to the others. It was sexhibited that at power application of 1.0 V exoelectrogens/electrogens (*Geobacter* spp.) overtook the bioanode, while mutually dependent communities of hydrogen-producing bacteria (i.e., *Bacteroidetes* and *Firmicutes*) and hydrogen-consuming methanogens (i.e., *Methanobacterium* sp.) were discovered in the biocathode. It was suggested that appropriate voltage supplementation strengthened specific microbial groups on the anode and cathode for increment of methane generation [100].

It can be concluded that the amount of the voltage supplied to the MECs affects the efficiency of the MECs in terms of biogas or hydrogen generation, organic material removal, current generation and coulumbic efficiency [95-100]. These results mainly occur due to the electron transport rates that change the oxidation and reduction reaction rates, determine the distribution of microbial consortia and classification. Electron transfer also can also cause to destruction of cell membrane and inhibition of microbial activity at higer voltages and insufficient electron transportation at lower voltages [95-100].

2.9. Potential Application of Microbial Electrolysis Cell and Anaerobic Digestion

For the last 20 years Bioelectrochemical Systems (BES) have been studied extensively for its potential applications to recover energy and value-added products from various organic waste streams. A couple of years after the discovery of first bioelectrochemical systems, Microbial Electrolysis Cell was invented by two distinct group at the same time, Liu et al. [44] and Rozendal et al. [51], in purpose of hydrogen production. Early studies on hydrogen production in MECs reported that methane was also produced as a side product [35, 38, 44, 51]. In light of these findings, Clauwaert et al. [30] and Cheng et al. [31] showed for the first time that methane could be generated effectively by using biocatalyzed electrolysis cell in a two chamber MEC and by electromethanogenesis in a

single cell MEC respectively. In both of the systems acetate were used as the substrate. The studies conducted on hydrogen production by employing voltage application to MECs led the way to generation of valuable goods as in methane, hydrogen peroxide, alcohols, acetate, ethanol, formic acid, and etc [19, 21]. Methane production in MECs is one of the most promising area that is being investigated nowadays. There is a strong expectation that MEC technology can overcome the obstacles arise in conventional anaerobic digestion process which aim methane generation.

Microbial electrolysis cells and anaerobic digestion processes can be combined together or can be employed in series to fulfill various operations that could help to save and produce energy and recover value-added chemicals or remove inhibitory chemicals. Inhibitory effect of inorganics such as total ammonia (NH_3, NH_4^+) and salts such as K^+ and Na^+ and hydrogen sulphide (H_2S) can take place in ananerobic process at certain pH levels and temperatures. Low carbon to nitrogen ratio (C/N) of the substrate and recalcitrant organics may cause inbition in anaerobic digestion [78, 100]. MECs can be used for removing or recovering inorganics that have inhibitory effects to anaerobic processes, therefore it can be utilized to stabilize the process [100, 101]. Extraction of total ammonia, K^+ and Na^+ from the streams can be implemented by the use of bioelectrochemical systems (MFC, MEC) equipped with a cation exchange membrane that only permits these ions pass through. The electrical current used in these systems enable transportation of the ions from the anolyte to the catholyte where they can be recovered in the form of ammonia gas or salt compounds [100, 102, 103]. Removal of sulfide through utilization of sulfide as an electron donor/substrate by microorganisms in MECs is reported by some researchers. Beside with sulfide removal, concomitantly hydrogen and methane production was reported by Jiang et al. [104] and Dong et al. [105]. The recovery of phosphorus is related to the resource gaining issue because it is an useful and valuable nutrient for many processes. Phosphate recovery can be realized in cathode of MECs in the form of precipitated struvites with Ca^{2+} and Fe^{2+} due to the increased pH of the catholyte as a result of the hydrogen dissipation [100, 106].

VFA is the intermediate product in biomethane production. During the process, any imbalances such as undesirable temperature change, abrupt pH variation and substrate overloading as well as presence of toxic compounds can affect the methanogenic consortia first in anaerobic process. As a result of these imbalances, accumulation of the

toxic VFA can take place with the rapid pH decrease [72, 100]. Removal of excess VFA is critical to sustain biogas production. There are some ways to ensure the removal of excess VFA such as lowering the OLR and increasing the HRT, elimination of potentially inhibiting compounds from the feedstock, alkalinity supplementation and etc [101]. In this regard, MECs can provide the necessary stabilization through removal of the excess VFA produced and polishing the effluent of the anaerobic digestion processes even at ambient temperatures and low organic concentrations [107, 108]. MECs can be used in series with AD as in pre-treatment or post-treatment of the stream to stabilize the VFA or polish the effluent respectively. It can also be used as side stream of a main AD process to stabilize the process parameters. Other than being a separate modul, MECs can be combined with AD in one single reactor that can provide short HRTs and high OLRs for the process instead of common operational parameters used in AD [100]. In case of a high VFA concentration or in a possible VFA accumulation situation (>10 g COD/L), extraction is another potential application that can be materialized through an anion exchange membrane suited electrolysis cell and a side stream esterification unit [109]. Electrolyzed VFAs which are negatively charged can be transported from cathode to anode through the membrane and further to a separate unit equipped for esterification.

Biogas produced from organic substrates through AD consists of CH_4 (40-75%) and CO_2 (25-60%). But in order for biogas to be injected into the existing gas grid as a fuel, the ratio of CH_4 in biogas needs to be increased to 96 % or more [66, 72, 100, 110]. Upgrading biogas can be carried out with several different processes which are: removing CO_2 from biogas via physical-chemical post processing (absorption and adsorption); supplying a reduced substrate (e.g., hydrogen gas) to the digestor or feeding biogas to a seperate unit to convert CO_2 in biogas to CH_4 biologically (using hydrogenotrophic methanogens and algae for CO_2 fixation) [110]. Recently bioelectrochemically biogas upgrading process was introduced as an alternative. There are two ways of methane generation through bioelectrochemical method that were reported. One way was proposed by Cheng et al. [31] and Villano et al. [32] that CO_2 could be converted into CH_4 biologically by the process of electromethanogenesis; which is the reduction of CO_2 to CH_4 directly by accepting electrons from electrodes via methanogens. The second way is the indirect CH_4 generation through CO_2 (electron acceptor) reduction by H_2 as the electron donor which is also generated by exoelectrogen microorganisms [32]. Biogas can be upgraded by removing CO_2 using an MEC or an external MEC. Xu et al [111] studied upgradig of

biogas in *situ* in an integrated MEC (anode was sealed with a membrane in the MEC) and AD at set cathode potential of -700 mV. Influent COD of the synthetic wastewater used as the substrate source in the integrated system was 400 mg COD/L. The HRTs were 3.2 and 1.6 days at two distinct operational mode. It was reported that CO_2 content in the biogas was lower than 10% for all cases owing to hydrogenotrophic methanogenesis (*mathanobacterium petrolearium*) and alkali production with CO_2 absorption. Another study that was conducted by Jin et al. [112] for biogas upgrading, included a three chamber MEC at room temperature which had a CO_2 regeneration chamber beside the anode and cathode chambers separated by membranes. It was stated that CO_2 was separated efficiently from the raw biogas (60% CH_4, 40% CO_2) and the CH_4 ratio of the biogas in the outlet of cathode reached as high as 97 % at applied voltage of 1.2 V and raw biogas flow rate of 19.6 mL/h.

2.10. Previous Studies on Combined MEC+AD System

It was first pointed out by Clauwaert et al. [30] and Cheng et al. [31] that methane could be produced efficiently in MECs along with the waste/wastewater treatment. Although, at present, conventional biogas production technology is greatly in use, there are some challenges that must be tackled. Challenges such as high volumed tanks due to the long HRTs, unstable processes and VFA accumulation due to high OLRs, ammonia inhibition due to low C/N ratio (<15/1), pH dependent imbalances, low methane yields and failure of the process due to the toxic and inhibitory substances set back the efficiency of the AD process [40, 66, 69, 72, 77]. These drawbacks led the way for researchers to investigate methane production in MEC technology. The studies so far revealed that, MECs are superior to AD processes regarding the methane yield, biogas production, organic removal efficiency, shorter period of HRTs, stability, functionality at low and high OLRs [27, 40, 100]. MEC studies that focused on methane production so far used different types of reactor configurations, operational parameters and substrates to demonstrate the results of the new technology. Single and two chamber MECs, integrated MEC and AD reactors and other type of reactors were used for MEC technology. Different applied voltages from 0.2 V to 1.5-2.0 V were used in these studies to reveal the effects of the voltage application. Influence of high and low OLRs and HRTs on the MEC technology were studied in these experiments. A wide variety of substrates including synthetic wastewater and real waste streams were used as substrate and carbon source. Ambient temperature, mesophilic temperature as well as thermophilic temperature ranges were used to search

the effects of temperature on the MEC microbiome and process outputs. In this context, Table 2.4 was prepared to illustrate the studies conducted on methane production in MECs. In Table 2.4, construction type of MECs, reactor volumes, anode and cathode materials, operational parameters, substrate type with influent COD/VS concentration and OLR or HRT were given for the summarized works. The results of the summarized studies were presented in COD/VS removal rate, methane production rate and methane yield and current production. Specific applications or treatments were also explained briefly for every one of the studies.

Table 2.4. Previous studies conducted on methane production in MECs.

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	
TC-0.225 L total, CEM was used	Continuous mode, 22ºC. Synthetic medium in anode and cathode. Feeding acetate OLR 1.6 kg COD/m^3 total anodic compartment. HRT=2.2 h. Voltage was 0.6 V.	Graphite granules (d:1.5-5 mm) graphite rod (d:5 mm)	41.25 cm^2 graphite woven web with 5 g Pt/m^2 on it and it was fixed to a graphite rod	0.41 mole CH_4/ mole acetate (%57 CH_4 and %42 H_2). 1.85 mol H_2/ mol acetate	46±8% (Acetate conversion to H_2)	6 - 6.8A/m^2	Anode and cathode medium were refreshed by continuous recirculation. Acetate was dosed to the anode batch wise when it was depleted in anode medium. The acetate oxidation in anode was not hindered by NH_4-N conc. up to 5 g N/L.	[30]
TC-0.3 L each chamber, 0.6 L total	Batch, 30ºC. Anode and cathode medium was buffer solution only. Anode didn`t have any biofilm on it but cathode did. CO_2 was sparged to cathode chm. as e- donor. Set cathode potentials between -0.7 V and -1.2 V (vs Ag/AgCl).	Abiotic anode, graphite fiber brush (diameter:5 cm, lenght: 7 cm)	Carbon cloth cathode (area:14 cm^2) coated with a carbon layer on one side (2.5 mg/cm^2) and no metal catalyst	656 mmol CH_4/d/m^2 (cathode geometric surface area) at set -1.2 V potential			Membrane (d=2.9cm) was used to separate the chambers. CO_2 was reduced to CH_4 using a TC MEC containing both biotic anode and cathode, without any metal catalysts. Since there was no acetate in MEC, there was not any CH_4 production from acetoclastic methanogenesis, on the contrary CH_4 was produced from direct e- transfer. $CO_2+8H^++8e\rightarrow CH_4+2H_2O$	[31]
TC-0.3 L total	Batch, 35ºC. A pretreated PEM(3 cm^2) was used for separating the chambers. Feed to both chambers: 30% CO_2 by volume in a gas mixture of N_2 and CO_2. 8 h cycles were applied. Set cathode potential btw. -0.65 and -0.9 V (vs SHE).	Glassy carbon rod(7 cm^2), 150 mL mineral medium	Carbon paper (8 cm^2), 75 mL source culture and 75 mL mineral medium	2.6 meq CH_4/d at -0.9 V set potential		0.5-0.6 mA at -0.75 set cathode potential	Source culture was prepared earlier by feeding H_2 in a different reactor at 35ºC. Hydrogenotrophic methanogens were aimed to reproduce. The medium of the chambers were explained in chamber columns.	[32]
SC-1100 L rectangular, h: 0.7 m, w: 0.64, l: 2.3 m	Continuous, 31ºC. Substrate was winery WW HRT=1 day, Feed conc. was in the range of 0.7−2.0 g SCOD/L. pH was controlled (pH>6). Acetate amendment was used at first 65 days to enhance the exoelectrogen microorg. conc. Applied voltage was 0.9 V.	Each module contained 6 anodes, graphite fiber brushes (d= 5.1 cm, h=66 cm)	Each module contained 6 cathodes, SS 304 (mesh #60, w=7.6 cm, l=66 cm)	Total gas (CH_4 and H_2) production of 0.15-0.28 L/L/day (86±6 % CH_4)	SCOD removal 62±20% (days btw. 60-100)	Max. 7.4 A/m^3 (after 100 days of operation)	144 electrode pairs in 24 parallel modules were used in the MEC. Enrichment of the MEC finished about 60 days after. Mixing the reactors, chemical and substrate addition to the reactor was achieved by a recirculation flow at the rate of 0.82 m^3/day.	[48]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	
SC-0.9 L (MEC unit in AD)	Batch, 35ºC. 1:1 ratio of food waste and inoculum used at the start. TS and VS of the mixture of food waste and inoculum were 6.8% and 4.9% respectively. Initial COD was 15.64 g/L in all reactors. Applied voltage was 0.9 V.	11 graphite plates of 1.5x15x0.1cm^3 seperated by thin rubber bands inserted in stainless steel cylinder	Stainless steel cylinder covering the anode with rubber bands separating them. Cathode was the outer cylinder.	0.59m^3/m^3/day (CH_4+H_2) or 12.8 L (CH_4+H_2)/L in 23 days	57% and 82.7 % (at the end of 8 and 23 days respct.)	17.3 A/m^3 (based on total AD-MEC)	MEC was constructed as the cylinder pipe that had holes on it for water exchange from outside to inside. The CH_4 and H_2 production amount was 0.59 L/L/d based on the avarage of total biogas divided by 23 days. The overall efficiency of AD-MEC over the electrical input energy for running the MEC exceeded 400%.	[49]
Combined AD+MEC; 20 L, cylindrical	Sequencing batch (SBR), 35ºC. Food waste; OLR=2-10 kg/m^3/d ; HRT=20 days, 15days for OLR 10 kg/m^3/d; 0.3 V.	Three 15x30 cm^2 graphite carbon meshes coated with Ni	Three 15x30 cm^2 graphite carbon meshes coated with Ni, Cu and Fe	19-76 L/day due to the OLR 2-10 kg/m^3/d; 0.3-0.36 L CH_4/g $COD_{rem.}$	68-76%		OLR was changed between 2-10 kg/m^3/d. At 10 kg/m^3/d, MEC still stably produced CH_4. The distance between electrodes were <3mm. Non-woven fabric was placed between electrodes to prevent short circuit.	[54]
SC-0.18 L, cylindrical	Batch, 37ºC. Thermal alkaline pretreated sludge (150 mL) and inoculum (30 mL) mixture had TCOD of 15.84 g /L (6 gVSS/L). Voltages of 0, 0.6, 0.8, 1.3, 1.8 and 2.3 were applied; 0-2.3 V (5 different voltages).	4x5x 0.2 cm^3 mesh plates fabricated from Ti/Ru alloy.	4x5x0.2 cm^3 mesh plates fabricated from Ti/Ru alloy.	0.26 L total in 21 days, 0.2 L CH_4/g VSS (for 1.8 V)	59% (VSS) for 1.8 V		The pH of concentrated sludge was first set to 12.0 by using 10 M NaOH and then treated for 30 min. at 175ºC. And pH was adjusted to 7 again with HCl.	[55]
Combined AD+MEC; 0.5 L, cylindrical	Batch, 35ºC. Fresh incineration leachate, feed conc. was changed from 4.8-21 g COD/L; Cycle time 11-12 days. At the beginning 12 mL sludge and 188 mL diluted leachate were added to all MECs. Applied voltage was 0.7 V.	Graphite rod (d=0.3 cm x h: 5 cm)	Graphite rod (d=0.3 cm x h: 5 cm)	Cumltv. of >25 mmol and >33 mmol CH_4 for feed conc. of 10 and 15.9 g COD/L respct.	>90%		High COD concentration of 21 g/L influent inhibited the AD+MEC process. AD+MEC was superior to anerobic process in terms of methane production, COD removal and recovering time period.	[57]
SC-0.15 L, cylindrical	Batch, 37ºC. Substrate and inoculum was sewage sludge. 148 mL sludge and 2 mL mineral medium were added to the reactors. Sludge`s TCOD:11.75 g/L, VSS:6g/L. Applied voltages were 1.4 and 1.8 V.	Anode (4x5x0.2 cm^3) were made of Ti/Ru alloy mesh plate	Cathode (4x5x0.2 cm^3) were made of Ti/Ru alloy mesh plate	CH4 cumulative production of 130- 165 mL over 30 days	VSS removal 61-62 %		The distance btw. the anode and cathode was 2 cm. H_2 and CH_4 production enhanced by 1.7-5.2 fold and by 13.6-11.4 fold in the MEC with added 1.4 V and 1.8 V respct. compared to the control reactors. CH_4 production started after 5th day in the MECs.	[58]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
SC- 2 L, cylindrical	Batch, 35ºC. Raw sludge from WW treatment plant had 77 g VS/L and 114 g TCOD/L. The mixture of sludge and inoculum had 63 g VS/L and 107 g TCOD/L. pH of the mix. was 7.5. Applied voltages were 0.3 and 0.6 V.	Fe tube electrode (d:10 cm, h: 18 cm)	Graphite pillar electrode (d:8 cm, h:18 cm). The graphite pillar electrode was located in the axes of Fe tube electrode.	170.2 L/ kg VS at 0.3 V after 22 days of operation.		4.3 A/m^3 for 0.3 V	Inoculum was obtained from an UASB reactor. The raw sludge and seed sludge were mixed. At the end of 22 days of operation pH of the MEC was 8.3 (0.3 V) and 9.1 (0.6 V). Alkaline pH inhibited methanogens at 0.6 V application. VFA formation was enhanced in MEC with 0.3 V application.	[59]
SC-1 L UASB (d:7 cm, h:26 cm)	Continuous, 35°C. Synthetic WW with glucose, NH_4Cl and $KHPO_4$ was fed to the MEC. COD was changed from 1 g/L to 7 g/L. HRT of 6 h was used for all feeding period. For 7 g COD/L, OLR was 28 g COD/L/d. Applied voltage was 1.0 V.	7 graphite electrodes (d:1 cm, h:15 cm) anode	1 graphite electrode (d:1 cm, h:15 cm) cathode	44.6 mL/h for 1g COD/L; 248.5 mL/h for 7 g COD/L	89.2% for 1 g COD/L; 71.0% for 7 g COD/L	7.9 to 51.4 A/m^2 increased with the OLR	The distance between the electrodes was 2.5 cm. Inoculum was taken from a laboratory-scale UASB reactor. The influent COD was from 1 g/L to 7 g/L for two reactors during the 100 days operation period. COD:N:P ratio of 200:5:1 was adjusted.	[60]
SC-2.5 L, cylindrical	Batch, 30, 35 and 40ºC. Sewage sludge and inoculum mix. ratio 7:3. Total COD conc. of the mix. was 29 g COD/L. 6 days cycle; 0.3 V	Graphite felt (3 cm width, 9 cm length)	Graphite felt (3 cm width, 9 cm length)	1.11 L CH_4/L (at 35 ºC) or 139 L CH_4/kg $VSS_{rem.}$	Max. 44% (at 40 ºC)	1.63 ± 0.11 A/m3 (at 35ºC)	Electrode spacing was 1.6 cm. 30, 35 and 40ºC temperatures were tried out for the study. Any additional chemical was not used in the experiments.	[71]
SC-0.02 L, cylindrical (d:3 cm, h:4 cm)	Batch, 10ºC. Batch cyle of 2 weeks. 15 mL anaerobic sludge inoculum(5.9 g VSS/L) and 5 mL concentrated medium were mixed. After mixing, 3.2 g/L CH_3COONa was the acetate conc. In every batch cycle the medium was freshed. Fixed potential of cathode was -0.9 V (vs Ag/AgCl).	Granular activated carbon (1.5 g, 875 m^2) in a cylindrical cage (d:3.0 cm, h:0.6 cm) at one flat side of the r.	Granular activated carbon(1.5 g, 875 m^2) in a cylindrical cage (d:3.0 cm, h:0.6 cm) at other flat side of the r.	20-30 mg CH_4-COD/g VSS	Acetate removal of 45%	-10 A/m^3 (cathodic current)	Electrodes were separated from the chamber with textile spacers on the inner surface of the flat sides. A graphite rod was placed in both anode and cathode chambers. CH_4 productiın in AD at 30ºC was 28-33 mg CH_4-COD/g VSS.	[75]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
TC- 0.5 L anode + 0.5 L cathode, H type	Batch. Temperatures of 15, 20, 35, 50 and 70ºC were tried. Mixed culture inoculum (33.4 g VSS/L) was used in cathode. 0.5 L of different synthetic medium was used in anode and cathode. Batch cycle period was 50 h. Set cathode potential of −0.9 V (vs Ag/AgCl) was used.	Carbon-felt (10 cm × 6.8 cm^2)	Carbon-felt (10 cm × 6.8 cm^2)	0.157 mmol CH_4/L/h and max. 2.06 mmol CH_4 at 50ºC. H_2 was also produced.		0.35 - 1.52 A/m^2 at 50ºC	CEM was used to separate the chambers. Distance between electrodes was 6 cm. Cathode pH was adjusted to 6.8 in each experiments. Buffer was used. 5 g/L $NaHCO_3$ was added to catholyte to represent CO_2. 42.23 g/L $K_4[Fe(CN)_6]\cdot 3H_2O$ was used in anolyte as e- donor(Fe(II)→ Fe(III)).	[76]
SC-0.27 L, cylindrical	Batch, 35ºC. Substrate was glucose at conc. of 2 g/L. The feed medium were made by mixing the seed sludge (TCOD: 19.4 g/L) with synthetic media at a ratio of 1:1 (V/V). Supplied voltages were 0.5, 0.7, 1.0 and 1.5 V.	Carbon fiber brush (2.5×4.0 cm^2) winded by stainless steel wire	Carbon fiber brush (2.5×4.0 cm^2) winded by stainless steel wire	Cumulative 219 mL CH_4; 408 mL CH_4/g COD glucose (at 1.0 V application)	86.6%	5.06 mA (at 1.0 V)	The study was carried out with repeated batch cyles. The results were from the 4th or the last cycle. The effluent of anaerobic digestion was used as inoculum. Batch cyle period was 6 days.	[95]
TC- 2 x 0.4 L each chamber, cubic	Batch, 35ºC. HRT (1 cycle period)=3 days. Sythetic WW was prepared for both chms. Inlet flows of anode and cathode chm. included acetate and saccharose respectively. The COD conc. was 2–3 g/L. App. voltages were 0.4 , 0.6, 0.8, 1.0, 2.0 V.	Carbon felt (7x3x0.3 cm^3)	Carbon felt (7x3x0.3 cm^3)	62.8 mL at 0.8 V, 625- 629 mL CH_4/g $COD_{rem.}$at 0.8 and 1.0 V.	~80% for 0.8 V and 1.0 V	1.25-1.35 mA for 0.8 and 1. V	PEM was used for separating the chambers. Secondary sedimentation tank sludge was used as inoculum (200mL, 8-10 g VS/L) for both chambers. Over the app. voltage of 0.8 V, anode microorg. community was inhibited (deteriorated) due to cell membrane breakdown.	[96]
SC-0.27 L, cylindrical	Batch, 35ºC. Acetate as feed substrate at 2 g/L concentration. Each cycle was 8 days. Application of different voltages of 0.5, 1 and 1.5 V.	Carbon brush electrode without any catalysor (depth 2.5 cm, height 4 cm)	Carbon brush electrode without any catalysor (depth 2.5 cm, height 4 cm)	0.351 L CH_4/g COD at 1.0 V. 66% of CH_4 in biogas	83.6% (Soluble COD)	19.5 mA at 1.0 V	Inoculum were mixed with growth medium at a ratio of 1:1 and acetate was added as substrate at 2 g/L conc. Buffer was used. All MEC results were superior compared to control AD reactor.	[99]
TC-2x0.24 L	Batch. Anolyte was asynthetic WW with added $C_6H_{12}O_6$. Catholyte was same with anolyte but without $C_6H_{12}O_6$. Catholyte was purged with pure CO_2. Study period was 70 h. Applied voltage was 0.7 V.	Carbon felt, 7x7 cm^2	Carbon felt, 7x7 cm^2	0.354 mL CH_4/L/h	Glucose removal, 43 %	1 - 5 mA	Chambers were separated by CEM. Anaerobic sludge and mixed hydrogenophilic methanogenic culture were fed as inoculum into both chambers respectively. 0.2 g/L conc. of sulfide($Na_2S.9H_2O$) was added to anode. 72% of sulfide removal was accomplished by the MEC.	[104]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
TC- 0.5 L Anode + 0.265 L Cathode	Continuous, 23ºC, CEM 14x12 cm^2. Pig slurry, 7.87 kgCOD/m^3/d and 0.44 kgN/m^3/d. HRT_{anode}=32.4 h, $HRT_{cathode}$=14.1 h. 0.8 V set potential vs. SHE.	Carbon felt, 14x12 cm^2; thickness: 3.18 mm and 14x12 cm^2 SS mesh to collect electrons	Granular graphite with diameter from 1 to 5mm and 14x12 cm^2 SS mesh to collect electrons	79 L CH_4/m^3/d for MEC; 450 L CH_4/m^3/d for CSTR	24±8 % for MEC; 54±8% for CSTR; 30±6% N-NH_4 for MEC	0.4 A/m^2 (anode area)	A thermophilic anaerobic 4 L CSTR was connected in series with a TC MEC (1 L). Effluent of CSTR was filtered and given to the anode of MEC and cathode was fed with synthetic solution. Cathode was inoculated with 30 mL resuspension of an UASB reactor.	[107]
SC-0.256 L total, cubic	Continuous, 22ºC. Substrate was acetic acid solution(8.5 mL/d) added to medium in recircul. loop. HRT=5.3 h, OLR= 1.38 g COD/L/d. A nonwoven cloth was used to prevent contact btw. anodic and cathodic graphite granules. App. voltage was 0.8 V.	Graphite granules (diameter btw. 1.5 and 5 mm), graphite rod inserted	Graphite granules (diameter btw. 1.5 and 5 mm), graphite rod inserted	0.33 and 0.28 L CH_4/L/d with and without recirculation loop respectively	98% and 93% with and without recirculation loop respct.	~110 and 70 A/m^3 MEC with/without recirculation loop respectively	MECs were operated under dif. conditions of OLR and with/without recirculation loop of fresh medium. Recircul. loop to both anode and cathode enhanced the CH_4 production. Max. perform. was 0.75 L CH_4/L/d with OLR of 4.13 g COD/L/d with recircul. loop. pH buffer was used in all experiments.	[108]
SC- 0.8 L, cylindrical, integrated MEC	Continuous, 37ºC. Influent feed: ethanol and organic acids with a total COD of 400 mg/L and rate was:0.25 and 0.5 mL/d. Synthetic medium for cathode and anode were placed. Set cathode potetial was -0.7 V (vs SHE).	Graphite stick (2.5x7.5x1.3 cm^3), anode electrode was rolled with a CEM inside the MEC.	Graphite stick (2.5x7.5x1.3 cm^3). Cathode chamber was the digester itself.	~50% removal of CO_2 compared to raw biogas`s CO_2 from control reactor		Max. 3 A/m^2	Chambers were separated by CEM. CO2 content of the continuous MEC was lower than the control reactor. Inoculum was anaerobic granular sludge.	[111]
Three Chamber MEC-0.2 L total	Batch, 22ºC. Acetate added domestic WW with 2 g COD/L conc. was the anolyte. It was recirculated with an external anolyte source of 0.25 L. The regeneration and cathode chms. were filled with 0.05 M NaCl solution. Synt. biogas (CH_4/CO_2: 60/40: v/v) was introduced to the cathode at dif. flowrates. App. voltage was 1.2 V.	Carbon brush (d:5 cm, h:5 cm)	Titanium woven wire mesh (4×5cm, 0.15mm aperture) coated with 0.5 mg/cm^2 Pt.	CH_4% of raw biogas increased from 60 to 88-98% at all gas flowrates. When the flowrate increased, CH_4 content decreased.	98.2% at raw gas flowrate of 19.6 mL/h.	0.8-1.9 A/m^2, it decreased with the COD consumption at all raw gas flowrates	The MEC consisted of anode (50 mL), regeneration (50 mL) and cathode(100 mL) chambers separated by 25 cm^2 of BPM and AEM respectively. Anode electrode was preacclimated earlier in an MFC. Effluent gas of the cathode was also recirculated in cathode for mixing and CO^2 capturing.	[112]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
SC-0.13 L	Batch, 30ºC. Sodium acetate, 1 g/L; (At start up, 50:50 mixture of inocula and wastewater was used); Applied voltage was 0.8 V.	Graphite brush with 24 bunches of graphite fiber, each 4 cm length, 40 mg	7 cm^2 carbon cloth with 0.5 mg/cm^2 platinum catalyst	84-93 L/m3/d CH_4; Max. 0.33 L/g COD.		Max. 72±5 A/m^3, Ave. 66±5 A/m^3	Total operation time of all cycles was 2500 h including start-up. There was 95% CH_4 in biogas during the cycles. The medium was replaced with the new one when current was less than 1 mA in the reactors.	[113]
SC-0.4 L	Batch, 38ºC. Inoculum and synthetic wastewater ratio was, I/S=0,6 . COD of the subst.=15.35 g/L; C/N ratio of substrate=20; 0.8 V (1000 Ω).	4x3 cm^2 carbon cloth, cobalt phosphorous catalysts deposited electrodes	4x3 cm^2 stainless steel mesh, cobalt phosphorous catalysts deposited electrodes	146-152 mL CH_4/g VS	64-65%	~0.3 A/m^2 (anode area)	Distance between electrodes was 2 cm. Cobalt phosphorous (CoPi) catalysts were deposited on the electrodes to increase endogenous hydrogen utilization.	[114]
Combined AD+MEC; 20 L, cylindrical	Sequencing batch (SBR), 35ºC. Food waste; 60 g TCOD/L; OLR=3 kg/m^3/d, HRT=20days; 0.3 V	Three 15x30 cm^2 graphite carbon meshes coated with Ni	Three 15x30 cm^2 graphite carbon meshes coated with Ni, Cu and Fe	17 L CH_4/d; 0.34 L CH_4/g $COD_{rem.}$	76% COD; 73 % VS		OLR was 3 kg/m^3/d. AD and combined AD+MEC reactors were contrasted due to methane production for start-up, intermediate and final stages. Electrode construction was same in [54].	[115]
SC-0.3 L, cylindrical	Batch, 32ºC. Cheese whey (CW), 19.9 g COD/L; Cycle period=2 days, pH was set to 7.	60 cm^2 graphite felt, distance of electrodes was 4 cm. Anode was taken from a 2 weeks working MFC.	71 cm^2 stainless steel	Ca. 0.37 L biogas / L. %41 H_2 and %45 CH_4		0.13-0.23 mA/cm^2 (CW was first treated in CSTR and DFR)	Raw CW was directly fed to the MEC, 75 mL phosphate buffer and the rest 225 mL of CW were used in MECs. After the cyle, pH of the CW was 3.8 due to the VFAs accumulation which was needed to be treated.	[116]
SC-15 L (d:28 cm, h: 41 cm), cylindrical	Fed-batch (SBR), 35ºC. Food waste, TCOD : 63,000 mg/L, VS :37,000 mg/L. OLR= 2 kg VS/m^3/d, HRT=20 days. Applied voltage was 0.3 V.	15x30 cm^2, 6 sets of anodes composed of graphite mesh coated with Ni	15x30 cm^2, 6 sets of graphite mesh cathodes coated with Ni and complex metal catalyst (Fe, Cu, Mn)	0.56 L CH_4/g $VS_{rem.}$or 0.34 L CH_4/g $COD_{rem.}$			Anodes and cathodes were combined together with a non-woven fabric(1mm thick) placed btw. electrodes. In MEC, CH_4 production enhanced due to the increased bacterial populations, especially *Methanosarcina thermophila* and *Methanobacterium formicicum* compared to AD.	[117]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
Upflow-1L (empty bed volume)	Continuous, 24ºC. Acetate based synthetic WW and NH_4-N rich synthetic WW, flowrate 1.6 L/d. HRT=14 h. Influent COD:1-1.2 g COD/L, 1.3 V was applied for acetate based WW. 0.5-0.65 g COD/L (NH_4-N rich WW) 0.6-0.75 V was applied for NH_4-N rich WW.	Anode was made up of 10 pieces of carbon felt put (05.x1x1 cm^3) on top of each other to form 10 cm height of compartment. Titanium rods inserted in them to collect e-.	Cathode was made up of 10 pieces of crb. felt put (05.x1x1 cm^3) on top of each other to form 10 cm height of compartment. Titanium rods inserted in them to collect e-.	0.27 L CH_4/g COD for acetate WW, 0.22 L CH_4/g COD for ammonium rich WW	81-87%	32±5mA/L for acetate fed MEC at 1.3 V. 15±5mA/L for NH_4-N rich WW fed MEC at 0.6-0.75 V.	Inoculum for MECs was the effluent of an MFC.The carbon felt pieces (0.5x1x1 cm^3) make 10 cm heigth of two anodes and two cathodes compartments seperated with geotextile. At the condition of non-voltage application (only AD), methane production decrased. COD removals were not effected from the voltage application. The lowest methane production occured when real domestic WW was used.	[118]
TC- H type bottles, 0.2 L each chamber	Batch cycles, 35ºC. Cathode was sparged with CO_2 for about 10 min. for %100 CO_2 saturation in catholyte as substrate (e- donor). In every cycle, solutions of both chambers, membrane and abiotic anode were replaced (new-fresh). Set cathode potential -0.7 V (vs SHE) was used.	Graphite felt, 4x2x0.5 cm^3 for the anode.Synthetic buffer and mineral solutions at both chambers.	Graphite felt 4x1x0.5 cm^3 for the cathode.Synthetic buffer and mineral solutions at both chambers.	97.7% conversion of CO_2 to CH_4 after 863 hour (5.41 mmol CH_4). And max. yield 384 mmol CH_4/m^2.d	10-98% removal of CO_2 over different cycle periods	2-5.5 mA over four different cycles	A nafion PEM (6.6cm^2) was used to separate the chambers. Buffer and synthetic solution were used at both chambers. Only cathode chamber was inoculated. CO_2 was fed to the reactors as substrate. *Methanobacterium* was the dominant species among the cathode methanogens. Most archaeal sequences (>89%) were assigned to a *Methanobacterium palustre*- related OTU.	[119]
TC-1.3 L (Cathode-0.7 L, anode 0.6 L)	Batch, 35ºC. The COD(acetate) and SO_4^{2-} conc. of the cathode medium was fixed at 3 g/L and 1.5 g/L respectively and mixed with inoculum. Anode medium had COD (acetate) conc. of 1 g/L and no inoculum. pH was adjusted to 7 at the beginning and buffer solution was used. Cycle time: 36 h. Set cathode potential (-0.8 V vs SHE).	Carbon felt, anode chamber was the outer cylinder.	Carbon felt covered with a Pt catalyst layer (0.5 mg-Pt/cm^2) on one side. Cathode chamber was the inner chamber.	Max. 0.91 m3 CH_4/m^3 reactor	86±3% at the cathode, 76±3% at the anode	9.5A/m^3 (based on total AD-MEC volume)	The chambers were cylindrical and the cathode chamber was placed in the anode chamber. CEM seperated the chambers. Electrodes were next to chamber walls. Cathode medium was the anerobic digestion zone with the inoculum and acetate mixture. Anode zone constitued organic solution. H_2S (sulfide) inhibition did not occur in MEC-AD but it oocured in AD only.	[120]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
SC-0.18 L	Fed-batch, 22-23ºC. Raw sludge conc. was 7.9±2 g COD/L. SRT of 7, 10 and 14 days were applied. Feeding was done by the replacement of a cal. amount of treated waste with the raw waste in every 7 days to maintain the needed SRT. 1.2 V.	3 carbon fiber brush(2 cm diameter x 2.5 cm lenght)	Stainless steel mesh with total projected area of 135 cm^2, wrapped around the MEC interior wall	95 % of the biogas was CH_4. Calculated production was 25.6 to 14.0 mL CH_4/d for SRT 7, 10, 14 d.	30-34 % at all SRTs	15 - 25 A/m^3 for 7 days SRT	Carbon fiber brushes were pretreated. No catalyts were used on cathode.The reactors were started with an influent containing 50% digested sludge and 50% waste activated sludge (WAS).	[121]
MEC-Stacked, 33 L	Batch, 22ºC. Feed: waste activated sludge, average COD of 12 g/L. 0.01 M phosphate buffer of pH=7 was used. 0.75, 1.5 and 2 V was used.	10 casette type reticulated vitreous carbon mm(20x1.8x 13 cm^3)(2.9m^2 per anode)	9 casette type nickel steel (19x13x0.2 cm^3)	Cumultv. biogas of ~5.3 L at 70 days. 68.7% CH_4 at 2 V, 62.5% CH_4 at 1.5 V.	82.6% at 2 V 20.2% at 1.5 V	5.5-6.5 mA	The anode and cathode casettes were placed in 33 L tank and operated as a single chamber MEC without using any membrane. No hydrogen were detected in biogas although high voltage (1.5-2 V) application.	[122]
Upflow-MEC, 0.6 L, l=35 cm, d=5 cm	Continuous, 30ºC. Phosphate buffer added to artificial beer wastewater. COD conc. of 1.5-2.0 g/L. HRT=24 h., Applied voltage was 0.8 V.	Granular graphite	Stainless steel (SS), nickel and copper meshes were used as cathodes	143 mL/g COD (Ni cat.), 367 mL CH_4/L/d.	85%-Ni cat., 79% SS cat., 69% Cu cat.	8.6 mA in MEC with Ni cathode.	Distance btw. electrodes was 3 cm. Stainless steel(SS), nickel and copper meshes were compared in three dif. reactors. Ni cathode was superior compared to others. 10 mL of domestic WW was used as inoculum.	[123]
SC-0.008 L, cylindrical	Batch, 60ºC. 8 mL of *M. thermautotrophicus* cell suspension was inoculated into the MECs. NO_2 and CO_2 mxture (80/20:v/v) was sparged to the MECs. Voltages of 0.5 to 1.0 V were applied.	Plain carbon paper, 3 cm2.	Plain carbon paper, 3 cm2, coated with carbon layer (2.5 mg/cm2)	87.9 mmol/d/m^2 (cathode surface area)		0.2-0.5 mA/m2	A nylon filter placed btw. anode and cathode to prevent contact of electrodes to each other. *Methanothermobacter thermautotrophicus* strain was specially cultured earlier for use.	[124]
TC-2x0.86 L, rectangular: 17×17×3 cm^3	Continuous flow mode for anode, 24-26ºC. In anode: synt. WW with acetate, OLR=1.08 g COD/L/d, HRT=0.6 d. Synt. WW was flushed with N_2/CO_2 (70/30) mixture. In cathode: synt.medium without acetate. Catholyte was flushed with N_2/CO_2 (70/30) mix. and catholyte was recirculated. The anode was controlled at +0.2 V (vs. SHE).	Graphite granules, diameter btw. 2 and 6 mm. Graphite rod was inserted into the granules.	Graphite granules, diameter btw. 2 and 6 mm. Graphite rod was inserted into the granules.	0.28 L CH_4/ L/d (91.2 meq/L/d)	Acetate removal of 94 ± 1%	Average 90-120 mA/d	PEM was used to separate the chambers. After granules were placed in chambers, a bed porosity of 0.48 was occured. 103 mL of catholyte was removed everyday because the same amount was coming from the anode through PEM. Electrodes and PEM were pretreated and pH was controlled at the begining. pH control in catholyte was maintained by N_2/CO_2 mixture.	[125]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
TC-2x0.86 L, rectangular: 17×17×3 cm^3	Batch, 21-25ºC. Recirculation of both anolyte and catholyte. In anode: synthetic medium with acetate(10-15 mM). In cathode: synthetic medium. The anode was controlled at +0.5 V (vs. SHE).	Graphite granules, diameter btw. 2 and 6 mm. Graphite rod was inserted into the granules.	Graphite granules, diameter btw. 2 and 6 mm. Graphite rod was inserted into the granules.	0.018 L CH_4/L/d		6 - 9 mA	Anode and cathode chambers were inoculated with a mixed hydrogenophilic methanogenic culture and a *G. sulfurreducens* culture during the study. Anolyte and catholyte medium were refreshed by 15% weekly. The pH was adjusted at both chambers using $NaHCO_3$ or HCl.	[126]
Tubular TC, 25 mL anode + 40 mL cathode	Batch, 25ºC. Synthetic medium with 12.2 mM sodium acetate was placed in anode inoculated with earlier MFC study. Synthetic medium and WW plant sludge mixture were placed in cathode. App. voltages of 0.1, 0.2, 0.3, 0.5 and 0.7 V were tried.	Graphite fiber (inner chamber)	Porous graphite felt (outer chamber)	0.113 ± 0.000 mol CH_4/mol COD (at 0.2 V)		34.2 A/m^3 (at 0.7 V)	A cylindrical CEM (d:4 cm, h:6.4 cm) was centrally located inside, forming an outer cathode and an inner anode chambers. Both the anolyte and the catholyte were renewed every 2–3 days. Cobalt, Co(II), was also introduced to cathode chm. at dif. conc. Co(II) reduction of 88% was achieved in biocathode.	[127]
SC, 0.5 L, cylindrical	Batch, 25ºC. 50:50 mixture of the domestic WW and a medium with acetate and buffer was used at the start up and steady state period. 0.7 to 1.3 V of voltages applied to the MEC.	Carbon cloth (30x10 cm^2)	Nickel foam (30x10 cm^2)	0.08 to 0.17 L/L/d	62±4% to 76±5; 0.15 to 0.34 g COD/L/d	46–132 mA/L between voltages of 07 and 1.3 V.	A membrane electrode assembly (MEA) was made by putting an AEM between the electrodes. The MEA was rolled into a compact structure and fitted into the reactor. Energy balance of the methanogenic spiral-wound-electrode MEC was at highest at 0.7 V. Optimal app. voltage was determined by balancing the COD removal and energy efficiency that was 0.95 V.	[128]
SC-0.55 L, cylindrical	Batch, 20ºC. Substrate: pretreated waste activated sludge with 15.65 g TCOD/L. 40 days of batch operation. Applied voltage was 0.8 V.	Graphite brush (d:4 cm × h:8 cm; 1.375 m^2)	Carbon cloth (d:3 cm; 7 cm^2 (0.5 mg Pt/cm^2 on one side))	39.0 ± 14.0 mL/d; 56.4 mL CH_4/L/d until day 18th (phase 1)	41.2% TCOD; 48.5% VSS at the end of 40 days	1-3.5 mA	The distance btw. the electrodes was 2 cm. The anode brush was enriched prior to use. WAS was pretreated by alkali coupled with ultrasonic treatment. The volume of WAS was 500 mL and inoculation was 50 mL. In phase 2 (18-40 days) CH_4 production decreased.	[129]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
SC- 3 L total volume, cylindrical	Continuous fllow, 21°C. Synthetic WW with acetate (450 mg COD/L) and domestic WW (78 mg COD/L)were fed to the MEC with HRTs of 4, 8, 12 and 24 h at different stages of the experiment. Applied voltage of 1.0 V.	21x10x1 cm^3 one layer of carbon felt	21x10 cm^2 stainless steel electrode	0.061 L CH_4/L/d for synthetic WW at 8 h HRT. 0.012 L CH_4/L/d for domestic WW at 24 h HRT.	~50% for synt. WW and real WW at 8 h and 24 h HRTs respct.	Synthetic WW and domestic WW were utilized as feed flow for the MEC at different periods. One piece of polyester cloth (0.6 mm thick) was placed strictly between the anode and the cathode to prevent short circuit. Net energy balance: 0.7 kWh/kg $COD_{rem.}$ (Synthetic WW); -0.1 kWh/kg $COD_{rem.}$(Domestic WW) at 24 h HRT.		[130]
TC-Integrated (0.7 L catho.+0.5 L anode)	Continuous, 25°C at HRT of 1 day. Acetate(1.5 g/L) and glucose(2.5 g/L in 50 mMPBS) were used as the carbon sources in the anode and cathode respectively. OLR=2.5 $kg/m^3/d$ of glucose. Cathode was the AD unit for CH_4 production. App. voltage to the MEC was 0.8 V.	The anode was made of carbon brush, placed in the external tube.	Stainless steel mesh, placed close to the AEM in the inner cylinder.	0.07 L CH_4/L/d for glucose; 0.247 L CH_4/L/d for SFL	40-60% for for both glucose and SFL	9-11 mA	AEM was used to separate the chambers. The cathode was placed in the inner cylinder and the anode was the outer cylinder. Reactors were inoculated with WAS. Sludge fermentation liquid(SFL) containing acetate, polysaccharide, protein and VFAs was also used for cathode subsequently. OLR of SFL was 3.8 $kg/m^3/d$.	[131]
TC-2x0.86 L, rectangular: 17×17×3 cm^3	Continuous flow mode for anode, 25°C. In anode: synt. WW with acetate, OLR=1.08 g COD/L/d, HRT=0.6 d. Synt. WW was flushed with N_2/CO_2 (70/30) mixture. In cathode: synt.medium without acetate. Catholyte was flushed with N_2/CO_2 (70/30) mixture. And catholyte was recirculated. Anode potential was set at values btw. +0.2 V and −0.2 V (vs. SHE)	Graphite granules, diameter btw. 2 and 6 mm. Graphite rod was inserted into the granules.	Graphite granules, diameter btw. 2 and 6 mm. Graphite rod was inserted into the granules.	Changed from 4.2 mmol/L day 9.7 mmol/L day when anode set potential arranged btw. -0.2 V to -0.1 V	Acetate removal changed from 35% to 88% when set potential varied from -0.2 V to -0.1 V	Changed from 37 mA to 91 mA when set potential changed from -0.2V to -0.1V	PEM was used to separate the chambers. The min. distance btw. graphite granules at the anode and cathode chambers was less than 0.5 cm. 103 mL of catholyte was removed everyday because the same amount was coming from the anode through PEM resulting in an HRT of 8.35 d. Electrodes and PEM were pretreated. pH was controlled in catholyte by gas mixture of N_2/CO_2. And it was also the carbon source for catholyte.	[132]
SC-0.625 L, d:10 cm, h:10 cm, cylindrical	SBR, 35°C. Fed with acetate and discharged once daily. The OLR and HRT were 2.0 kg $COD/m^3/d$ and 20 days respct. Electrodes were placed at distances of 1, 3 and 5 cm from each other. Mixing velocity was also changed btw. 30 and 60 rpm. Applied voltage was 0.3 V.	Graphite carbon coated with nickel was used as the anode (64 cm^2).	Graphite carbon coated with copper, iron, and nickel was used as the cathode (64 cm^2).	0.33-0.34 L CH_4/g $COD_{rem.}$(0.26-0.27 L/L/d) for 1 cm distance.	71-78% at all MECs, but higher in small distances	0.69-0.72 A/m^2 for 1 cm distance; 0.06-0.15 A/m^2 for 5 cm distance	Reactors were inoculated with anaerobic sludge. Small distances btw. electrodes were very crucial in terms of performance. Higher mixing velocity enhanced the CH_4 production at higher electrode distances (3-5 cm). Small distance of electrodes had the best CH_4 production and performance.	[133]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
SC(0.35 L), TC(0.5 L anode+0.35L cathode)	SBR, 55°C. 3.5 mL glucose(1 M) was fed as substrate to MECs daily. In TC anode chm. 350 mL of 150 mM $NaHCO_3$ solution was used as the electrolyte. 350 mL of thermophilic inoculum was filled to both SC and cathode of TC MEC. Set cathode potential of -0.8, -1.0 and -1.2 V (vs.Ag/AgCl) were applied.	TC: grapthite rod(h:15 cm, d:2.5 mm); SC: grapthite rod(h:15 cm, d:2.5 mm)	TC:carbon felt (w: 6 cm, l:22 cm); SC:carbon felt (w: 6 cm, l:22 cm)	TC: 75-93% CH_4, ~0.7 L/L/d; SC: 54% CH_4, ~0.5 L/L/d at -1.2 V. TC: 60-77% CH_4, ~1.0 L/L/d; SC: 56% CH_4, ~1.0 L/L/d at -1.0 V		TC:10 mA, SC: 2 mA at -1.0 V; TC: btw. -20 and -40 mA at -1.2 V	SC and TC reactors were compared in terms of their performances. CEM was used to separate the chambers of TC MEC. The distance between TC electrodes was 10 cm. Thermophilic anaerobic inoculum was obtained from a full-scale commercial anaerobic digester. CH_4 content was higher in TC and SC MECs compared to control.	[134]
SC- 2.4 L (26.7cm x 11.5cm x 8.8cm)	Continuous flow, 30°C. The medium contained 1 g/L acetic acid with buffer. HRT was 1 day. Anodes and cathodes were grouped into four each with two pairs of anode and cathode. 0.9 V was applied.	8 graphite brushes (d: 6 cm, h:7 cm) preacclimated. Half of the brushes were cut into half cylinder.	8 pieces of stainless steel 304 mesh 8.5x9 cm^2.They were cascade folded. Total area was 0.153 m^2.	0.118 L CH_4/L/d	Between 31-47%	181 mA (1.18 A/m^2, cathode surface area; 74 A/m^3	Pairs of electrodes were distinguised by 4(7.5x11 cm^2) separators with holes on it. At the first 10 days of operation there was significant H_2 production from MEC. On the 3rd day max. H_2 production was 0.53 L/L/d. However it decreased later on and became very few.	[135]
SC-0.17 L, barrel shaped stainless steel MEC	Batch, 30°C. 150 mL medium using acetate (10 g/L) and 20 mL of WAS were added to the MECs. Applied voltages were 0.4 and 1.0 V.	Anode was carbon felt of 2.0×5.0 cm^2 pretreated	Stainless steel cathode (d:5.0 cm; h: 9.2 cm), the wall of the MEC	Cumulative CH_4 of 293±7 mL for 0.4 V, 340±11 mL for 1.0 V.	100% COD removal in 72 hours in MECs	20-25 mA for 1.0 V, 0-3 mA for 0.4 V	The wall of the MEC was stainless steel. pH of the all reactors was adjusted to 7 at the begining. CH_4 content of 98% was achieved in MECs without upgrading the biogas. H_2 was also produced at the 1.0 V applied MEC but not in the 0.4 V applied MEC.	[136]
SC-0.23 L, barrel shaped stainless steel MEC	Batch, 25°C. Feed was 10 g/L acetate with buffer. The MEC was inoculated with WAS(2 mL), Geobacter containing inoculum(2 mL) and *Methanosarcina sp.* culture(2 mL). Voltage input was 1.0 V.	Anode was carbon felt 5.0×5.0 cm^2, pretreated	Stainless steel cathode (d:10.0 cm; h: 7.6 cm), the wall of the MEC	360.2 mL/g COD for co-cultivation of Geobacter and Methanosarcina	100% COD removal in 72 hours in MECs	50-80 mA	Co-cultivating *Geobacter* with *Methanosarcina* in ain integrated MEC and AD system achieved 24% more CH_4 production compared to same MEC without bacteria selection. Microorganism anlysis showed that *Geobacter* and *Methanosarcina* could cohabit together in the biofilm.	[137]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
SC-2.1 L, cylindrical CSTR	Batch, 22ºC. A mix. of dextrin/peptone stock solution was fed in every 3 or 4 days (cyle period), resulting a conc.of 1.03/1.37 g COD/L. OLR=0.343 g COD/L/d, HRT=21 d. Voltage applications of 0.5, 1.1 and 2 V were compared.	4 Carbon felt strips(15.24x2.54 x2.54 cm^3) attached to the SS rod collectors (d:6 mm)	4 Carbon felt strips(15.24x2.54x 2.54 cm^3) attached to the SS rod collectors (d:6 mm)	0.7 L CH_4 at OC; 0.7 L CH_4 at 0.5 V; 0.88 L CH_4 at 2.0 V all in 4 days	%80 removal at both applied potential	Max. 30 mA at 2.0 V	The results were obtained after a batch cycle. Suspended biomass and biofilm in the hybrid system had a greater ability to survive a shock organic loading than only suspended biomass. CH_4 productions of MECs at the first 2 days of 4 days batch cycle were at least 30% more than control reactors.	[138]
TC-0.85 L (d:10cm x h:16.5cm)	Continuous flow, 37ºC. Inoculum: anerobic sludge. Synthetic medium with acetate (differing from 0.25 to 0.6 g COD/L) was used. The influent flow rate to cathode was 600 mL/d with the HRT of 1.4 d. The cathode was poised at −0.5 V (vs SHE).	Graphite rod anode (d:0.6cm, h: 0.8cm) was wrapped by a CEM(d:3 cm, h:13cm) and put in cathode chamber	One graphite plate (6×2.5×0.6 cm^3)	CH_4 content increased to >90 (4-6 mmol/L) and CO_2 content decreased to 4.6% after power application	COD removal increased to >95% after power application		Anode was in the cathode chamber in SC-MEC. Synthetic medium without acetate was placed in anode and it was continuously bubbled with a slow stream of N_2/CO_2 (80:20). At the period of power application flow conc. was 0.6 g COD/L. *Methanothrix* was the most significant member of the archaeal community (%77) at all conditions.	[139]
SC-1 L, cylindrical	Batch, 55ºC (thermophilic). 0.8 L WAS (34g TCOD/L; 19 g VS/L) and 0.2 L inoculum was used in reactors. MECs with/without biochar was compared due to performances. Applied voltage was 0.6 V.	Carbon felt 8×8 cm; thickness: 5 mm	Carbon felt 8×8 cm; thickness: 5 mm	CH_4 yield of biochar added MEC was %25 (~85 $mL/VS_{add.}$) more than AD in 22 days	~41% and ~%39 VS removal with and without biochar respct.	0.8–3.5 A/m^2 in MEC with biochar	Distance between electrodes was 1 cm. WAS was pretreated chemically, physically to make biochar powder with diameter of 5 µm to enhance CH_4 production in the MECs. CH_4 yield of MEC without biochar was ~80 $mL/VS_{add.}$in 22 days.	[140]
TC-0.2 L	Batch, 35ºC. Both chambers were filled with synthetic medium with yeast extract. After each cylces synthetic medium was refreshed. Different carbon based electrode material all with approx. 11 cm2 surface area were used. Cathode potential was set to −0.9 V vs. Ag/AgCl.	Platinum(d:0.5 mm, h: 23.0 cm) was used as the anode in all experimental scenarios	Carbon stick(CS), CS twined with Ti wire, CS covered with carbon fiber, CS wrapped in graphite felt, CS packed by carbon cloth	31.00 mL/L/d at the first cycle, 75.7 mL/L/d in the cycle 12 for CS wrapped in graphite felt (CS-GF)	Almost 90% of CO_2 was removed at the 12. cycle (CS-GF)		PEM (working surface area=4 cm^2) was used to separate the chambers. The distance btw. electrodes was 4 cm. Cathodes were inoculated with CH_4-producing culture. The cathode chamber was flushed with 0.3 L/min pure CO_2(99.99%) as the carbon source for 30 min in each batch cycle (24 h/cycle).	[141]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
SC-0.115 L, cylindrical	Batch, 25ºC. Phosphate buffer and different amounts of 2,4,6-trichlorophenol (TCP) was used as substrates. The MEC-AGS reactor were inoculated with 3.13 g AGS after star-up period. The applied voltage was 0.8 V.	Porous carbon felt (3×2.5×2 cm^3). Anode was set in the center of the MEC.	Stainless steel mesh (35×2.5 cm^2). Cathode was attached to the inner wall of the MEC.	57 mL total for MEC-AGS and 18 mL total for MEC in 5 days.	23-46% of TCP removal for MEC-AGS and 15-38% for MEC	40-90 A/m^3 in MEAGS; 40-120 A/m^3 in MEC. Higher the TCP conc., lower the current dens.	An ordinary MEC and MEC coupled with AGS system was compared. The distance between electrodes was 3 cm. Municipal WW was the inoculum. AGS was pretreated before feeding to the MEC. Higher the TCP was, higher the inhibition on the MEC-AGS and MEC.	[142]
SC-0.42 L cylindrical (d:8, h:10)	Batch, 35ºC. Diluted sludge hydrolysate was used as the substrate, COD: 2.4 g/L. After start-up, in each cycle 420 mL substrate was fed to the MECs. Wollastonite was added to the MECs to see the effects. Batch cycle period:4 days Applied voltage was 0.8 V.	Carbon fiber brush (d:6 cm, h:8 cm) was fixed in the center of MEC.	Carbon cloth (l:25.5cm, w:10 cm) coated with Pt-carbon catalyst (0.5 mg/cm^2) fixed on the inner wall of the MEC.	188−200 CH_4 mL in each cycle, average 282 mL/g COD, CH_4 content in biogas: 88.5–90.4 %	64.9 %–72.0 % in each cyle (4 days)	4-12 mA with wollastonite	Wollastonite (19g/L) was placed in the MECs to determine the effects. $^{12}CO_2$ and $^{13}CO_2$ were fed to the MECs for microbial culture identification. Wollastonite enhanced COD removal at 8.4% rate and decreased CO_2 content at 4.1% rate.	[143]
SC-0.8 L, cylindrical	Batch, 38ºC. Swine manure(SM), TS=23.6%, VS=19.5%, C/N=16:1. 228.8 g sample of SM, 160 g inoculum (20% m/m), and 411.2 g pure water were placed in MECs. 24 days of batch operation. Different mode of voltage application was briefed in explanation column.	Carbon felt (3x6 cm^2), pretreated	Titanium mesh (3x6 cm^2), pretreated	VGG:12 L CH_4 cumulative (222 m^3/t dry SM), GG: 9.1 L CH4 cumltv. (168.4 m^3/t dry SM)	Dissolved COD removal for VGG: 59.4%; GG: 53.9%		Inoculum (TS=6.3%, VS=4.35%, C/N=22:1) was taken from an anaerobic full-scale CSTR. The distance btw. electrodes was 1 cm. App. voltage was 2.5 V for 1 h/day for an MEC(VGG) (24 days). 24 hours of 2.5 V was app. just once prior to operation for another MEC(GG). Graphene was in-situ formed in MECs by 2.5 V app.	[144]
SC-0.52 L, cylindrical	Batch, 25ºC. MECs were fed with 0.2 L of supernatants of the raw WAS, WAS-Fenton and two diff. AD(3 day) effluents (inoculum+WAS and inoculum+WAS-Fenton) and 0.3 L of PBS. 6 days of batch operation. Applied voltage was 1.0 V.	Carbon brush	Stainless steel mesh with a projective area of 78 cm^2 (13x6)	Highest in MEC(WAS-Fenton-AD):~162 mL CH_4/L/d (178 mL biogas/L/d)	Highest SCOD removal in MEC (WAS-Fenton-AD) 26.12%	Highest 24 mA in MEC (WAS-Fenton-AD)	WAS was pretreated with Fe(III)/PCA/H_2O_2 at circumneutral pH (WAS-Fenton), and WAS without pretreatment was used as control. At the start up of the MECs, MFC effluent was used as inoculum and acetate was used as substrate with phosphate buffer.	[145]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
SC-12 L, cylindrical (d:24 cm)	SBR, 35ºC. Substrate was mixed sewage sludge (TCOD:36.6g/L, VS:28.8 g/L). HRT was 20, 15, 10, and 5 days (by increasing of the sewage sludge feeding rate). OLR were in the range of 1.44 to 5.76 kg VS/m^3/d. Applied voltage was 0.3 V.	Modified graphite fiber fabric (GFF) was screen printed with a mixture paste of the MWCNT and exfoliated graphite (EG)	GFF modified with a multiwall carbon nanotube (MWCNT) and nickel	CH_4 yield was 369 (20 days) to 479 mL CH4/g $COD_{rem.}$(10 days); Max. Production:1.34 L/L/d at 5 days HRT	64% to 39% decreasing by decreasing HRT from 20 to 5 days.		The separator and electrode assembly (SEA, 6×24 cm^2) was prepared by stacking the anode, a polypropylene nonwoven sheet as a separator, and the cathode in order. SEAs were constructed inside of the reactor. At the start up, 40% of the whole medium was inoculum (15.6 g TS/L; 10.6 g VS/L), the rest was sewage sludge.	[146]
SC-12 L, cylindrical (d:24 cm)	SBR, 25ºC. Substrate was sewage sludge (TCOD= 31.7-47 g/L and VS=43,3–51 mg/L) HRT=20 days. Applied voltages were 0.3, 0.5 and 0.7 V.	Modified GFF with MWCNT and (EG)	GFF modified with MWCNT and nickel	370 mL/L/d for 0.3 V. 346 mL/L/d for 0.5 V. 350 and 330 mL/g $COD_{rem.}$for 0.5 V and 0.3 V respectively	~55% COD and 64-66% VS removal for both 0.3 and 0.5 V		The separator and electrode assembly(SEA, 6×24 cm^2) was prepared by stacking the anode, a polypropylene nonwoven sheet as a separator, and the cathode in order. SEAs were fixed inside of the reactor. The dominant species of planktonic anaerobic bacteria was *Cloacamonas* at 0.3 V and 0.5 V.	[147]
SC-0.8 L, cylindrical	Fed batch mode(3 times feeding in a week), 34ºC. Anaerobic sludge from WW plant was the inoculum (10 g VSS/L). SRT was 20 days. Molasses was as a biorefinery sidestream was the substrate (OLR=2 g COD/L/D). Applied voltages were 0.5, 1.0 V and open circuit.	Carbon felt (60cm^2), projected surface area to volume ratio of 0.015 m^2/L reactor.	Carbon felt (60cm^2), projected surface area to volume ratio of 0.015 m^2/L reactor.	0.57-0.59 mL CH_4/L/d for MECs including open circuit MEC also.		3.4±3.3 - 6.44±4.77 A/m^2 for both applied voltages	The distance btw. electrodes was 1 cm. Working electrodes after 60 days in an MEC, were moved to another control reactor to make a new MEC to determine the biomass effect on performance. *Methanosaeta* was the dominant acetotrophic methanogen on the electrodes free of power supplementation, indicating the importance of biomass retention.	[148]
SC- 0.5 L, (d:7 cm, h:18 cm), cylindrical	Batch, 20-25ºC. The substrate was WAS(pretreated by applying ultrasonic energy density) with COD conc. of TCOD:17.5 g/L. 0.5 L of WAS was placed in the MEC. Batch operation for 35 days. Applied voltage was 0.8 V.	The anode was a graphite brush enriched with biofilm (d:4 cm h:8 cm; 1.01 m^2 surface area)	The cathode was a carbon cloth (d:4 cm)	138 mL CH_4/L reactor/d, ~175 mL CH_4/g VSS	48 % of VSS removal	10-12 mA	Anode was enriched previously in a different MEC. Cathode was new. The distance between bottom of the cathode and top of the anode brush was 1 cm. Hydrogenotrophic methanogens and acetobacterium were substantially enriched in cathode biofilm.	[149]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
SC-0.7 L, cylindrical	Batch, 35ºC. Synthetic beer industry wastewater, composed of beer, PBS, and trace elements. COD: 1.125 g/L; NH_3-N: 28 g/L. Batch cyle was 48 hour. Applied voltage changed between 0.5 to 0.9 V.	Graphite fiber brushes with a spatial volume of 78.5 cm3 (d: 5 cm, h: 4 cm)	Cathode was made up of different layers of circular SS mesh(d: 5 cm) connected in series with 5 mm interspace.	0.14 L/L reactor; 257 mL CH_4/g COD for 0.9 V application	65-80% for all MECs	415 mA/m^2 for 0.9 V; 664 mA/m^2 for 0.7 V	The stacked cathode layers were different for different reactors: 2 layers, 5 layers and 8 layers. The distance between electrodes 3 cm. MECs were started with mixture of WAS and fed with synthetic medium (2 g/L NaAc., 50 mM phosphate buffer).	[150]
SC-0.5 , cylindrical	Batch, at 35ºC. All MECs were inoculated with 100 mL of anaerobic sludge (35°C; 8.7 gVS/L; 19.6 g COD/L) and 400 mL of swine manure (5.8 gVS/L; 8 g COD/L) obtained from a swine manure treatment plant. Voltages of 0.1, 0.3, 0.5, 0.7, and 0.9 V were used.	Graphite felt (6×6 cm^2) was used as the anode and the cathode. A stainless steel bar (d: 0.1 mm, l: 20 cm) was used as the collector.		2.92 L CH_4/L for 0.7 V application.	COD: 75-79 % and VS: 50-55 % for all voltages		Different temperatures and different voltage supplies were used for MECs. A CSTR control reactor and an MEC with open circuit were used as control reactors. When different temperatures of 25, 35, 45 oC were applied, the best performance of 3.7 L CH_4/L was obtained at 45ºC.	[151]
Upflow MEC- 5.6 L, cylindrical	SBR, 35ºC. Synthetic brewery wastewater (65.3 g COD/L). OLR and HRT were 5.8 g COD L/d and 5.6 d respectively. Applied voltages were 0.5 and 1.0 V.	Graphite rod electrodes (d:6 mm×h:300 mm)	Graphite rod electrodes (d:6 mm×h:300 mm)	0.91 and 1.16 L/L/d for reactors with GAC and PAC respct. at 0.5 V.		6.1–7.9 mA at MEC with PAC	Two types of coal-based activated carbon with different particle sizes (5 g/L), GAC (0.84–2.00 mm) and PAC (powdered activated carbon)(75–177 μm) were added into both reactors to compare their performances.	[152]
SC-1 L cylindrical	Batch, 35ºC. Inocula was the effluent of an MEC. Reactor start-up was made through synthetic WW with acetate. The substrates were changed to 0.7 L pretreated WAS (14 g VSS/L) in the operation phase. Applied voltage was 0.8 V.	The anodes were carbon fiber brushes (d:5 cm, h: 5 cm). Surface area of 1.02 m^2	Stainless steel (304 type), copper (purity >99%) and nickel (purity > 99%)	59.2 mL CH_4/gVSS for Ni cathode		9 A/m^2 with Ni mesh cathode	3 types of cathode were investigated. All cathodes were at diameter of 2.5 cm, thickness of 1 mm, and pore diameter of 75 μm. The initial pH of WAS was adjusted to 10. Ni was the best cathode material amongs the others. Low anode potential was needed for Ni cathode. *Methanobacterium* was observed on cathode electrodes. They can use both electrons and H_2 to form CH_4.	[153]

Reactor type/ volume	Operation; Substrate; OLR/HRT/Feed cycle; Applied voltage	Anode	Cathode	Methane production	COD Removal	Energy/ current production	Explanation	Ref.
Combined AD+MEC; 20 L, cylindrical	SBR, 35ºC. Food waste; 171± 38 g TCOD/L; OLR=8 kg TCOD/m^3/d, HRT=20days; Electrodes had biofilm on them readily when the reactors were settled up. Inocula was from an earlier MEC and AD study. Applied voltage was 0.3 V.	Three 8x25 cm^2 graphite carbon meshes coated with Ni. Electrodes had biofilm them readily.	Three 8x25 cm^2 graphite carbon meshes coated with Ni, Cu and Fe	62±2 L/d and 0.36 L CH_4/g $COD_{rem.}$ (at the stabilized period)	Cal. ~88 % of TCOD removal at the stabilized period		AD and AD+MEC reactors were compared in terms of CH_4 yield and CH_4 production for dif. periods. Electrode construction was same as in [54]. AD+MEC stabilized 40 days faster than AD only.	[154]
SC-0.35 L cylindrical	Batch, 35ºC. Synthetic WW with glucose concentration of 2, 4, 8 and 10 g/l. Inoculum sludge (VSS: 8.7±1 g/l, TCOD: 13.1±1 g/l,) was mixed with the synthetic WW at the ratio of 1:1. One cycle was 8 days. Applied voltage was 1.0 V.	Carbon fiber brush (d: 2.5 cm, h: 12 cm) Stainless steel wire used to connect the electrodes	Carbon fiber brush (d: 2.5 cm, h: 12 cm) Stainless steel wire used to connect the electrodes	Max. CH_4 yield was 0.34 L CH_4/g COD. This result was obtained at glucose concentrations of 2 and 4 g/l.	SCOD removal: 85.3% for 4 g/l; 79.9% for 2 g/l	Changed btw. 44-53 A/m^3 for 2, 4 and 8 g glucose/L concentrations	The inoculum was collected from the CH_4 fermentation tank of an WW treatment plant. Different concentration of glucose was added to the synthetic WW. pH of the mixture of inoculum and synthetic WW was adjusted to 7 at the beginning.	[155]

Abbreviations: MEC: microbial electrolysis cell; AD: anaerobic digestion; COD: biochemical oxygen demand; TC: two chamber; CEM: cation exchange membrane; OLR: organic loading rate; HRT: hydraulic retention time; conc.:concentration; chm.:chamber; PEM: proton exchange membrane; SHE: standart hydrogen electrode; btw: between; SC: single chamber; SCOD: soluble COD; AD-MEC (MEC+AD): combined MEC and anerobic digestion; mix.:mixture; VSS: volatile suspended solid; rem.: removed; SBR: sequencing batch reactor; TCOD: total COD; Cumltv.: cumulative; respct.: respectively; microorg.: microorganism; WW: wastewater; UASB: upflow anaerobic sludge blanket reactor; app.:applied; recircul.:recirculation; dif.:different; perform.:performance; synt.:synthetic: BPM: bipolar membrane; AEM: anion exchange membrane; subst.:substrate; C/N: carbon to nitrogen ratio; MFC: microbial fuel cell; DFR: dark fermentation reactor; OTU: operational taxonomic unit; cal.: calculated; WAS: waste activated sludge; OC: open circuit; add.:added; PBS: phosphate buffer solution; AGS: anaerobic granular sludge; VGG: voltage graphene group; GG: graphene group

3. MATERIALS AND METHODS

In this study cattle manure (cow dung) was selected as the sole waste to feed the combined MEC and anaerobic digester (MEC+AD)for biogas production. As far as we know cattle manure was not utilized as substrate in MECs that aims biogas production. Pig slurry, food waste, leachate and all kinds of synthetic waste/wastewater were selected as substrate in studies conducted with MEC technologies for biogas production. These substrates are presented in Table 2.4. In our study, it was aimed to enhance the biogas production from manure in a combined MEC+AD reactor at different operational conditions. HRT and OLR of the MEC+AD reactors were changed at different voltage supplementation.

Cattle breeding is an important agricultural industry for meat and dairy products such as milk, cheese, yogurt and meat derived products. It is estimated that meat production increased four folds for the last 50 years in the world, being around 330 million tones in 2017. Comprising 22% of the total meat production of in the world, cattle breeding industry has nearly one billion cattles in 2020 [156]. If it is assumed that a cattle can generate 9–15 kg dung/day [157], it can be understood that massive amount of manure is generated worldwide that is a potential pollution source for the environment. And it is also known that 18 % of green house gas emissions is originated from animal husbandry industry including cattle breeding which cause climate change through global warming [158]. In this context cattle manure was selected as waste feed for the combined MEC+AD system in this study.

3.1. Inoculum and Cattle Manure Preparation

The cattle manure utilized as the substrate in this study was delivered from the influent unit of the De Solar 7 Biogas Energy Plant located in Anayurt, Sincan district of Ankara. The biogas production plant is in the Sincan Organized Animal Husbandry Zone which makes the transportation of manure as fresh as it is new. The anaerobic sludge utilized as inoculum in this work was obtained from 2nd stage anaerobic CSTR of a two stage anaerobic process operated at 35^{o}C. TS, VS and TCOD concentration analysis of the raw manure and anaerobic sludge inoculum were conducted as soon as they were brought to the laboratory. The characteristicsof raw cattle manure and anaerobic sludge inoculum are depicted in Table 3.1. Fed manure column shows the range of TS nd VS

concentrations of the manure that was diluted to feed to the MECs at desired VS concentartions. In this study VS concentration was the reference point for diluting the manure.

Raw cattle manure obtained from the biogas production plant was carried with 5 L volumed plastic bottles to the laboratory and they were placed into a refrigerator at the temperature of-18^{o}C to prevent biological degredation until it was used.

Table 3.1. Characteristics of the inoculum and raw cattle manure.

	Inoculum	**Raw manure**	**Diluted Manure**
TS, g/L	77.5±2.0	113±3	41.1±2 – 87.5±3
VS, g/L	51±2	89.5±2.5	29.8±1.5 – 60.1±1.5
TCOD, g/L	65±2	98±3	48±3 – 91±3
TN (g/L)			3.4±0.2 (for 3% VS)
TCOD/TN			14.1±0.3 (for 3% VS content manure)
pH	7.30±0.1	7.0±0.2	7.25±0.15
EC (mS)	12.3	>20	>13
ORP (mV)	-345	-175	-170
VS/TS %	66±2%	77±3 %	71±3 %

When raw maure was needed to prepare the feed manure for the MECs, desired amount of bottles was removed from refrigerator one day earlier to be defrosted at ambient temperature. Then manure taken from the plant, was grinded with a blender for large particles to become small enough to pass through the plastic hoses of the MECs. Following the blendering, manure was screened by a sieve that has holes of 1 mm of diameter. Finally prior to feeding the manure to the MECs, it was diluted to the desired VS concentrationby adding tap water depending on the TS and VS concentrations of the screened manure.

VS content adjusted manure was kept in another refrigerator at the temperature of 4oC during feeding to the MECs. The VS concentration of the fed manure adjusted to between 29.8±1.5 – 60.1±1.5 g VS/L during the entire study. A photo of feed cattle waste and anaerobic sludge is presented in Figure 3.1.

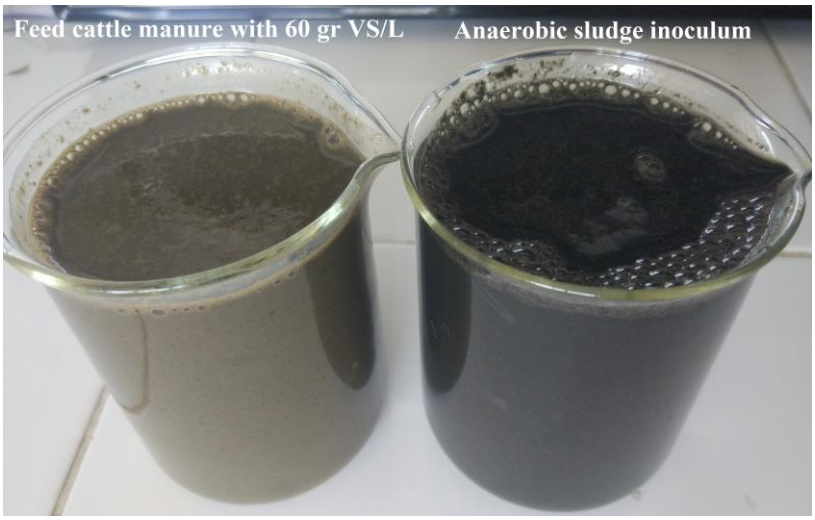

Figure 3.1. A photo of anaerobic sludge and feed cattle manure used in this study.

3.2. Configuration of Combined MEC+AD and Experimental Setup

In this study, four identical borosilicate glass reactors (inner diameter: 9 cm, height: 17 cm) with 1 L capacity were used as one control anaerobic reactor and three combined MEC and anerobic digestion reactors (MEC+AD).All the reactors had an inlet and an outlet hole of diameter of 3 to 4 mm (d: 3-4 mm) at the opposite sides of the cylindrical surface of the borosilicate glass reactor. Inlet hole was close to the bottom of the reactor and the outlet hole was at the upper side of the cylindrical surface of the reactor. Substrate drawing and feeding to the reactors was achieved through the inlet hole of the reactor only by scaled plastic syringes. After drawing a given volume of waste from the reactors, the same volume of substrate (untreated waste) was fed to the reactors consecutively from the inlet hole by scaled 100 mL plastic syringes.

All MEC+AD reactors were membraneless single chamber MECs. Anode and cathode electrodes were made up of carbon fiber cloth and activated carbon pellets respectively. Carbon based materials were selected as anode and cathode materials due to conductivity, chemical and biological stability, availability and cheapness [19, 21].All electrode materials were pretreated in an oven at 450 °C for 30 min to carbonize combustible organics and to remove the impurities from the surface, then it was washed with deionized water and finally dried in a stove at 105 °C [142].The carbon fiber cloth was purchased from My Warm House, Guangdong, China and activated carbon pellets with diameter of 2,5 to 3 mm and length of 2 to 7 mm were purchased from a firm that operates on sales of water and wastewater treatment systems and chemicals.Three pieces of carbon fiber

cloth with length of 20 cm and width of 10 cm were cut from the purchased carbon cloth. They were folded in two and placed flat in a plastic net (22 cm x 11 cm)with 1 mm diameter voidswhich are used in household windows for avoiding flies and bugs from the houses. The edges of the of the plastic net was siliconed to stabilise the carbon fiber cloth inside the plastic net. 50 gr of activated carbon pellets were placed into a cylinder plastic net with diameter of 2.5-3 cm and length of 13-15 cm which was the same net used for anode preparation. Titaniumwire with diameter of 1.5 mm was passed through the nets from the bottom of the net to the 20 cm above of the top of the net. Excess length of 20 cm titanium wire was needed to connect the wires to the power supplier at the outside of the MEC+ADs. Titanium wire was tightened at the bottom and in the middle of the plastic net cells to assure the contact between the electrode materials and the wires for electron transfer. Anode and cathode electrodes and their materials are shown in Figure 3.2.

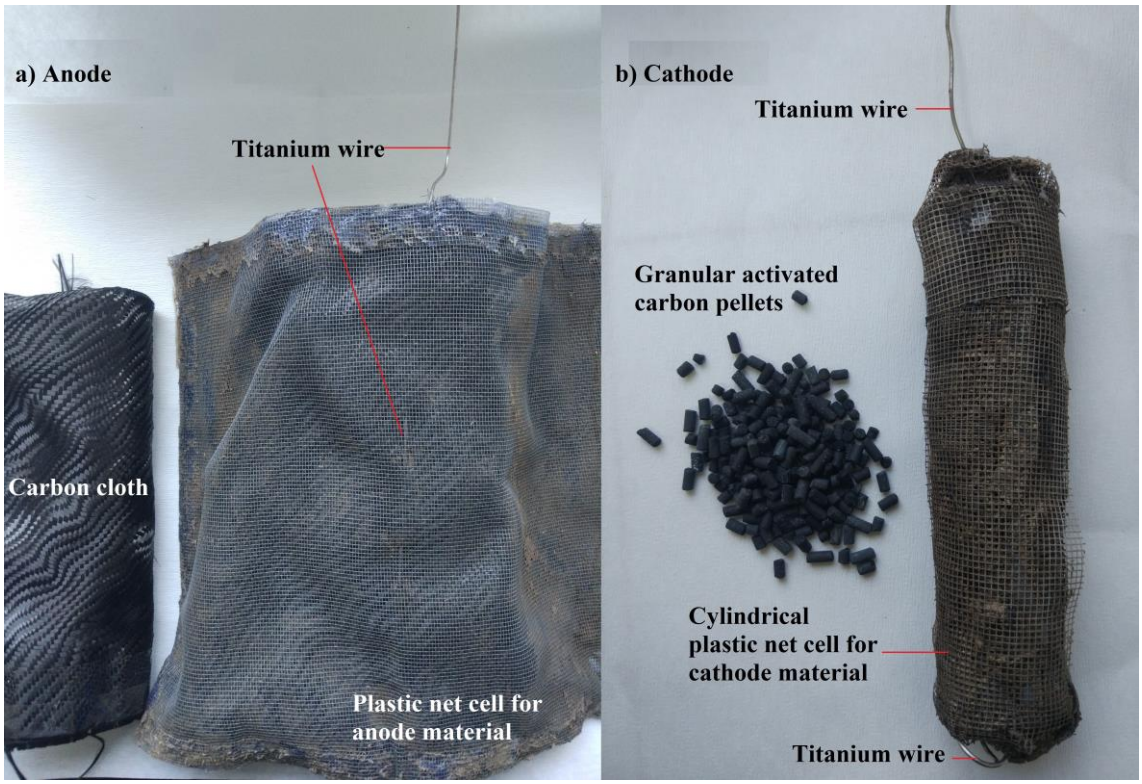

Figure 3.2 Anode and cathode electrodes and their configuration

Three electrode pairs (anode and cathode) were replaced in 3 borosilicate glass reactor that had 1 L of capacity in order to construct the identical MEC+AD reactors. The replacement was as following; the anode electrode was introduced into the reactor from the top of the reactor by wrapping the carbon cloth as tight as it could fit from the 3 cm opening at the top. Once the anode electrode was inside the reactor it was opened flat and

placed on the surface of the inner wall of the cylindrical reactor. The excess 20 cm titanium wire was sticked out from the outlet holethat was close to the top of the reactor to make the electrical connections. The excess titanium wire of the cathode electrode was screwed from one end to and beyond the other end of a rubber stopper that was used to cap the reactor air tight at the opening top. Thus the cathode electrode was hanging in the middle of the reactor and was not touching to the base of the reactor. The distance between the anode and cathode electrodes inside the reactor was about 2 cm. Another hole was made on the rubber stopper for biogas collection from the top of the reactor. All the wire and hose exits from the reactors was sealed with parafilm to ensure the air tightness. Figure 3.3 presents a schematic drawing of the MEC+AD reactors from the lateral section and top view.

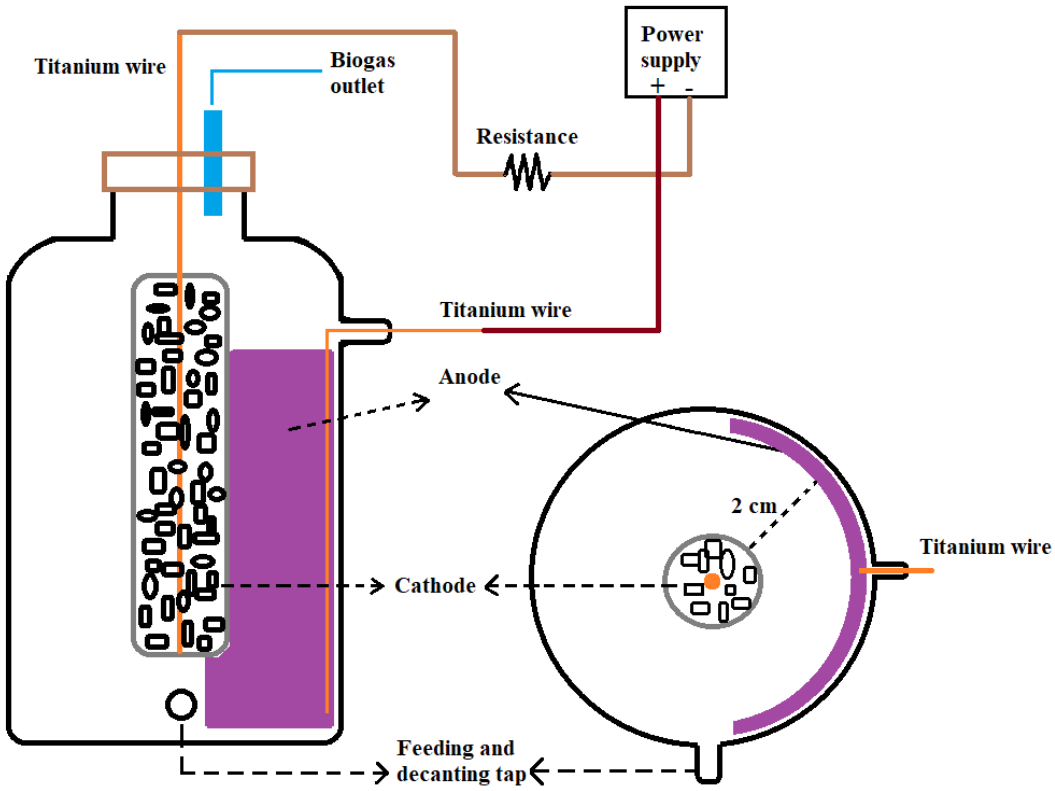

Figure 3.3. A schematic presentation of the MEC+AD reactor from lateral section and top view.

In order to apply the power to the MEC+AD reactors, excess part of the titanium wires that was outside of the MECs, was connected to the power supply unit as it is described following. Positive end of the power supply unit was connected by electric cable to the titanium wire of theMEC+AD reactor`s anode end which was negative. In Figure 3.4, the

red colored electrical cable reaching out to the back side of the reactor is the wire connecting positive end of the power unit to the negative end of the reactor. Negative end of the power supply unit was connected to the cathode electrode`s titanium wire which was positive end of the reactor. There was a 10 ohm (Ω) resistance in series between the these ends [95, 99, 113]. It can be seen from Figure 3.4 that yellow electric cable connected to the top of the reactor is coming from the negative end of the power unit. And there is a small 10 Ωresistance connected in series between the ends. The voltage produced by the MEC+AD reactor was recorded by a multimeter connected across the both ends of the resistance.

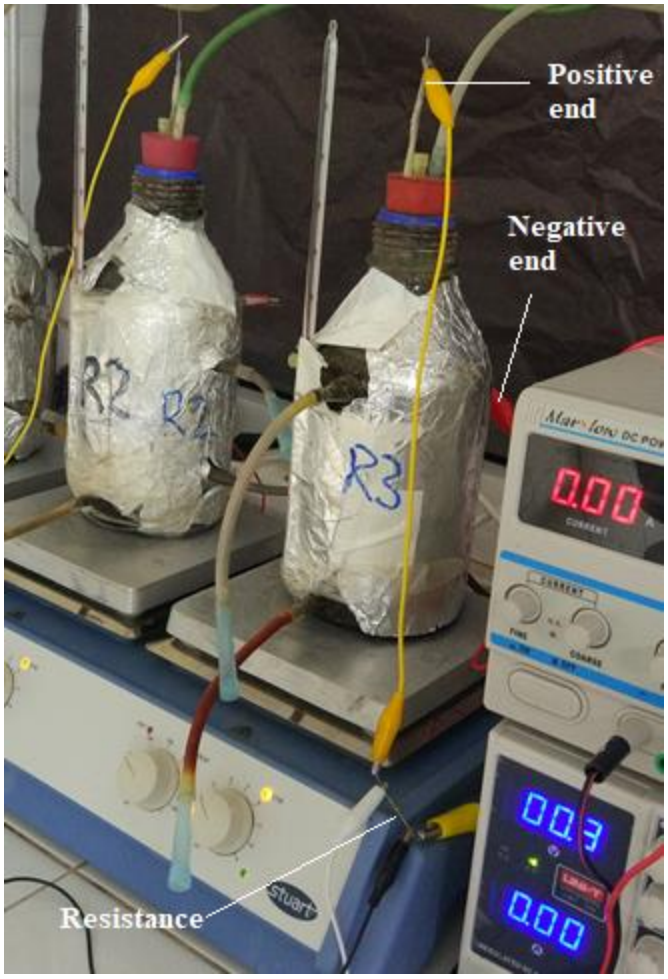

Figure 3.4. A presentation of the MEC+AD reactor`s connection to the power supply unit.

Three MEC+AD reactors and a control reactor were heated and stirred at 250-300 rpm by magnetic stirrers. The hoses and connection points of the hoses to the reactors were controlled for airtightness under water and soap foam by pumping air to the hoses and reactors. There were only two external power suppliers (Uni-T UTP 3315 TFL) that were

powering the MECs. Therefore it was only possible to apply two different voltages to the reactors at the same time. For example, when two of the reactors were applied either by 0.3, 0.6 or 1.0 V, the remaining reactor could be givendifferent voltage but not necessarily. When one set of studywas finished, the voltage amount given to the specific reactors at the next set could be changed according to the missing combination of the operational conditions. One set of study was finalized after 15 to 20 days at every set of study. By this way all reactors had been operated under different applied voltages at different periods of the study. However to ensure the results of the study, all experimental combinations were conducted dublicate at different reactors. Figure 3.5 presents the MEC+AD reactors, magnetic mixing and heating instrument, power suppliers, biogas collection units, voltagemeters and etc.

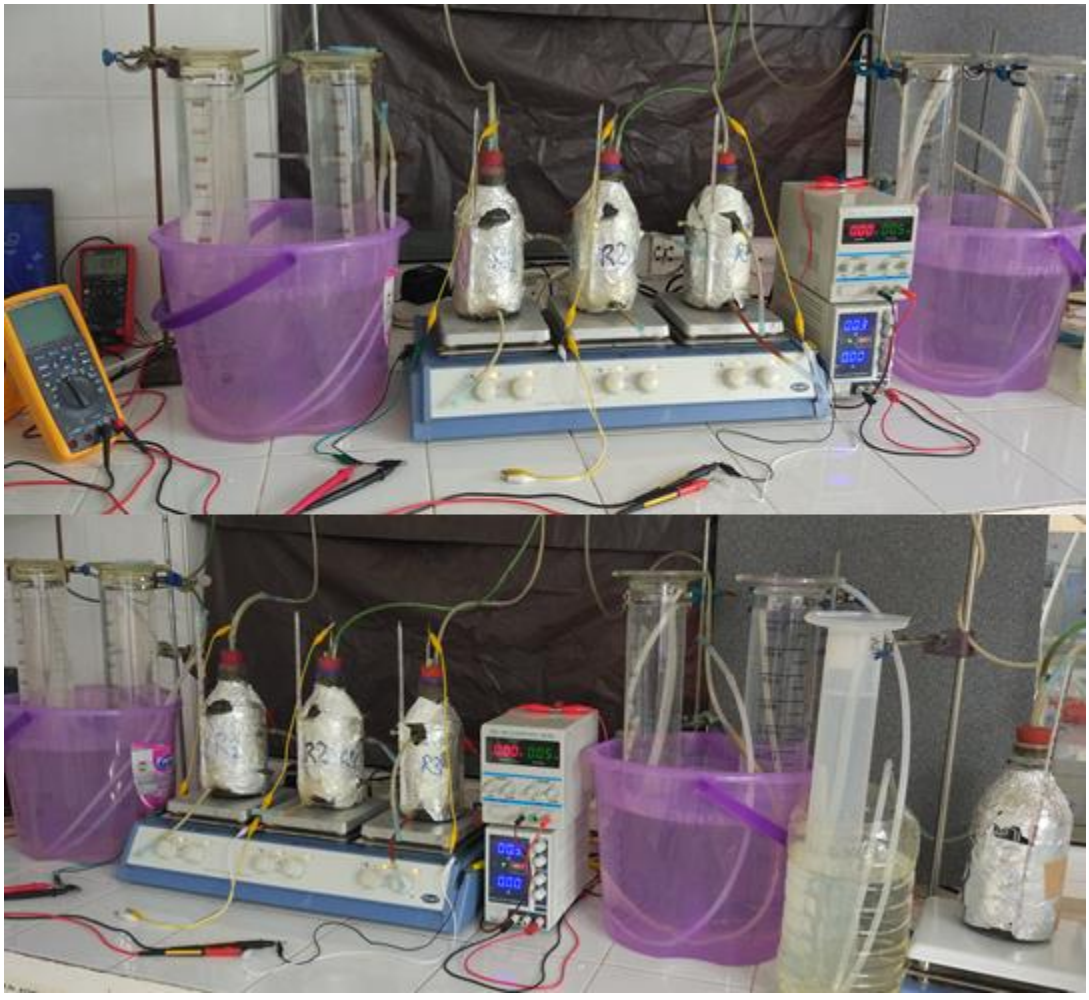

Figure 3.5. Experimental setup of the MEC+AD reactors and control reactor.

3.3. Start-up Period and Operational Procedure of the Combined MEC+AD

Three MEC+AD reactors were first inoculated with 1 L of inoculum that was obtained from De Solar 7 Biogas Energy Plant located in Anayurt, Sincan district of Ankara. For about two weeks the reactors were operated under mesophilic temperature (37±2 ^{o}C) and open circuit conditions. During this time MEC+ADs were fed with 100 mL of synthetic wastewater or cattle manure at 35-40 g VS/L concentrations once a week for microorganisms to stay active. Biogas production was observed during this period.

In this study two sets of batch experiments were conducted. First batch experiments were conducted after the start-up period by feeding the MEC+AD reactors with 250 mL cattle manure including 33.5±1 g VS/L (3.35 % VS : w/w) and inoculum at a ratio of 1:3. The effluents of the MEC+AD reactors (250 mL each) were used to set a control reactor with the additional 250 mL of cattle manure. The batch cyles contiuned 10 days until the biogas production was as low as 50 mL a day. The second set of batch experiment was conducted during the semi continuous operational mode. Following the batch experiments cyle, MEC+AD reactors were operated on semi continuous mode by feeding once a day (sequencing batch reactor). Seven sets of experiments were conducted in the part of the study that reactors were operated on semi continuous mode. Cattle manure with VS concentrations varying from 30 g VS/L (3% VS: w/w) to 60 g VS/L (6% VS: w/w) were fed to the reactors at varying HRTs (6, 4, 3, 2, and 1 days). Table 3.2 presents the operational conditions of MEC+AD reactors based on HRT and OLR. At every set, reactors were also operated at applied voltages of 0.3, 0.6 and 1 V respectively.

Table 3.2. Operational conditions of the combined MEC+AD reactors based on HRT and feed VS concentration.

At cattle manure concentration of 30 g VS /L					
	Set I	Set II	Set III	Set IV	Set V
HRT (day)	6	4	3	2	1
OLR (g VS/ L.d)	5	7.5	10	15	30
At 2 days HRT					
	Set IV (repeat)	Set VI	Set VII		
VS conc. (g VS/L)	30	45	60		
OLR (g VS/ L.d)	15	22.5	30		

In this study two power supply equipments were used to supply external voltage to the three MECs at the same time. At these circumstances, same amount of voltage either 0.3 or 0.6 or 1.0 V was given to two MECs, and the third MEC was supplied with different amount of voltage other than the other two MECs. This operational method was used to show the impacts of the various input voltages on the same MEC+AD reactors in terms of biogas production and process performances. Reactors were first fed with low VS including manure (30 g VS/ L or 3 % VS) at highest HRT (6 days) in this study at all voltage supplementations (0.3, 0.6, 1.0 V). All trials were carried out for 15 to 20 days to observe the stable periods of the experiments in which biogas production and organic removal come to a steady state. For example when 0.3 V was applied to an MEC+AD reactor fed with 3 % VS including manure at 6 days HRT, the process was contiuned around 15-20 days to observe the steady state results. In addition, same trial such as feeding 3 % VS including manure to an MEC+AD reactor at 6 days HRT and 0.3 V power supplementation was conducted once more at one of two remaining MEC+AD reactors at the following trials to make sure the experiments were dublicate. All the trials in this study were dublicates and all sets of experiments were contiuned more than 3 HRT periods.

The HRT of reactors were decreased from 6 days to 1 day step by step when feeding the 3 % VS content manure by weight. Thereby, when the HRT of the MEC+AD reactors was 1 day, OLR of the reactors increased from 5 g VS/ L.d to 30 g VS/ L.d automatically. Later on, to observe the effects of the VS concentration of the manure on biogas production and process performance, manure concentration was first changed from 30 g VS/ L to 45 g VS/ L and then to 60 g VS/ L at the constant HRT of 2 days with different voltage supplementation. All three voltage supplementations was applied to the reactors and the trials were conducted dublicate with at least 15-20 days of periods. In these trials, power applications to every one of the MEC+AD reactors were also changed, such that one individual reactor was operated under 3 different power supplementation at different substrate concentrations to observe the power supply effect on the reactor performance. Consequently all three MEC+AD reactors were operated under different power applications and different manure concentrations.

3.4. Analytical and Calculation Methods

In this thesis, total solids (TS) and volatile solids (VS) analysis, measurement of the biogas production and determination of the biogas content as well as measurement of COD, pH and ORP were conducted to observe the process performances during the entire study. Also voltage production of the MEC+AD reactors were recorded to calculate the current production by the reactors.

3.4.1. Total Solids (TS) and Volatile Solids (VS) Analysis

TS and VS analyzes of the inocula, raw cattle manure, feed manure and effluent manure were conducted according to Standart Methods [159]. The aim of the these analyzes were to determine the approximate total solids content and the organic content of the raw and feed cattle manure. Also it was aimed to feed the MEC+AD reactors with known content of VS or TS. Effluent manure VS and TS content were analyzed to calculate how much VS and TS were degraded by the microorganism. At least three TS and VS experiments were conducted for every change in OLR or HRT and voltage application. The procedure was as following: first of all, the porcelain dishes that were going to be used were cleaned and dried accordingly. After that, the weight of the empty porcelain dishes (tare) were measured with a precision scale and noted. The next step was to measure the weight of 50 mL sample and porcelain dishes together. Afterwards, dishes with the samples were dried at 105°C in a stove for one day to ensure the removal of water in solid particles in the manure. Following, the weight of the TS included dishes was measured with the precision scale after cooling. Lastly, to determine the VS content of the manure, TS included dishes were placed in the oven at 550°C at least 1 hour to make sure all the cumbustible organic matter in solid were removed. Finally when the dishes cooled down to room temperature in a desiccator, the weight of the dishes with the inert matter were measured in a precision scale. Following equations shows the calculation of the TS and VS content and concentration of the samples respectively.

$$TS\ \% = \frac{(\mathrm{C}-\mathrm{A})}{(\mathrm{B}-\mathrm{A})}\mathrm{x}100 \qquad \text{Equation 3.1}$$

$$gr\ {}^{TS}/_{L} = \frac{(\mathrm{C}-\mathrm{A})}{\mathrm{V}}\mathrm{x}1000 \qquad \text{Equaiton 3.2}$$

$$VS\ \% = \frac{(\mathrm{C}-\mathrm{D})}{(\mathrm{B}-\mathrm{A})}\mathrm{x}100 \qquad \text{Equaiton 3.3}$$

$$gr\,{}^{VS}/_{L} = \frac{(C-D)}{(B-A)}x1000 \qquad \text{Equation 3.4}$$

In Equations from 3.1 to 3.4: A= weight of the tare of the porcelain dish (gr), B= the weight of the porcelain dish with 50 ml sample (gr), C= the weight of the porcelain dish and the dry TS together after drying in the stove at 105^{o}C (gr), D= the weight of the porcelain dish and the residual inert matter after combusting in the oven at 550^{o}C (gr), V: volume of the sample (mL).

3.4.2. Chemical Oxygen Demand (COD) Analysis

COD analyzes of the influent and effluent manure and the inoculum that was used in the present study, was performed to detect the effect of voltage application and OLRs on the COD removal rates of the MEC+AD reactors. The tests were conducted twice a week by applying LCK 514 cuvette tests (100-2000 mg O_2/L) in Hach Lange spectrophotometer with the model name Cadas 200. In Figure 3.6 Hach Lange spectrophotometer and LCK 514 cuvette tests are represented. The tests were conducted according to the instructions on the test kits. Before the analysis, samples were diluted to a certain level. After the cuvette tests were cooled down to room temperature, they were placed into the spectrophotometer which was at 620 nm wavelenth for COD analysis.

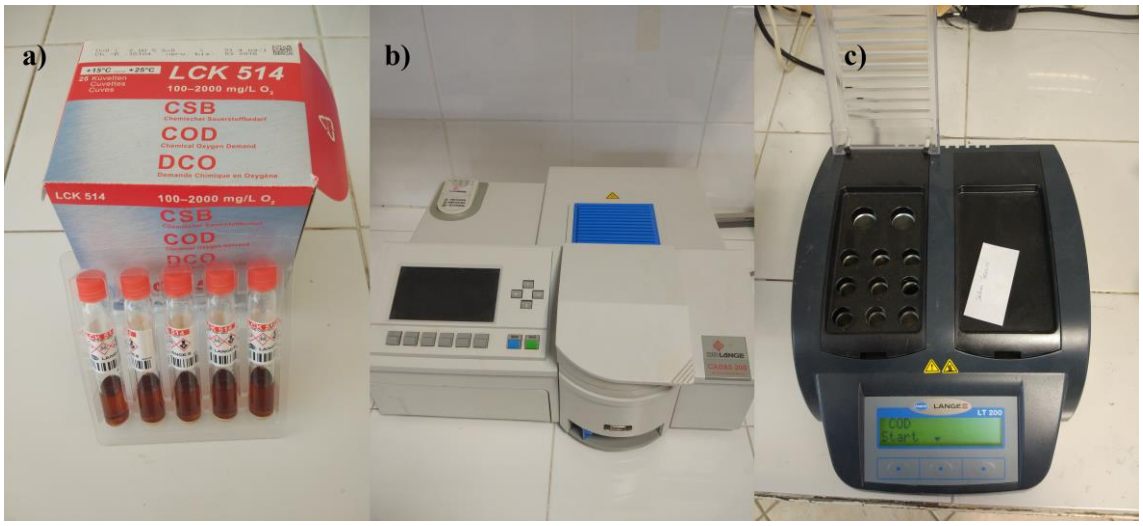

Figure 3.6. COD analysis apparatus: a) LCK 514 cuvette test, b) CADAS 200 spectrophotometer and c) thermostate

In order to calculate the COD removal efficiency, the difference of influent and effluent stream`s COD concentration was divided by influent COD concentration and multiplied by 100 %: [($COD_{inf.}$ – $COD_{eff.}$)/$COD_{inf.}$]x100%.

3.4.3. Measurement of Biogas Production and Analysis of Biogas Content

The biogas produced by the MEC+AD reactors were measured daily by using the water displacement method with graduated cylinders previous to manure decanting and feeding to reactors respectively. The biogas measurements were standardized according to conditions of standart temperature (0 ^{o}C) and pressure (1 atm, 1.013 bar) by using Ideal Gas Law equations shown in Equations 3.5 and 3.6.

$$P_0.V_0 = n.R.T_0 \qquad \text{Equation 3.5}$$

$$P_1.V_1 = n.R.T_1 \qquad \text{Equation 3.6}$$

In Equations 3.5 and 3.6; V_o is the volume of 1 mole of biogas at standart temperature and pressure, V_1 is the volume of 1 mole of biogas measured in mL. P_o is the ambient pressure at STP (1 atm or 1.013 bar), P_1 is the known pressure of Ankara at average latitude of 850-950 m (accepted as 0.91 atm), T_o is the ambient temperature at STP (0°C, 273 K), T_1 is the ambient temperature in the graduated cylinders (Average temperature of water that biogas bubbles through, accepted as 20°C, 293 K).

Using Equations 3.5 and 3.6, we can convert the volume of biogas that is known at the sea level atmospheric pressure (1 atm) and at the temperature of 273 K (22.4 L) to volume that would be measured in laboratory conditions (0.91 atm and 293).

$$P_0.V_0/T_0 = P_1.V_1/T_1 \qquad \text{Equation 3.7}$$

When the given variables is placed into Equaion 3.7, V_1= 26.42 L would be calculated. According to these findings, any measured volume in the laboratory conditions in this study should be converted to STP volume by dividing the measured volume by 26.42/22.4=1.18. This is the correction coefficient used in this study.

In this study, the content of biogas was determined with the same method that Gelegenis et al. [160] and Ergüder et al. [161] used in their studies. Biogas content is an important parameter that shows the stability and effectiveness of the anaerobic process. In this study, CH_4 content of the biogas was determined in every two or three days by the method

specified below. Volumetric determination of the CH_4 content in the biogas was obtained by the absorption of CO_2 and other trace gases in a concentrated NaOH or KOH solution. Afterwards remaining gas was measured by water displacement method using the apparatus shown in Figure 3.7. The absorption of CO_2 was realised by using 3 molar NaOH or KOH solution. More than half volume of a 250 mL borosilicate bottle was filled with 3 M NaOH or KOH. A known (50 or 100 mL) amount of biogas was vacuumed from the graduated cylinders with a scaled 100 mL syringe. And it was pressurized into the borosilicate bottle which contained 3 M or more NaOH solution. The cap of the bottle was tightened for airproofing. Afterwards the bottle containing pressurized air and biogas was shaken manually for 3 or 4 minutes for the absorption of CO_2 and other trace gases with NaOH solution. At the end of the shaking process, the gas in the bottle was composed of CH_4 and air. The remaining gas in the bottle was still pressurized due to the excess CH_4 in the bottle. The pressurized gas in the bottle was linked to a 100 mL graduated cylinder through a fit hose. The 100 mL graduated cylinder that was used to measure CH_4 content of the biogas performed according to the water displacement method shown in Figure 3.7.

Figure 3.7. Water displacement method to measure the methane rate of the biogas.

The ratio of the volume (V_2) of the gas in the 100 mL graduatedcylinder to the volume (V_1) of the biogas enjected to the 500 mL determines the CH_4 content of the biogas that is shown in Equation 3.8.

$$\% CH_4 = V_2/V_1 \qquad \text{Equation 3.8}$$

Using the biogas production measurement and methane content of the biogas, methane production and methane yield of the reactors can be calculated. Methane production is the methane amount that the reactor can produce for a given time (day), per reactor volume and it is shown as: mL CH_4/L/d. Methane yield is defined as the amount of methane produced for a given quantity of organic matter as in VS or COD concentration in the reactor and it is shown as: mL CH_4/ g VS (COD). If the methane yield is calculated according to the removed VS concentration, then it is shown as : mL CH_4 /g $VS_{rem.}$.

3.4.4. pH, Temperature and Oxidation Reduction Potential (ORP) Measurements

pH is a crucial parameter due to the excess or low hydrogen ion concentration in the medium that can inhibit the process if not controlled. In this study, pH adjustment were not applied to the MEC+AD reactors due to the high alkalinity and buffering capacity of the cattle manure [162]. pH and ORP measurements of the MEC+AD reactors were measured in every 3 days by Hanna brand HI 83141 model pH meter. Temperature of the MEC+AD reactors were measured with the glass thermometers sticked to the outer lateral surface of the reactors and it is controlled at 37 ^oC by magnetic stirrer with heater. pH measurements were applied by submerging pH meter probe into a beaker which includes the effluent of the MEC+AD reactors prior to feeding. pH meter were calibrated according to the user manuel every two weeks.

The oxidation reduction potential (ORP) is an evaluation of oxidizing and reducing potential of an aqueous environment. ORP is sensitive to the presence of O_2 in an aqueous solution [163, 164]. It can also be used as an indicator for the control of anaerobic digesters because CH_4 production mostly takes place between ORP values of -175 to -400 mV range [163, 164]. In this study ORP measurements were also conducted with Hanna brand HI 83141 model pH meter applying the same procedure with the pH measurement.

3.4.5. Bioelectrochemical Calculations

Strength of current flow between the electrodes (anode-cathode) determines the effectiveness of the MEC process. Current flow in MECs contribute to H_2 production which is converted into CH_4 by hydrogenotrophic methanogens [27, 32, 63]. In bioelectrochemical studies, performance is characterized in terms of current density in reactors. The current density can be determined by dividing the current flow by either the volume of the reactor or the cathode chamber (A/m^3), or by the cathode electrode area (A/m^2) [71, 108, 133].

In this study, influence of power applications of 0.3 V, 0.6 V, and 1.0 V were observed on the CH_4 generation by feeding cattle manure as the organic matter. Uni-T UTP 3315 TFL power supply equipment was utilized to supply the aimed external voltage. The cathode and anode electrodes were attached to the negative and positive edges of the power supply unit respectively, with an external resistance of 10 Ω [95, 113, 150]. At regular time intervals of 15 min. the voltage across the resistance was measured with the a multimeter and it was saved into a computer. All currrent measurements recorded in a day were reduced to an average current value. The current (I) was calculated by Ohm's law shown in Equation 3.9 [71, 108] and to find the current density, the current was divided by the combined MEC+AD reactor volume (0.6-1 L) as shown in Equation 3.10. In Equations 3.9 and 3.10, R_{ex} is the external resistance (10 Ω) of the lead circuit, V_{act} is the actual voltage measured across the circuit (across the resistance), and V is the MEC+AD reactor volume (m^3).

$$V_{act} = I.R_{ext} \qquad \text{Equation 3.9}$$

$$I\ (density) = V_{act}/(R_{ext}.V) \qquad \text{Equation 3.10}$$

Coulombic efficiency (CE) is another electrochemical analysis that can evaluate the MEC+AD reactors` performances. It shows how much current is captured from the theoritical available current that can be produced from the substrate [30, 121, 126]. It is represented as the ratio of electrons measured in electric current to the available electrons in the removed substrates [60, 150]. It is calculated by the Equation 3.11 as following:

$$CE = \frac{I\ .\ t}{n\ F\ (C_i - C_e)\ V/M} x100\% \qquad \text{Equation 3.11}$$

In Equation 3.11; C_i and C_e are the COD concentrations (g/L) of the influent substrate and effluent waste respectively, V is the liquid volume of the MEC+AD reactor (L), F is the Faraday constant (96485 C/mol of e−), M is the molar weight of oxygen (32 g/mol), I is the current (A) calculated by Ohm`s Law, n is the moles of electrons transferred from the organic matter oxidized per 1 mol of oxygen (4 mol of e−/mol of oxygen), t is the time of one cycle.

3.4.6. Assesment on Energy Balance of the Combined MEC+AD

In order for the engineering processes to be materialized, it is significant to practise the feasibility of the process. Most of waste treatment processes focus on the energy efficiency which is the rate of the recovered energy from system to the energy input for the operational activities. For the MEC reactors it is crucial for the process to be energy efficient because an energy input will be required all the time for the voltage supplementation and/or heating and mixing. The overall energy efficiency can be determined by the ratio between the energy obtained from the MEC in the form of methane and the energy added to the system as the external power supply with the energy needed for heating and mixing [71, 107, 121, 150]. Energy efficiency due to the electricity supplied to the reactors is given in Equation 3.12 where r_{EE} is the energy efficiency, W_{CH4} is the additional energy obtained in the form of CH_4 in biogas that is produced as a result of voltage application in MEC process, W_V is the energy given to the MEC+AD reactors by external power supply [60, 125, 129, 150].

$$r_{EE} = \frac{(W_{CH4})e}{W_V} x100\% \qquad \text{Equation 3.12}$$

W_{CH4} is the additional energy recovered from the methane that is produced by the methanogens in the MEC+AD reactors as a result of substrate break down. It is determined by the Equation 3.13 where n_{CH4} is the total moles of methane produced in a given period of time and ΔH_{CH4} is the the energy content of CH_4 based on the heat of combustion value (890.8 kJ/mol) [60, 71, 126, 150]. On the other hand energy consumed for voltage application (W_V) can be calculated with the Equation 3.14 [60, 126].

$$W_{CH4} = n_{CH4}\,\Delta H_{CH4} = \Delta t\,\Delta H_{CH4}\left(\frac{V_{MEC}-V_C}{22.4\ L/mol}\right) \qquad \text{Equation 3.13}$$

In this study to determine the methane originated from bioelectrochemical process separately, second part of the Equation 3.13 can be used. It is only valid if there is a control reactor used in the study. Here, Δt is the HRT of the experiment (for this study it was 10 days for batch studies, and 6 days to 1 day for continuous studies). V_{MEC} is the total cumulative CH_4 generation of the combined MEC+AD reactor (L/day), V_C is the total cumulative CH_4 generation of the control reactor (L/day).

$$W_V = I\ V_{ap}\Delta t \qquad \text{Equation 3.14}$$

In Equation 3.14, I is the average current (A), V_{ap} is the applied voltage (V) and Δt is the period of time for voltage application (s). In this study produced biogas was measured daily by water displacement method. Methane content of the biogas was determined by using concentrated NaOH solution in every 2 days. Therefore moles of CH_4 in Equation 3.13 can be estimated via $n_{CH4} = \frac{V_{MEC}-V_C}{22.4\ L}\ \%CH_4$.

In MEC studies, a diifferent energy efficiency can be calculated using proprotion of the energy content of produced biogas to energy content of the degraded substrate is also used as a performance indicator. Equation 3.15 represents the ratio of the energy produced in methane from the reactor and the energy content of the removed organic material [71, 135, 146].

$$r_S = \frac{W_{CH4}}{W_S} x100\% \qquad \text{Equation 3.15}$$

Here $W_S=\Delta H_S.m_S$ in which ΔH_S is the heat of combustion of fed cattle manure (3900 kcal/kg for 97.2 % dry manure which has bulk density of 700 kg/m^3 [165]) and m_s is the amount of total COD or VS removal of substrate in grams (COD_{in} - COD_{out} or VS_{in}-VS_{out}) (g).

In MEC reactors methane can be produced from each one of the following pathways: reducing acetate (acetoclastic-acetotrophic methanogenesis, Equation 2.17), reducing methyl (methylotrophic methanogenesis, Equation 2.19), reducing carbondioxide via using the electrons directly that are added to the system (Equation 2.8) or reducing carbondioxide with hydrogen (Equation 2.18) that is formed with electrons combined with protons [30-32, 63].

3.4.7. Optimization of the Operational Parameters

In this study to optimize the operational parameters of the MEC+AD reactors for efficient biogas production, Response Surface Methodology (RSM) was applied. RSM is a collection of mathematical and statistical techniques for empirical model building of various engineering and designing applications [166]. The objective of RSM is to optimize an output variable which is influenced by several independent input variables [166, 167]. It was introduced by Box and Wilson in early 1950s [166]. It has essential implementations in design, evolvement, and discovery of new products, as well as in the refinement of existing product layouts [166, 167].

In this thesis statement, optimization of the biogas production from cattle manure in MEC+AD reactors was studied by applying RSM. The influence of operational factors such as HRT, OLR and supplied voltage on biogas production and methane yield were examined by applying RSM based on Central Composite Design (CCD). CCD can signify the interactions between the operational parameters and can evaluate the optimum conditions of independent variables in order to maximise the biogas production. Also CCD is known for its fit for numeric factors. Thus, three factors, HRT (from 1 to 6 day), OLR (from 15 to 30 kg VS/L.d) and applied voltage (0.3 to 1.0 V) and three levels (-1, 0, +1) were used to run the CCD experiments that were presented in Table 3.3. A fitted regression model presented the relationship between the real effects of the these dependent variables and the response by simulating the experiment results in Design-Expert (13) software. CCD was simulated in Design Expert 13 with 21 runs.

Table 3.3. Variables and levels of the biogas production

Independent variables	**Coefficient Symbol**	**Coded and actual levels**		
		-1	0	+1
HRT (day)	A	1	3.5	6
OLR (g VS/L.d)	B	15	22.5	30
Voltage (V)	C	0.3	0.6	1

4. RESULTS AND DISCUSSION

Main objective of this study is to determine the biogas/methane production yields in combined MEC+AD reactors at different operating conditions. The effects of different HRT, OLR and voltage addition on biogas production and methane rate in the biogas were demonstrated in the present study. An anaerobic digester (AD) and MEC+AD reactor were compared in terms of their biogas productions, methane yields and VS/COD removal efficiencies to show the effect of the external voltage supply. It is important to state that all methane yields obtained in this study were calculated according to fed (added) VS contentrations.

In this chapter, the data gained from the experimental study are given in an organized way to explain and evaluate the results in detail. In Section 4.1. the outputs of Biochemical Methane Potential (BMP) study were presented. BMP of cattle manure was evaluated in MEC+AD reactor and AD only (control) operated at batch condition. Sections 4.2 and 4.3 focus on the results of the semi continuous (sequencing batch) operating mode of the MEC+AD reactors and control reactor at the same feeding conditions. Biogas productions and methane yields, TS and VS removal as well as COD removal in MEC+AD and AD reactors were also investigated in order to compare the results. Current production of the MEC+AD reactors were evaluated regarding the current density and coulombic efficiency. In Section 4.4, a wider look on the results with the summary of the study are presented. In Section 4.5, optimum operating conditions of the combined MEC+AD reactor are analyzed using Central Composite Design of the Response Surface Methodology. The studies focus on the effects of operational conditions and effect of interaction of operational conditions on the results. Finally energy assesment of the MEC+AD reactors is carried out in order to clarify the issues on feasibility in Section 4.6.

4.1. Biochemical Methane Potential (BMP) of Cattle Manure in MEC+AD and AD

Two sets of batch experiments were carried out in order to determine BMP of cattle manure in MEC+AD and AD reactors respectively in this part of the study. First batch experiment was carried out prior to operating the reactors on semi continuous mode. The second batch experiment was also conducted during the study to observe the effect of bacteria accustomed to cattle manure and operating conditions such as temperature and

voltage addition. All the reactors were operated at dublicates and results were expressed as average values.

4.1.1. Batch Experiments-Set I

The reactors used in the first set of batch experiments were operated for two weeks for acclimation to the conditions before the batch experiment. Start up of the reactors were explained in Section 3.3. In this set, MEC+AD reactors (R_1, R_2) and the control reactor (R_C) were fed with 250 mL of cattle manure at concentration of 33.5±1 g VS/L (3.35 % VS; 4.59 TS; w/w) for BMP test. The effluents of the MEC+AD reactors were used as inoculum for commencing the control reactor operation. 250 mL of cattle manure was placed to the control reactor as well. Total working volume of each reactor was 1 L in the first batch experiment. Voltage of 1.0 V was applied to R_1 and R_2 in order to determine the voltage effect on the MEC+AD reactors. Two identical MEC+AD reactors (R_1, R_2) and a control reactor free of electrodes (R_C) were operated simutaneously. The batch cyle continued 10 days until the daily biogas production decresed 50 mL a day. Figure 4.1 shows the daily biogas production of R_1, R_2 and R_C (A), methane content of the biogas (B), and cumulative biogas production (C) of the reactors.

All measured biogas volumes were expressed as in standart temperature and pressure (STP; 273 K, 1 atm) in this study. All three reactors, R_1, R_2 and R_C produced most of the total biogas in the first days of the batch study. R_1 and R_2 which were applied 1.0 V of energy, produced approximately 63 % and 68 % of their cumulative biogas production respectively, in the first three days of the startp up. However, R_C produced 53 % of its total biogas production at the same period of time. And it was only able to produce 69 % of its total biogas production at the end of the fourth day. It is stated that energy addition in MECs can enable hydrogenotrophic methanogenesis take place in the reactor in a very short time, for example couple of hours, that can lead to shorten the hydraulic retention time contrasted to AD [31, 71, 121]. It is suggested that bioelectrochemical degradation of organic materials took place faster than anaerobic digestion [149, 155, 168].

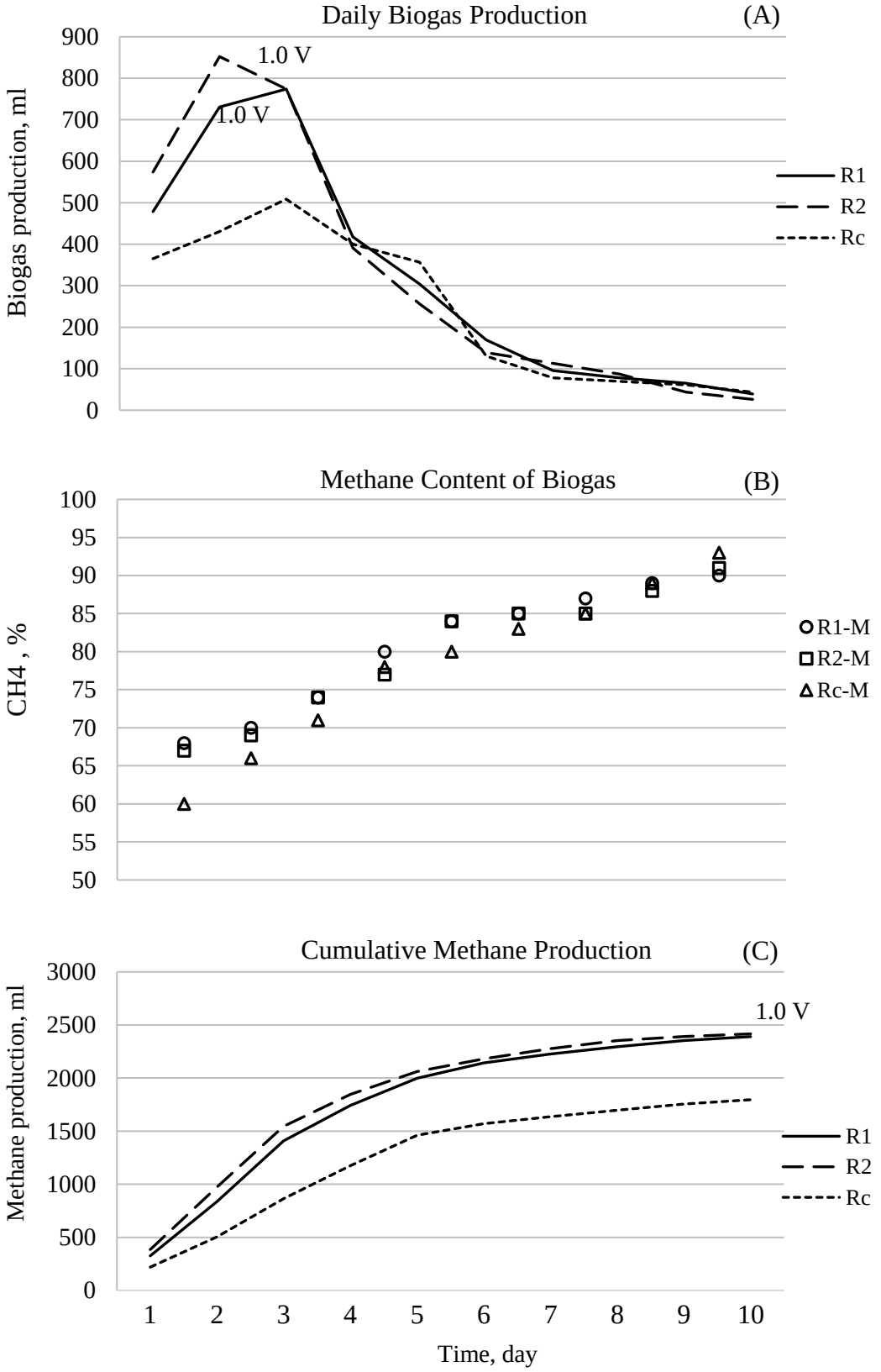

Figure 4.1. Biogas production (A), methane percentage of biogas (B), and cumulative methane productions (C) in R_1, R_2, and R_C at batch experiment Set I.

Average volumetric biogas production of R_1, R_2 and R_C were 315±20 mL/L/d, 325±22 mL/L/d and 243±17 mL/L/d respectively. Methane contents of the biogas obtained from R_1, R_2 and R_C increased from 68, 67 and 60 % to 90, 91 and 93 % in ten days. Methane content of biogas produced in R_C was higher than the usual methane content (55-70 %) found anaerobic digestion. The reason of this might be the inoculation of R_C with the effluent of MEC+AD reactors at the beginning of the study. Methane production of R_1, R_2 and R_C at the batch test were calculated as 239±9 mL/L/d, 242±12 mL/L/d and 180±8 mL/L/d respectively. And consequently methane yields of R_1, R_2 and R_C were 285 mL/g VS, 288 mL/g VS and 214 mL/g VS respectively. Methane productions in MEC+AD reactors were compatible with other studies and even higher in some cases. Ahn et al. [71] used sewage sludge as substrate in a single cell MEC batch study. They reported methane production of 1.11 L CH_4/L and 139 L CH_4/kg $VSS_{rem.}$ at 6 days of cyle period and under similar conditions with this study. Yu et al. [151] reported methane production of 2.92 L CH_4/L which was higher than the present study`s methane production, 2.4 L CH_4/L. In their study, swine manure to inoculum ratio was 4 folds which caused higher methane production. However, methane yield and methane production (285 mL/g VS and 2.39 L CH_4/L) obtained in the present study were competent with the other studies [54, 55, 58, 59, 95, 99, 114, 115, 144, 150, 151] carried out with single cell MECs or integrated MEC and AD reactors. Various kind of substrates such as artificial and real wastewater, waste sludge and animal wastes and artificial food waste were used in the previous studies. These studies were conducted at different voltage addition varying from 0.3 to 2.3 V and at 35-40 ^oC. The reactors used in these studies had volume range of 0.27 L to 20 L and in some of these studies special and high cost electrode configurations were used. Stainless steel, graphite/carbon brush, graphite/metalalloy plates and metal catalysts coated electrodes were used to increase the electron transfer and enhance the methane production eventually [54, 55, 58, 59, 95, 114, 115, 150]. Detailed information about the reference works is depicted in Table 2.4 which shows the previous MEC studies on methane production.

In this study, low cost carbon cloth and granular activated carbon pellets were used as electrodes with Ti wire connection. pH buffering and manure pretreatment were not applied. Nevertheless, combined MEC+AD reactors exhibited high performance on producing methane gas. In Figure 4.2, cumulative methane productions of R_1, R_2 and R_C were presented. At the end of the batch study R_1 and R_2 produced nearly 33-34 % higher

methane amounts compared to R_C. These differences can be attributed to the addition of 1.0 V to the R_1 and R_2 which enables CO_2 reduction with electrons given to the MEC by electrical circuit resulting CH_4 formation. Voltage addition to the system also resulted in hydrogen formation and finally utilization of hydrogen and CO_2 by hydrogenotrophic methanogens for CH_4 formation [27, 30-32]. It is known that in energy applied MECs, hydrogenotrophic methanogenesis can proceed very fast and hence, enhance the methane production [31, 71, 121]. In MECs exoelectrogens oxidize the substrate and release electrons to anode, and then electrons are transferred to cathode, finally at cathode electrons are used to produce methane [32]. In cathode, methane is produced rapidly from cathodic reduction of CO_2 with hydrogen ions during decomposition of organic matter by hydrogentrophic methanogens which mostly found in abundance on cathode electrode in MECs [31, 59, 142, 168].

Figure 4.2 presents COD, TS and VS removal rates of R_1, R_2 and R_C. As it was expected, R_1 and R_2 showed higher removal rates in COD, TS and VS than R_C possibly due to the voltage application to R_1 and R_2 reactors.

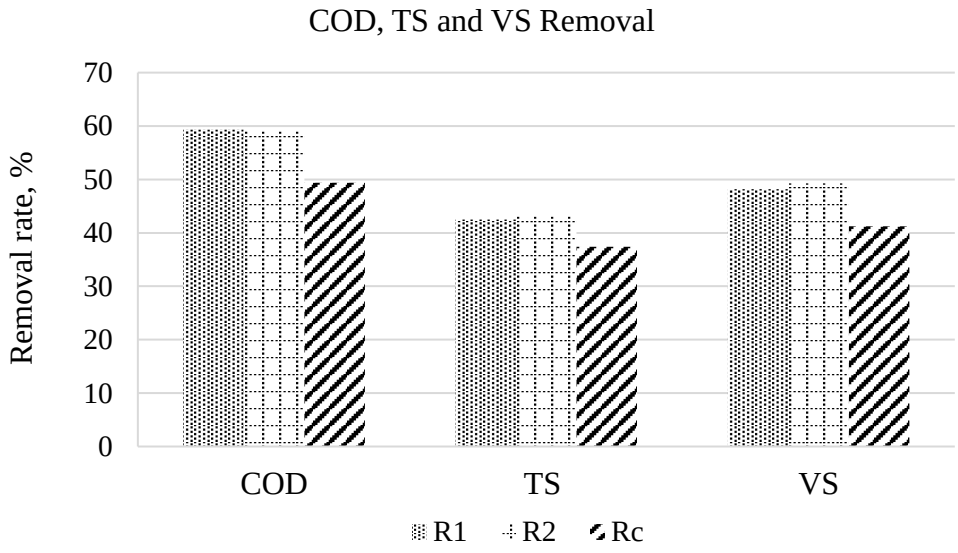

Figure 4.2. COD, TS and VS removal rates of R_1, R_2 and R_C.

COD removal of R_1 and R_2 were nearly 60 % which is almost 18 % higher than COD removal of R_C. Likewise, VS and TS removal of R_1 and R_2 were superior to R_C by 16 and 13 % respectively. This can be explained by 1.0 V of energy addition to R_1 and R_2 which stimulated reduction of CO_2 with electrons and combination CO_2 and H_2 by hydrogenotrophic methanogens for CH_4 formation [31, 32]. It is also worth to indicate

that methane rate of the biogas generated in R_1 and R_2 were higher than methane content of R_C in the first 3 days of batch experiment as it was shown in Figure 4.1. This can be explained by the reduction of CO_2 with electrons and occurence of hydrogenetrophic methanogenesis in this phase that led to methane formation. A study carried out with an AD reactor including MEC inside of the AD, resulted in 57 % COD removal at the 8th day of the study. The study carried out at 35 ^oC and applied voltage of 0.9 V with initial COD of 15.64 g/L in the ractors [49]. Swine manure, pure water and inocula mixture (Ca. 6.45 % VS) was treated in a single cell MEC at varying voltage applications at mesophilic temperature. COD removal efficiencies of 59 % and 54 % were reported with two different kinds of electrode materials in 24 batch days [144]. It can be understood that COD and VS removal rates in this study were appropriate with other studies.

Figure 4.3 represents the volumetric current density and Coulombic efficiency (A), ORP (B), and pH (C) values of the reactors. Current density and Coulombic efficiency were calculated according to the Equations 3.10 and 3.11 respectively. Current density of R_1 and R_2 started from 5.5 A/m^3 and 6.2 A/m^3 from first day with the organic loading to the reactors and it decreased over time and became stable around 3 A/m^3 at the end of the experiment. The sudden drop in current density from 5.5 and 6.2 A/m^3 to around 4 A/m^3 after the first 3 or 4 days can be explained by the rapid VS reduction and utilization of organic material via fermentative bacteria and methanogens immediately. VS reduction and current density decrease in the first days were correlated with the rapid CH_4 formation respect to remaining days of the experiment [48]. Current density became stable around 3-4 A/m^3 at the following days of the study due to the stable exoelectrogen activity [96]. Organic matter degradation and CH_4 production shows the scale of current production in the process [48, 125]. Current production of MEC+AD reactors were at the range of 2.8 mA and 6.2 mA which was higher than other studies conducted with single and two chamber MECs using synthetic and real wastewater as substrate at different applied voltages [32, 96, 104, 122, 129]. However, the current production trend were similar with these studies which were high at the begining of the process and decreased towards the end of the cycles. The current density (3-6 A/m^3) calculated in this study was appropriate with the other studies [48, 59, 121].

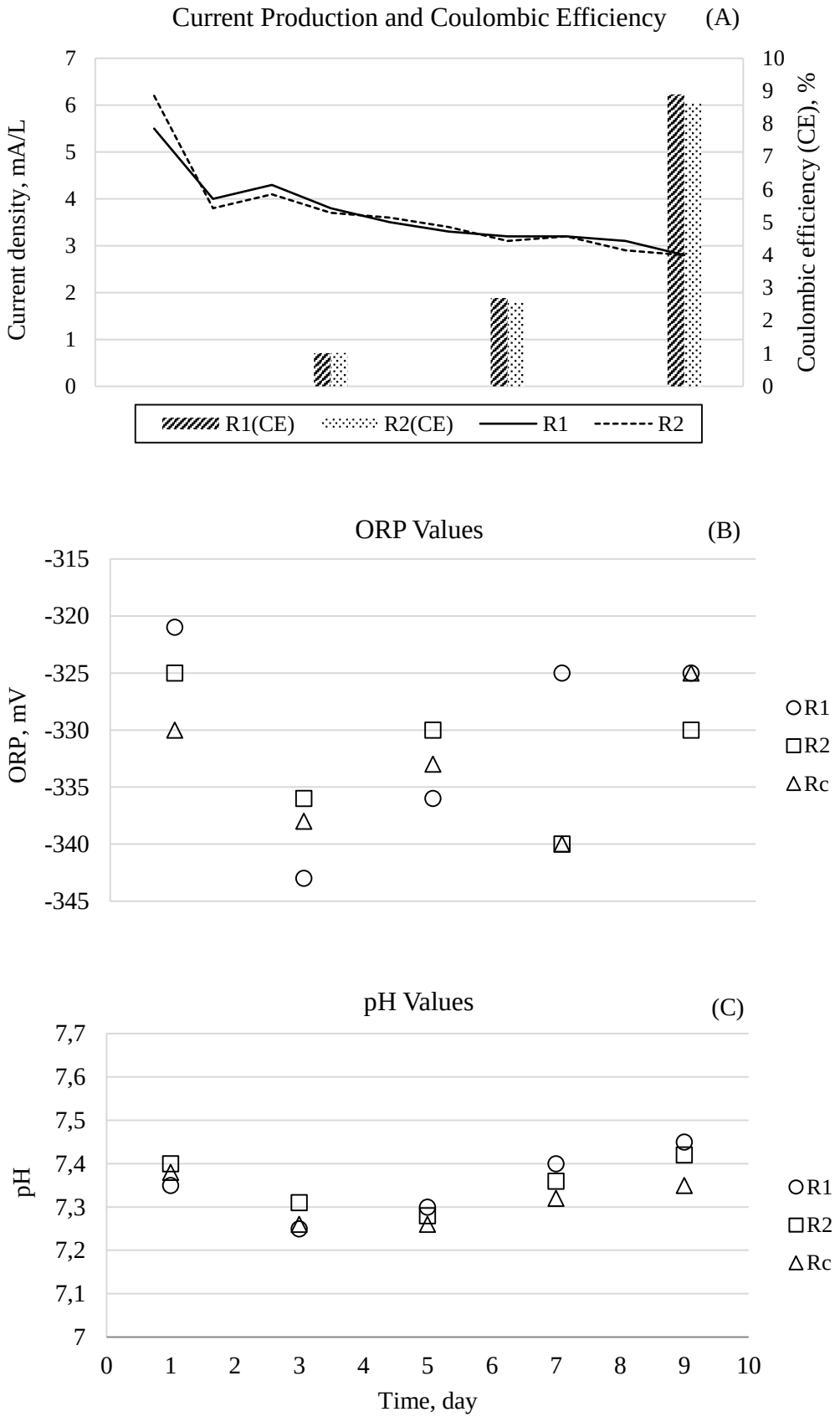

Figure 4.3. Current production and coulombic efficiency (A), measured ORP values (B) and pH values (C) of R_1, R_2 and R_C respectively.

Cusick et al. [48] designed a pilot scale continuously operated single chamber MEC with specially constructed 144 electrode couples in 24 capsules. They reported a maximum current density of 7.4 A/m^3 treating winery wastewater (0.7−2.0 g SCOD/L) with 0.9 V application and pH adjustment. Another study conducted by Feng et al. [59] claimed current density of 4.3 A/m^3 with a single chamber MEC operating at mesophilic temperature and 0.3 V application. Waste activated sludge (63 g VS/L) was the substrate in their study and process was inhibited due to the alkaline pH values [59].

Coulombic efficiency (CE) shows how much current is captured from the theoritical available current that can be produced from the substrate [30, 121, 126]. It is represented by the proportion of electrons calculated in the produced electric current and available electrons in the removed organic matter [60, 150]. CE of the reactors R_1 and R_2 were calculated according to COD removal rates and average current production at that interval using Equation 3.11. Coulombic efficiencies were given in cumulative values at the final stage of whole batch study. CE of R_1 and R_2 were 8.90 % and 8.67 % respectively. CEs calculated in batch experiment were lower than the CE results of other studies that ranged between 28 % and 154 %. Those studies were conducted with acetate and synthetic wastewater as the readily available substrate for exoelectrogens, at varying applied voltages and with pH adjustment or specially designed electrodes and units [57, 75, 99, 118, 128, 130, 133]. The amount of supplied voltage, dominant microorganism type, substrate type and substrate concentration, cell design, temperature, electrode material and distance between electrodes can effect methane and current production and CE significantly [95, 133, 137, 151, 155]. Nevertheless, CE results obtained in Set I were similar to several other studies` results changing between 4.3 % and 14.3 % [59, 60, 99, 107, 123, 150, 154]. The results were even higher than some other studies` results. In those studies, reported CEs were between 2.1 % and 8.1 % [59, 107, 150, 154]. A study conducted by Cerrillo et al. [107] had an CSTR and an separate two chamber (TC) MEC operated in series. Pig slurry was fed to the CSTR first and then to the anode of the TC-MEC. Maximum CE was 3.5 % at the lowest COD contentration influent and at set potential of 0.8 V. Guo et al. [150] showed that the increase in ratio of anode surface area to volume increased the CE from 5 % to 9.5 % at applied voltage of 0.5 V when treating beer wastewater in a single chamber MEC designed with specific cathode and anode electrodes. Lower CE obtained in the present study can be attributed to the fact that a major part of the biodegredable material has been converted to carbondioxide and

methane by fermantative bacteria and methanogens faster than the exoelectrogens due to the bulk sludge mixing [57, 59, 118, 123]. In other words, it can be concluded that there was a race between exoelectrogens and methanogens over the oxidized organic matter which caused current generation decline [98]. It is also suggested by Zhao et al. [169] that microorganism such as bacteria and archaea that live in symbiosis in the biofilm or bulk sludge, can metabolize organic materials and produce methane which in turn cause decreasement in electron transfer through the electrodes [154, 169]. Another assumption made by Li et al. [60] was that hydrogenotrophic methanogenesis in the suspended sludge used a major part of the substrate and contribute to a substantial percentage of CH_4 generation and as a result a smaller part of the organic matter was left for current generation by the exoelectrogens in the biofilm [60, 170].

In Set I, methane production of MEC+AD reactors were 25 % higher than the control reactor. This indicates that approximately 75% of the methane was produced by the methanogens in the bulk sludge and only 25 % of the methane was produced by the microorganisms on the electrode biofilm which could composed of methanogens, exoelectrogens and fermentative bacteria. COD reduction in R_1, R_2 and R_C were 59.3 %, 59 % and 49.4 % respectively. Coulombic efficiency of approximately 9 % in R_1 and R_2 and 25 % of all methane production occured via electrode biofilm in the MEC+ADs. It was calculated that approximately 14-15 % of the COD reduction was achieved by the biofilm on electrodes. This assumption was convenient with the methane production and the COD removal rates in Figure 4.1 and 4.2 respectively.

Oxidation reduction potential (ORP) was around -315 and -345 mV in all three reactors in this study. ORP can be used as an indicator for the control of anaerobic digesters. CH_4 production mostly takes place between ORP values of -175 to -400 mV range [163, 164]. It is also claimed in detail that acidogenesis and methanogenesis take place at optimum ORPs between -250 : -300 mV and -300 : -360 mV respectively [163]. The ORP values measured in this study were in the range of methanogenesis.

pH is a crucial parameter due to the excess or low hydrogen ion concentration in the medium that can inhibit the process if not controlled. In this study, pH adjustment with chemical additives were not applied to the MEC+AD reactors and AD reactor due to the high alkalinity and buffering capacity of the cattle manure [162]. pH measurements of the R_1, R_2 and R_C were presented in Figure 4.3. In Set I, pH of the all reactor effluents changed

between 7.25 and 7.5 which was in the suitable range for anaerobic digestion and methanogenesis [66, 77, 163].

4.1.2. Batch Experiments-Set II

Batch experiments-Set II aimed to observe the effect of the full settled and acclimated biofilm and consortia on the electrodes of the MEC+AD reactors on methane production. In Set I, batch experiments were conducted just after the start-up period. However, in Set II, batch experiments were conducted during the study, after an acclimation period for biofilm on the electrodes. In Set II, two MEC+AD reactors with dublicates (R_{1V} and $R_{0.3V}$ with applied voltages of 1.0 and 0.3 V respectively) and two control reactors (R_{C1}: present control reactor-used in Set I, R_{C2}: a new control reactor-inoculated with fresh inocula obtained from a full scale anaerobic digester) were operated for the batch experiments. The MEC+AD reactors ($R_{1.0V}$, $R_{0.3V}$) and one control reactor (R_{C1}) were filled with 320 mL of cattle manure including 30±2 g VS/L (3 % VS; 4.10 TS, w/w; 48±3 g COD/L) and 480 mL of inocula that was already in the reactors remained from the previous sets. Only the newly prepared control reactor, R_{C2}, was filled with 320 mL of cattle manure and 420 mL fresh anerobic inocula obtained from biogas plant.

Two identical MEC+AD reactors ($R_{1.0V}$, $R_{0.3V}$) and two control reactors free of electrodes (R_{C1}, R_{C2}) were operated simutaneously. Total working volume of each reactor was 0.8 L in Set II. The reason of decreasing the working volume of the reactors from 1 L to 0.8 L was that a part of the volume in combined MEC+AD reactors were occupied by the biofilm on and inside of the electrode materials in this case GAC and carbon fiber cloth. Therefore in order to prevent foaming and overflowing in the MEC+AD reactors, the liquid volume in each reactor was reduced to 0.8 L. The batch cyle contiuned 10 days until the daily biogas production of the reactors decreased to 100 mL a day. Figure 4.5 shows the daily biogas production, methane content of biogas and cumulative biogas production in $R_{1.0V}$, $R_{0.3V}$, R_{C1} and R_{C2} at the given conditions.

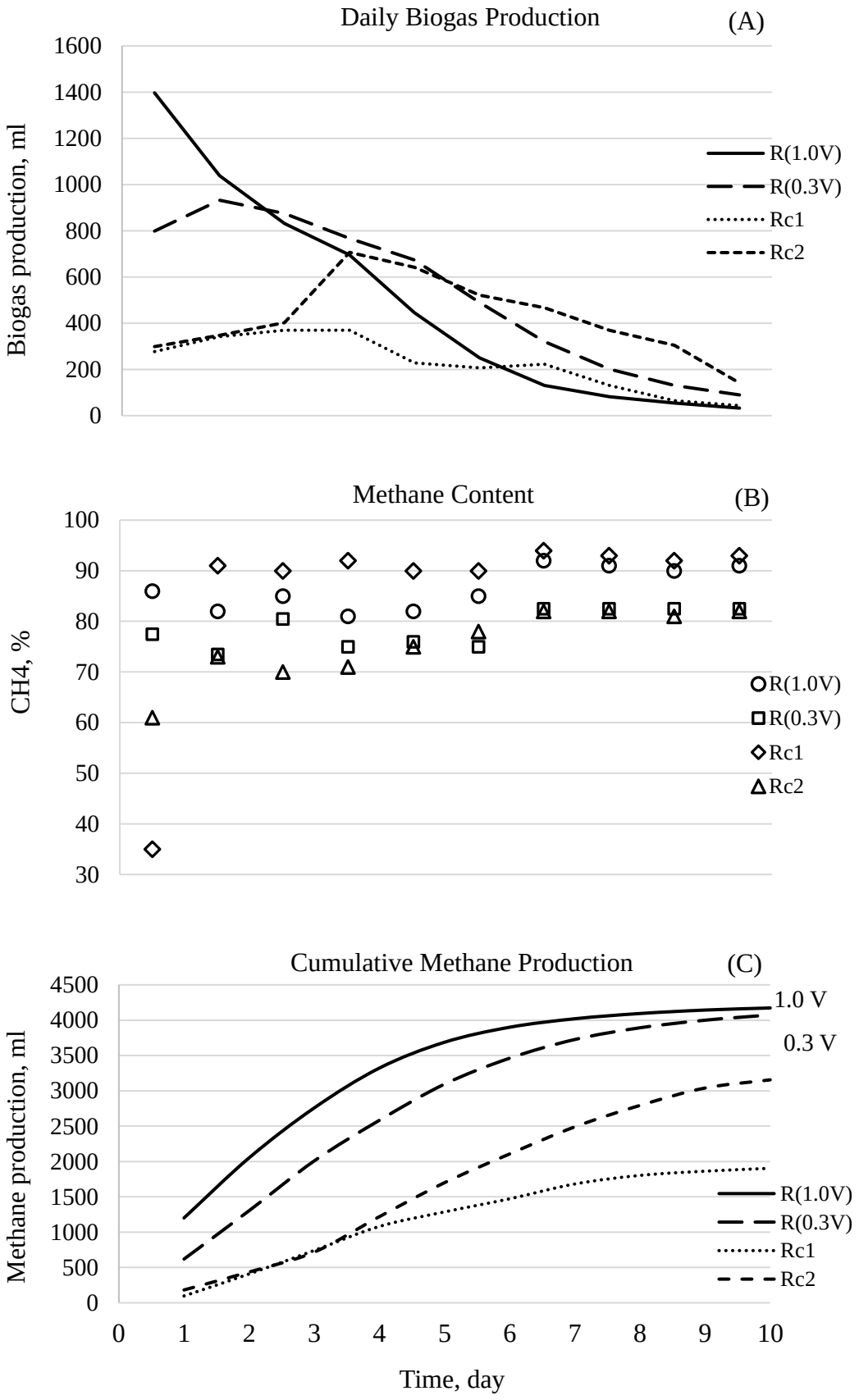

Figure 4.4. Biogas production (A), methane percentage of biogas (B) and cumulative methane productions (C) in $R_{1.0V}$, $R_{0.3V}$, R_{C1} and R_{C2} at batch experiment Set II.

Both of MEC+AD reactors, ($R_{1.0V}$, $R_{0.3V}$) produced most of the total biogas in the first days of Set II. $R_{1.0V}$ and $R_{0.3V}$ produced approximately 80 % and 64 % their cumulative biogas production respectively in the first four days. However R_{C1} and R_{C2} produced 60 % and 42 % of their total biogas production respectively at the same period of time. Cumulative biogas production of R_{C1} was very low compared the other reactors. $R_{1.0V}$ produced 80% of its total biogas at the end of 4th. Day. The fast and high biogas production were achieved in MECs when higher voltages of 0.8-1.0 V were applied compared to low voltage (< 0.5 V) applications [94, 95, 98]. Eventually, it can be concluded that bioelectrochemical degradation of organic materials took place faster than anaerobic digestion [31, 149, 155, 168].

Average volumetric biogas production of $R_{1.0V}$, $R_{0.3V}$, R_{C1}, and R_{C2} were 496±11 mL/L/d, 528±13 mL/L/d, 226±9mL/L/d and 420±110 mL/L/d respectively. Methane contents of the biogas obtained from $R_{1.0V}$, $R_{0.3V}$, R_{C1}, and R_{C2} ranged between 86-92 % ($R_{1.0V}$), 72-83 % ($R_{0.3V}$), 35-94 % (R_{C1}) and 61-82 % (R_{C2}) respectively during Set II. Methane contents of the biogas produced in each reactor had an increasing tendency from day one to day ten. High methane content in MEC+AD reactors ($R_{1.0V}$, $R_{0.3V}$) can be attributed to the voltage application which may have resulted in higher activation of hydrogentrophic methanogens for methane production. In MECs applied voltage leads to CO_2 reduction with electrons and also hydrogen formation via combination of electrons and protons. As a result, utilization of hydrogen and CO_2 by hydrogenotrophic methanogens form CH_4 [27, 30-32]. It is known that in energy applied MECs, hydrogenotrophic methanogenesis can proceed very fast and hence, enhance the methane production [31, 71, 121]. On the other hand high methane content of 35-94 % in R_{C1} can be attributed to the domination of the reactor by different microbiome (eg. hydrogentrophic methanogens) unlike R_{C2}. It was mentioned earlier that R_{C1} was inoculated by the effluent of the MEC+AD reactors prior to Set I batch experiment. However, R_{C2} was inoculated by a fresh sludge obtained from a full scale biogas plant. Although methane content of R_{C1} was very high during Set II, total methane production was the lowest compared to other reactors. This can be an indicator of different dominant microorganism community in the reactor.

Average methane productions of R_{1V}, $R_{0.3V}$, R_{C1}, and R_{C2} at Set II batch experiment were calculated as 417±11 mL/L/d, 407±11 mL/L/d, 190±7mL/L/d and 316±9 mL/L/d respectively. Specific methane productions of the reactors were calculated according to

the total methane production and feed VS concentration (30±2 g VS/L). Methane yields of $R_{1.0V}$, $R_{0.3V}$, R_{C1}, and R_{C2} were 435 mL/g VS, 424 mL/g VS, 198 mL/g VS and 329 mL/g VS respectively. Methane productions and methane yields of $R_{1.0V}$, $R_{0.3V}$, and R_{C2} reactors increased compared to the Set I batch experiment conducted prior to the semi-continuous studies. In Set I, MEC+AD reactors supplied with 1.0 V of power resulted in average methane production and methane yield of 240 mL CH_4/L/d and 287 mL CH_4/g VS respectively. It can be stated that when electrogens and methanogens fully developped and acclimatized on the MEC+AD electrodes, methane production and yield increased % 73 and % 51 resulting in 417±14 mL/L/d and 435 mL/g VS respectively for MEC+AD reactor ($R_{1.0V}$) which was supplied with 1.0 V of energy. Importance of acclimatization and enrichment of microbial community as well as pre-acclimatized electrodes on methane generation are pointed out in several studies [112, 127, 135, 152]. One way for high rate electromethanogenesis in anaerobic digestion combined with MECs is inoculating the reactor with hydrogenotrophic methanogens dominated culture. The other way for a quick start-up in the reactor or to recover a failed reactor is to use pre-acclimatized electrode materials in [171]. Acclimations period may vary according to the operational conditions (pH, temperature, substrate) and inoculum selection [171]. For example, Xu et al. [152] maintained a 80 days of acclimation period to ensure the symbiotic microorganism development while others reported one month for acclimation period [127]. Acclimation period of MEC+AD reactors used in the present study in Set I prior to Set II was rather shorter compared to those studies [127, 152]. Therefore, it can be suggested that enrichment of the electrodes and acclimation of microbiome on the electrodes were not fully completed in Set I batch experiment. Similarly, when fresh anerobic inocula obtained from biogas plant was used in the newly control reactor (R_{C2}), methane production and methane yield increased at a rate of 76 % and 54 % respectively compared to the results of the control reactor in Set I batch experiment. However, in Set II batch experiment, methane production and methane yield (190 mL/L/d; 198 mL/g VS) of the control reactor (R_{C1}) already in use, were similar to the results (180 mL/L/d; 214 mL/g VS) obtained in Set I batch experiment. These outcomes indicate that fresh inocula obtained from a biogas plant operated at longer HRTs (20-30 days) had a fully developped bacteria and methanogenic consortia which led to higher methane production and yields [172, 173]. It is stated that HRT play a key role in shaping microbial structure and on process performances [172]. Although it is desired to shorten the HRTs, it is likely, short HRTs can cause the wash out of the active bacterial population, hence lead to VFAs

accumulation and reduction in methane production and yield [64, 174]. It is also pointed out by several studies that HRT changes can shift the bacterial and methanogenic community and change the community diversity [172, 174, 175]. The control reactor, R_{C1}, used in Set II, was operated at HRTs of 3 to 4 days before Set II. Therefore lower CH_4 production and CH_4 yield of R_{C1} compared to R_{C2} can be attributed to short HRTs that could have led VFA accumulation and bacterial and methanogenic community shifts and diversity of the microorganism community. For instance, acetotrophic and hydrogenotrophic methanogens function in syntrophic relationship with acetogenic bacteria (hydrogen producing and hydrogen comsuming acetogens and acetate forming homoacetogens) [63, 66, 176]. Acetotropic methanogens are more sensitive to environmental changes such as HRT shortening than hydrogenetrophic methanogens [172, 173]. This could be the reason of the decrease in methane production and yield in R_{C1} which was operated at short HRTs prior to Set II batch experiment. Also shortening HRT could have led to hydrogen accumulation in the system that could have caused the hydrogen consuming acetogens got inhibited and limited producing acetate for acetotrophic methanogens. Consequently methane formation via acetoclastic pathway might have decreased. To sum up, it is suggested that compared to Set I, higher methane productions and methane yields obtained in MEC+AD reactors in Set II, can be attributed to fully developped biofilm on electrodes and acclimation of the microorganisms to operational conditions.

Methane productions and methane yields obtained from $R_{1.0V}$ and $R_{0.3V}$ after the acclimation of the electrodes were 417±11 mL/L/d, 407±11 mL/L/d and 435 mL/g VS and 424 mL/g VS respectively. These results were appropriate with the results of other studies. Hassanein et al. [49] operated a batch MEC at 35^{o}C which composed of 11 graphite plates anode electrodes inside a stainless stell cathode chamber. The TS and VS contents of mixture of inocula and food waste were 6.4 % and 4.9 % respectively in the 0.9 L MEC+AD reactor. They reported a biogas production of 0.59 L/L/d in 23 days with the addition of 0.9 V of power. The results obtained (417±11 mL/L/d, 435 mL/g VS) in Set II batch experiment was sufficient enough if considered the batch cycle (23 d) and VS of mixture used in the study conducted by Hassanein et al. [49]. Choi et al. [95] employed a laboratory scale 0.27 L of batch MEC integrated with anaerobic digester that had two carbon fiber brushes (high surface area) as electrodes. The reactor was operated by feeding 2 g glucose/L as substrate at 35^{o}C and various applied voltages. They reported

408 mL CH_4/g COD glucose at applied voltage of 1.0 V which was lower than the result of the present study. Integration of anaerobic digestion with MEC in a 0.35 L reactor operated at 35^oC and 1.0 V of power application was fed with synthetic WW at different concentrations at 8 days cycle. Methane yield was 0.34 L CH4/g COD at the maximum which was lower than the present study [155]. In another study, a 0.18 L of single chamber MEC contained mesh plates electrodes fabricated from Ti/Ru alloy and was operated at 37^oC and 1.8 V application over a period of 21 days. Methane yield of 0.2 L CH_4/g VSS was reported at most by feeeding thermal alkaline pretreated sludge as substrate [55]. The results of that study [55] was also lower compared to present study. After all, it can be seen in Table 2.4 that methane generation and yield obtained in Set II batch experiment of this study were significantly higher than the other studies conducted with single chamber, two chamber or integrated MECs operated at the temperatures of 35 to 40 ^oC and at different voltage supplementations and substrate loadings [54, 58, 59, 99, 115, 144, 150, 151] The reactors used in some of those studies had special and high cost electrode configurations [54, 58, 59, 115, 150].

Cumulative methane productions of $R_{1.0V}$, $R_{0.3V}$, R_{C1} and R_{C2} are presented in Figure 4.4 (C). At the end of the Set II batch study, $R_{1.0V}$ and $R_{0.3V}$ produced nearly and 119 % and 114 % higher methane compared to R_{C1} and 32 to 29 % higher methane compred to R_{C2} respectively. These differences can be attributed to the addition of power to the $R_{1.0V}$ and $R_{0.3V}$ which enables direct CO_2 reduction with electrons given to the MEC by electrical circuit. Power application can also enable H_2 production by the combination of electrons and protons in the cathode of the reactor. Then, H_2 and CO_2 are used by hydrogenotrophic methanogens for CH_4 formation at the cathode. It is explained earlier that in MECs, hydrogenotrophic methanogenesis can reproduce and operate rapidly and hence, enhance the methane production [31, 71, 121]. These methane formation pathways make MEC technology superior to conventional anaerobic digestion in terms of methane production. Exoelectrogens oxidize the substrate and release electrons to anode, and then electrons are transferred to cathode to produce methane [32]. In cathode, methane is produced by cathodic reduction of CO_2 with hydrogen ions through hydrogentrophic methanogens which mostly found in abundance around cathode in MECs [31, 59, 142, 169]. When electrogens and methanogens fully developped and acclimatized on the MEC+AD electrodes, methane production and yield increased [14, 112, 127, 135, 152]. In the

present study, enrichment and acclimation period was more than two or three weeks according to the Set I and Set II results.

COD, TS and VS removal rates of $R_{1.0V}$, $R_{0.3V}$, R_{C1} and R_{C2} are presented in Figure 4.5. As it was expected, $R_{1.0V}$ and $R_{0.3V}$ showed higher removal rates in COD, TS and VS than R_{C1} and R_{C2} due to the voltage application to $R_{1.0V}$ and $R_{0.3V}$ reactors. COD removals of $R_{1.0V}$ and $R_{0.3V}$ were 56 % and 53 % respectively. COD removal rates of R_{1V} and $R_{0.3V}$ were 19 % and 12 % higher than COD removals of R_{C2} which was 46 %. Even though inoculum taken freshly from a biogas plant which operated at HRT of 20-30 days, was placed into R_{C2}, its COD removal rate was lower than R_{1V} and $R_{0.3V}$ due to bioelectrochemically active microorganisms, electrogens. Mixed sewage sludge was treated in a 12 L integrated MEC with specially designed electrodes which were graphite fiber fabric. The electrodes were amended by multiwall carbon nanotube and nickel which enhanced methane generation [146]. The COD removal rate being between 64% and 39%, decreased when HRT reduced from 20 to 5 days at 35^{o}C and input voltage of 0.3 V. Ahn et al. [71] operated an integrated anaerobic digestion and MEC treating sewage sludge at 40^{o}C and applied voltage of 0.3 V reported a COD removal of maximum 40 % during cycle of 6 days. As it can be seen from Table 2.4, COD removal rates obtained in the present study arein line with the previous studies that were conducted with integrated MECs treating various waste streams at similar operational conditions to present study [49, 114, 144]. It is suggested that voltage application to MEC+AD reactors ($R_{1.0V}$ and $R_{0.3V}$) enhanced the activity of electrogens and methanogens, thereby increased methane production and organic removal efficiency compared to anaerobic digestion reactors (R_{C1} and R_{C2}).

Likewise, VS and TS removal of $R_{1.0V}$ and $R_{0.3V}$ were superior to R_{C1} and R_{C2}. TS and VS removal rates of $R_{1.0V}$ and $R_{0.3V}$ were 38 % and 39 % (TS) and 44 % and 47 % (VS) respectively. On the other hand TS and VS removal rates of R_{C2} were 33 % and 41 % respectively. Better removal rates of $R_{1.0V}$ and $R_{0.3V}$ can be explained by the voltage application and by the symbiotic relationship between electrogens and methanogens acclimatized on the electrode biofilm. It thought that electron application to MEC+AD reactors stimulated reduction of CO_2 with electrons and formation of methane. Voltage application could have also stimulated the reduction of CO_2 by H_2 that can be formed through electron and proton combination. Higher electron flow enhanced the electrogen

and hydrogenotrophic methanogen activity which resulted in higher organic removal compared to control reactor [27, 30-32, 119, 151]. It is also worth to indicate that percentage of methane in the biogas obtained from $R_{1.0V}$ and $R_{0.3V}$ were higher than the methane content of R_{C2} almost all along the batch experiment as it was shown in Figure 4.5. This can be a sign to that of methane production by direct reduction of CO_2 with electrons (electromethanogenesis) and efficient methane production via hydrogenotrophic methanogenesis due to voltage application.

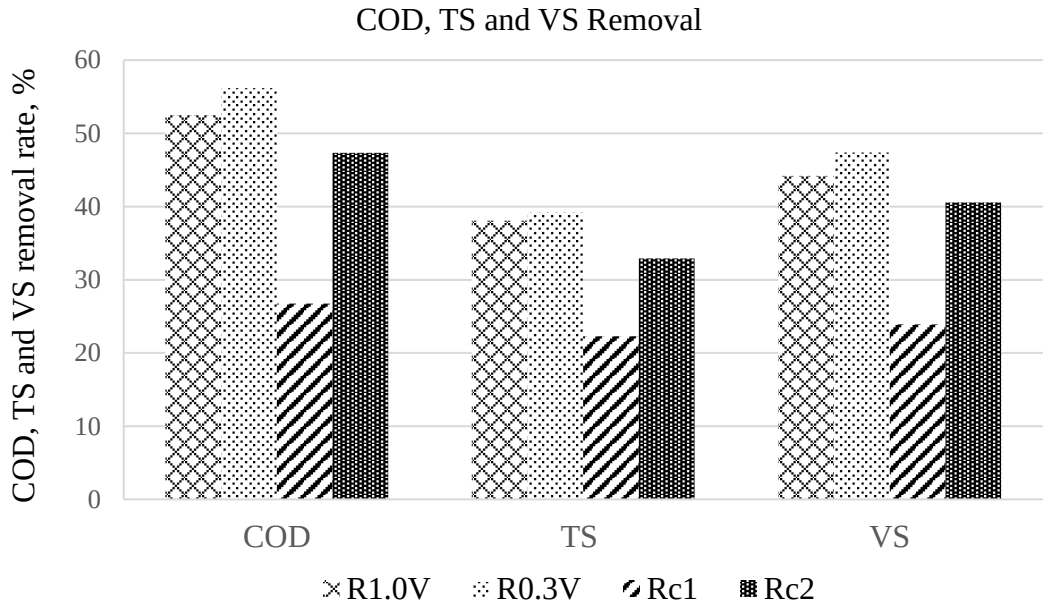

Figure 4.5. COD, TS and VS removal rates of $R_{1.0V}$, $R_{0.3V}$, R_{C1} and R_{C2}.

A batch study with 40 days cycle period, was carried out with an integrated anaerobic digestion and MEC reactor treating pretreated waste activated sludge (15.65 g TCOD/L) at ambient temperature ant applied voltage of 0.8. They reported 48.5 % VSS and 41.2 % TCOD removals at the end of the batch cycle [129]. In another study, single chamber MEC was fed with thermal-alkaline pretreated sludge (15.84 g TCOD/L) at various applied voltages and at 37^{o}C [55]. VSS removal at input voltage of 0.8 V was 49.6. Ti/Ru alloy electrode materials were used in that study to enhance the electron transfer. It can be seen that TS and VS removal rates in the present study were appropriate with the previous studies.

Despite the fact that methane production increased in Set II batch experiment which was conducted after the electrodes were acclimatized to the operational conditions, the COD, TS and VS removal results (53 %, 38 %, 44 % respectively) of MEC+AD reactors

decreased slightly compared to Set I batch experiment which was conducted at the very beginning of the study. This can be accredited to the biomass and biofilm development period on the electrodes of the MEC+AD reactors that could have been taking place during Set I batch experiment [14, 154].

Figure 4.6 shows the volumetric current desity (A), ORP (B) and pH (C) values of $R_{1.0V}$, $R_{0.3V}$, R_{C1} and R_{C2}. Volumetric current density and Coulombic efficiencies of $R_{1.0V}$ and $R_{0.3V}$ were calculated according to Equations 3.10 and 3.11. Current densities of $R_{1.0V}$ and $R_{0.3V}$ started from 11.25 A/m^3 and 1.4 A/m^3 respectively from first day with the manure loading to the reactors. The currenty density of $R_{1.0V}$ was higher respect to the currrent density of $R_{0.3V}$ due to the given voltage of 1.0 V [59, 95]. However it decreased over time and became stable at around 3.5 A/m^3 at the end of the experiment due to the stable exoelectrogen activity [96]. Current denstiy decreased from 11.25 A/m^3 to 3.5 A/m^3 and it can be explained by the COD reduction and usage of organic material via the fermentative bacteria and methanogens. COD breakdown and higher current density in $R_{1.0V}$ were correlated with the CH_4 formation during the experiment [48]. When the exoelectrogenic activity was high, in other words when the current density was high, methane production was also high as the VS removal rate. The decrease in the current density and the methane production occured simultaneously indicating that current density and methane production were directly proportional in $R_{1.0V}$ [28, 95, 149]. The methane production of $R_{1.0V}$ in the first 3 to 4 days was 29 % higher compared to $R_{0.3V}$ due to the high current density which occured as a result of 1.0 V of power application. At the end of the Set II, methane production of $R_{1.0V}$ was 2.5 % higher compared to $R_{0.3V}$. This can be an indication of higher methane production from CO_2 reduction with electrons given to the MEC by electrical circuit [27, 30-32]. Current density in $R_{0.3V}$ was around 1.4 A/m^3 and 1.9 A/m^3 at the beginning of the Set II batch experiment. Then current density of $R_{0.3V}$ decreased to below 1.0 A/m^3, and finally current density of $R_{0.3V}$ was around 0.5 A/m^3 at the end of Set II batch experiment due to the reduced organic material and 0.3 V voltage application.

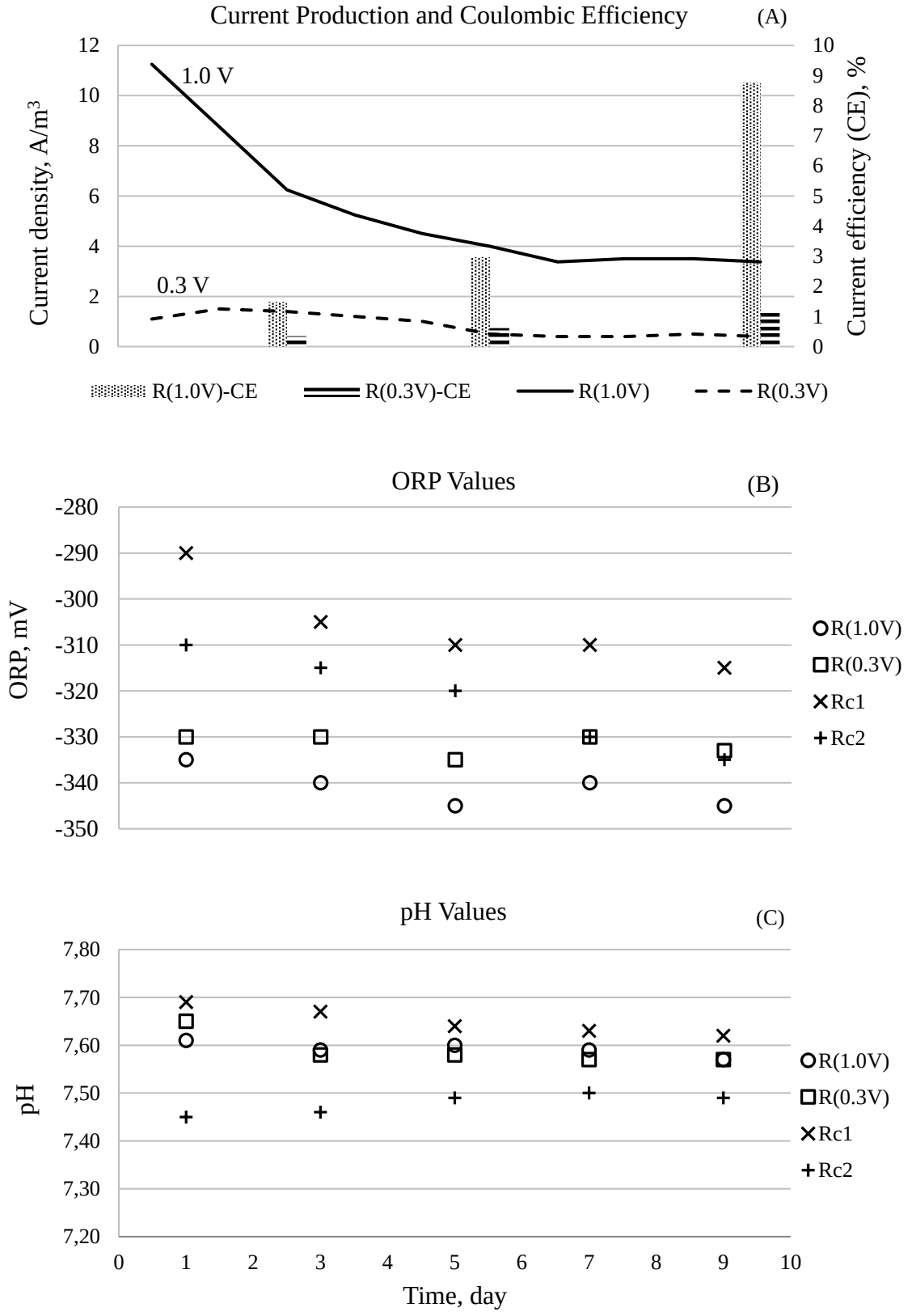

Figure 4.6. Current production and coulombic efficiency (A), measured ORP values (B) and pH values (C) of $R_{1.0V}$, $R_{0.3V}$, R_{C1} and R_{C2} respectively.

Current production of $R_{1.0V}$ was at the range of 9 mA at the begining and it decreased to around 3 mA towards the end of the Set II and stabled at that values. For $R_{0.3V}$, current production was around 1.5 mA and it declined to 0.5 mA towards the end of the set. Current density of $R_{1.0V}$ was appropriate with the previous batch studies conducted in single chamber MECs supplemented with 1.0 V of energy or similar values of voltages at mesophilic temperature [49, 95, 150, 153]. In some cases the results of the present study are better than the other studies [99, 104, 114, 120]. For example, Hagos et al. [114] obtained 0.3 A/m^2 (Ca. 1.8 A/m^3) current density when treating synthetic WW in a single chamber MEC which contained cobalt phosphorous catalysts deposited carbon cloth electrodes. The applied voltage was 0.8 V and the operation temperature was 38^oC in that study. Current density reported by Hagos et al. [114] was lower than the current density obtained in the present study. Current densities (0.5-1.88 mA/L) obtained from $R_{0.3V}$ in Set II was also convenient with the previous studies conducted with supplied voltage of 0.3 V. Ahn et al. [71] reported a current density of 1.63 A/m^3 with a 2.5 L single chamber MEC when treating sewage sludge at applied voltage of 0.3 V and 35^oC. The current densities obtained in this study (0.5 - 11.25 mA/L) at different supplemental voltages were appropriate with the reference studies indicating that electrodes were functional and tranfering the electrons.

Coulumbic efficiencies (CE) of the reactors $R_{1.0V}$ and $R_{0.3V}$ were calculated according to COD removal rates and average current production at that interval using Equation 3.11. At the end of the Set II, coulombic efficiencies were given in cumulative values. CE of $R_{1.0V}$ and $R_{0.3V}$ were 8.77 % and 1.18 % respectively. CEs calculated in Set II batch experiment were lower than some other studies that were ranging between 28 % and 154 % due to the higher current production and COD removal rates in the reactors. However, those studies that obtained higher CE compared to the present study, were conducted with acetate or synthetic wastewater as readily available substrate for exoelectrogens, at varying applied voltages and with pH adjustment. In some of those studies waste pretreatment were applied or specially designed electrodes were used in the units [57, 75, 99, 118, 128, 130, 133]. It was mentioned earlier that structural and operational conditions affect methane and current production and CE significantly [95, 133, 137 151, 155]. Considering substrate type and low cost structural and operational maintainance in the reactors used in this study, CE results (8.77 % and 1.18 % for R_{1V} and $R_{0.3V}$respectively)

obtained were similar to CE results of several previous studies that varied between 2.1 % and 14.3 % [59, 60, 99, 107, 123, 150, 154].

Lower CE obtained in this study can be attributed to that of most of the organic materials were used by fermentative bacteria and methanogens to form CO2 and methane [57, 59, 118, 123]. This might have led to a reduced current production by exoelectrogens that only perform on the biofilm on electrodes along with the other bacteria and archea. It is claimed that microorganism such as bacteria and archaea that live in symbiosis in the biofilm or bulk sludge, can metabolize organic materials and produce methane which in turn cause decreasement in electron transfer through the electrodes [154, 170]. In Set II batch experiment, methane production of $R_{1.0V}$ and $R_{0.3V}$ reactors were 29 % higher than the control reactor inoculated with fresh inocula, R_{C2}. This indicated that approximately 70 % of the methane was produced by the methanogens in the bulk sludge and 30 % of the methane was produced by the microorganisms on the electrode biofilm which could composed of methanogens, exoelectrogens and fermentative bacteria. COD reduction in $R_{1.0V}$, $R_{0.3V}$ and R_{C2} were 53 %, 57 % and 47 % respectively. Coulombic efficiency of 8.77 % and 1.18 % were obtained from $R_{1.0V}$ and $R_{0.3V}$ respectively, and 30 % of all methane production occured via electrode biofilm in the MEC+ADs. It was calculated that approximately 14 % of the COD removal in MEC+AD reactors were achieved by the biofilm on electrodes. This assumption is in consistent with the methane production and the COD removal rates (Figure 4.4 and 4.5).

Oxidation reduction potential (ORP) of $R_{1.0V}$ and $R_{0.3V}$ were averaged as -330 and -350 mV in this Set II. CH_4 production mostly takes place between ORP values of -175 to -400 mV range [163, 164]. It is also reported in detail that acidogenesis and methanogenesis take place at optimum ORPs between -250 : -300 mV and -300 : -360 mV respectively [163]. The ORP values measured for $R_{1.0V}$ and $R_{0.3V}$ were in convenient range for methanogenesis. On the other hand ORP values of R_{C1} and R_{C2} changed between -290 and -315 mV and, -310 and -335 mV respectively. ORP values of R_{C1} being on the edge of ORP values for methanogenesis, shows that higher negative values of ORP are more reduced conditions for methanogenesis. On the other hand ORP values of R_{C2} was in the methanogenesis range. Methane production of R_{C2} was superior to R_{C1} as it is shown in Figure 4.6, indicating that methanogenic community was already in the reactor. And we know that, inocula of R_{C2} was taken freshly from a biogas plant operated at HRTs

of 20-30 days and mesophilic temperature. pH measurements of the $R_{1.0V}$, $R_{0.3V}$, R_{C1} and R_{C2} were presented in Figure 4.6. pH of the reactor effluents changed between 7.45 and 7.7 during the batch experiment. The results were appropriate for the methanogenic process [66, 77, 163].

4.1.3. Energy Efficiency of the Batch Experiments

Engineering processes should be energy efficient regarding the sustainability and environmental concerns. In MEC technologies energy is needed for voltage supplementation and for heating, mixing and other operational activities. However energy optimization must be applied for sustainability and energy efficiency. To present the energy balance of the combined MEC+AD reactors, energy efficiencies regarding the electrical energy given to the system and energy content of substrate were calculated according to the Equations 3.12 and 3.15 respectively. These efficiencies were submitted into Table 4.1 in detail. It is important to indicate that in some studies, energy efficiencies were calculated based on the additional methane production in MECs compared to control anaerobic digesters [118, 120, 129]. In other studies energy efficiencies were calculated based on the total methane production of MEC reactors [60, 121, 125, 126, 150]. The energy of the oxidized substrate was also used in overall energy efficiency calculations in other studies [71, 113, 146]. The calculation methods and equations used in energy efficiency assesments with the input values were given in Table 4.1. Energy efficiencies relative to electrical input (r_{EE}) of the MEC+AD reactors were calculated according to Equation 3.12. r_{EE} were found as high as 836 % due to the efficient methane production in MEC+AD reactors. These results were appropriate with those studies conducted at single chamber MECs with different constructional and operational parameters [48, 60, 118, 120, 128, 129, 131, 135, 150]. An UASB MEC reactor succeeded r_{EE} of more than 1200% with synthetic wastewater at 7 g COD/L concentration at 6 h HRT and 1.0 V application [60]. Guo et al. [150] designed a specially stacked cathode in a MEC which treated synthetic beer brewery wastewater at 35 ^{o}C. They reported r_{EE} values of between 378 and 1584 % at various applied voltages. In a batch study at 20^{o}C, alkali and ultrasonic pretreatment was applied to waste activated sludge that concentration of 15.65 g TCOD/L. r_{EE} of more than 250 % was gained at power application of 0.8 V [129]. If the ratio of energy value of methane to energy value of electrical input is more than 100%, it indicates that under those operational terms the process is economically profitable

Table 4.1. Energy efficiencies in MEC+AD reactors and control reactors relative to diferent parameters.

Total energy obtained from methane	Energy given to the system in voltage	Energy content of the substrate removed	Energy of methane originated from voltage application
W_{CH4}	W_V	W_S	$(W_{CH4})e$
$n_{CH4}\ \Delta H_{CH4}$	$I\ V_{ap}\Delta t$	$\Delta H_S.m_S$	$\Delta H_{CH4}(\frac{V_{MEC}-V_C}{22.4\ L/mol})$
ΔH_{CH4}=890.8 kJ/mol, $n_{CH4}=\frac{V_{CH4}}{22.4\ L}$	I_{av} = X Ampere, V_{ap}= 1.0 V, 0.3 V; $\Delta t = HRT = 10\ days = 240\ h = 3600\ x\ 240\ s$	ΔH_S =3900 kcal/kg or 16302 kJ/kg for 97.2 % dry manure -TS) (700 kg/m^3), VS/TS=0.75, (m_s=VS_{in} - VS_{out})	V_{MEC}, V_C : Cumulative CH_4 production of MEC+AD and control reactors respectively.
R_1= 95.08 kJ	3.5 kJ (I_{av}:4 mA)	22.4 kJ/ g VS x 8.5 g VS x %49 (or %41 for R_C) g $VS_{rem.}$x 1.1 kg = 102.45 kJ (or 85.8 kJ for R_C)	R1= 23.66 kJ,
R_2= 96.12 kJ	3.5 kJ (I_{av}:4 mA)		R2= 24.70 kJ
R_C= 71.42 kJ			
R_{1V} = 165.95 kJ	4.84 kJ (I_{av}:5.6 mA)	22.4 kJ/ g VS x 9.6 g VS x %45.5 (or %41 for R_{CN}) g $VS_{rem.}$x 1.1 kg = 107.63 kJ (or 96.98 kJ for R_{CN})	R_{1V}=40.44 kJ
$R_{0.3V}$=162.01 kJ	0.39 kJ (I_{av}:1.5 mA)		$R_{0.3V}$=36.51 mL
R_{C2} = 125.51 kJ			
Cumulative CH_4 production of the reacors: R1= 2391 mL, R2= 2417 mL, R_C= 1796 mL; R_{1V}= 4173 mL, $R_{0.3V}$= 4074 mL, R_{C2}= 3156 mL, m=m_s=1.1 kg			

	Energy efficiency relative to the electrical input	Energy efficiency relative to energy content of the removed substrate and energy input
	r_{EE}	r_S
Reactors	$\frac{(W_{CH4})e}{W_V}x100\%$	$\frac{W_{CH4}}{W_S}x100\%$
R_1 (1V)	676 %	92.81 %
R_2 (1.0 V)	705 %	93.82 %
R_C	-	83.24 %
R_{1V}	836 %	154.19 %
$R_{0.3V}$	-	150.52 %
R_{C2} =	-	129.42 %

ΔH_S =3900 kcal/kg or 16302 kJ/kg for 97.2 % dry manure -TS [165] ; 1.0 Volt.ampere.second = 1 joule
22.4 kJ/ g VS= 16302kJ/kg/0.972/1000/0.75

It is shown in Table 4.1 that approximately 75-77 % of methane produced in MECs could have been originated from the bulk sludge or biofilm on the electrodes due to the commonly suggested pathways of acetotrophic and hydrogenotrophic methanogenesis without the effect of applied power. With the effect of power supply, MEC+AD reactors surpassed methane production of control reactor by 23-25 %. It is estimated that roughly 30 % of methane produced in anaerobic process originates from hydrogentrophic methanogenesis [63, 177, 178]. However addition of power to MEC reactors can enhance the growth of hydrogenotrophic methanogens faster than acetotrophic methanogens especially at low HRTs [55, 60, 144]. Thereby contribution of hydrogen to methane formation can exceed 30 %. In this study electrical energy efficiency (r_{EE}) of MEC+AD reactors were over 100 % owing to the direct conversion of CO_2 into methane via electron capturing and occurence of hydrogenotrophic methanogenesis in the biofilm or suspended sludge.

Proportion of the energy content of methane produced in reactors and energy content of the removed organic material (r_S) of R_1, R_2 and R_C in Set I batch experiment were 92.8 %, 93.8 % and 83.24 % respectively. And proportion of the energies obtained from methane and removed substrate respectively for $R_{1.0V}$, $R_{0.3V}$ and R_{C2} in the second batch experiment were 154.2 %, 150.5 % and 129.4 % respectively. Voltage application to the MEC+AD reactors, increased the substrate removal by 12 % an 18 % in both Set I and Set II compared to the control reactors. Energy efficiency relative to energy content of removed subsrate for MEC+AD reactors was appropriate with the previous studies [136, 145] and it was rather higher than the others [113, 124, 127, 135]. A pilot scale single chamber MEC operated semi continuously at 0.3 V voltage application and mesophilic condition succeeded overall efficiency of 90 % and more at OLRs between 1.44 to 5.76 kg VS/m3/d. with mixed sewage sludge as the substrate [147]. Song et al. [146] used specially designed graphite fiber fabric electrodes which enhanced the MEC performance. In another study, Yin et al. [137] reported 75 % of overall energy efficiency with a single AD–MEC co-cultivating *Geobacter* with *Methanosarcina* which achieved 24% more CH_4 production at 25ºC compared to same MEC without bacteria selection. They used acetate as the carbon source and the supplied power was 1.0 V.

It can be seen from Table 4.1 that energy output of additional methane production in MEC+AD reactors due to the external power, was six to eight times higher than the energy input in both sets. Also electricity supply increased the energy efficiency due to the organic matter removed compared to the control reactors.

4.2. Sequencing Batch Operations at Different HRTs

In this part of the study, MEC+AD reactors and a control reactor were operated at 37±2 ^{o}C in sequencing batch mode/semi continuously at diferent HRTs (6, 4, 3, 2, 1 days) with varying external power supplementations (0.3 V, 0.6 V, 1.0 V). Once a day, a specific volume of waste according to HRT was removed from the reactors and same amount of fresh cattle manure was fed to the reactors. Feeding and removing the manure was carried out by plastic scaled syringes. No external chemicals were added to the reactors for the control pH of the reactors and no pretreatment were applied to the fed manure.

The reactors were fed with cattle manure including 3±0.03 % VS and 4.15±0.05 % TS (w/w) in this part of the study. HRTs of 6, 4, 3, 2 and 1 day respectively (5 sets) were applied for the operation of the reactors. Various external power supplementations (0.3, 0.6 and 1.0 V) were applied to the reactors. All operational combination was applied in dublicates, one with a MEC+AD reactor, the second one with another MEC+AD reactor. Experiments of continuous operation were conducted at least 15 days or 3 HRT periods. It was stated by many researchers that MEC process was superior to the anaerobic digesiton processes when compared the methane generations and substrate removal efficiencies. In the present study, anaerobic digestion and MEC+AD process were compared in terms of biogas production performance and organic removal efficiency. Comparison of the MEC+AD and control reactors was made for the operational HRTs of 6, 4 and 3 days. All the results obtained from MEC+AD and control reactors at different HRTs and applied voltages are presented in a table at Section 4.4.

It is essential to note that volume of the MEC+AD reactors were 0.8 L in the beginning of the continuous experiments. When the operational HRTs were decreased from Set I to Set V, during the study, reactors were exposed to foaming and the gas ports were clogged by the manure particles. Therefore, the operational volume of the MEC+AD reactors were decreased to 0.6 L step by step throughout the study. MEC+AD reactors were operated for 5 Sets for 5 different HRTs in this part of the study.

4.2.1. Set I - HRT of 6 Days

Following the batch experiments, three reactors [$(MEC+AD)_{0.3V}$, $(MEC+AD)_{0.6V}$, $(MEC+AD)_{1.0V}$] were operated under 6 days of HRT at 0.3, 0.6 and 1.0 V of power applications in Set I. A control reactor was also operated to compare to MEC+AD reactors. Control reactor was the same reactor used in the batch experiments. Cattle manure concentration was between 28.6-31.6±1 g VS/L (2.95±0.15 % VS; 4.1±0.1 % TS; w/w) and OLR was 5 g VS/L/d. The MEC+AD and control reactors had working volume of 0.8 L. To obtain 6 days of operating HRT, reactors were fed with 133 mL of cattle manure once a day as it was described in the earlier sections. The study continued more than 3 HRT periods in Set I. The biogas productions (A) and methane content of biogas (B) obtained in this study are depicted in Figure 4.9.

It can be seen from Figure 4.7 (A) that biogas productions of MEC+AD reactors operated under different voltage applications at HRT of 6 days were significantly different from each other. Also biogas productions of all MEC+AD reactors were significantly higher than biogas production of control reactor. Average daily biogas production rates in MEC+AD reactors operated at 0.3 V, 0.6 V and 1.0 V voltage application and in control reactor were 1.23, 1.53, 1.29 and 1.0 L/L/d respectively in Set I. Methane percentage of the biogas obtained in MEC+AD reactors were 77, 78 and 79 % in the order of supplied voltages of 0.3, 0.6 and 1.0 V respectively in Set I. On the other hand methane content of biogas produced in the control reactor was 74 %. There were no significant difference between the methane contents of the biogas produced in MEC+AD reactors operated under different applied voltages.

Methane yield of the reactors were 0.19, 0.24, 0.20 and 0.15 L CH_4/ g VS respectively in $(MEC+AD)_{0.3V}$, $(MEC+AD)_{0.6V}$, $(MEC+AD)_{1.0V}$ and control reactors. Methane yield of $(MEC+AD)_{0.6V}$ reactor was 26 % and 20 % higher than $(MEC+AD)_{0.3V}$ and $(MEC+AD)_{1.0V}$ respectively. Methane yield of control reactor was about 26 % lower than the MEC+AD reactors applied with different voltages. The differences in biogas production and methane yield of MEC+AD reactors and control reactor can be attributed to the dominant presence of hydrogenotrophic methanogenesis and electromethanogenesis that take place in MEC+AD reactors [27, 30-32]. Voltage application to MECs cause rapid hydrogentrophic methanogenesis and electromethanogenesis and enhance the biogas production [119, 151]. Voltage

application to MECs can enable direct CO_2 reduction with electrons and form CH_4. Also electrons given to MECs can combine with protons and form H_2. Following, H_2 is used by hydrogenotrophic methanogens to produce CH_4 [30-32].

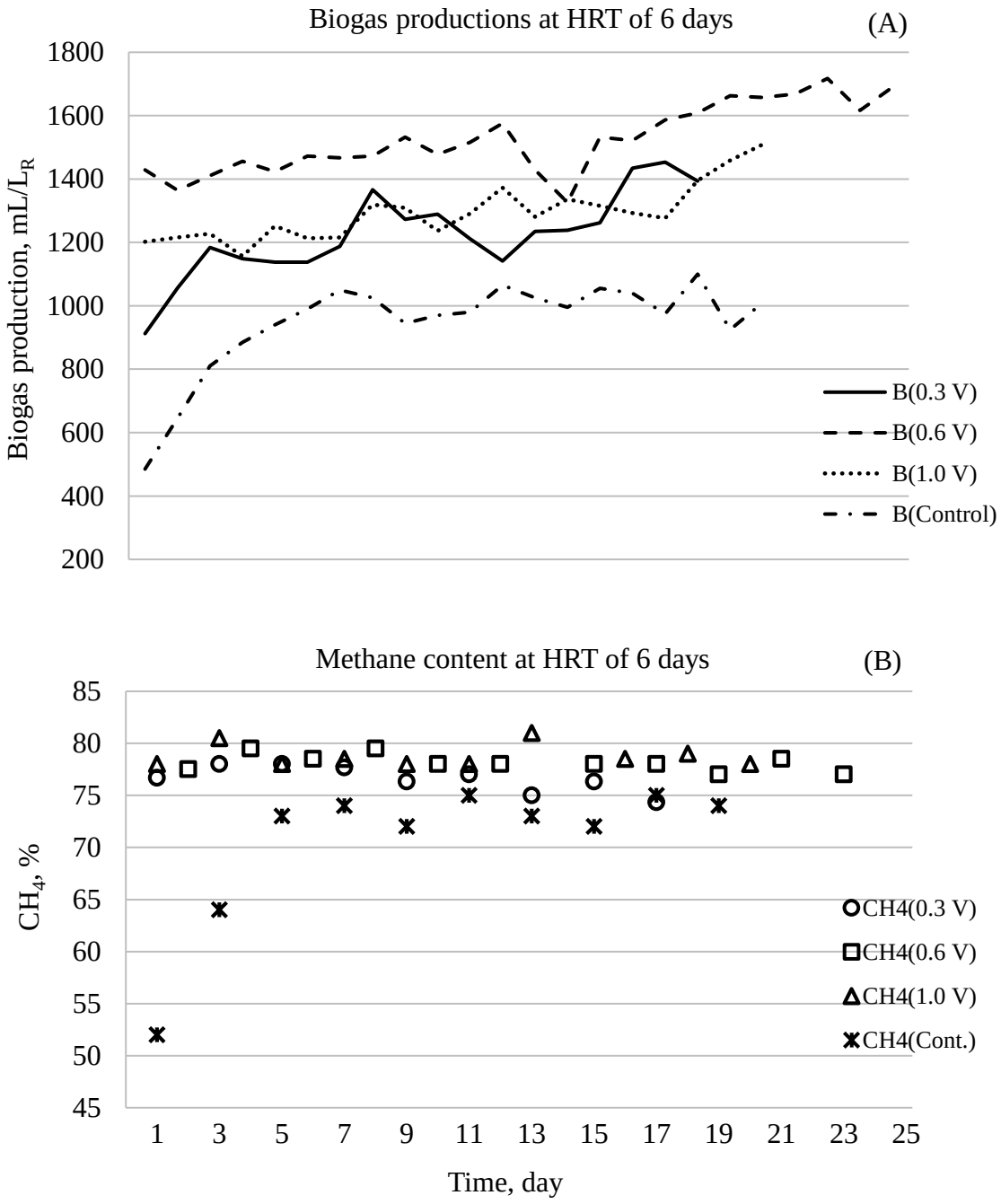

Figure 4.7. Biogas production (A) and methane content of biogas (B) in MEC+AD and control reactors at HRT of 6 days.

The results obtained in Set I are in consistent with the other studies operated under similar conditions. Song et al. [146] treated mixed sewage sludge in a 12 L combined MEC and AD reactor equipped with specially designed electrodes with carbon nano tubes. The reactor was operated at power supplementation of 0.3 V and at 35^{o}C. The operational OLR and HRT in the study were of 5.76 g VS/L/d and 5 days respectively. Methane production and yield were 1.34 L/L/d and 0.41 L/ g $COD_{rem.}$ respectively. In our study methane production was lower than that obtained by Song et al. [146]. This can be attributed to the substrate type and the well designed electrodes that could enhance the electrogen activity. In addition, methane yields were calculated in terms of removed COD in that study where as the yields were expressed in fed VS in the present study. Xu et al. [152] treated synthetic brewery wastewater (65.3 g COD/L) at OLR and HRT of 5.8 g COD L/d and 5.6 d respectively in an upflow MEC packed with coal based granular (GAC) and powdered activated (PAC) carbon and graphite rod. The biogas production results were between 0.91 and 1.16 L/L/d for reactors with GAC and PAC respectively at 0.5 V of applied voltage and 35^{o}C. The results of the present study were significantly superior to that of Xu et al. reported [152].

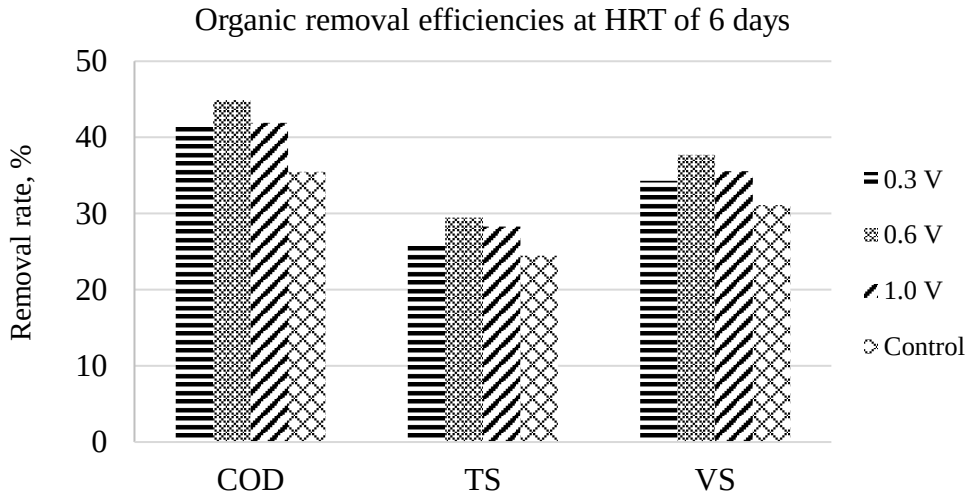

Figure 4.8. Organic removal rates of MEC+AD reactors and control reactor at HRT of 6 days.

Methane productions in anaerobic reactors originates from the degradation of organic substrates in the reactors. Organic removal rates of MEC+AD and control reactors operated at HRT of 6 days are illustrated in Figure 4.8. In Set I, highest removal efficiency of COD, TS and VS were achieved by the reactor operated at voltage supplementation of 0.6 V. Organic removal rates of $(MEC+AD)_{0.6V}$ were 44.9 % (COD), 29.5 % (TS), and

37.7 % (VS) respectively. On the other hand organic removal efficiencies in other MEC+AD reactors operated at 0.3 and 1.0 V power applications were slightly lower than the results of the $(MEC+AD)_{0.6V}$. Therefore we can conclude that power application was not significantly effective on the organic removal rate. The COD, TS and VS removal efficiencies in control reactor were 35.5, 24.5 and 31.1 % respectively. Organic removal efficiencies of MEC+AD reactors operated at any applied voltages were higher (16 % for COD, 10 % for VS, and 7 % for TS) than the control reactor. It can be attributed to the voltage application to MEC+AD reactors which enhanced methanogenic activity. Organic removals obtained in Set I were in consistent with the other studies conducted at similar HRTs. Song et al. [146] treated mixed sewage sludge in a combined MEC and AD reactor and achieved 39 % of COD removal efficiency at 35^{o}C and at OLR and HRT of 5.76 g VS/L/d and 5 days respectively. Waste activated sludge was used as substrate in another study conducted with single chamber MEC operated at ambient temperature and solid retention time of 7 to 14 days. The organic removal efficiencies at 1.2 V power application were between 30-34 % during the study [121].

Current production in reactors are dependent on the applied voltage as well as the electrogenic activity of the reactors. Higher applied voltage does not necessarily result in higher current production at the reactors. Microorganisms can be inhibited from high voltages [95]. Also magnitude of the applied voltage can effect the variety and dominance of microbial community [147, 151].

Current productions of MEC+AD reactors operated at three different voltage applications are shown in Figure 4.9. MEC+AD reactor applied with 1.0 V of power exhibited the highest current production which varied between 2.7 and 4.7 A/m^3 in Set I. On the other hand current productions in MEC+AD reactors applied with 0.6 V and 0.3 V of power varied between 1.4 - 2.6 A/m^3 and 0.9 – 1. 8 A/m^3 respectively. Applied voltage amount significantly effected the current production similarly with the previous works [96, 179].

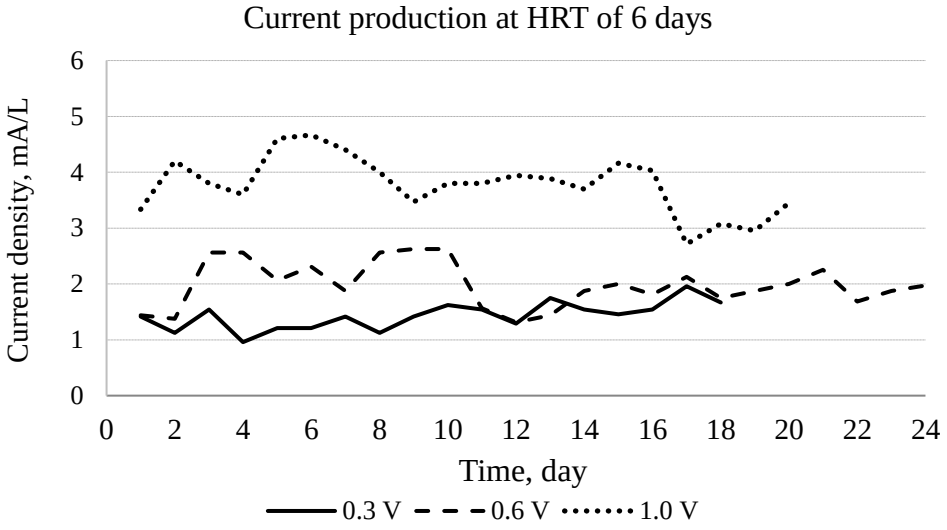

Figure 4.9. Current production in MEC+AD reactors at HRT of 6 days.

4.2.2. Set II - HRT of 4 Days

Comparison of the control reactor with the MEC+AD reactors continued at 4 days of HRT. Cattle manure concentration was between 29.8-30.2 g VS/L (2.98±0.02 % VS; 4.13±0.3 % TS; w/w) and the OLR was 7.5±0.05 g VS/L/d in Set II. The MEC+AD reactors had working volume of 0.8 L. To obtain 4 days of operating HRT, reactors were fed with 200 mL of cattle manure once a day. The experiments were conducted more than 3 HRT periods. Biogas productions (A) and methane content of biogas (B) obtained in Sett II are depicted in Figure 4.10. It can be seen that biogas productions of the $(MEC+AD)_{0.6V}$ was slightly higher than the biogas productions of MEC+AD reactors operated at 0.3 and 1.0 V of voltage applications. All three MEC+AD reactors were superior to control reactor in terms of biogas production. At the steady state period of the study, average daily biogas production rates in reactors operated at 0.3 V, 0.6 V and 1.0 V power applications were 1.75, 2.05 and 1.75 L/L/d respectively in Set II. Methane contents of the reactors were 77, 76 and 78 % in the same order. Average daily biogas production of control reactor was 1.28 L/L/d and the methane content was 76 %. There were no significant difference between the methane contents of the biogas produced in MEC+AD reactors and control reactor. Methane yields of the reactors were 0.18, 0.21, 0.18 and 0.13 L CH_4/g VS respectively in $(MEC+AD)_{0.3V}$, $(MEC+AD)_{0.6V}$, $(MEC+AD)_{1.0V}$ and control reactor. Methane yield of $(MEC+AD)_{0.6V}$ reactor was 17 % higher than the other two MEC+AD reactors and 62 % higher than the control reactor.

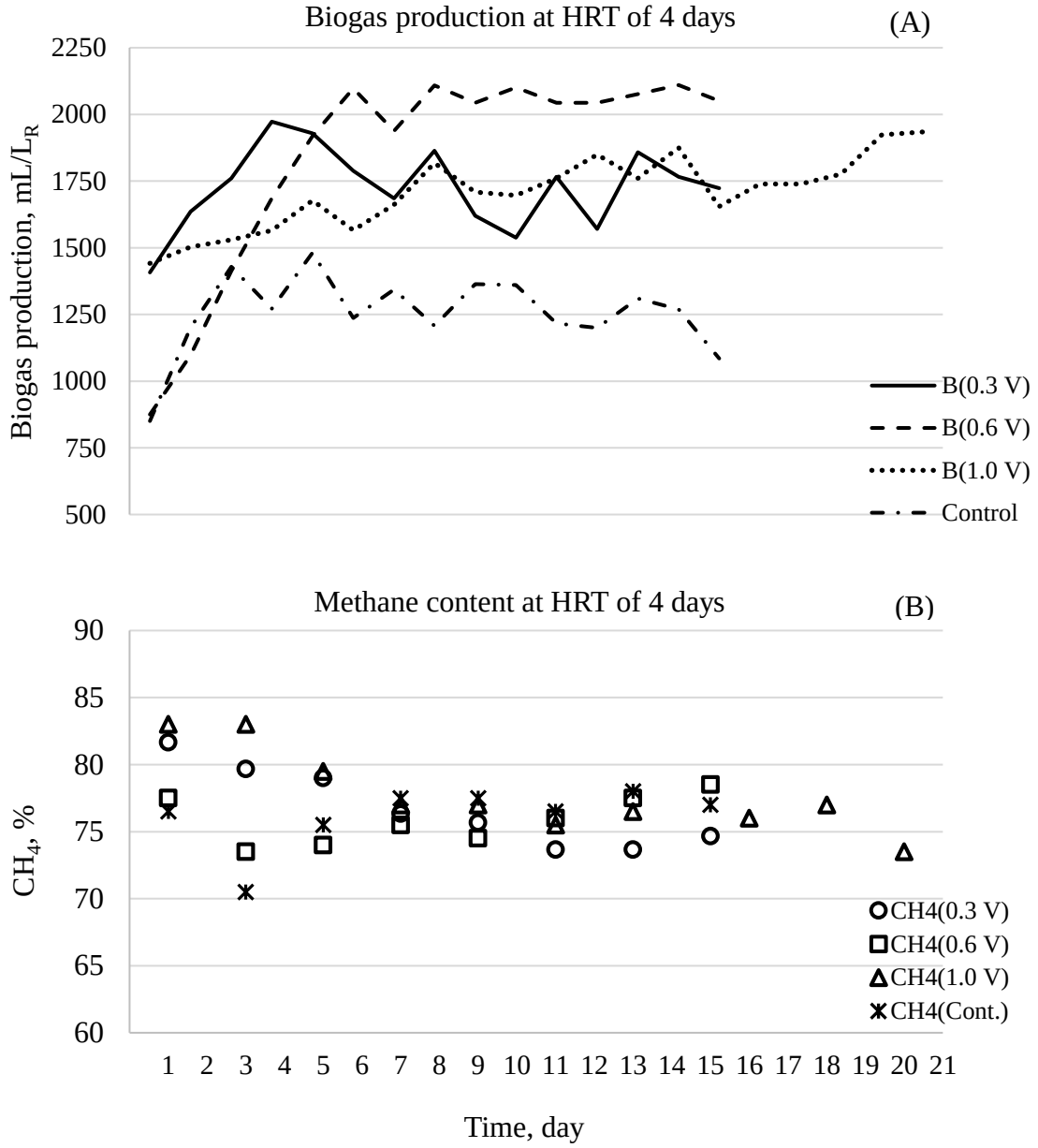

Figure 4.10. Biogas production (A) and methane content of biogas (B) in MEC+AD reactors at HRT of 4 days.

The biogas production results obtained in Set II were higher than the Set I due to the inreased OLR. However methane yield was lower in Set II compared to Set I due to the higher OLR (7.5 g VS/L/d) and shorter HRT (4 days). These results were still superior to the results obtained at other anaerobic digestion processes conducted at similar conditions. Bi et al. [180] co-digested cattle manure and food waste in a CSTR operated at 37 ºC and different HRTs and OLRs. The highest methane production (1.48 L/L/d) was achieved at an HRT of 5 days and OLR of 12 g VS/L/d in the study. However methane yields decreased at 10 days of HRT and shorter. Complete process failure due to volatile

fatty acids accumulation and microorganisms wash out was reported at HRT of 4 days (OLR:15 g VS/L/d). Another study was conducted in a two stage anaerobic digestion system operated at thermophilic and mesophilic conditions respectively. Poultry manure was treated in that two stage anaerobic system [181]. At similar conditions with the present study, such as total HRT of 4.5 day and OLR of 7.9 g VS/L/d, methane yield was 0.15 L/g COD that was slightly higher than the results obtained at control reactor in this study. The results of MEC+AD reactors in this study were higher than those studies that were operated at similar operational conditions (temperature, HRT, OLR) with two stage reactors or co-digestion of different wastes or pretreatment of the wastes.

COD, TS and VS removal performances of MEC+AD and control reactors at 4 days of HRT operation are illustrated in Figure 4.11. At 4 days of HRT, highest organic matter removal were achieved by the MEC+AD reactor operated at voltage supplementation of 0.6 V. Organic removal rates of $(MEC+AD)_{0.6V}$ were 38.7 % (COD), 26 % (TS) and 32.8 % (VS) respectively. On the other hand organic removal efficiencies in the other MEC+AD reactors operated at 0.3 and 1.0 V power applications were slightly lower than the results of the $(MEC+AD)_{0.6V}$. Therefore we can conclude that power application was not significantly effective on the organic removal rate in Set II. Organic removal obtained in Set II was convenient with the other studies conducted at similar HRTs [121]. COD, TS and VS removal efficiencies of the control reactor were 29.5 %, 21.7 % and 28.6 % respectively at 4 days of HRT. It is obvious that organic removal efficiencies in MEC+AD reactors were higher than the the control reactor at all different power application modes.

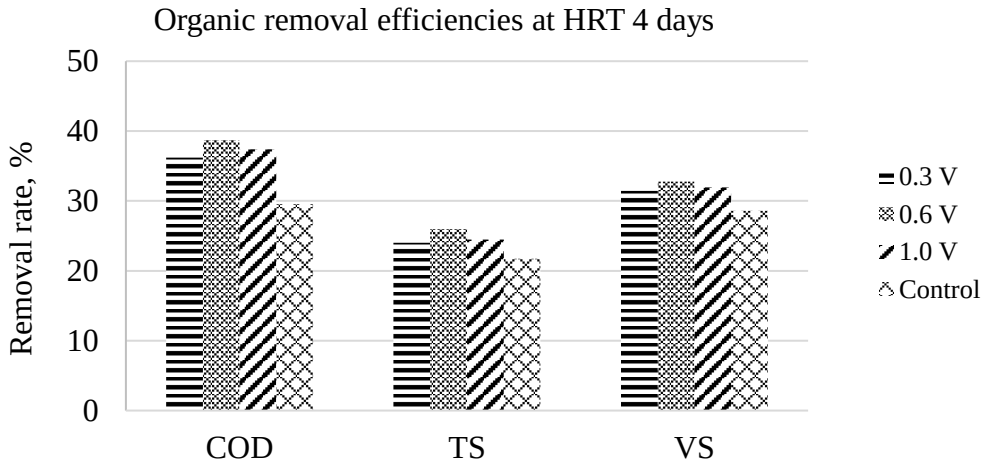

Figure 4.11. Organic removal rates of MEC+AD reactors and control reactor at HRT of 4 days.

Current productions of MEC+AD reactors operated at three different voltage applications and at 4 days of HRT are shown in Figure 4.12. MEC+AD reactor applied with 1.0 V of power exhibited the highest current production which varied between 4 and 6.5 A/m^3, mostly being between 5-6 A/m^3, in Set II. On the other hand current productions in MEC+AD reactors applied with voltage of 0.6 V and 0.3 V, varied between 2.5 - 4.4 A/m^3 and 0.9 – 1.8 A/m^3 respectively. Applied voltage significantly effected the current production as it was reported in other studies [96, 179]. However current production was not correlated with the methane production since the highest methane production was obtained in MEC+AD reactor operated at applied power of 0.6 V.

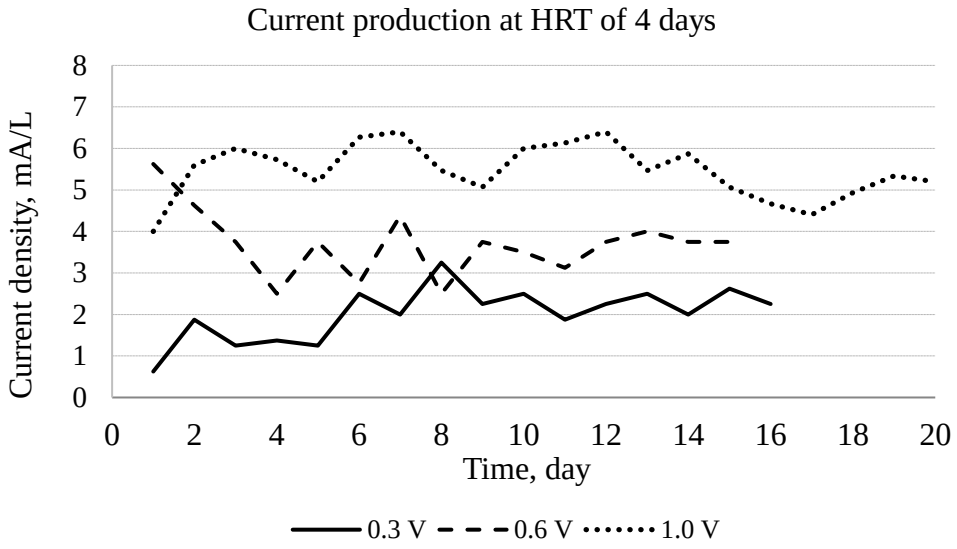

Figure 4.12. Current production in MEC+AD reactors at HRT of 4 days.

4.2.3. Set III - HRT of 3 Days

Comparison of the control reactor and MEC+AD reactors continued at 3 days of HRT. In Set III, cattle manure concentration was the same with the previous set (29.8-30.2 g VS/L; 3±0.03 % VS; 4.15±0.3 % TS; w/w). The reactors were operated at 3 days of HRT by feeding 267 mL of cattle manure once a day. Thus operational OLR inreased to 10 g VS/L/d in Set III. Biogas productions and methane percentage of biogas obtained in Set III are illustrated in Figure 4.13. It can be noticed that biogas production rate of $(MEC+AD)_{1.0V}$ was slightly higher than the other MEC+AD reactors. Daily average biogas productions of the MEC+AD reactors with supplied voltages of 0.3, 0.6 and 1.0 V were 2.46, 2.46 and 2.68 L/L/d respectively in Set III. Methane rate of the biogas generated in MEC+AD reactors were 77, 77 and 76 % respectively. Thus methane yield

of the MEC+AD reactors were 0.19 L/g VS, 0.19 L/ g VS and 0.2 L /g VS for voltage applications of 0.3, 0.6 and 1.0 V respectively. Methane productions of the MEC+AD reactors were very close to each other in Set III and in Set II as well. This can be attributed to the operation of the all three reactors under different voltages at different sets. This type of voltage application strategy may have resulted in various microbial communities acclimatized to different voltage applications which can lead to a strengthened microbial culture. Yu et al. [151] stated that specific microbial communities found in the suspension and in the cathode biofilm of a combined MEC and anaerobic system were at various dominance due to the different voltage applications. In another study it was reported that dominance of phylum, class and species of planktonic microbial communities differentiated from voltage of 0.5 V to of 0.7 V which led to different methane production rates [147]. In that study, a combined MEC+AD reactor were applied voltages of 0.5, 0.7 and 0.3 V respectively during the study, similar to voltage application strategy in the present study. Microbial communities at applied voltage 0.7 V found to be different compared to other applied voltages.

All three MEC+AD reactors were superior to control reactor when biogas production performances compared in Set III. At HRT of 3 days, it is thought that control reactor failed due to the acidification as a result of short HRT. Biogas production and methane content of the control reactor decreased sharply to around 0.45-0.5 L/L/d and 35-40 % respectively. Methane yield of the control reactor decreased to 0.02 L CH_4/g VS which shows the failure of methanogenic process. The performance of the control reactor became stable around these values as if it was an acidogenic reactor at 3 days of HRT and 10 g VS/L/d of OLR which might have arisen due to the insufficient time for acetogens and methanogens in order to operate fully [77, 182]. In a similar study, hydro-thermal pretreatment (at 180^{o}C and 1.2 MPa) was applied to blended sewage sludge and waste activated sludge that was used to feed an anaerobic reactor operated at HRT of 3 days and OLR of 13.9 g VS/L/d at 37^{o}C. Compared to result at 5 days HRT, methane yield decreased from 0.21 L/g $VS_{in.}$ to 0.12 L/g $VS_{in.}$ at 3 days HRT as a result of VFA accumulation and process inhibition which arose due to insufficient retention time and microorganisms washout [174]. Consequently, in the present study, biogas productions obtained from MEC+AD reactors in Set III were higher than Set II due to the inreased OLR. However methane yields in Set III was similar with the previous one indicating that

MEC+AD reactors can operate effectively at OLR of 10 g VS/L/d and HRT of 3 days without any instability.

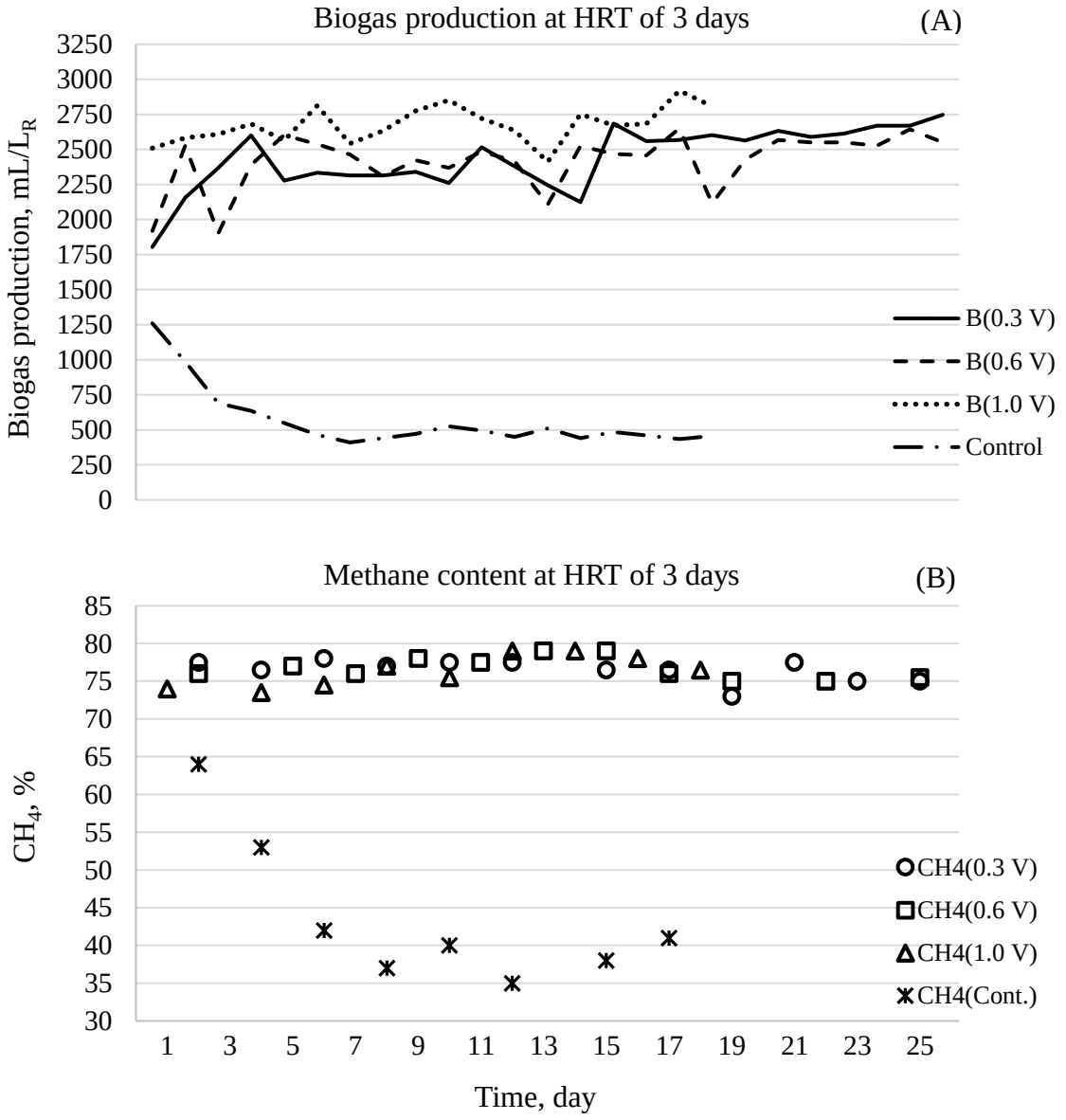

Figure 4.13. Biogas production (A) and methane content of biogas (B) in MEC+AD reactors at HRT of 3 days.

Organic matter removal rates of MEC+AD reactors and control reactor in Set III are shown in Figure 4.14. At 3 days of HRT, highest removal rate of COD, TS and VS were achieved by the MEC+AD reactor operated at applied power of 1.0 V. Organic removal rates of the reactor operated with input voltage of 1.0 V were 34.8 % (COD), 23.4 % (TS) and 29.5 % (VS) respectively. On the other hand organic removal efficiencies in other MEC+AD reactors operated at 0.3 and 0.6 V power applications were slightly lower than the results of the $(MEC+AD)_{1.0V}$. Therefore we can conclude that power application

was slightly effective on the organic removal rate. Organic removal rates of the control reactor were 13.8 % (COD), 7.7 % (TS) and 9.2 % (VS) respectively at HRT of 3 days. It was obvious that control reactor failed due to the high OLR and short HRT. Short HRTs can cause wash out of the active bacterial population, hence lead to VFAs accumulation and reduction in organic removal rates and methane production [64, 174]. On the other hand, organic removal efficiencies in MEC+AD reactors were higher than the control reactor at all three voltages. Organic removal efficiencies in MEC+AD reactors were slightly lower than the efficiencies obtained in Set II indicating that operational conditions were convenient for an MEC+AD reactor.

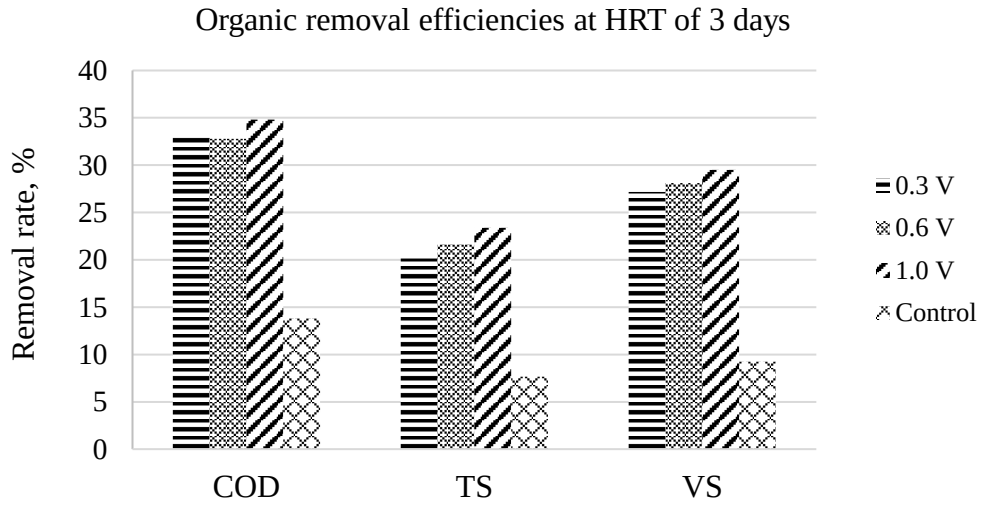

Figure 4.14. Organic removal rates of MEC+AD reactors and control reactor at HRT of 3 days.

Current productions of MEC+AD reactors operated at three different voltage applications and at HRT of 3 days are shown in Figure 4.15. MEC+AD reactor applied with 1.0 V of power exhibited the highest current production which varied between 3.30 and 5 A/m^3 in Set III. On the other hand current productions in MEC+AD reactors applied with voltage of 0.6 V and 0.3 V, varied between 2.2 - 3.5 A/m^3 and 1.1 – 2.6 A/m^3 respectively. Applied voltage significantly effected the current production as it was reported in other studies [96, 179].

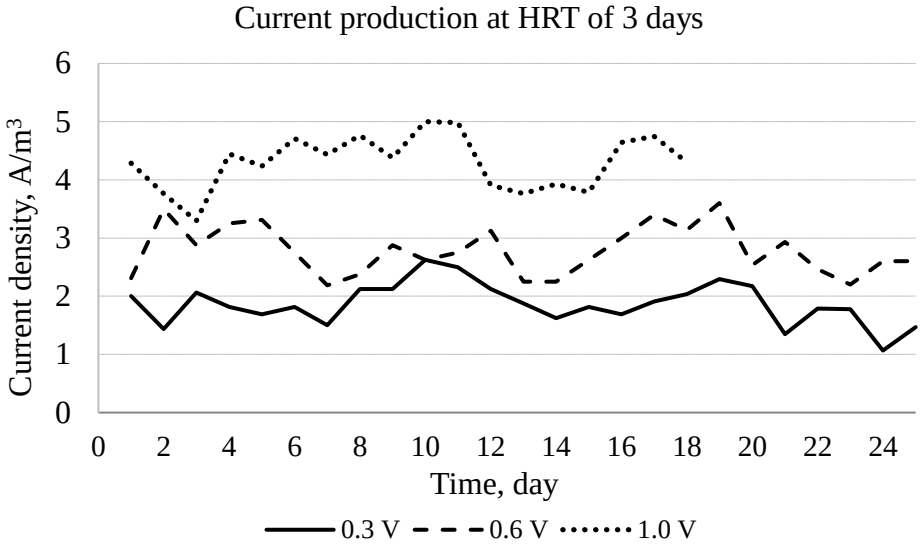

Figure 4.15. Current production in MEC+AD reactors at HRT of 3 days.

4.2.4. Set IV - HRT of 2 Days

In Set IV, cattle manure concentration was the same with the previous sets (29.8-30.2 g VS/L; 3±0.03 % VS; 4.15±0.3 % TS; w/w). The reactors were operated at HRT of 2 days by feeding 350 mL of cattle manure once a day. Effective volume of the reactors were reduced from 800 mL to 700 mL to prevent the clogging of the gas ports. At high concentration of manure and at high OLR, reactor medium tended to foam and clog the ports by the particles in the foam. This clogging can mislead the measurements of the biogas. By lowering the HRT from 3 days to 2 days, operational OLR inreased to 15 g VS/L/d in Set IV. Biogas productions and methane content of biogas obtained in Set IV are illustrated in Figure 4.16. It can be noticed that biogas production rate of $(MEC+AD)_{1.0V}$ was higher than MEC+AD reactors applied with voltages of 0.3 and 0.6 V. Daily average biogas productions of MEC+AD reactors with supplied voltage of 0.3, 0.6 and 1.0 V were 3.08, 3.06 and 3.42 L/L/d respectively at HRT of 2 days. Biogas production of $(MEC+AD)_{1.0V}$ was about 11 % higher than the others. Percentage of mehane in the biogas produced from the MEC+AD reactors were 76, 75 and 76 % respectively. Thus methane yield of the MEC+AD reactors were calculated as 0.156 L/g VS, 0.153 L/g VS and 0.173 L /g VS for voltage applications of 0.3, 0.6 and 1.0 V respectively in Set IV. Methane yield of the MEC+AD reactor with input voltage of 1.0 V was higher than MEC+AD reactors applied with voltages of 0.3 and 0.6 V. This can be ascribed by higher electrogen microorganism actions and electron transport between electrodes and electrogens. Hence it will lead to increase in oxidation and reduction

reaction rates [95, 96, 100]. Methane productions and methane yields of the MEC+AD reactors were not so distinct from each other in Set IV and in Set III. It is thought that operation of all three reactors under different voltages at different sets led to formation of similar microbial communities on the biofilm of the electrodes.

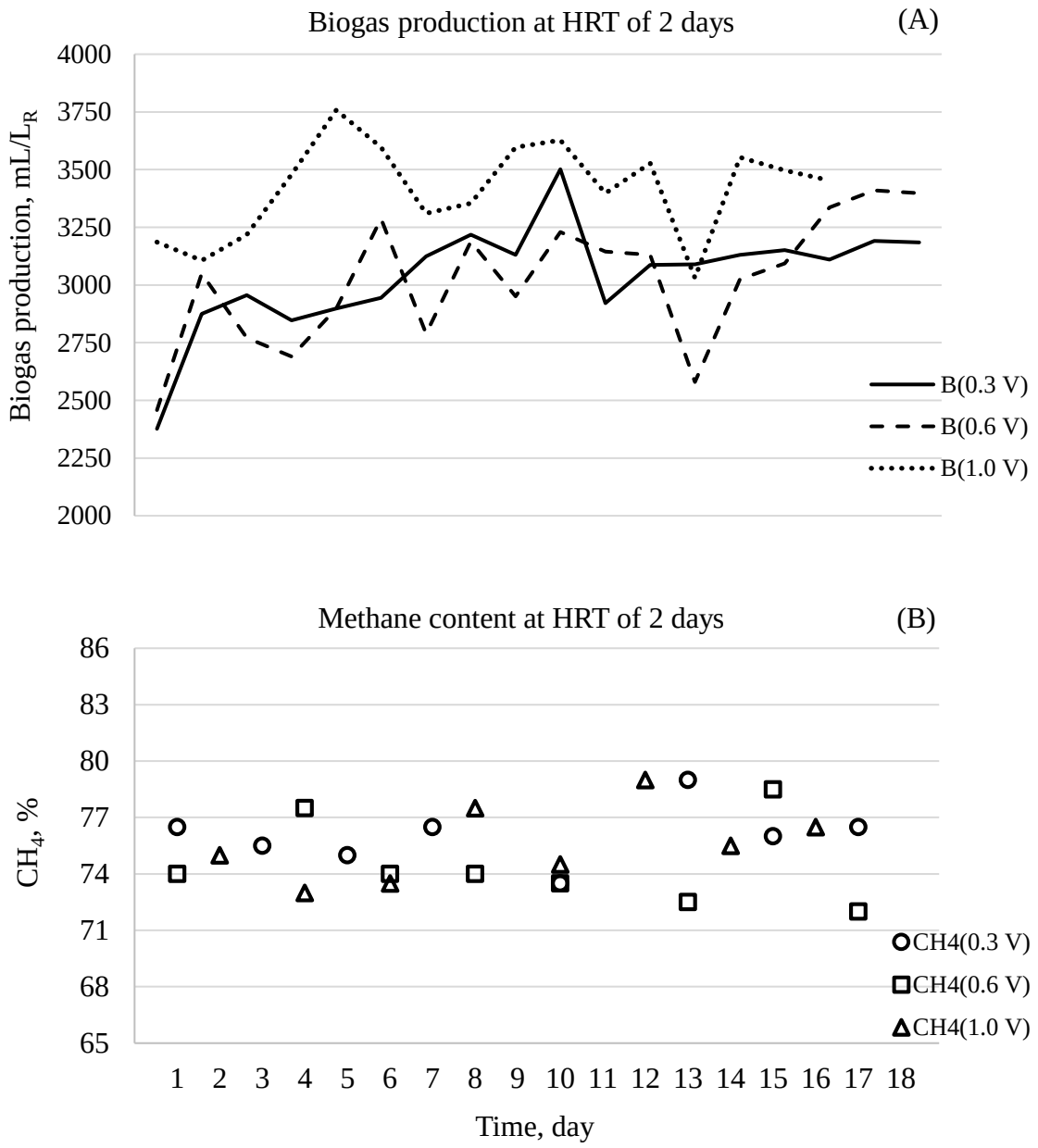

Figure 4.16. Biogas production (A) and methane content of biogas (B) in MEC+AD reactors at HRT of 2 days.

When the HRT was reduced from 3 days to 2 days in Set IV, daily biogas productions increased about 25-28 % compared to Set III. However due to the increased OLR, methane yield of the reactors decreased from 0.19-0.2 L/ g VS range to 0.15- 0.17 L/g VS. Even though the methane yield in Set IV decreased about 15-20 % compared to Set

III, methane contents of the biogas were similar which indicate that methanogenic process was still stable and effective. The results obtained in Set IV were convenient with other studies conducted at similar HRT and OLR applications. Arriagada et al. [181] operated a two phase process composed of two UASB reactors treating poultry manure. The reactors were operated at thermophilic and mesophilic temperatures respectively. The produced biogas gas was used to stir the reactors. The reported methane yield was 0.14 L CH_4/g VS at total HRT and OLR of 2.74 days and 8.31 g VS/L/d respectively [181]. These results were lower than the results obtained in the present study. Very similar reactor to the one used in the present study was operated by Quashie et al. [183] in which food waste was used as substrate. An integrated MEC+CSTR reactor used in the study was operated continuously at HRT and OLR of 2 days and 10 g COD/L/d respectively with an applied voltage of 1.2 V. Biogas production was between 3-4 L/L/d with methane content of 70 % approximately in that study. The results obtained in the present study was consistent with the results obtained by Quashie et al. [183].

COD, TS and VS removal rates of MEC+AD reactors at HRT of 2 days are shown in Figure 4.17. Highest removal rate of COD, TS and VS were achieved by the MEC+AD reactor operated at applied voltage of 1.0 V. Organic matter removal rates of $(MEC+AD)_{1.0V}$ were 31.1 % (COD), 21.4 % (TS) and 25.6 % (VS) respectively. On the other hand organic removal efficiencies in other MEC+AD reactors operated at power supplementation of 0.3 and 0.6 V were slightly lower than the results of $(MEC+AD)_{1.0V}$. Therefore we can conclude that voltage application was not significantly effective on organic removal rate. Compared to Set III, organic removal efficiencies of the reactors were not significantly different in Set IV which may indicate that MEC+AD reactors can be operated at 2 days of HRT without inhibition.

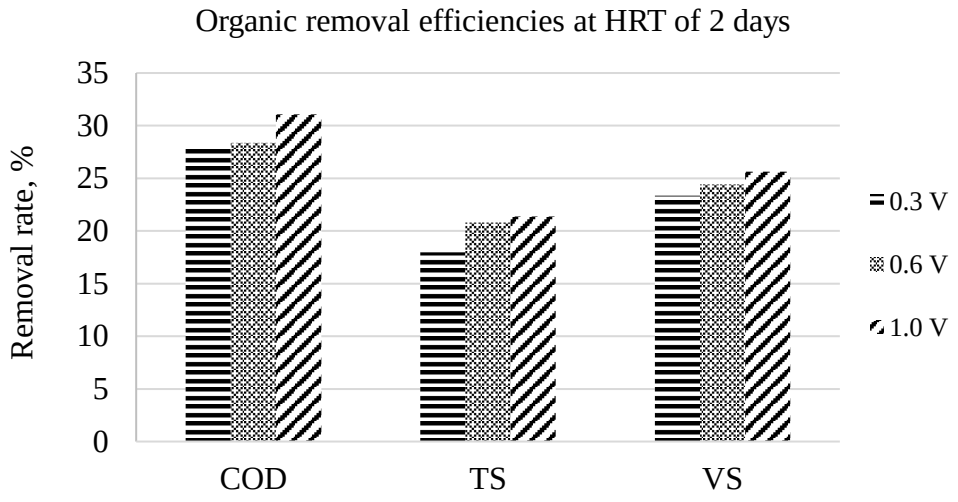

Figure 4.17. Organic removal rates of MEC+AD reactors at HRT of 2 days.

Current productions of MEC+AD reactors operated at three different voltage applications and at HRT of 2 days are shown in Figure 4.18. MEC+AD reactor applied with 1.0 V of power, exhibited the highest current production which varied between 3.90 and 6.3 A/m^3. The current production was mostly between 5-6 A/m^3 in Set IV. On the other hand current productions in MEC+AD reactors with applied voltages of 0.6 V and 0.3 V, varied between 1.7 - 3.1 A/m^3 and 1.2 – 1.9 A/m^3 respectively. Voltage application significantly effected the current production as it was reported in other studies [96, 179].

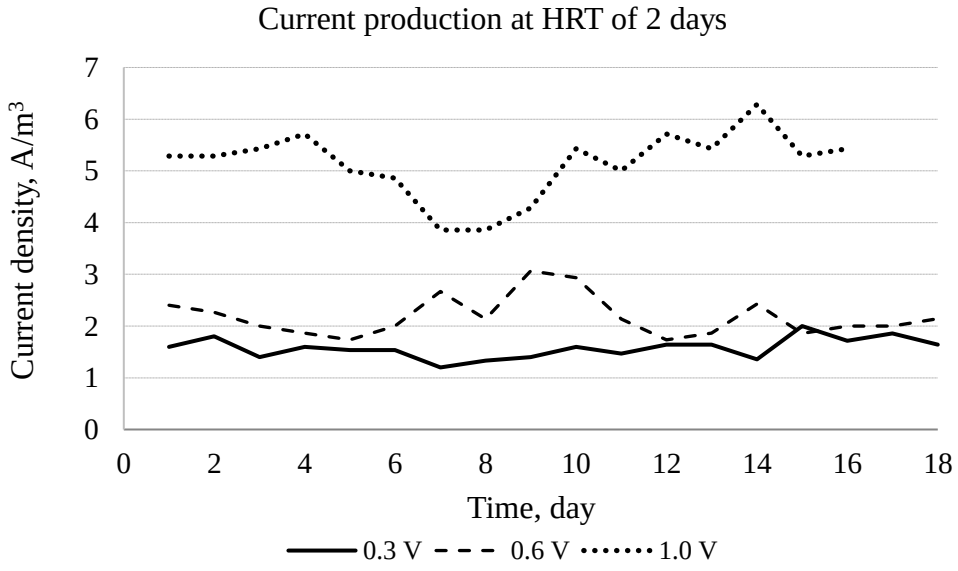

Figure 4.18. Current production in MEC+AD reactors at HRT of 2 days.

4.2.5. Set V - HRT of 1 Day

In Set V, cattle manure concentration was the same with the previous sets (29.8-30.2 g VS/L; 3±0.03 % VS; 4.15±0.3 % TS; w/w). However reactors were operated at the lowest HRT of 1 day. Liquid volume of the reactors were reduced from 700 mL to 600 mL to prevent the clogging of the gas ports. Prior to feeding 600 mL of cattle manure to the reactors, all the liquid in the reactors were drawn every day. By lowering the HRT from 2 days to 1 day, operational OLR inreased from 15 g VS/L/d to 30 g VS/L/d in Set V. Biogas productions and methane content of biogas obtained in Set V are illustrated in Figure 4.19. It can be noticed that biogas production rates of $(MEC+AD)_{0.6V}$ and $(MEC+AD)_{1.0V}$ were similar to each other. However biogas production of $(MEC+AD)_{0.3V}$ was slightly lower than these two MEC+AD reactors. Daily average biogas productions of the MEC+AD reactors with supplied voltages of 0.3, 0.6 and 1.0 V of were 3.22, 3.56 and 3.48 L/L/d respectively at HRT of 1 day. Methane content of the biogas produced from the MEC+AD reactors were 81, 79 and 79 % respectively. Thus methane yield of the MEC+AD reactors were calculated as 0.089L/ g VS, 0.095 L/ g VS and 0.093 L /g VS for power applications of 0.3, 0.6 and 1.0 V respectively. Methane yield of the MEC+AD reactors were very similar to each other. It is thought that the strategy of operation of every one of the reactors under different voltages at different sets led to formation of similar microbial communities on the biofilm of the electrodes. All reactors produced similar methane yields as a result of this strategy. It can be concluded that methane production of $(MEC+AD)_{0.6V}$ and $(MEC+AD)_{1.0V}$ reactors were slightly higher than $(MEC+AD)_{0.3V}$ reactor at high OLR (30 g VS/L/d) due to high electro flow to the reactors.

When HRT was reduced from 2 days to 1 day in Set V, daily biogas productions increased slightly. However due to the increased OLR, methane yield of the reactors decreased from 0.15- 0.17 L/g VS range to 0.09-0.095 L/ g VS. Methane yield in Set V decreased about 40-45 % compared to Set IV. Methane rate of the biogas produced in MEC+AD reactors were at the range of 79-81 % which indicate that methanogenic process was still stable and effective. The results obtained in Set V exhibited different results with other studies conducted at similar HRT and/or OLR conditions at MEC reactors.

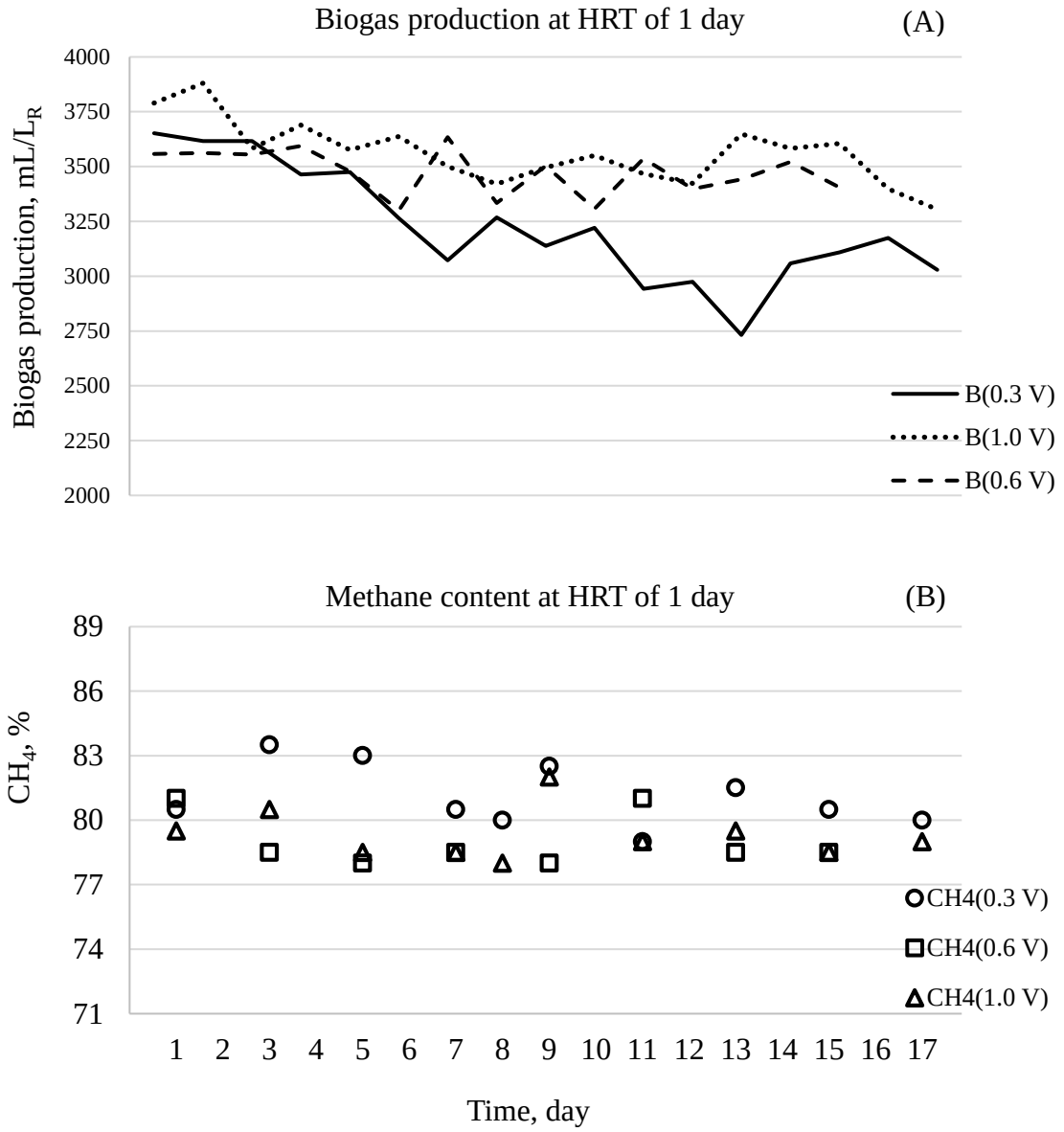

Figure 4.19. Biogas production (A) and methane content (B) of MEC+AD reactors at HRT of 1 day.

Quashie et al. [183] treated food waste in an integrated MEC+CSTR reactor at meophilic temperature. The reactor was operated continuously at HRT and OLR of 1 day and 20 g COD/L/d respectively with an applied voltage of 1.2 V. Biogas production at HRT of 1 day was around 6 L/L/d with methane content of 70-75 % [183]. The results obtained in that study was higher than the results obtained in the present study (3.22-3.56 L/L/d). The reason of this can be the substrate type used in this study. Food waste is known by its easily degradable content [40]. Cusick et al. [48] treated winery WW in a specially designed MEC constructed with 144 electrode pairs. The operational conditions in the study as in HRT, OLR, applied voltage and temperature were 1 day, 2 g SCOD/L/d, 0.9

V and 31ºC respectively. The methane production was reported as 0.24 L/L/d in that study. This was very low compared to the results obtained in the present study. In another study, UASB and MEC combined system was used to treat synthetic WW at OLR of 28 g COD/L/d [60]. The operational conditions as in HRT, temperature and applied voltage were 6 hours, 35ºC and 1.0 V respectively. The highest methane production obtained in that study was almost 6 L/L/d which was two folds of the results obtained in the present study. In that study, high rate reactor system (UASB) and synthetic WW were the two important factors that enhanced the methane production. However in an another UASB–MEC integrated system the results obtained were lower than the present study. The reactor was fed with artificial beer WW at HRT and OLR of 1 day and 2 g COD/L/d. Applied voltage and temperature in the reactor were 0.8 V and 30ºC respectively. Methane yield and methane production obtained in the study were 0.14 L/g COD and 0.37 L/L/d respectively [123].

COD, TS and VS removal efficiencies of MEC+AD reactors at HRT of 1 day are shown in Figure 4.20. Organic matter removal rates of the three MEC+AD reactors were 16-17 % (COD), 12-13 % (TS) and 14-16 % (VS) respectively. The similar removal efficiencies were obtained in these reactors in terms of biogas production and methane yield. Therefore it can be suggested that at high loadings voltage application was not significantly effective on the organic removal rate. On the other hand, operating each of the reactors under different voltage applications during different periods of the study, enhanced biogas production at every reactor.

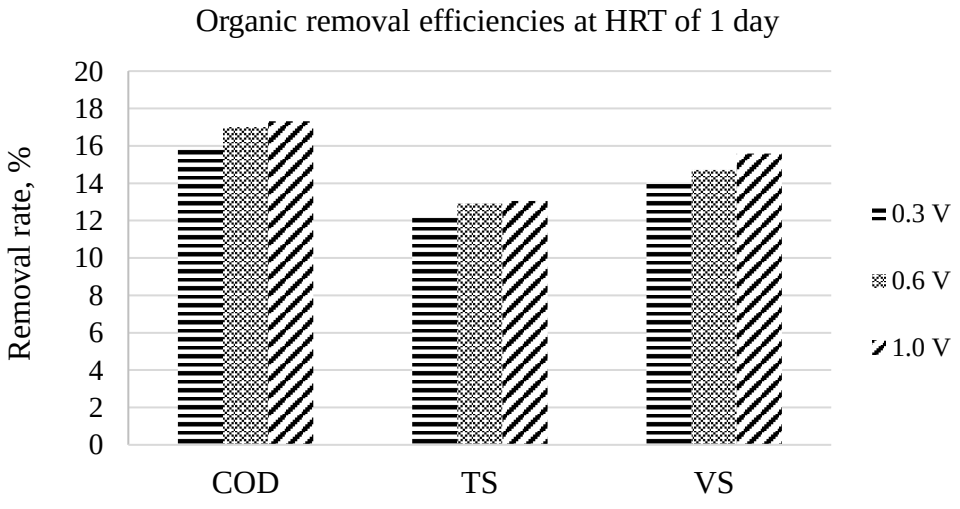

Figure 4.20. Organic removal rates of MEC+AD reactors at HRT of 1 day.

Therefore, biogas productions and organic removals efficiencies were similar in all three applied voltages. The results indicate that MEC+AD reactors can be operated successfully at HRT of 1 day without sign of inhibition.

Current productions of MEC+AD reactors operated at three different voltage applications and HRT of 1 day are shown in Figure 4.21. MEC+AD reactor applied with 1.0 V of power exhibited the highest current production which varied between 3.60 and 5.4 A/m^3. The current production was mostly between 4-5 A/m^3 in $(MEC+AD)_{1.0V}$. On the other hand current productions in MEC+AD reactors with input voltages of 0.6 V and 0.3 V, varied between 2.1 – 4.3 A/m^3 and 1.4 – 2.8 A/m^3 respectively. It has been observed that voltage application significantly effected the current production as it was reported in other studies [96, 179].

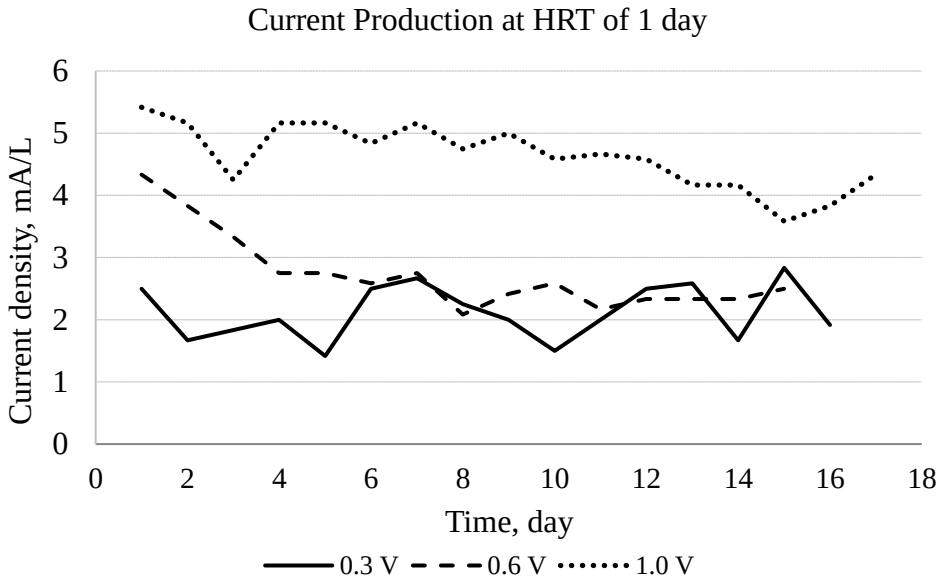

Figure 4.21. Current production in MEC+AD reactors at HRT of 1 day.

4.2.6. pH and ORP Variations of MEC+AD and Control Reactors at Different HRTs

pH is one of the crucial factors that should be observed in anaerobic process. pH can inhibit the anaerobic process due to the excess or low hydrogen ion concentration in the medium. VFA accumulation and ammonia inhibition in anerobic process are related to pH value [78]. So it is crucial to monitor the pH changes in the reactor and neutralize the pH if needed. In this study, pH adjustment with chemical additives were not applied to the MEC+AD and AD reactors. pH control was not required due to high alkalinity and buffering capacity of the cattle manure [162, 184]. In MECs, H^+ ions combine with

electrons and form H_2 and H_2 can be used for methane formation. Therefore, utilizaiton of H^+ ions in the medium also influence pH value. pH and ORP changes of MEC+AD reactors applied with different voltages and different HRTs were presented in Figure 4.22 and Figure 4.23 respectively.

pH of the MEC+AD reactors changed between 7.15 and 7.70 during the study. The reactor pH did not vary depending on different HRTs and voltages applied to the MEC+AD reactors. Cattle manure used as substrate in this study had a total COD and total nitrogen ratio (TCOD/TN) of 14.1±0.3. It is suggested that cattle manure had a similar C/N ratio which could have served as a buffering capacity for pH. Also utilization of H^+ ions for H_2 and methane production in the MEC+AD reactors might have prevented pH decrease in reactors at high OLRs. Therefore, it is thought that pH of MEC+AD reactors during the study was not affected from HRT and OLR changes. One In a long term study conducted by Park et al. [54], food waste was treated in a combined MEC and anaerobic digestion system at different OLRs of 2-10 g COD/L/d and HRTs of 15-20 days. Similarly, pH was stable around 8 and was not affected significantly from the OLR changes in that study. In another study, four different voltages between 0.6 and 1.8 were applied to a single cell MEC fed with thermal alkaline pretreated sludge. pH change was not notable at different applied voltages and at control reactor used in that study [55]. Therefore, it can be concluded that the results obtained in the present study were appropriate for the methanogenic process [66, 77, 163]. On the other hand, pH of the control reactor changed between 6.5 and 7.4. At HRTs of 6 days and 4 days, pH of the control reactor were in the range suitablefor methanogenic activity. However, when HRT decreased to 3 days, pH of the control reactor declined from 7.2 to around 6.5. Therefore, decrease in methane production of the control reactor in parallel with pH decline can be attributed to VFA accumulation originated from insufficient time for acetogens and methanogens in order to convert VFA into acetate and methane [182].

Oxidation reduction potential can be a valuable sign for the inspection of anaerobic processes. It is an indicator of the capacity of the molecules in the medium of a reactor to release or gain electrons (oxidation or reduction, respectively).

It is difficult for methane-forming bacteria to produce methane at ORP values higher than -300 mV due to strong competitiveness of fermentation bacteria at ORP values greater than -300 mV [164].

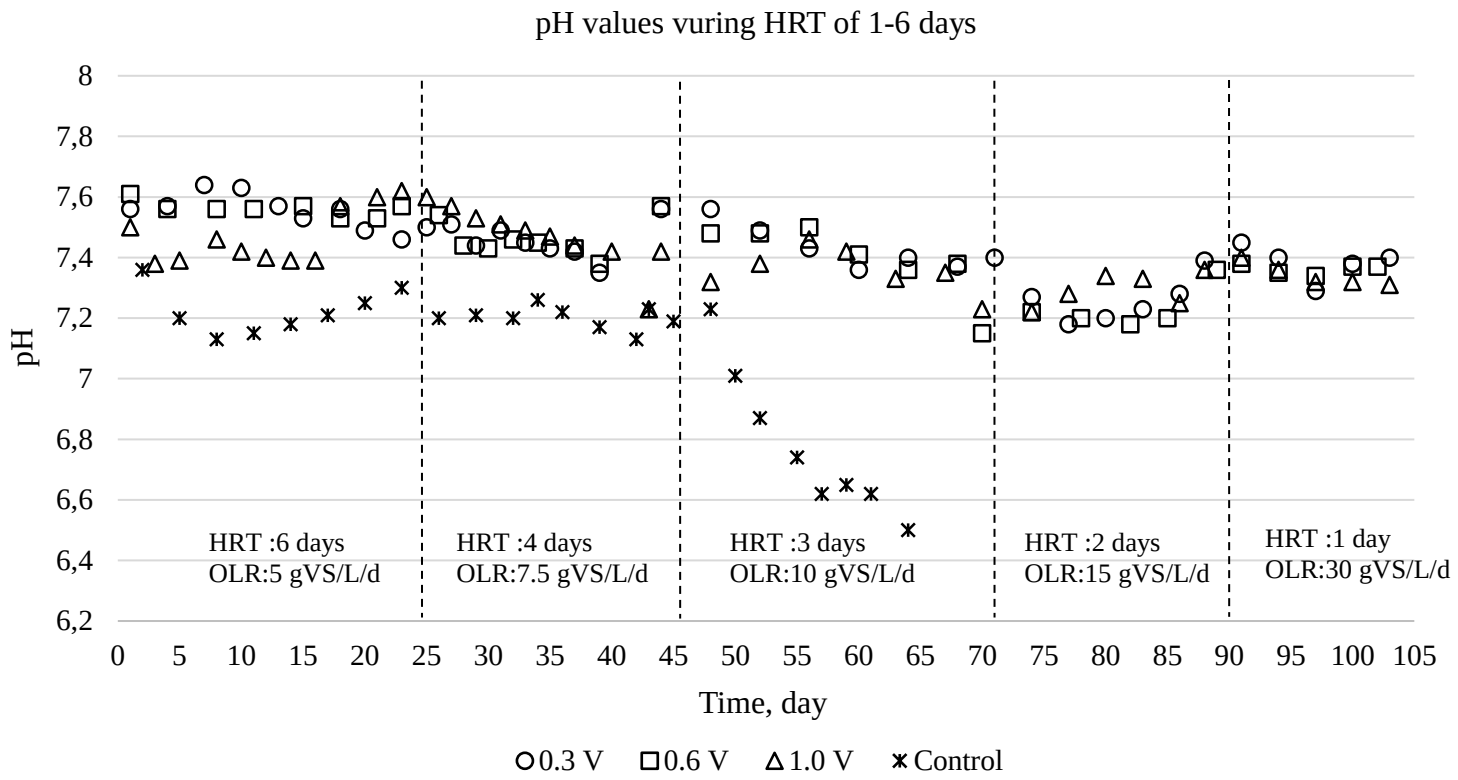

Figure 4.22. pH variations in MEC+AD and control reactors at HRTs of 6 to 1 days.

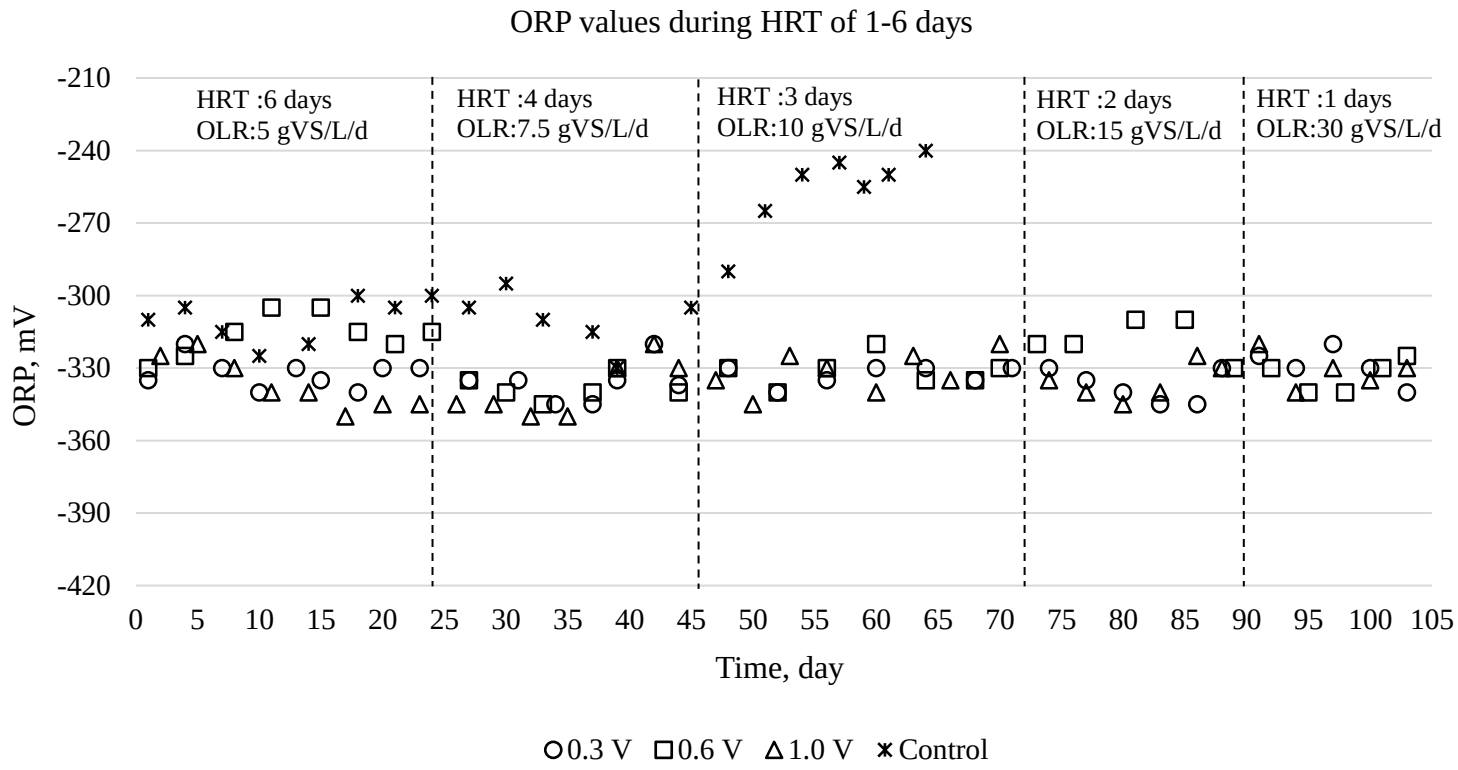

Figure 4.23. ORP variations in MEC+AD and control reactors at HRTs of 6 to 1 days.

It is also claimed in detail that acidogenesis and methanogenesis take place at optimum ORPs between -250 : -300 mV and -300 : -360 mV respectively [163, 164]. In the present study, ORP of MEC+AD reactors ranged between -305 and -350 mV at all 5 sets indicating a stable methanogenic activity during the study. Methane production and pH variations in MEC+AD reactors demonstrate the stable methanogenic activity as well. On the other hand ORP of the control reactor varied between -295 and -330 mV in Set I and II in which reactors were operated at HRTs of 6 and 4 days respectively. However ORP of the control reactor increased from –330 mV to around -245 mV at the 3rd set when the HRT was reduced to 3 days. The ORP values of the control reactor at the 3rd set show that the methanogenic process was inhibited due to the overloading. ORP increase in control reactor was in parallel with the decrease in pH and methane production in Set III. It is suggested that ORP was only dependent to the phase of the anaerobic digestion process. It is thought that due to the failure of the control reactor, methanogenic activity was replaced with acidogenic activity in the control reactor because of the short HRT (3 days). Methane production and methane content of the biogas decreased sharply. As a result, ORP of the control reactor increased from the range of methanogenic activity to ORP of the acidogenic activity (-250 mV).

4.3. Sequencing Batch Operations at Different OLRs

In this part of the study, MEC+AD reactors were operated for 2 sets (Set VI and Set VII) at constant HRT of 2 days in sequencing batch mode at diferent OLRs and varying external power supplementations. Operational procedure and feeding method of the reactors were explained earlier in Section 4.2. VS and TS content of the cattle manure fed to the MEC+AD reactors were increased step by step from 3 % VS to 4.5 % VS first and then to 6 % VS in two sets. The MEC+AD reactors were fed with manure at both VS content at least 15 days same in the previous sets. Power supplementations of 0.3 V, 0.6 V and 1.0 V were applied to the reactors in the mean time. All process combinations was applied twice, one with a MEC+AD reactor, the second one with another MEC+AD reactor.

4.3.1. Set VI - Manure Including 4.5 % VS

In Set VI cattle manure concentration was increased to 44-46 g VS/L (4.5±0.02 % VS; 6.55±0.05 % TS; w/w) and HRT was kept constant at 2 days. Liquid volume in the reactors were 0.7 L. To obtain operating HRT of 2 days, reactors were fed with 350 mL of cattle manure once a day. Thus MEC+AD reactors were operated at OLR of 22-22.5 g VS/L/d in Set VI. MEC+AD reactors were supplemented with voltages of 0.3, 0.6 and 1.0 V. Biogas productions and methane content of biogas obtained in Set VI are depicted in Figure 4.24. Daily average biogas productions of the $(MEC+AD)_{0.6V}$ and $(MEC+AD)_{1.0V}$ were very similar to each other being 4.59 L/L/d and 4.76 L/L/d respectively. On the other hand biogas production of $(MEC+AD)_{0.3V}$ was slightly lower (3.97 L/L/d) than those obtained in $(MEC+AD)_{0.6V}$ and $(MEC+AD)_{1.0V}$ reactors. Methane contents of the reactors applied with voltages of 0.3, 0.6, 1.0 V were 75, 77 and 75 % respectively, indicating that methane content was independent of applied voltage. As a result, methane yield of the reactors were 0.132, 0.157 and 0.159 L CH_4/g VS at input voltages of 0.3, 0.6 and 1.0 V respectively. Methane yield of $(MEC+AD)_{0.6V}$ and $(MEC+AD)_{1.0V}$ reactors were 20 % higher than the methane yield of $(MEC+AD)_{0.3V}$ reactor. In Set VI, applied voltage amount had influence on biogas production such that at applied voltages of 0.6 and 1.0 V, biogas productions were higher. It can be concluded that at high OLRs of 20 g VS/L/d and more, applied voltage has influence on biogas production.

In Set IV reactors were operated at HRT of 2 days and OLR of 15 g VS/L/d, and daily average biogas productions of the MEC+AD reactors with applied voltage of 0.3, 0.6 and 1.0 V of were 3.08, 3.06 and 3.42 L/L/d respectively, methane yields being 0.156 L/ g VS, 0.153 L/ g VS and 0.173 L /g VS. However, in Set VI, when OLR was increased to 22.5 g VS/L/d by increasing the manure concentration, the daily average biogas productions of the reactors also increased. However, methane yields of the reactors decreased slightly to the range of 0.13 - 0.16 L CH_4/g VS when compared to the results obtained at Set IV. According to the results obtained in Set VI, we can conclude that MEC+AD reactors were still efficient at HRT of 2 days and OLR of 22.5 g VS/L/d.

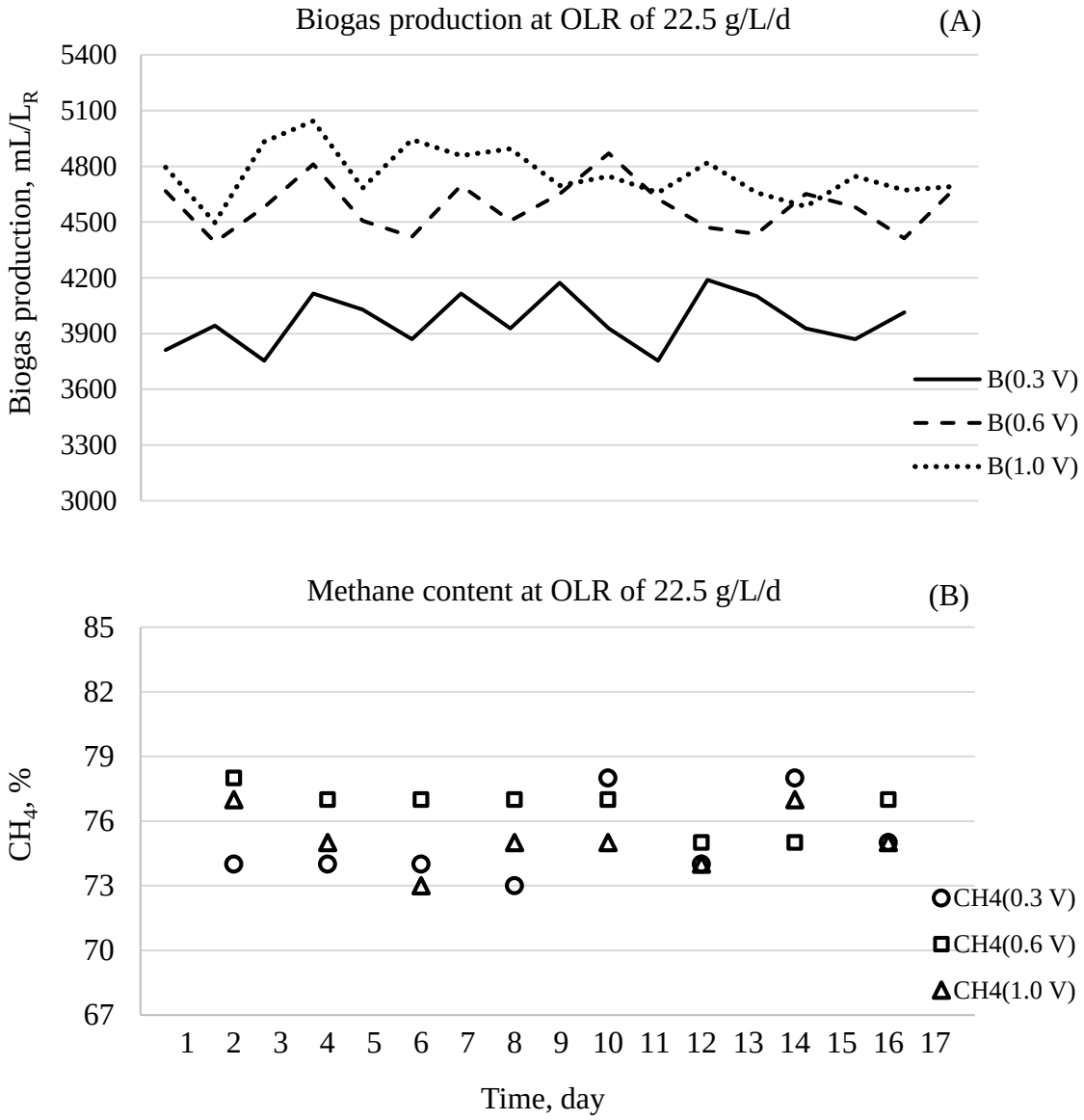

Figure 4.24. Biogas production (A) and methane content (B) of MEC+AD reactors at OLR of 22.5 g VS/L/d.

On the other hand organic removal efficiencies of $(MEC+AD)_{0.6V}$ reactor were similar to those results obtained at $(MEC+AD)_{1.0V}$. Similarly, biogas production of MEC+AD reactors with input voltage of 0.6 and 1.0 V were close to each other. If the organic removal efficiencies found in this set were compared to those obtained at operational conditions of HRT and OLR of 2 days and 15 g VS/L/d (COD:31.1 %, TS:21.4 %, VS:25.6 %), it can be observed that organic removal efficiencies decreased due to the high OLR. The decrease in organic removal rate was in parallel with decrease in methane yield in this set. According to the results, applied voltage was slightly effective on organic removal efficiencies. Power applications of 0.6 and 1.0 V resulted in higher biogas generation and organic removal rate in MEC+AD reactors.

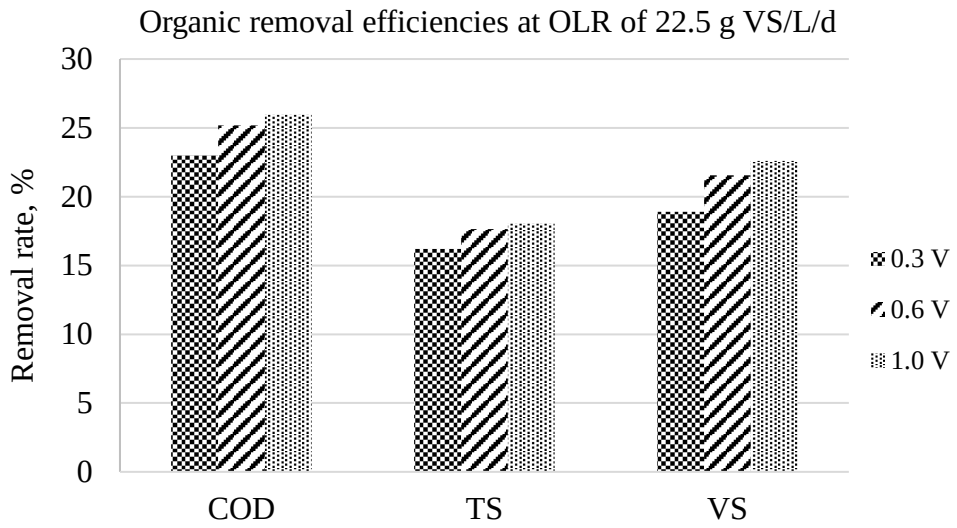

Figure 4.25. Organic removal rates of MEC+AD reactors at OLR of 22.5 g VS/L/d.

Current productions of MEC+AD reactors operated at three different voltage applications are shown in Figure 4.26. At operational conditions of OLR and HRT of 22.5 g VS/L/d and 2 days respectively, MEC+AD reactors presented various current densities as a result of different voltage applications. MEC+AD reactor supplied power of 1.0 V exhibited the highest current production which varied between 3.3 and 6.0 A/m^3. The current production was mostly between 4-5 A/m^3 in Set VI. On the other hand lower current productions were observed in MEC+AD reactors with supplied voltages of 0.6 V and 0.3 V, varied between 2.3 - 3.6 A/m^3 and 0.7 – 1.2 A/m^3 respectively. Voltage application significantly effected the current production.

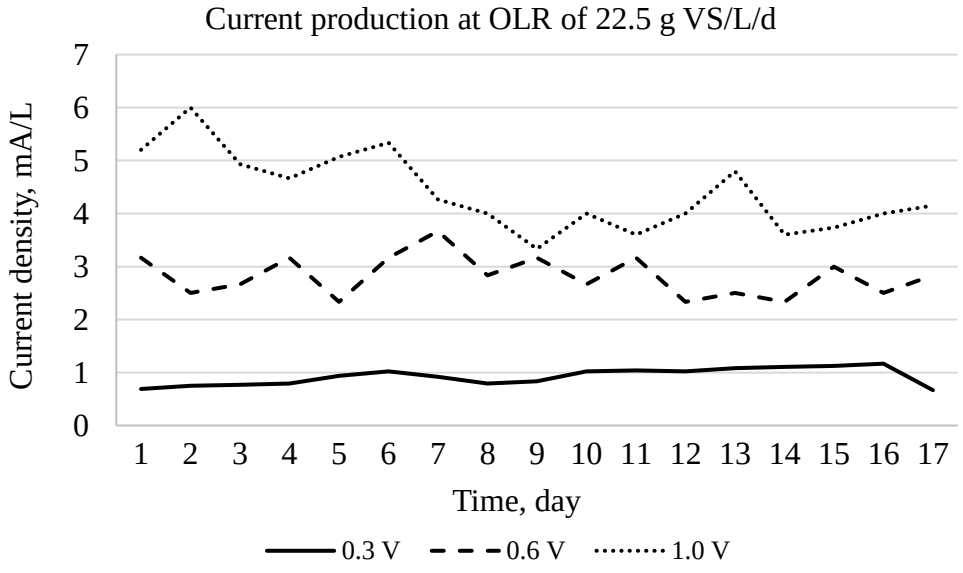

Figure 4.26. Current production in MEC+AD reactors at OLR of 22.5 g VS/L/d.

4.3.2. Set VII - Manure Including 6 % VS

In Set VII, cattle manure concentration was increased to 58-61 g VS/L (6±0.15 % VS; 8.7±0.2 % TS; w/w) and HRT was kept constant at 2 days. Liquid volume in the reactors were set to 0.6 L. To obtain operating HRT of 2 days, reactors were fed with 300 mL of cattle manure once a day. Thus MEC+AD reactors were operated at OLR of 29-30 g VS/L/d in Set VII. MEC+AD reactors were supplemented with voltages of 0.3, 0.6 and 1.0 V. Biogas productions and methane content of biogas obtained in this study are depicted in Figure 4.27. Daily average biogas productions of the $(MEC+AD)_{0.6V}$ and $(MEC+AD)_{1.0V}$ were very similar to each other being 4.81 L/L/d and 5.11 L/L/d respectively. On the other hand biogas production of $(MEC+AD)_{0.3V}$ was lower (4.02 L/L/d) than those obtained in $(MEC+AD)_{1.0V}$ and $(MEC+AD)_{0.6V}$ reactors. Methane contents of the reactors applied with voltages of 0.3, 0.6, 1.0 V were 76, 76 and 78 % respectively, indicating that methane ratio of the biogas was not affected significantly by the amount of given voltage. As a result, methane yield of the reactors were 0.104, 0.124 and 0.135 L CH_4/g VS at voltage supplementations of 0.3, 0.6 and 1.0 V respectively.

Methane yield of $(MEC+AD)_{1.0V}$ was 9 % and 30 % higher than $(MEC+AD)_{0.6V}$ and $(MEC+AD)_{0.3V}$ reactors respectively in Set VII. This can be attributed to the given voltage of 1.0 V which may have enhanced methane production at OLR of 30 g VS/L/d. It can be concluded that at high OLR of 20 g VS/L/d and more, applied voltages of more than 0.6 V were more effective on biogas production. It is thought that higher voltage application enhanced methane production through electromethanogenesis and hydrogenotrophic methanogenesis due to enhanced electron transfer. Higher OLR could also have caused an increment in acetoclastic methanogenesis in the anode and in the bulk sludge. Daily average biogas productions of the MEC+AD reactors found in Set VII were slightly higher than the results obtained in the previous set due to the OLR increase. However when the OLR increased to 30 g VS/L/d, the methane yields of the MEC+AD reactors decreased around 15-21 % compared to Set VI. Considering the results obtained in Set VII, we can conclude that MEC+AD reactors were still efficient at HRT and OLR of 2 days and 30 g VS/L/d respectively. Methane production and methane yield found in this set are convenient with the results reported in other studies conducted with MEC reactors. Food waste at OLR of 10 g COD/L/d was treated in a combined MEC+CSTR operating at temperature of 35 ^{o}C and HRT of 2 days with an applied voltage of 1.2 V [183]. The biogas production reported in that study was around 3.5-4.5 L/L/d (60-70 % CH_4) which

was slightly lower than the results obtained in the present study. However, it was also reported in the same study [183] that, when the OLR was increased to 20 g COD/L/d by decreasing HRT to 1 day, biogas production increased to 6 L/L/d with methane content of 70-75 %. Therefore, it can be concluded that the results obtained in the present study was convenient with the previous studies.

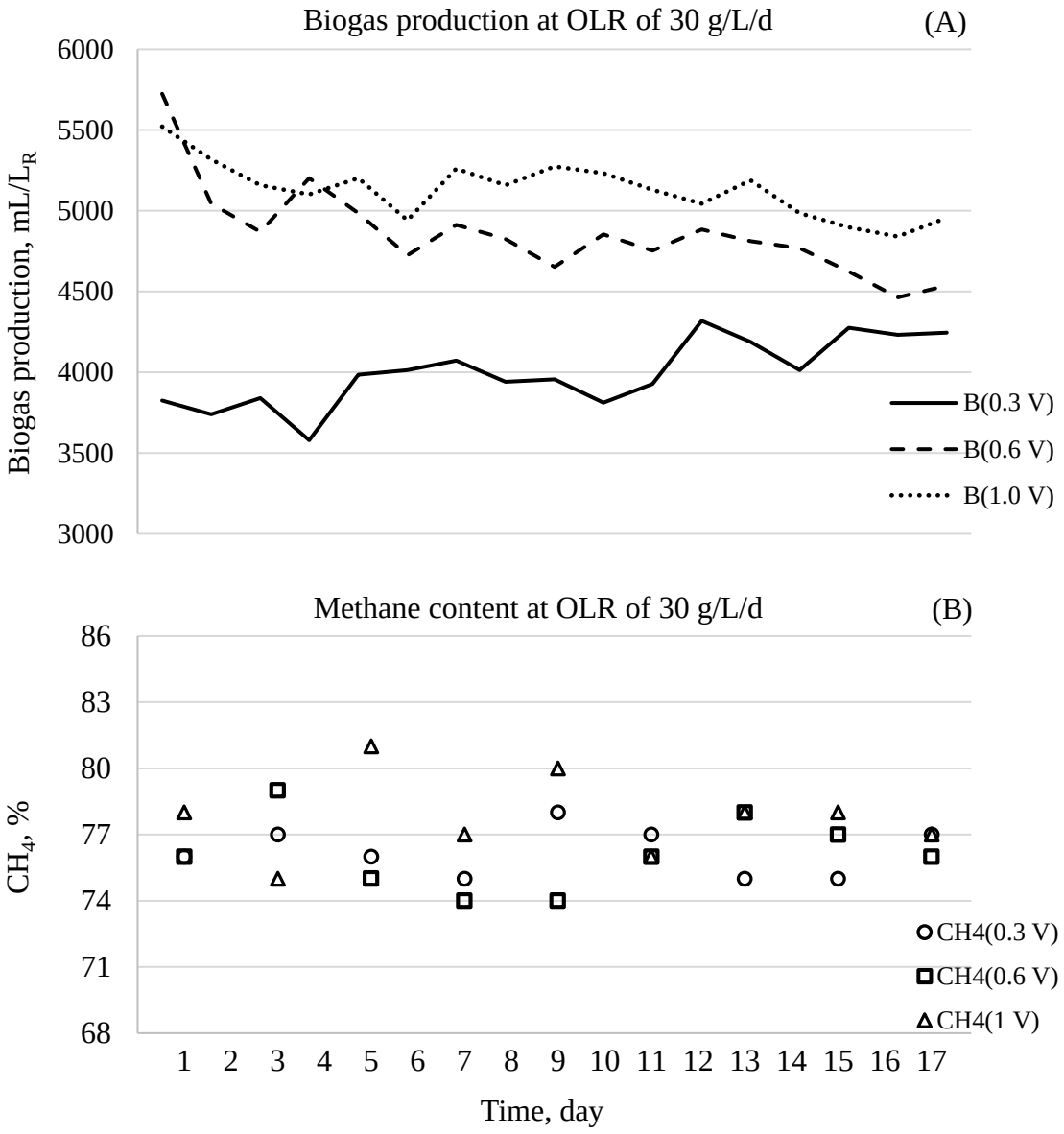

Figure 4.27. Biogas production (A) and methane content (B) of MEC+AD reactors at OLR of 30 g VS/L/d.

In an UASB treating sugar refinery wastewater at of 35 ºC and OLRs of between 12-54 g COD/L/d, methane yield was reported between 258–827 L CH_4/gVSS/d [185]. The results were higher than the results obtained in the present study due to the different treatment technology (UASB vs. MEC+AD) and waste stream. Sugar refinery wastewater

is known for its high carbon content [186]. Krishnan et al. [187] conducted a two stage bioprocess reactor (fermentation CSTR - MEC at 0.5 V) that were operated at 55 ^{o}C and 37 ^{o}C respectively. Palm oil mill effluent in CSTR effluent was fed to the MEC as the second stage process at OLR and HRT of 25-35 g COD/L/d and 4-12 days respectively. Methane production and yield reported in that study were between 0.93 - 2.37 L/L/d and 115 - 265 mL CH_4/gCOD repectively [187]. The results were similar to findings of the present study, however operational conditions were more challenging in the present study.

COD, TS and VS removal rates of MEC+AD reactors at OLR of 29-30 g VS/L/d are shown in Figure 4.28. Highest organic removal rates were achieved by the MEC+AD reactor operated at supplied power of 1.0 V. Organic removal rates of $(MEC+AD)_{1.0V}$ were 20.4 % (COD), 15.5 % (TS) and 15.9 % (VS) respectively. On the other hand organic removal efficiencies of $(MEC+AD)_{0.6V}$ and $(MEC+AD)_{0.3V}$ reactors were similar to each other being around 14-15 % for VS. The organic removal rates in these reactors were slightly lower than that obtained in $(MEC+AD)_{1.0V}$. Compared to Set VI, organic removal rates of MEC+AD reactors decreased at all applied voltages in Set VII. It is suggested that, organic removal efficiencies decreased due to the high OLR in this set. The decrease in organic removal rate resulted in lower decrease in methane yields in Set VII. Applied voltage was slightly effective on organic removal efficiencies. Applied voltage of 1.0 V to the MEC+AD reactor resulted in higher biogas productions and organic removal efficiency in the reactor.

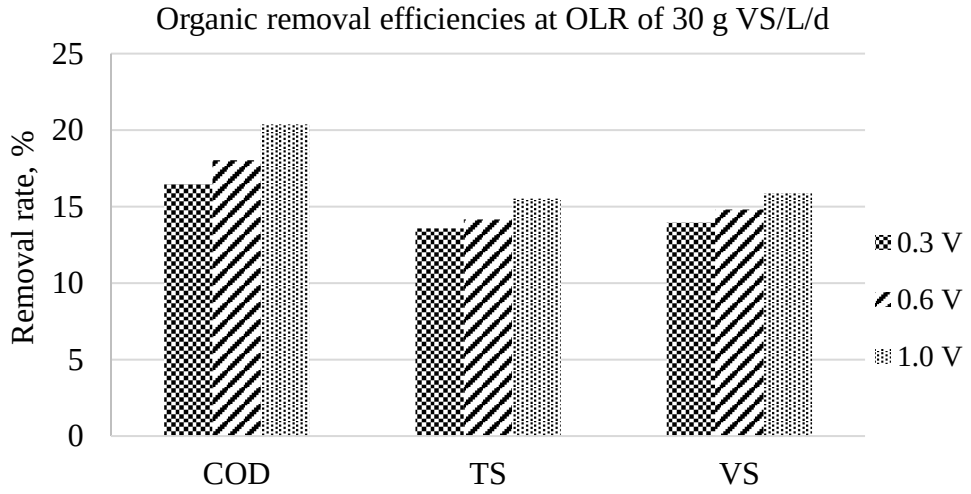

Figure 4.28. Organic removal rates of MEC+AD reactors at OLR of 22.5 g VS/L/d.

Current productions of MEC+AD reactors are shown in Figure 4.29. MEC+AD reactors presented various current densities as a result of different voltage applications under 2 days of HRT and 30 g VS/L/d of OLR. MEC+AD reactor with power application of 1.0 V exhibited the highest current production which varied between 4.4 and 6.5 A/m^3. This shows that higher electron transfer from anode to cathode took place in $[MEC+AD]_{1.0V}$ compared to other two reactors. This could have resulted in higher methane production as well. On the other hand current productions in MEC+AD reactors with applied voltages of 0.6 V and 0.3 V, varied between 2.3 – 4.4 A/m^3 and 0.5 – 1.2 A/m^3 respectively. Voltage application significantly effected the current production and methane production in the reactors.

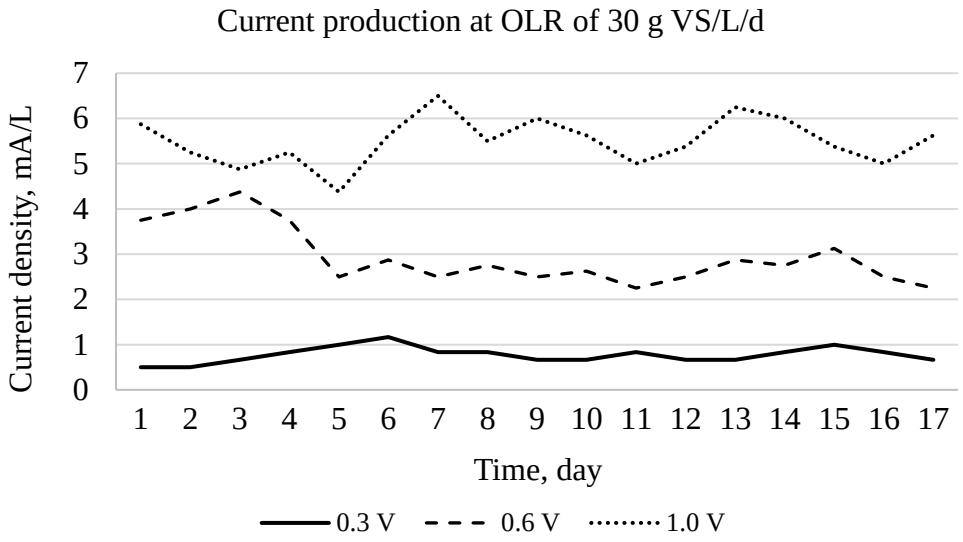

Figure 4.29. Current production in MEC+AD reactors at OLR of 30 g VS/L/d.

4.2.6. pH and ORP Variations of MEC+AD Reactors at Different OLRs

pH and ORP changes of MEC+AD reactors applied with different voltages and different OLRs are presented in Figure 4.30. pH of the MEC+AD reactors changed between 7.17 and 7.52 during the study. Considering the results, pH was not affected from the change in OLRs and voltages applied to the MEC+AD reactors. The results obtained in the present study were appropriate for the methanogenic process [66, 77]. In the set that OLR was 15 g VS/L/d, pH of the reactors were lower compared to the pH values obtained in Sets VI and VII. This difference was because of the characteristics of the cattle manure. However this difference did not affect the reactor performances. Also amount of voltage applied to the MEC+AD reactors were not effective on pH variations. Cattle manure used

in the present study had a total COD and total nitrogen ratio (TCOD/TN) of 14.1±0.3. It is suggested that cattle manure had a similar C/N ratio which could have served as a buffering capacity for pH. Also utilization of H^+ ions for H_2 and methane production in the MEC+AD reactors might have prevented pH decrease in reactors at high OLRs. Therefore, it is thought that pH of MEC+AD reactors during the study was not affected from HRT and OLR changes.

It is difficult for methane-forming bacteria to produce methane at ORP values higher than -300 mV due to strong competitiveness of fermentation bacteria at ORP values greater than -300 mV [164]. It is also claimed in detail that acidogenesis and methanogenesis take place at optimum ORPs between -250 : -300 mV and -300 : -360 mV respectively [163, 164]. In Set VI and VII, ORP ORP values of MEC+AD reactors at different OLRs and applied voltages ranged between -320 and -355 mV indicating a stable methanogenic activity in MEC+AD reactors during the study. Changes in applied voltage and OLR were not effective on ORP values obtained in the reactors due to the similar ORP values obtained from all MEC+AD reactors.

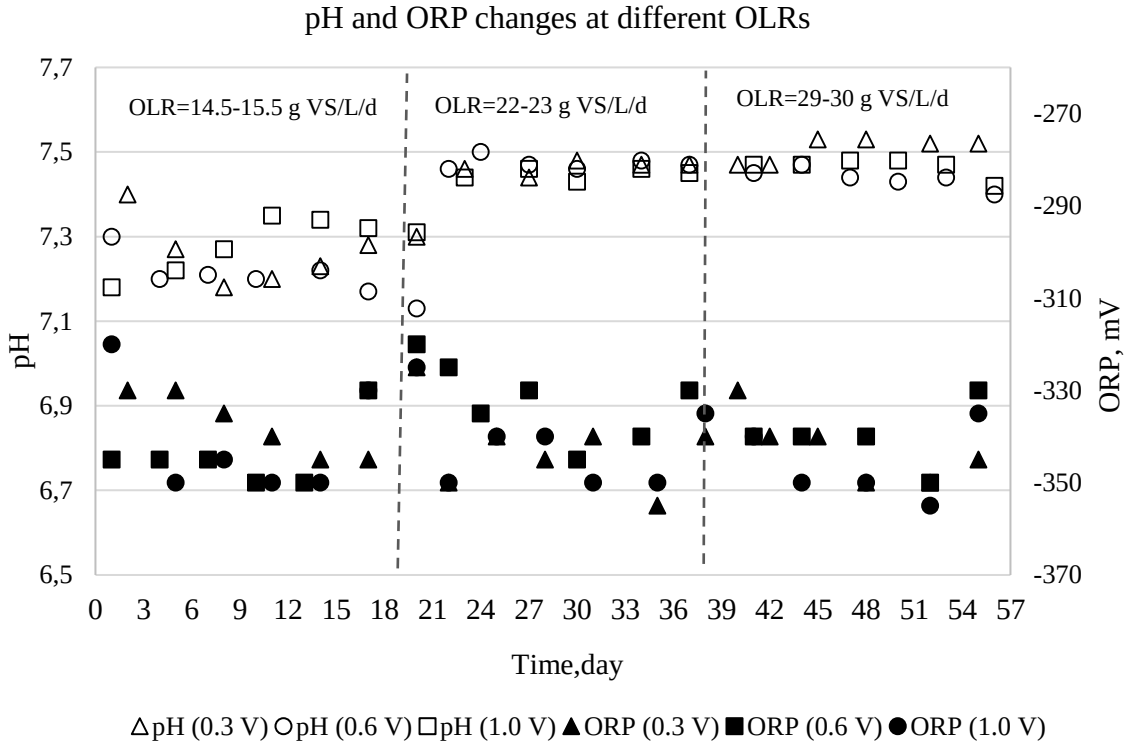

Figure 4.30. pH and ORP variations in MEC+AD reactors at different OLRs.

4.4. Summary of the Results Obtained at Different Operational Conditions

MEC+AD reactors and control reactors used in this study were operated under various conditions. At the first part of the study (5 sets), the reactors were operated at 5 different HRTs between 1 and 6 days by feeding cattle manure (3 % VS). MEC+AD reactors were operated at various supplied voltages (0.3, 0.6 and 1.0 V). In the second part of the study (2 sets), MEC+AD reactors were employed at different OLRs of 22.5 and 30 g VS/L/d by changing VS concentration of cattle manure. In this part of the study, reactors were applied with similar voltages. Consequently, MEC+AD reactors were activated at OLRs between 5 and 30 g VS/L/d with different voltage applications. Table 4.2 and Table 4.3 summarize and compare the results of the experiments conducted in this study. The results of the experiments were also summarized for comparison in Figure 4.31. Biogas productions and methane yields were also compared under different operational conditions.

Biogas productions and methane contents obtained from MEC+AD and control reactors during the entire study are presented in Figure 4.32. It can be seen that increasing OLR from 5 g VS/L/d (at HRT of 6 days) to 30 g VS/L/d, biogas generation improved consistently. Daily average biogas productions and methane contents of biogas at every different OLRs and HRTs are presented in Table 4.2 and Table 4.3. Biogas productions in MEC+AD reactors changed between 1.23 L/L/d and 5.11 L/L/d depending on the HRT, OLR and applied voltage. The percentage of methane in the biogas produced from MEC+AD reactors were in the range of 75-80 %. The methane rate of biogas was found independent of the applied voltage and the applied HRTs and OLRs in this study. However, biogas production was related to OLR and HRT. It was observed that biogas production was not affected from the applied voltage amounts at low OLRs. There is no correlation between applied voltage and biogas/methane productions at low OLRS (< 15 g VS/L/d). However in Sets V, VI and VII, when the OLR was increased further to 22.5 g VS/L/d and more, the effect of applied voltage was more pronounced. So, it can be concluded that, valtage supplementation of 0.6 and 1.0 V was notably effective on biogas productions at high OLRs such as 20-30 g VSL/d.

Table 4.2 The outputs of MEC+AD and control reactors at different HRTs and applied voltages.

	Set I				**Set II**				**Set III**				**Set IV**			**Set V**		
Operation condition	MEC+AD			Cont.	MEC+AD			Cont.	MEC+AD			Cont.	MEC+AD			MEC+AD		
HRT (day)	6				4				3				2			1		
OLR (gVS/L/d)	5				7.45-7.55				9.5-10.5				14.5-15.5			29.5-30.5		
Voltage(V)	0.3	0.6	1.0	-	0.3	0.6	1.0	-	0.3	0.6	1.0	-	0.3	0.6	1.0	0.3	0.6	1.0
Biogas pro. (L/L/d)	1.23	1.59	1.53	1.01	1.75	2.05	1.75	1.28	2.46	2.46	2.68	0.5	3.08	3.06	3.42	3.22	3.56	3.48
CH_4 content (%)	77	78	79	74	77	76	78	76	77	77	76	40	76	75	76	81	79	79
CH_4 yield (L/g VS)	0.19	0.24	0.20	0.15	0.18	0.21	0.18	0.13	0.19	0.19	0.2	0.02	0.16	0.15	0.17	0.089	0.095	0.093
Current density	1-2	1.5-2.5	3-4	-	1-3	2-4	6-8	-	1-2	2-3	4-5	-	1-2	2-3	4-6	1.5-2.5	2-3	4-5
COD Rem. (%)	41.4-44.9			35.5	36.2-38.7			29.5	32.8-34.8			13.8	27.9-31.1			15.9-17.3		
VS Rem. (%)	34.3-37.7			31.1	31.5-32.8			28.6	27.2-29.5			9.2	23.4-25.6			14.1-15.6		
TS Rem. (%)	26.1-29.5			24.5	24-26			21.7	20.1-23.4			7.7	18-21.4			12.3-13.1		
ORP (mV)	-305 : -350			-305: -325	-315 : -350			-295: -325	-320 : -345			-240 : -305	-310 : -345			-320 : -340		
pH	7.35-7.65			7.1-7.4	7.35-7.65			7.1-7.3	7.3-7.6			6.5-7.25	7.15-7.35			7.3-7.45		

Table 4.3 The outputs of MEC+AD at different OLRs and applied voltages.

	Set IV(from Table 4.2)			**Set VI**			**Set VII**		
Operation conditions	MEC+AD			MEC+AD			MEC+AD		
HRT (day)	2			2			2		
OLR (gVS/L/d)	14.5-15.5			22-23			29.5-30.5		
Voltage	0.3	0.6	1.0	0.3	0.6	1.0	0.3	0.6	1.0
Biogas pro. (L/L/d)	3.08	3.06	3.42	3.97	4.59	4.76	4.02	4.81	5.11
CH_4 content (%)	76	75	76	75	77	75	76	76	78
CH_4 yield (L/g VS)	0.16	0.15	0.17	0.13	0.16	0.16	0.10	0.12	0.14
Current density	1-2	2-3	4-6	0.7-1.2	2-4	4-6	0-1	2-4	3-6
COD Rem. (%)	27.9-31.1			23-25.9			16.5-20.4		
VS Rem. (%)	23.4-25.6			18.9-22.6			14-15.9		
TS Rem. (%)	18-21.4			16.2-18			13.6-15.5		
ORP (mV)	-310 : -345			-320 : -355			-330 : -355		
pH	7.15-7.35			7.3-7.5			7.4-7.55		

There are different results on the most effective voltage amount that can be applied to the MECs in the literature. In a study it is claimed that 0.7 V was the optimum voltage to apply to the MECs for highest methane yield [151]. In other studies, it is emphasized that 1.0 V of power application enhanced methane generation the most [95, 99, 128]. Moreover, Xiao et al. [55] stated that methane production was higher at applied voltage of 1.8 V. Feng et al. [147] pointed out that optimal voltage was between 0.3-0.5 V when treating sewage sludge. Different results reported on optimal voltage amount can be attributed to the operating conditions, substrate type and reactor design. Biogas production of $(MEC+AD)_{0.3V}$ reactor was lower than the other two MEC+AD reactors operated at higher voltages.

During the entire study, biogas productions and methane yields of MEC+AD reactors were significantly higher than the control reactors at all HRTs applied. The differences in biogas production and methane yield of MEC+AD reactors and control reactor can be attributed to the hydrogenotrophic methanogenesis and electromethanogenesis that take place in MEC+AD reactors as a result of voltage application [27, 30-32, 119, 151]. Voltage application to MECs can enable direct CO_2 reduction with electrons that are given to the MEC by electrical circuit. Also, under suitable conditions, electrons in MECs combine with protons and form H_2. Later on H_2 is used by hydrogenotrophic methanogens [30-32]. As a result CH_4 formation occur through hydrogenotrophic methanogenesis and electromethanogenesis in MECs. It was reminded earlier that hydrogenotrophic methanogenesis can take place rapidly in MECs and enhance methane production [31, 71, 121]. Methanogenesis in MECs can be explained briefly as the following: exoelectrogens oxidize the substrate and release electrons to anode, and then electrons are transferred to cathode to produce methane [32]. In cathode, methane is produced by cathodic reduction of CO_2 with hydrogen ions through hydrogentrophic methanogens which mostly found in abundance around cathode in MECs [31, 59, 142, 169].

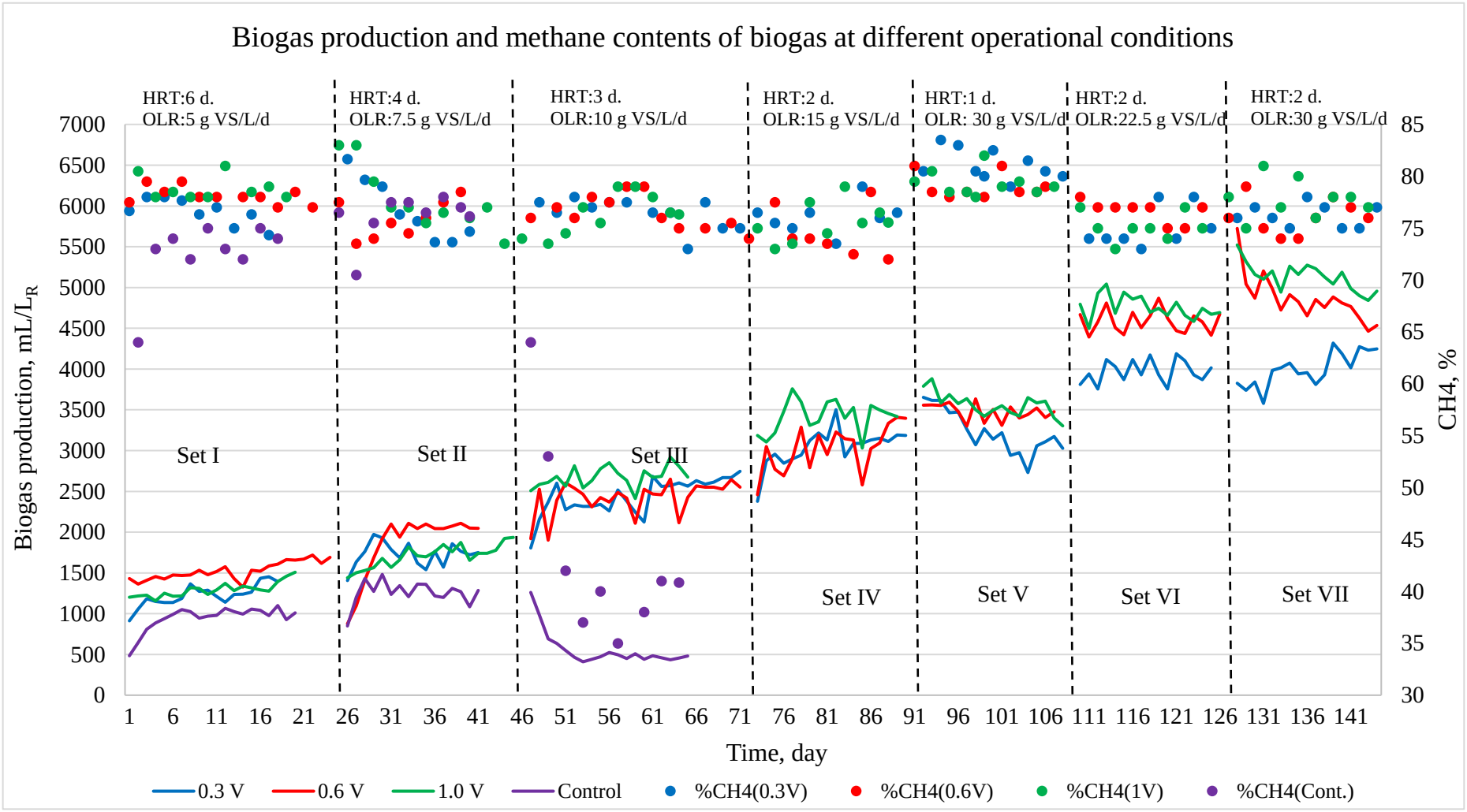

Figure 4.31. Biogas production and CH_4 content in MEC+AD and control reactors at different operation conditions.

Biogas productions and methane yields of the reactors respect to HRTs and OLRs are presented in Figure 4.32. It can be seen that highest biogas productions weres obtained at the OLR of 22.5 and 30 g VS/L/d and at HRT of 2 days. As can be seen that biogas production and methane yield of the reactors operated at HRT of 1 day and OLR of 30 g VS/L/d was lower than that of obtained at HRT of 2 days. It can be suggested that, although there is no signs of system failure, operational HRT should be higher than 1 day. Methane yield of the reactors varied between 0.089 and 0.24 L CH_4/g VS at these conditions. Methane yield of the MEC+AD reactors decreased as the OLR was increased (as the HRT was reduced). The decrease in methane yield was due to exposure of the reactor medium to higher OLRs at low HRTs. Highest methane yield was obtained at 4 to 6 days of HRT. Biogas production of control reactor increased when the HRT decreased from 6 days to 4 days, hence methane yield decreased. Control reactor failed to convert organic material into methane when HRT decreased to 3 days.

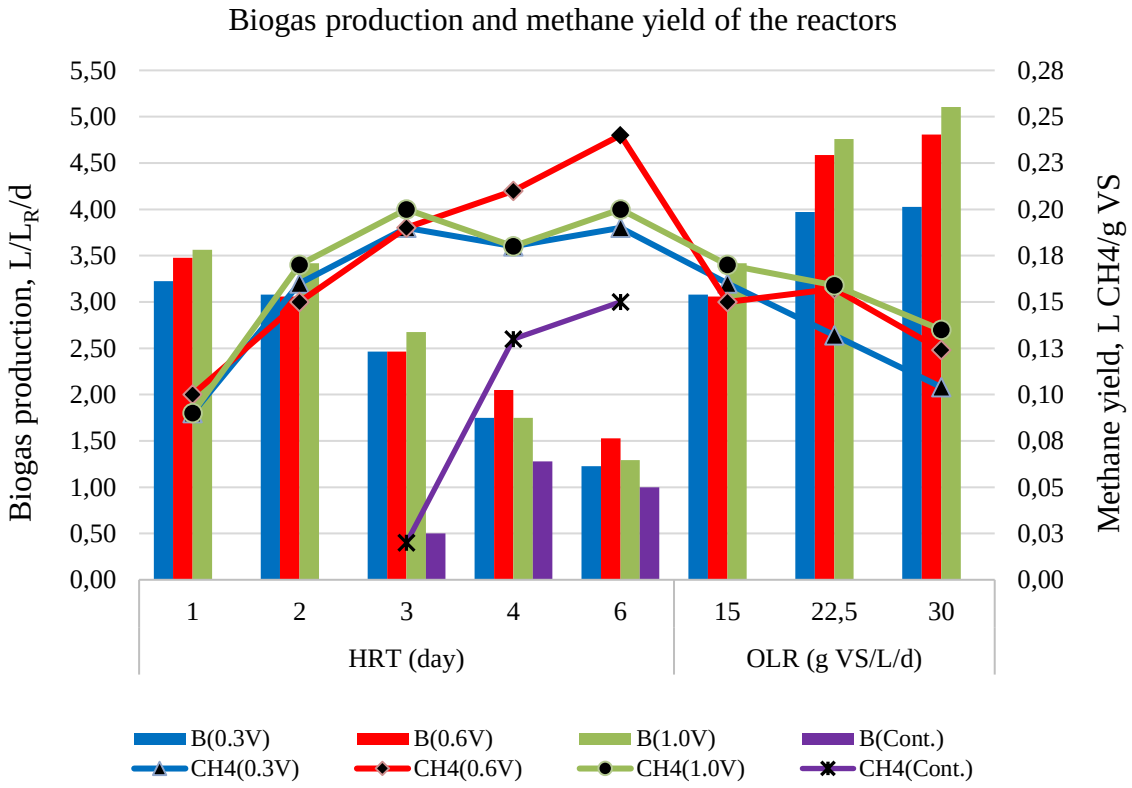

Figure 4.32. Biogas productions and CH_4 yield respect to HRTs and OLRs.

Biogas productions and methane yields found in this study are convenient with the reference works. Quashie et al. [183] treated food waste at OLRs of between 4.30 and 20 g COD/L/d and HRTs of between 1 and 2 days in a combined MEC+CSTR system. The

operation was carried out at 35 ^{o}C and applied voltage of 1.2 V. Methane productions reported in that study were in the range of 0.72 and 4.79 L/L/d. Methane rate of biogas in that study was close to the methane rate obtained (75-80 % CH_4) in the present study. A two stage reactor system composed of a CSTR fermentation unit and a methanogenic MEC unit respectively were used to treat palm oil mill effluents at high OLRs and 4 to 12 days HRT [187]. The effluent from CSTR was fed to MEC at OLRs of between 25 and 35 g COD/L/d and applied voltage was 0.5 V. Under 4 days of HRT and 35 g COD/L/d of OLR, methane production and yield of the MEC were 0.93 L/L/d and 0.115 L/g COD respectively. At higher HRTs, the peformance of the MEC increased. Those results were lower than the results reported in this study. Zhang et al. [60] treated synthetic WW in a well designed high rate UASB-MEC integrated reactor at HRT and applied voltage of 6 h and 1.0 V respectively. Applied OLR was in the range of 4 and 28 g COD/L/d in the high rate system. Methane productions in the integrated system were reported between 1 and 6 L/L/d for the given OLRs. The results obained from the present study were comparable with those results obtained from a high rate reactor that was fed with synthetic WW [60]. In the present study, combined MEC+AD reactor achieved high biogas productions compared to conventional anaerobic digestion process due to voltage application. Voltage application to MEC+AD rectors enhanced electron transfer through electrodes which resulted in CH_4 production through electromethanogenesisi and hydrogenotrophic methanogenesis by biofilm on the cathode. High OLR may have also led the way for acetoclastic methanogenesis compete with electrogens on the anode for organic matter utilizaiton.

MEC+AD reactors exhibited high rate biogas production at short HTRs varying from 6 days to 1 day in descending order. At the same time OLR was increased from 5 to 30 g VS/L/d by lowering HRT or by concentrating VS content of the cattle manure. Organic matter removal efficiencies in MEC+AD reactors are presented in Figure 4.33. The highest organic removal rates were obtained at HRT of 6 days and OLR of 5 g VS/L/d in MEC+AD reactors. The highest removal efficiencies were between 41.4 - 44.9 % for COD, 26.1 - 29.5 % for TS and 34.3 - 37.7 % for VS respectively. On the other hand, the lowest organic matter removal rates of MEC+AD reactors were obtained at HRT and OLR of 1 day and 30 g VS/L/d respectively.

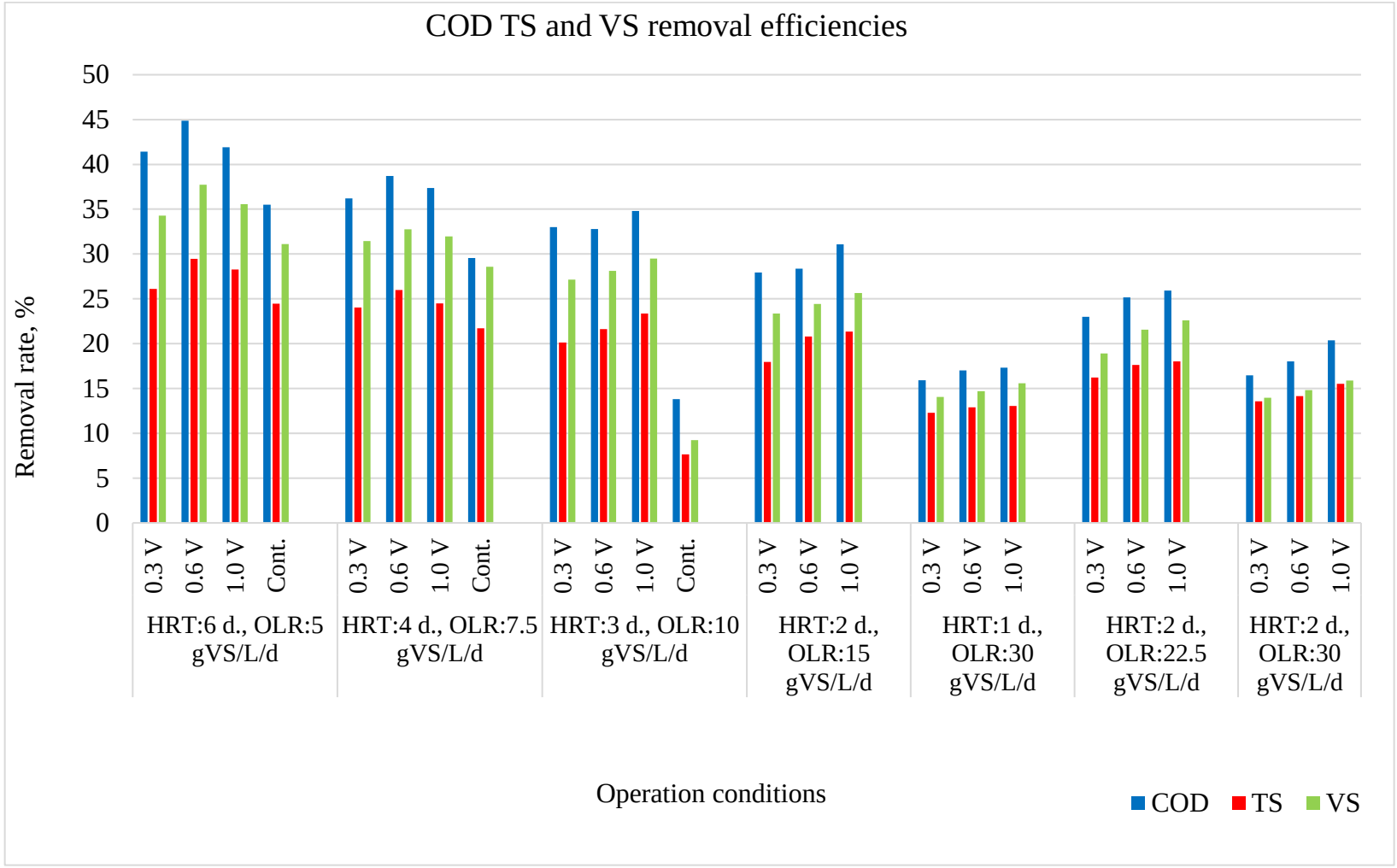

Figure 4.33. COD, TS and VS removal efficiencies in MEC+AD and control reactors at different operation conditions.

The removal efficiencies were between 15.9 - 17.3 % for COD, 12.3 - 13.1 % for TS and 14.1 – 15.6 % VS respectively. Substrate removal rates in control reactor followed the same route as in MEC+AD reactors. The highest COD, TS and VS removal rates were 35.5, 24.5 and 31.1 % respectively at OLR and HRT of 5 g VS/L/d and 6 days. When the HRT was decreased to 3 days (10 g VS/L/d), control reactor failed due to overloading resulted in COD, TS and VS removal rates of 13.8, 7.7 and 9.2 % respectively. Considering the organic removal rates of MEC+AD reactors, it can be concluded that power application was effective on the organic removal rates. At high OLRs such as 20 g VS/L/d and more, the reactors with applied voltages of 0.6 and 1.0 V exhibited more organic removal efficiency compared to the reactor with applied voltage of 0.3V. At lower OLR (<10 g VS/L/d: HRTs of 3 to 6 days) organic removal efficiencies in MEC+AD reactor with applied voltage of of 0.6 V was slightly higher than other two MEC+AD reactors. The effect of applied voltage amount on organic removal efficiencies were in parallel with the biogas productions of MEC+AD reactors. The higher the removal rate was in a particular MEC+AD, the higher the biogas production occured in the same MEC+AD reactor. Voltage applicaiton enhanced organic removal efficiencies in MEC+AD reactors. It is suggested that electrons that were given to the reactors were used to reduce CO_2 and hydrogen ions which are derived from the degredation of organic materials. Therefore organic removal efficiencies in MEC+AD reactors applied with high voltages (0.6 and 1.0 V) were higher than the MEC+AD reactor that ws applied with 0.3 V of voltage. Organic removal rates obtained in MEC+AD reactors were appropriate with other studies that were conducted with real wastewater/waste or manure. In a two stage CSTR-MEC system, pig slurry was first fed to thermophilic CSTR and then to anode of MEC in series at OLR and HRT of 7.9 g COD/L/d and 32.4 h respectively. At ambient temperature and set potential of 0.8 V vs. SHE, COD removal rates in MEC were in the range of 24±8 % [107]. Song et al. [146] treated mixed sewage sludge in a combined MEC and AD reactor operated at 35ºC and supplied voltage of 0.3 V. TCOD removal efficiency between 64% and 39% was achieved at OLRs and HRTs in of 1.44 to 5.76 g VS/L/d and 20 to 5 days respectively.

Microbial electrolysis cell operates with an external power supplementation to the cell. The idea is to overcome energy barriers limiting the chemical and biological reactions that are desired to occur. Methane production can be enhanced in MECs by voltage application. As a result of voltage application, various amount of current is produced in

MECs depending on the electrodes, reactor design, substrate and scale of the applied voltage. Current production is an essential indicator of bioelectrochemical activity in MECs. It demonstrates the capability of the MECs on electron recovery and transfer through bioelectrochemical activities [150]. Current density shows the activity of exoelectrogens that release the electrons to electrode derived from substrate breakdown. The higher the current production the higher substrate oxidation and methane production is expected in MECs [99]. Current production in reactors are dependent on the applied voltage as well as the electrogenic activity in the reactors. Higher applied voltage does not necessarily result in higher current production at the reactors. Microorganisms can get inhibited from high voltages [96]. Also magnitude of the applied voltage can effect the variety and dominance of microbial community [147, 151]

Current productions determined in MEC+AD reactors that were operated at different conditions are shown in Figure 4.34. MEC+AD reactors presented various current densities as a result of different voltage applications. The highest current productions were achieved at voltage supplementation of 1.0 V which varied mostly between 4 and 6 mA/L. On the contrary lowest current productions ranging between 1 and 2.5 mA/L were observed at 0.3 V voltage supplementation. Current productions in MEC+AD reactors were strictly depended on the applied voltage amount [96, 179]. Methane production in all MEC+AD reactors were similar to each other until the last two sets (Set VI, Set VII) of the study. In Set VI and VII, biogas production of $(MEC+AD)_{0.3V}$ was lower than the other two MEC+AD reactors operated at applied voltages of 0.6 and 1.0 V. If looked at Figure 4. 36 closely, it can be realized that current production of $(MEC+AD)_{0.3V}$ reactor in Set VI and VII decreased compared to Sets I to V. This decrease can be attributed to the OLR increase in reactors. High OLR can affect the current production through change in microbial community. As a result, the decrease in current production was followed by a smaller rise in biogas production in $(MEC+AD)_{0.3V}$ compared to $(MEC+AD)_{0.6V}$ and $(MEC+AD)_{1.0V}$ as it is shown in Figures 4.31 and 4.32. Biogas production in MEC+AD reactors were mostly similar to each other at different applied voltages. It is suggested that applied voltage and current production had a significant effect on methane production however, applied voltage amount was not significantly effective.

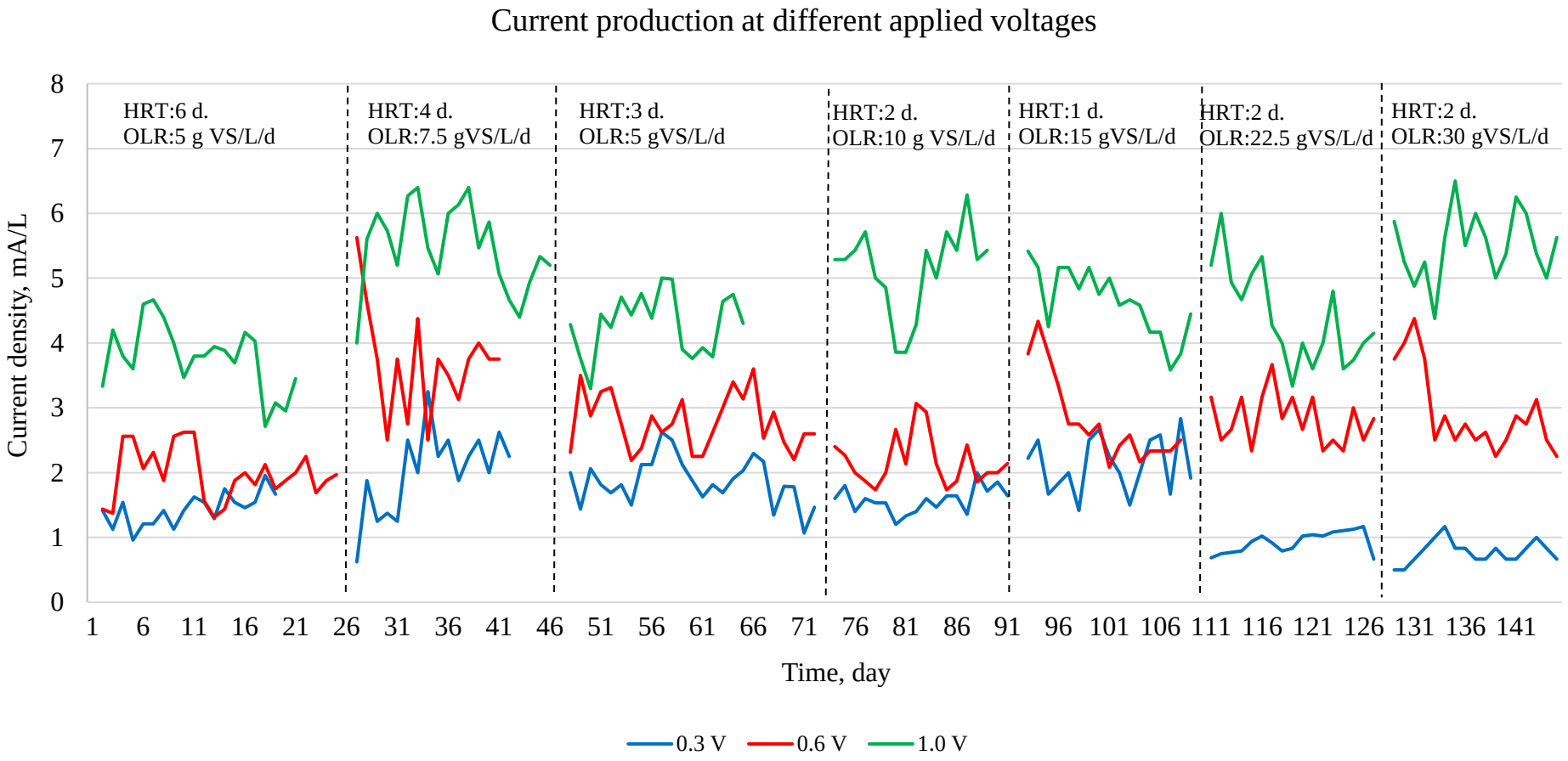

Figure 4.34. Current productions in MEC+AD reactors at different operation conditions.

Most of the studies conducted with continuously operated reactor reported higher current productions compared to results obtained in the current study. It is stated by some researchers that methanogens are more willingly to collect electrons from electrogens (*Geobacter*) on the anode instead of using acetate oxidation for metabolic activity. Electron flow from electrogens to methanogens but not to anode may have resulted in low current production in MEC+AD reactors. It is known that acetoclastic methanogenesis can compete electrogens over acetate utilization, and low organic concentrations are not favorable for them. Therefore acetoclastic methanogenesis can gradually increase and even replace anodic oxidation (electrogens) to become the dominant pathway to degrade organics at high VS concentrations of 30 g VS/L as in the present study [129, 169]. Finally growth of acetoclastic methanogens on anode may have suppressed electrogen growth on anode and as a result, current production decreased [148, 169]. Nevertheless, current production in the present study were convenient with some of the studies. For example, maximum current density of 7.4 mA/L was reported by Cusick et al. [48] in a study conducted with a 1100 L MEC treating winery WW at voltage supplementation of 0.9 V. HRT and OLR were 1 day and 0.7-2 g SCOD/L/d respectively in the study. Also MEC and elecrodes were very well designed with graphite fiber brush anodes and stainless steel mesh cathodes in that study. In another study, glucose fed single chamber MEC and two chamber MEC were compared in terms of biogas and current production. Graphite rod and carbon felt electrodes were used in the reactors which were operated at temperature of 55^{o}C and set potentials of ranging between -0.8-1.2 V (vs.Ag/AgCl). Current productions were reported between 2 and 10 mA in that study [134]. Current productions in a baffled reactor integrated with MEC were between 2 and 10 mA at applied voltages of between 0.6 and 1.0 V. The reactor was operated at mesophilic temperature and short HRTs of 2-3 day and it was fed with petrochemical wastewater [189]. Although the current productions obtained in the present study were appropriate with the studies mentioned above, they were lower compared to most of the studies. It is thought that methanogens and electrogens in the biofilm on the electrodes competed over the electrons and substrate. This competition caused to lower current productions in the MEC+AD reactors. On the contrary methane production not being affected from this competition may indicate the dominance of methanogens on the electrodes.

4.5. Response Surface Methodology Application for Optimization of Process

Response Surface Methodology (RSM) is an integration of some appropriate mathematical and statistical methods to find out a model for an answer that is influenced by several variables [166, 188]. The aim of the RSM is to optimize the result of the model to accomplish intended target [188]. In the case of biogas production from cattle manure in a combined MEC+AD reactor, it provides to examine the effects of HRT, OLR and voltage application and the interaction between these variables on biogas production and methane yield. In the case of this study, RSM can also be applied to examine the relationship between process inputs (OLR, HRT and applied voltage) and outputs such as organic removal (VS:%) and current production. The method enables to fit a model to explain the relationship between process variables and biogas production as well as methane yield and current production. The final goal in this study was to produce highest biogas production and methane yield. Central Composite Design (CCD) was commenced with three variables and three inputs for investigation of effects on biogas production and methane yield, current production and VS removal. Biogas production, methane yield, current production and VS removal rate analyzed independently because the effect of each variable on these outputs were different. The independent variables were chosen as HRT, OLR and applied voltages. Consequently, relationship between input variables and outputs could be defined. The levels of these variables were determined according to the experimental data given in Section 4.2 and 4.3. The variables used in experiments and results found in the study are presented in Table 4.2 and Table 4.3, in the previous section. Variables (factors) and results (responses) entered into RSM-CCD analysis are given in Table 4.4. In this part of the study, ANOVA was also applied to test outcomes of the variables (HRT, OLR, applied voltage) on the results of MEC+AD reactors. The variables of the analysis were as the following: for HRT: 1, 2, 3, 4 and 6 days, for OLR: 5, 7.5, 10, 15, 22.5 and 30 g VS/L/d and for applied voltage: 0.3, 0.6 and 1.0 V.

4.5.1. RSM Analysis on Biogas Production

RSM studies showed that different values of HRT and OLR had significant effects on biogas production. On the other hand, power applications of 0.3, 0.6 and 1.0 V had very little effect on biogas production.

Table 4.4 The factors and responses entered into design.

Run	Factor 1 A:HRT day	Factor 2 B:OLR g VS/L/d	Factor 3 C:Voltage V	Response 1 Biogas producti... L/L/d	Response 2 Methane yield L/g VS	Response 3 Current product... mA/L	Response 4 VS removal rate %
1	1	30	0.3	3.22	0.089	2	14.05
2	1	30	0.6	3.56	0.095	2.5	14.71
3	1	30	1	3.48	0.093	4.5	15.58
4	2	15	0.3	3.08	0.16	0.5	23.35
5	2	15	0.6	3.06	0.15	2.5	24.43
6	2	15	1	3.42	0.17	5	25.64
7	3	10	0.3	2.46	0.19	0.5	27.16
8	3	10	0.6	2.46	0.19	2.5	28.13
9	3	10	1	2.68	0.2	4.5	29.5
10	4	7.5	0.3	1.75	0.18	2	31.46
11	4	7.5	0.6	2.05	0.21	3	32.77
12	4	7.5	1	1.75	0.18	7	31.95
13	6	5	0.3	1.23	0.19	1.5	34.28
14	6	5	0.6	1.59	0.24	2	37.72
15	6	5	1	1.53	0.2	3.5	35.58
16	2	22.5	0.3	3.97	0.13	0.95	18.9
17	2	22.5	0.6	4.59	0.16	3	21.56
18	2	22.5	1	4.76	0.16	5	22.6
19	2	30	0.3	4.02	0.1	0.5	13.95
20	2	30	0.6	4.81	0.12	3	14.81
21	2	30	1	5.11	0.14	4.5	15.89

Figure 4.35 is the 3D surface model for biogas production regarding the HRT and OLR variations. The figure represents the interaction between biogas production and HRT and OLR. It is understood from Figure 4.37 that, at lower OLRs, biogas productions would be lower. On the other hand when the OLR is increased, biogas production would increase as well. When looked at the HRT effect on biogas production, lower HRTs would result in lower biogas production and higher HRTs would result in higher biogas production. It seems that OLR and HRT affect the biogas production in the same way. However there is a detail that should be clarified. It is obvious in Figure 4.35, OLR is more effective on biogas production compared to HRT. At lowest HRT and highest OLR, response is more than 3 L/L/d in the figure. However at lowest OLR and highest HRT, response is lower than 2 L/L/d. Since the blue parts of the surface of the figure represent the lowest biogas production rates, it is distinguishable that at all HRTs, biogas production can be at lower values if the OLR is low. In Figure 4.35, biogas production tend to increase with the increase of OLR and HRT. However, it is not clear at which point, biogas production would decrease. So this figure also say that MEC+AD reactors have potential of being operated at further conditions. In other words, OLRs and HRTs of more than 30 - 40 g VS/L/d and 6 days can be applied to the MEC+AD reactors to examine the response of biogas production.

Biogas Production At Different HRT And OLR

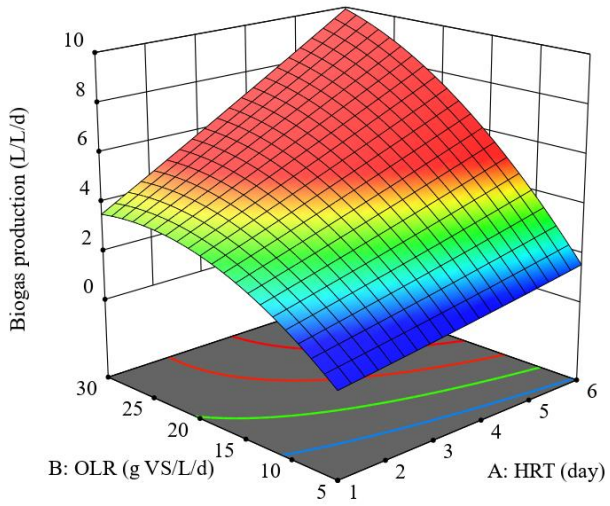

Figure 4.35. The interaction effect of HRT and OLR on biogas production.

Biogas Production At Different HRT And Applied Voltage

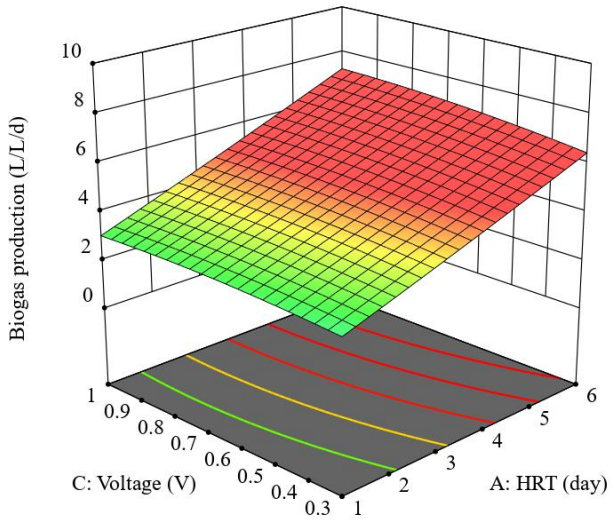

Figure 4.36. The interaction effect of HRT and applied voltage on biogas production.

In Figure 4.36, interaction effect of HRT and applied voltage on biogas production is presented. Figure 4.36 suggests that biogas production is not very much affected from the applied voltage however it is definitely affected by the variation of HRT. When the HRT is increased from low to high, there occurs a difference more than 2 folds in biogas production at lowest and highest HRT values respectively. However at the highest and

lowest voltages applied to MEC+AD reactors, the biogas productions do not seem to be affected at constant HRT.

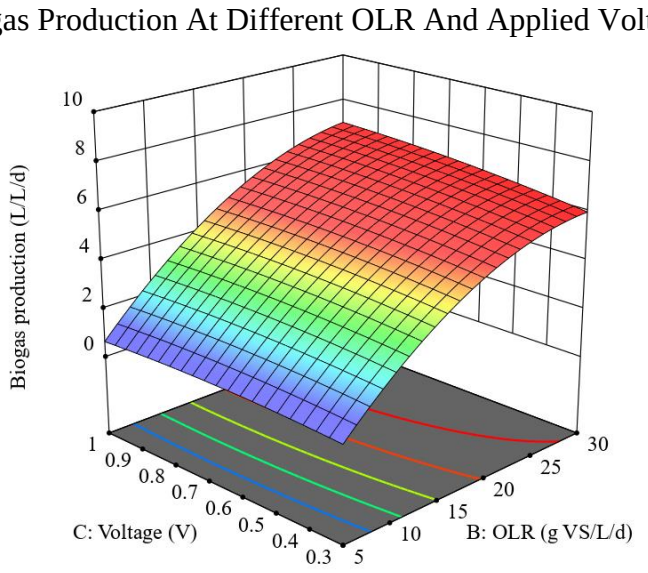

Figure 4.37. The interaction effect of OLR and applied voltage on biogas production.

Interaction effect of OLR and applied voltages on biogas production is shown in Figure 4.37. The effect of OLR and applied voltage on biogas production looks the same with the previous 3D surface graphic model of biogas production at different HRTs and applied voltages. Biogas production increased as the OLR increased independently from voltage application. However change in applied voltage does not affect the biogas production. The only difference between Figure 4.36 and 4. 37 is that, the increase in biogas production in Figure 4.36 is likely linear compared to parabolic (quadratic) increase in biogas production in Figure 4.37. This indicates that HRT can be increased further which would not affect biogas production negatively. However OLRs more than 30 g VS/L/d may cause a fall in biogas generation which needs to be searched.

Table 4.5. Quadratic model fitted by ANOVA for biogas production.

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001	0.7334	0.6679	
2FI	0.0004	0.9091	0.8500	
Quadratic	**00009**	**0.9729**	**0.9439**	**Suggested**
Cubic	0.0675	0.9902	0.9004	**Aliased**

The figures that were presented earlier clarify the effects of HRT, OLR and applied voltages on biogas production. ANOVA test for the biogas production suggested that best model was Quadratic model to fit to the process according to the factors and responses. In RSM it is aimed to regulate a model that determines the outputs (response) of a process according to the inputs (factor). Model determination in RSM is linked to selecting highest polynomial model with additional important parameters. The selected model should fit enough to estimate all coefficients. p-value is also important because it gives an information about the model values which can be same or greater than actual results. If p- value is low, then it can be concluded that the model is at more importance. According to the ANOVA test, quadratic model was suggested for biogas production at different operation conditions as presented in Table 4.5. Quadratic model involves the influence of variables one by one, interactions of two variables, and quadratic influence of variables (square of factor). In this study, the reason of the selection of quadratic model was because the Adjusted R^2 and the Predicted R^2 were maximum. The Predicted R^2 of 0.944 was consistent with the Adjusted R^2 of 0.973 and the difference was less than 0.2 which makes quadratic model fit best for representation of interaction effect of HRT, OLR and applied voltages on biogas production.

Analysis of variance (ANOVA) test carried out to determine the weight of independent HRT, OLR, and voltage. The results of ANOVA test showed that, for all operational conditions, the models were determined to be at great importance which means that operational conditions were effective on biogas production. Especially HRT and OLR were significant on biogas production due to the p-values of less than 0.0001. However according to the ANOVA test, voltage application and quadratic effect of OLR were also in correlation with biogas production. A coefficient that is dedicated for a factor to be meaningful, the p- value should be less than 0.05 for that coefficient. Table 4.6 presents ANOVA of sum of squares, F and p values for fitted quadratic model made by RSM-CCD for biogas production. Model F-value of 80.67 indicate the model has a weight on the responses. In addition, if the p-values is less than 0.05, then it shows that model tools are respectable. Therefore the coefficients of A, B, C, B^2 are noteworthy simulation tools. ANOVA test suggests that biogas production was affected significantly by all variables and quadratic effect of OLR as well. On the contrary there was no a sign of mutual effect of any couple of variables on biogas production. Changing the factors one by one, had a significant effect as one factor on biogas production.

Table 4.6. ANOVA for Quadratic model fitted by CCD for biogas production.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	27.57	9	3.06	80.67	< 0.0001	Significant
A-HRT	1.77	1	1.77	46.50	< 0.0001	
B-OLR	5.61	1	5.61	147.70	< 0.0001	
C-Voltage	0.5723	1	0.5723	15.07	0.0026	
AB	0.1144	1	0.1144	3.01	0.1106	
AC	0.0371	1	0.0371	0.9779	0.3440	
BC	0.1548	1	0.1548	4.08	0.0685	
A^2	0.0003	1	0.0003	0.0085	0.9283	
B^2	0.4870	1	0.4870	12.82	0.0043	
C^2	0.1154	1	0.1154	3.04	0.1092	
Residual	0.4178	11	0.0380			
Cor Total	27.99	20				

ANOVA is useful to create a model for prediction of responses. Biogas production in MEC+AD reactors can be predicted by the equation below using coded factors. The coded equation is beneficial for describing the comparative effect of the input variables by matching against the variable coefficients. The equaiton and coefficients also show the effects of the factors alone and factors together on biogas production. According to the equation, effects of OLR, HRT and voltage application and quadratic effect of OLR were more significant on biogas production according to the coefficients of ANOVA test.

Biogas Production (L/L/d) = 5.02 + 1.96xA + 2.95xB+ 0.2601xC + 1.23xAxB + 0. 1507xAxC + 0.2467xBxCx + $0.0431xA^2$ - $1.29xB^2$ – $0.161xC^2$

Zinatizadeh et al. [190] studied the effect of HRT and OLR on methane production using RSM. The experimental data in that study was obtained from a continuously operated up-flow anaerobic sludge fixed film (UASFF) bioreactor. The substrate was palm oil mill wastewater. The reduced quadratic model showed that an increase in OLR (by decreasing HRT and increasing COD_{in}) caused an increase in methane production rate which was he same result found in the present study. It was reported in the study that at a fixed HRT, an uptrend in the methane formation was the case when OLR was increased. This situation is also presented in this study, in Figure 4.35. Impact of HRT on hydrogen production was studied by Liu et al. [191] in a process operated semi continuously and treating cow manure and food waste mixture. In their study, RSM analysis stated that HRT was identified as the factor that contributed the most to the hydrogen production rate before mixing ratio, and substrate concentration. As a result effect of OLR and HRT in the

present study can be attributed to the contact time due to the high HRT and higher ratio of degredable substance to biomass originated from high OLR.

4.5.2. RSM Analysis on Methane Yield

When the methane yield of the MEC+AD reactors are examined in RSM according to the variables, results showed that different values OLR had significant effect on methane yield. On the other hand, applied voltage and HRT had very little effect on methane yield. It should be noted that each factor (HRT, OLR, applied voltage) had affected the methane yield separately. The effect of each factor on methane yield was independent of other two factors. In Figure 4.38, 3D surface model graphic, interaction effect of OLR and HRT on methane yield is given. It can be understood from the figure that when the OLR is increased, methane yield decreases. On the other hand methane yield is almost same at different HRT values when OLR is kept constant. This result is understandable because when biomass to food ratio increases, mehane yield decreases as well. In other words, if less substrate is given to microorganisms, most of it would be degraded and turned into methane which result in high methane yield. In Figure 4.38, it can be seen that, at low OLR and high HRT methane yield tends to increase more compared to other possibilities because low amount of substrate can be degraded and used at higher rates in a longer time period. According to Figure 4.38, it can be concluded that OLR is the most important variable on the scale of methane yield. The figure also indicate that there is a high possibility of obtaining higher methane yields at higher HRTs than 6 days when MEC+AD reactors operated at further conditions.

It was found out in RSM studies that methane yield was only affected by the OLR. Neither of the other two factors affected methane yield. There were no interaction effect of any variables on the amount of methane yield. Therefore Figure 4.39 was prepared by RSM when aimed to show the effects of factors on methane yield separately. It can be understood from Figure 4.39 that, methane yield was mostly affected by OLR. As the OLR is increased, methane yield decreases. On the other hand HRT and voltage application seem to affect methane yield in a positive way which means, both of the variables cause in an increase on methane yield, when they are increased. However the effect of HRT and applied voltage seems to be very low on methane yield.

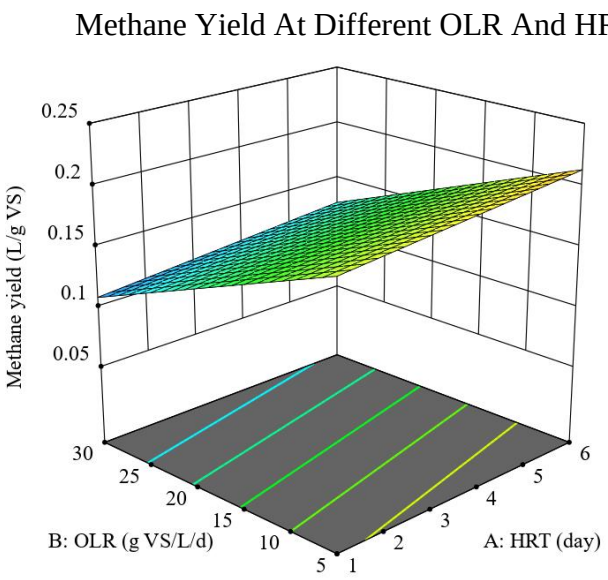

Figure 4.38. The interaction effect of HRT and OLR on methane yield.

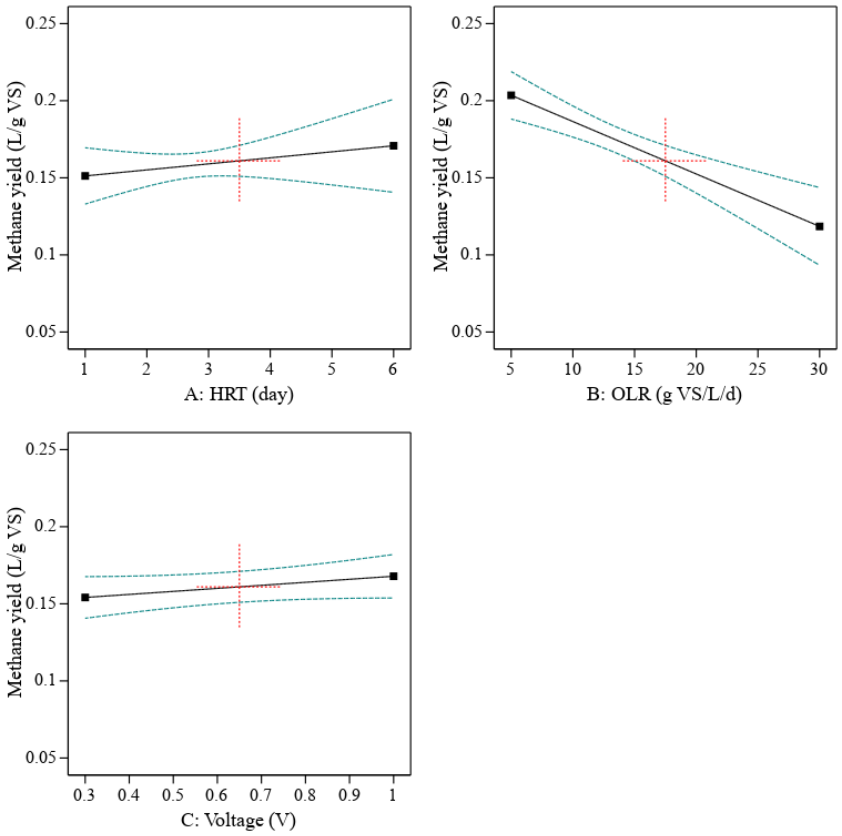

Figure 4.39. The effect of HRT, OLR and applied voltage solely on methane yield.

Table 4.7. Linear model fitted by ANOVA for methane yield.

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.8415**	**0.7781**	**Suggested**
2FI	0.1249	0.8706	0.7256	
Quadratic	0.3956	0.8729	0.6836	
Cubic	0.0784	0.9508	0.6805	Aliased

The figures that were presented earlier clarify the sole effects of HRT, OLR and applied voltages on methane yield one by one. There was no interaction effect of any couples of the factors on the methane yield. ANOVA test for the methane yield suggested that best model was Linear model to fit the the process according to the factors and responses as presented in Table 4.7. Linear model includes the effect of factors alone on responses. In this study, the reason of the selection of linear model was because the Adjusted R^2 and the Predicted R^2 were maximum. The Predicted R^2 of 0.842 was convenient with the Adjusted R^2 of 0.778. The difference between Predicted R^2 and Adjusted R^2 was not higher than 0.2. This make linear model fit best to show the relationship of methane yield with HRT, OLR and applied voltages.

To establish the importance of independent variables (HRT, OLR, voltage) on methane yield, ANOVA test was conducted. Due to the test, the models were notable for all operational parameter, meaning that operational conditions were effective on methane yield. Especially OLR was significant on methane yield due to the p-value of less than 0.0001. On the other hand according to the ANOVA test, HRT and voltage application were not in correlation with methane yield significantly due to the p- values higher than 0.05. Table 4.8 presents ANOVA of sum of squares, F and p values for fitted linear model made by RSM-CCD for methane yield. Model F-value of 36.4 indicate the model has a weight on the responses. ANOVA test suggests that methane yield was affected only by OLR. On the other hand OLR and applied voltage were not effective on methane yield nor any of interaction of the factors. Negative effect of OLR on methane yield can be attributed to the high VS amount that was fed to the reactor which included limited biomass in the bulk and on the electrodes of MEC+AD reactor. As a result biomass did not have enough time to breakdown all the VS that was fed to the reactor resulted in a decrease in methane yield.

Table 4.8. ANOVA for Linear model fitted by the CCD for methae yield.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0312	3	0.0104	36.40	< 0.0001	significant
A-HRT	0.0002	1	0.0002	0.857	0.3791	
B-OLR	0.0069	1	0.0069	23.99	0.0001	
C-Voltage	0.0007	1	0.0007	2.33	0.1449	
Residual	0.0049	17	0.0003			
Cor Total	0.0360	20				

ANOVA is a useful tool to create a model for prediction of responses. Methane yield in MEC+AD reacors can be predicted by the equation below using coded factors. The coded equation is helpful for describing the relative impact of the factors by comparing the factor coefficients. According to the equaion below, we can understand that HRT and applied voltage do not affect the methane yield significantly, however OLR affected the methane yield significantly and in the negative direction. Negative effect of OLR on specific methane production was also submitted by Safari et al. [192] in a study conducted batch wise by co-digestion of canola residues and cattle manure. The effect of inoculum (biomass) to substrate ratio on methane yield was investigated at different ratios. The result was methane yield increased up to a point by the increase of inoculum to substrate ratio which meant that sufficient amount of microroganism can increase the methane yield. Lower inoculum to substrate ratio than optimum values cause reduction of methane yield.

Methane Yield (L CH_4/g VS) = 0.161 + 0.0098xA – 0.0425xB + 0.0069xC

4.5.3. RSM Analysis on Current Production

According to the results of RSM analysis on current production, different values of HRT and OLR had no significant effect on current production. When looked at Figure 4.40, current productions do not change according to the change in OLR and HRT. The figure indicate that current was almost same at different HRTs and OLRs. The decrease or increase in HRT and/or OLR one by one or at the same time did not affect current production means that OLR and HRT had no significant effect on current production.

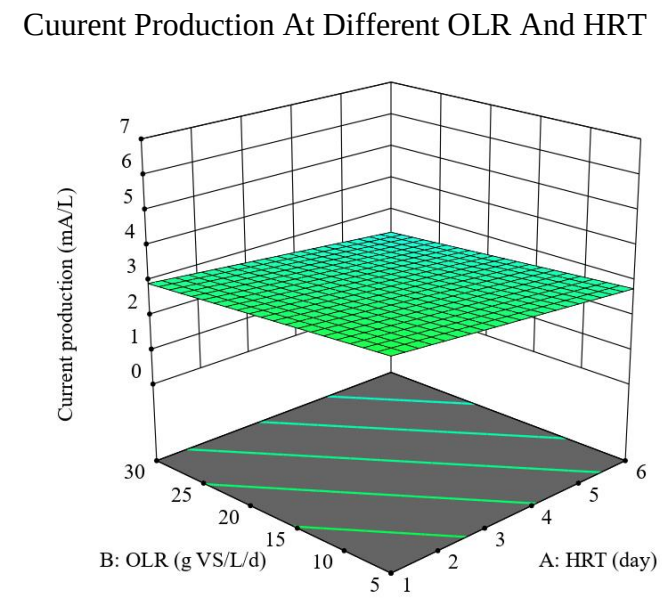

Figure 4.40. The interaction effect of HRT and OLR on current poduction.

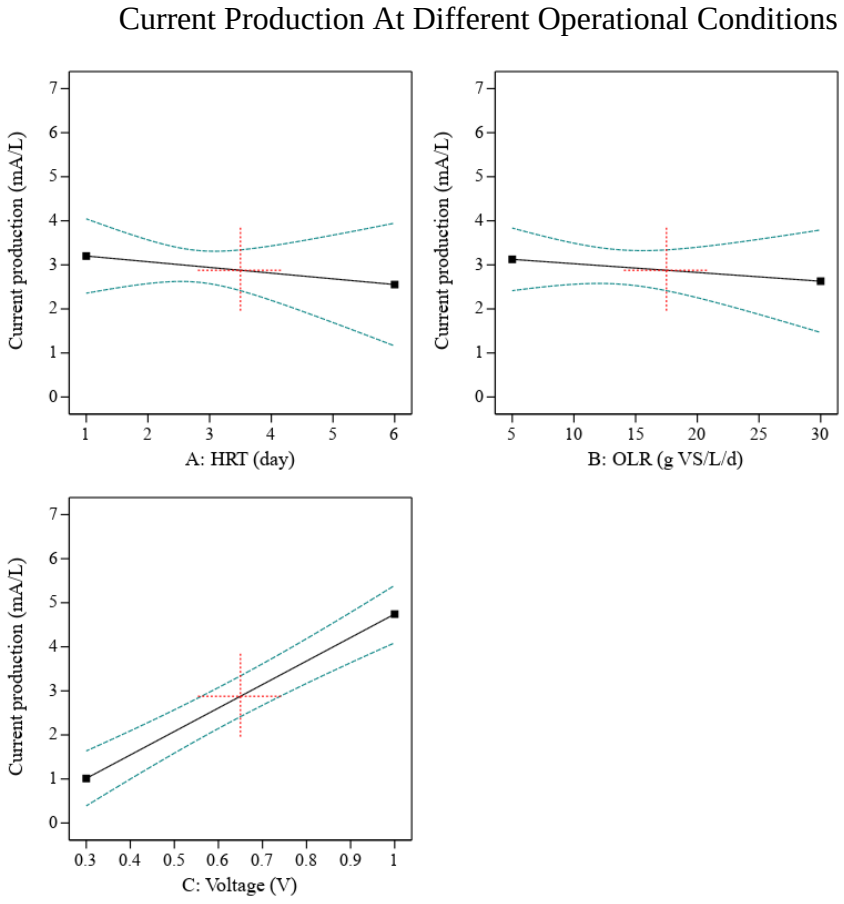

Figure 4.41. The effect of HRT, OLR and applied voltage solely on current production.

On the other hand applied voltages had significantly affected the current production. In Figure 4.41, it is obvious that current production of MEC+AD are dependent on the applied voltages to the reactor. This result is understandable because the higher the voltage applied to the reactors is, the higher the current can be produced by the reactors. It was found out in RSM studies that current production was affected only by applied voltages. There were no interactions between factors on current production. It can be understood from Figure 4.41 that, current production is around 3mA/L at all OLRs and HRTs indicating that none of those factors do not have any significant effect on current production.

ANOVA test suggested that best model was Linear model to fit for the current production according to the factors and responses. Table 4.9 presents the comparison between models due to current production results. The reason of selection of linear model was because the Adjusted R^2 and the Predicted R^2 were maximum at linear model. The Predicted R^2 of 0.796 was in reasonable aggreement with the Adjusted R^2 of 0.716 and the difference was less than 0.2 which makes linear model fit best for representation of relationship between current productions and the variables.

Table 4.9. Linear model fitted by ANOVA for current production.

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.7957**	**0.7162**	**Suggested**
2FI	0.5851	0.7830	0.5561	
Quadratic	0.1176	0.8347	0.5927	
Cubic	0.3030	0.8772	0.2445	Aliased

ANOVA test was conducted to find out the weight of HRT, OLR and voltage on current production. The result of the test showed that, the models were fit to be meaninful for all operational parameters. Voltage application was significant on current production due to the p-value of less than 0.0001. On the other hand according to the ANOVA test, HRT and OLR were not in correlation with current production significantly due to the p- values higher than 0.05. Table 4.10 presents ANOVA of sum of squares, F and p values for fitted linear model made by RSM-CCD for methane yield. As a result, Model F-value of 26.97 implies the model is significant. ANOVA suggests that current production was affected significantly by applied voltages. Current production in MEC+AD reacors can be predicted by the equation below using coded factors. According to the equaion below, we

can understand that HRT and OLR do not affect the methane yield significantly, however applied voltage affected the methane yield significantly and in the negative direction.

Current Density (mA/L) = 49.29 + 0.2554xA + 0.2335xB + 49.02xC

Table 4.10. ANOVA for Linear model fitted by the CCD for current production.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	49.29	3	16.43	26,97	< 0.0001	significant
A-HRT	0.2554	1	0.2554	0.4192	0.5260	
B-OLR	0.2335	1	02335	0.3833	0.5441	
C-Voltage	49.02	1	49.02	80.47	< 0.0001	
Residual	10.36	17	0.6092			
Cor Total	59.64	20				

4.5.4. RSM Analysis on Organic Removal Rate

RSM studies showed that different values of HRT and OLR had significant effects on VS removal rates. Conversely, applied voltages had very slight effect on VS removal rate.

VS Removal Rate At Different HRT And OLR

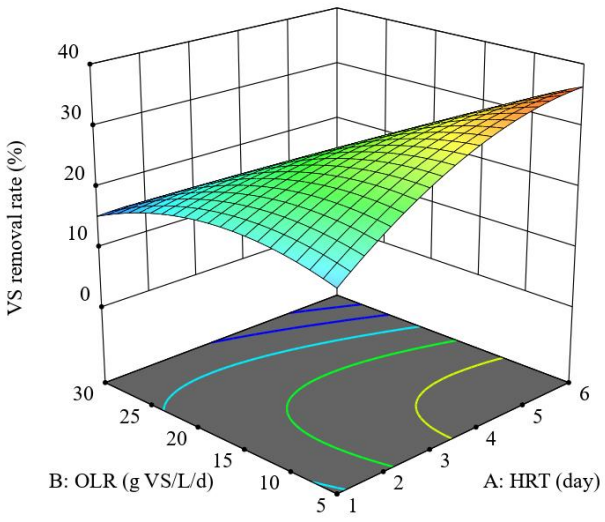

Figure 4.42. The interaction effect of HRT and OLR on VS removal rate.

Figure 4.42 is the 3D surface model for VS removal rate regarding the HRT and OLR variations. The figure illustrates the interaction impact of HRT and OLR on VS

degredation rate. It is understood from Figure 4.42 that, at lower OLRs, VS removal rates increases. When the OLR is increased, VS removal rate decreases. Effect of HRT on VS removal was the same with OLR however the effect of HRT was not as strong as the effect of OLR on VS removal rate. At lowest HRT and highest OLR, VS removal rate was around 15 %, however when the OLR decreased to lowest level and HRT increased to highest value, VS removal rate resulted at around 35 % in the figure. It seems that VS removal rate tends to increase with the decrease in OLR and increase in HRT. However, it is not clear at which point, VS removal rate would decrease on the other side. So this figure also depicts that MEC+AD reactors may be operated at further conditions such as longer HRTs and lower OLRs to increase the VS removal rates.

In Figure 4.43, interaction effect HRT and applied voltage on VS removal rate. Figure 4.45 suggests that VS removal rate was not affected from the applied voltage however it was affected from the variation of HRT at some points. At HRT of 3 and 4 days, VS removal rates seemed to increase but not effectively. It can be concluded that HRT and applied voltage together were not significantly effective on VS removal rate.

Interaction effect of OLR and applied voltage on VS removal rate is shown in Figure 4.44. The effect of OLR on VS removal rate was signigicant. When the OLR was lowered, VS removal rate increased. On the other hand voltage application had no effect on VS removal rate. It seemed that the interaction effect of OLR and voltage application was dependent on OLR variations but not applied voltages. At all applied voltages and constant OLR, VS removal was the same however at constant applied voltage and different OLRs, VS removal rates changed between 15 % and 35. Figure 4.44 indicates if OLR would be decreased further to lower than 5 g VS/L/d, VS removal rate can increase independent of voltage application.

The figures that were presented below clarify the effects of HRT, OLR and applied voltages on VS removal rate. ANOVA test for VS removal rate suggested that there were two models that fit best to represent the process as it is shown in Table 4.11.

VS Removal Rate At Different HRT And Applied Voltage

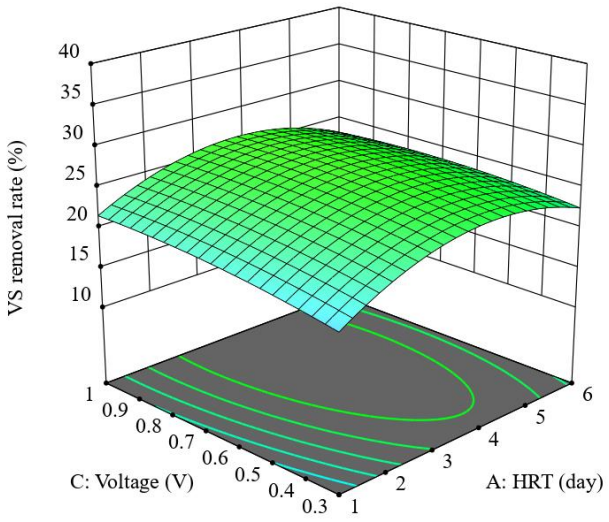

Figure 4.43. The interaction effect of HRT and voltage application on VS removal rate.

VS Removal Rate At Different OLR And Applied Voltages

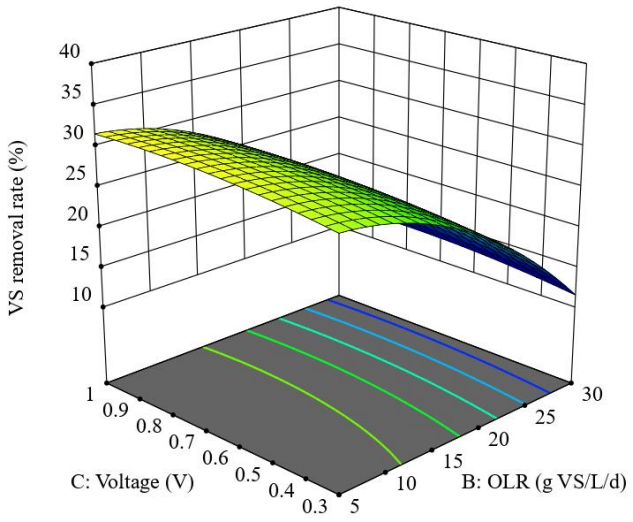

Figure 4.44. The interaction of HRT and voltage application on VS removal rate.

Linear and quadratic models fit the process according to the factors and responses. According to the ANOVA test, linear and quadratic models were suggested for VS removal rate due to maximum the Adjusted R^2 and the Predicted R^2 calculated in the models. In this section, quadratic model was chosen to apply for VS removal rate

prediction. The Predicted R^2 of 0.967 was in reasonable aggreement with the Adjusted R^2 of 0.989 and the difference was less than 0.2 which makes quadratic model fit best for representation of interaction effects of HRT, OLR and applied voltage on VS removal rate.

Table 4.11. Quadratic model fitted by ANOVA for biogas production.

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.9840**	**0.9780**	**Suggested**
2FI	0.6281	0.9827	0.9629	
Quadratic	**0.0420**	**0.9893**	**0.9687**	**Suggested**
Cubic	0.1126	0.9951	0.9665	Aliased

The results of the ANOVA test showed that for all operational conditions, the models were found to be high importance meaning that operational conditions were effective on VS removal rate. OLR was more significant on VS removal rate due to the p-values of less than 0.0001. However according to the ANOVA test, voltage application, quadratic effect of OLR and interaction of OLR and HRT were also in correlation with VS removal rate. Table 4.12 presents ANOVA of sum of squares, F and p values for fitted quadratic model made by RSM-CCD for VS removal rates. As a result of ANOVA, Model F-value of 205.6 indicate the model has a weight on the responses. In addition, if the p-values is less than 0.05, then it shows that some of the model tools are respectable on VS removal rate. Therefore the coefficients of B, C, B^2 and A*B are noteworthy simulation tools.

Table 4.12. ANOVA for Quadratic model fitted by CCD for VS removal rate.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1213.73	9	134.86	205.60	< 0.0001	significant
A-HRT	0.7999	1	0.7999	1.22	0.2930	
B-OLR	54.57	1	54.57	83.19	< 0.0001	
C-Voltage	5.95	1	5.95	9.07	0.0118	
AB	4.50	1	4.50	6.86	0.0239	
AC	0.4150	1	0.4150	0.6326	0.4432	
BC	0.0203	1	0.0203	0.0309	0.8636	
A^2	2.86	1	2.86	4.37	0.0607	
B^2	5.04	1	5.04	7.68	0.0182	
C^2	2.51	1	2.51	3.83	0.0761	
Residual	7.22	11	0.6559			
Cor Total	1220.95	20				

VS removal rate in MEC+AD reactors can be predicted by the equation below using coded factors. The equaiton and coefficients also show the effects of the factors alone and factors together on VS removal rate. According to the equation, effects of OLR and applied voltage alone and OLR and HRT together and square of OLR were more significant on VS removal rate according to the coefficients. The results of ANOVA test on VS removal efficiency were appropriate with the findings of study conducted by Zinatizadeh et al. [190] where interaction of OLR and HRT affected the VS removal rates significantly.

VS Removal Rate (%) = 1213.73 + 0.8A + 54.57xB+ 5.95xC + 4.50xAxB + 0. 415xAxC + 0.0203xBxCx + 2.86xA^2 + 5.04xB^2 – 2.51xC^2

4.5.4. Optimization of Operational Parameters

Optimization scenario was also conducted in RSM software to achieve optimum operational conditions for biogas production and methane yield. Two optimization scenarios were planned in order to find the optimum values of biogas production and methane yield. Therefore, for the first optimization scenario, input variables including HRT, OLR, and applied voltage were chosen as “in range” in the methodology which meant that experimental values were used in the calculation. For the outputs of the run, biogas production and methane yield were set to be maximized but importance of biogas production was at the highest level compared to importance of methane yield. The outputs of other variants (VS removal and current production) were expected to be in the range of experimental results. The weights of the upper and lower limtis of the variables and variants were set to be “1”. All the possible results and predictions are presented in Table 4.13. Also a suggested result was chosen and presented in Figure 4. 45. It can be seen from Table 4.13 that biogas production at desired conditions changed between 5.11 and 5.49 L/L/d. Methane yield was between 0.183 and 0.196 L CH_4/g VS.

The desirability fuction method is a practical tool that transform every variable to a single function. The individual single function can be accepted as the decision maker`s guide that ranges between zero an done. Optimization is a output of the desirability function, determines a point that shows the highest result. The specifications of a goal (maximum biogas production) may be changed by setting the importance. For responses and factors more than one, all targets are integrated into one desirability function. The goal of

optimization is to find a good set of conditions that will meet all the goals. In this study desirability of the first optimization run was “0.879” which means that maximum biogas production was the closest to the constraints. In Figure 4.45 we can see that all the variables were in the experimental range. Biogas production and voltage application were at the highest level and OLR was close to lowest level.

Table 4.13. Maximum biogas production and methane yield calculated at the optimization studies.

	Number	HRT	OLR	Voltage	Biogas production	Methane yield	Current production	VS removal rate	Desirability	
	1	**6.000**	**12.079**	**1.000**	**5.110**	**0.196**	**4.526**	**29.815**	**0.879**	**Selected**
	2	6.000	12.079	0.997	5.110	0.196	4.510	29.825	0.879	
	3	6.000	12.079	0.996	5.110	0.196	4.504	29.828	0.879	
	4	5.981	12.108	1.000	5.110	0.196	4.528	29.815	0.879	
	5	6.000	12.078	0.990	5.110	0.196	4.475	29.846	0.879	
	6	6.000	12.078	0.984	5.110	0.196	4.440	29.867	0.878	
	7	6.000	12.177	1.000	5.153	0.196	4.524	29.710	0.878	
	8	5.963	12.135	1.000	5.110	0.196	4.530	29.814	0.878	
	9	5.990	12.093	0.979	5.110	0.196	4.415	29.881	0.878	
	10	5.929	12.188	1.000	5.110	0.195	4.533	29.810	0.877	
	11	6.000	12.078	0.966	5.110	0.195	4.343	29.919	0.877	
	12	6.000	12.079	0.960	5.110	0.195	4.314	29.934	0.877	
	13	5.906	12.222	1.000	5.110	0.195	4.535	29.809	0.876	
	14	6.000	12.081	0.947	5.110	0.195	4.242	29.967	0.876	
	15	5.839	12.325	1.000	5.110	0.195	4.542	29.799	0.875	
	16	6.000	12.524	1.000	5.306	0.195	4.517	29.332	0.874	
	17	5.973	12.578	1.000	5.310	0.194	4.519	29.320	0.874	
	18	6.000	12.099	0.895	5.110	0.194	3.969	30.064	0.873	
	19	6.000	12.101	0.893	5.110	0.194	3.953	30.068	0.872	
	20	6.000	12.106	0.884	5.110	0.194	3.907	30.079	0.872	
	21	5.998	12.926	1.000	5.478	0.193	4.509	28.891	0.870	
	22	6.000	12.452	0.634	5.110	0.188	2.571	29.774	0.852	
	23	6.000	12.684	0.544	5.110	0.185	2.082	29.356	0.844	
	24	6.000	12.771	0.515	5.110	0.184	1.928	29.189	0.841	
	25	6.000	12.927	0.468	5.110	0.183	1.672	28.874	0.836	

In the second run it was aimed to maximize the biogas production only. Therefore, input variables were chosen as “in range” and outputs were chosen as “none” which meant that the output values were not important as long as biogas production was maximum. For the outputs of the second run, biogas production was chosen as “maximum”. Figure 4.46 illustrates the outcomes of the second optimization run in terms of biogas production, methane yield and other outputs. It can be seen from Figure 4.46 that all the outputs were in the range. On the other hand biogas production result was above the maximum range obtained from the experiments. The report of the second optimization run stated that biogas production was in the range of 5.13 and 9.64 L/L/d. However upper limit of 9.64 and similar results were not extensive.

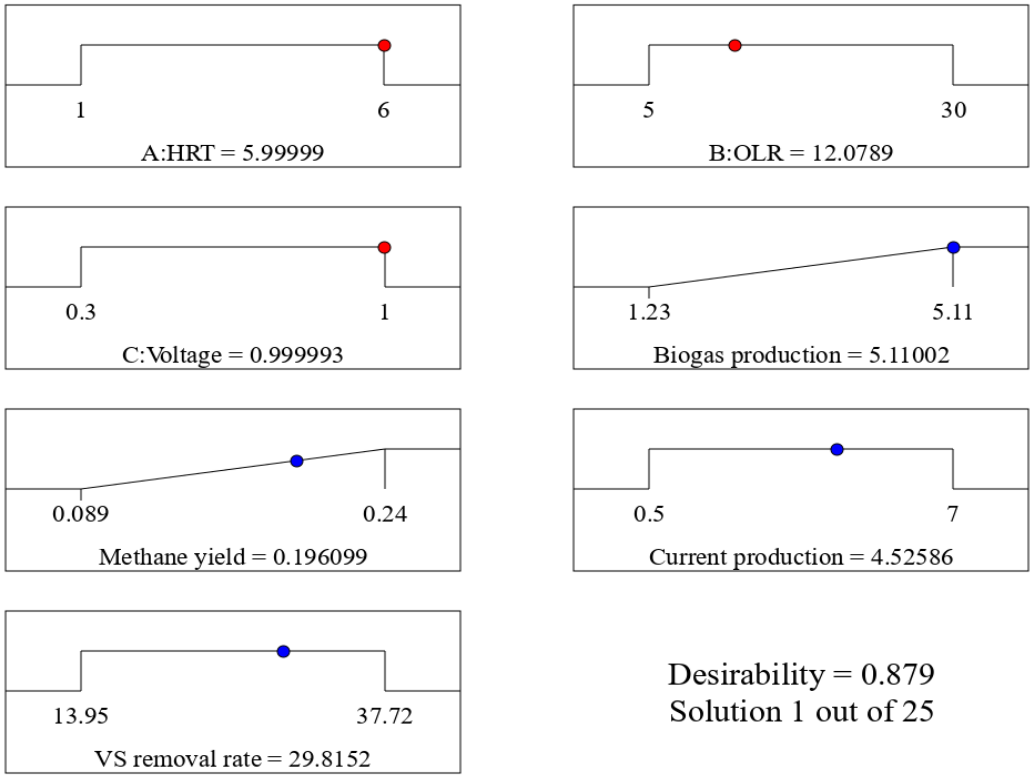

Figure 4.45. Optimization for biogas production and methane yield.

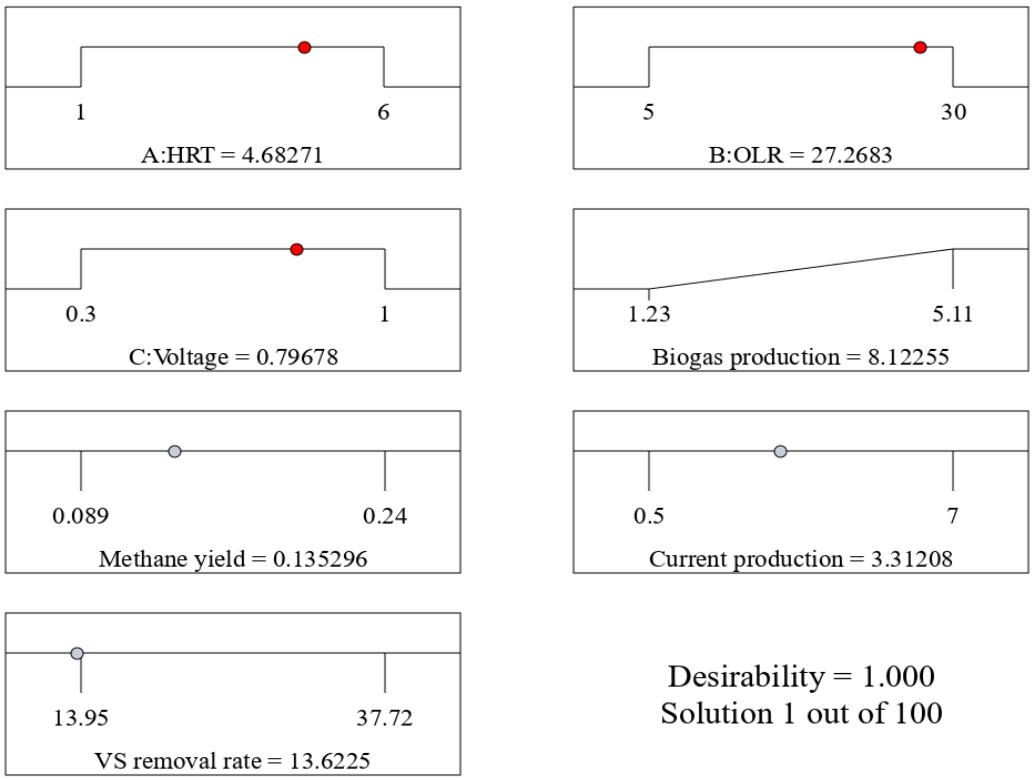

Figure 4.46. Optimization for maximum biogas production.

As can be seen from optimization results, for maximum biogas production and methane yield together, optimum HRT should be at high values (6 days) and optimum OLR should be closer to lower values (12 g VS/L/d). Also it seems that voltage application of 1.0 V is optimum for maximum production of target . In the case of maximum biogas production only, HRT and OLR values are important. Optimum HRT is around 4 and 5 day, and optimum OLR is around 25 and 30 g VS/L/d. At these operational conditions, biogas production will be calculated as more than 5.13 L/L/d.

4.6. Energy Recovery Assessment of the Combined MEC+AD

Energy is a major factor for countries and communities due to its usage areas that stimulate and support life standarts, economical growth and development. Since the excessive part of the global energy production is based on fossil fuels that are finite and being depleted, studies are directed towards investigating for new sources of energy [193]. Biogas is a renewable energy source that can be produced from a variety of organic materials. It can be utilized for heating, electric generation and as vehicle fuel purposes [194]. For a long time biogas has been produced efficiently through conventional anaerobic digestion technology with some problems and drawbacks as well. Biogas production technology is an engineering process that should be energy efficient regarding the sustainability and environmental concerns. From an engineering point of view, the energy efficiency in anaerobic digestion can be estimated as the ratio of energy that could be obtained theoratically as in methane from the biomass to the energy that is required for the operational needs. Therefore, in this section an assesment of energy recovery from cattle manure in a MEC+AD technology is performed.

MEC technologies need energy input as in voltage supplementation for the continuity of the process. The other operational factors that need energy are heating, mixing and etc. Therefore energy efficiency of MEC+AD process must be evaluated interms of energy inputs and energy outputs. When the energy inputs of the conventional anaerobic digetion and MEC+AD system are compared, the only difference would be application of voltage to the MEC systems. The other sources that require energy input in both of the systems are, heating, mixing, pumping and other operational activities such as biogas upgraging, digestate removal, transportation and etc. Regarding the heating energy of the reactors, it is a fact that, MEC+AD reactors can be operated effectively at ambient temperatures or at temperature range of 25 to 30 ^{o}C which is not the case for conventional anaerobic

digestors. However, in this energy efficiency assesment, the energy needed for heating, mixing and other operational activities will be assumed as equal in both reactors except the voltages applied to the MEC+AD reactor. Recovered energy from the anaerobic digestion and MEC+AD reactor is in the form of methane that is derived from the organic materials in digestion process.

Biogas plants can be grouped in two in terms of volume and capacity: large scale (industrial scale) and small scale (farming scale) plants. Large scale plants have a operation capacity of ≥20,000 tonnes of feedstock/waste (manure, waste sludge) per annum. They can characteristically generate approximately 2 million m^3 of biogas annually and have a installed capacity of ≥500 $kW_{el.}$. On the other hand small scale biogas plants has a operation capacity of ≤10,000 tonnes of feedstock with a biogas production and electrical installation capacity of 100 thousands – 1 million m^3 of biogas per year and ≤500 $kW_{el.}$.respectively [193]. The important operational parameters that are required to be determined for installation of a biogas production plant can be seen in Table 4.14. These are the parameters that need the most consideration because they determine the plant efficiency and sustainability due to their variable environment.

Table 4.14. Parameters should be considered in designing of biogas production plants [194].

Parameter	**Explanation**
Volume/capacity of the plant	Amount of the waste planned to be treated
Feedstock/waste type	Availability of the waste and economical value
Biogas plant technology	UASB, CSTR, ABR, EGSB, AF, Sequencing Batch
Biogas utilization channels	Electric production (CHP), external heat, supply to gas grid, vehicle fuel, stirling engine, cooling energy
Biogas upgrading	Biogas upgrading is needed if some of the utiliziation channels are considered
Digestate management	Seperation of solid and liquid fractions, spreading solid and liquid, composting of solid fraction, fertilizer production, residual biogas production, removal of non-treated digestate
Transportation distance of the feedstock/waste	Easiness of access to source and economical concerns determine the sustainability of process
Pretreatment requirement	Grinding, screening, physical and chemical pretreatment, fermentation in two stage process
Co-digestion alternatives	Co-digestion of wastes can increase biogas production

In this study, for comparison of MEC+AD and anaerobic reactor, CSTR technology was chosen due to the similarity between the lab scale reactors used in the current study and CSTRs. The investment cost of a MEC+AD reactor can differ from corrensponding anaerobic technology with maintainance of electrodes in the reactor and electrical connection elements to the power supply unit. Power supply unit is also need to be taken into consideration as another difference in investment cost. Electrode materials in this study were activated carbon pellets and carbon fibre cloth for anode and cathode respectively. However the cost of carbon originated materials changes from couple of dollars to one thousand dollar per square meter of carbon material. The cost differs acoording to the process that is used to produce it [16, 195]. It is expected for the materials cost to decrease in time. It is stated that nearly 85 % of total investment cost of MEC comprised of cathode material (expensive metal catalyzed cathode) and membrane [195]. As a result capital costs of AD and MEC+AD can be estimated as 0.01 and 0.04 €/kg COD respectively [151]. For the operational expenses, input energy of the voltage application to the electrodes in MEC+AD reactor is the only difference between operational costs of AD and MEC+AD. In literature, the operational cost of MEC was found as 0.05 Euro/kg of $COD_{rem.}$ (accepted as an energy cost of 0.06 Euro/kWh) [16] or an energy consumption under 0.9 kWh/kg of $COD_{rem.}$ would make a MEC usefull [196].

In this section, the cost originated from voltage supplementation to the MEC+AD reactors is calculated in terms of energy input. Then total energy production of MEC+AD reactors and AD reactor were compared with each other in terms merthane production. The additional energy production of MEC+AD reactors compared to AD reactor were evaluated according to the input energy of the reactors. The comparison was only made for 6 days of HRT because the methane yield of AD tends to increase at higher HRTs. The results were presented in Table 4.15.

Energy recovery of the MEC+AD reactors relative to electrical input $[(W_{CH4})_e/W_V]$ was calculated according to Equation 3.12. The energy obtained in the methane form was 60 to 200 folds higher respect to electrical energy input. The results were very promising owing to the voltage application. Addition of power to MEC reactors can enhance the growth of hydrogenotrophic methanogens faster than acetotrophic methanogens especially at low HRTs [55, 60, 144].

Table 4.15. Energy recovery in MEC+AD reactors and anaerobic reactor at HRT of 6 days

Total energy obtained from methane	Energy given to the system in voltage	Energy content of the substrate removed	Energy of methane originated from voltage application
W_{CH4}	W_V	W_S	$(W_{CH4})e$
$n_{CH4}\,\Delta H_{CH4}$	$I\,V_{ap}\Delta t$	$\Delta H_S.m_S$	$\Delta H_{CH4}(\frac{V_{MEC}-V_{AD}}{22.4\ L/mol})$
ΔH_{CH4}=890.8 kJ/mol, $n_{CH4}=\frac{V_{CH4}}{22.4\ L}$	I_{av} = X mA, $\Delta t = 1\ day = 24\ h = 3600\ x\ 24\ s$	ΔH_S =3900 kcal/kg or 16302 kJ/kg for 97.2 % dry manure -TS) (700 kg/m^3), VS/TS=0.75, (m_s=VS_{in} - VS_{out})	V_{MEC}, V_{AD} : Daily CH_4 production of MEC+AD and AD reactors respectively.

Parameters/Calculations	MEC+$AD_{0.3V}$	MEC+$AD_{0.6V}$	MEC+AD_{1V}	AD
HRT	6 days			
OLR	5 g VS/L/d			
Applied Voltage (V_{ap})	0.3	0.6	1	-
Biogas production L/L/d	1.23	1.59	1.53	1.01
Methane content, %	77	78	79	74
Methane yield L/g VS	0.19	0.24	0.2	0.15
Ava. current production (A)	$1.5x10^{-3}$	$2x10^{-3}$	$3.5x10^{-3}$	-
VS removal, %	34.3	37.7	35.6	31.1
$n_{CH4}\,\Delta H_{CH4}$ (kJ)	37.66	49.32	48.07	29.72
$I\,V_{ap}\Delta t$ (kJ)	0.039	0.104	0.302	-
$\Delta H_S.m_S$ (kJ)	30.66	33.69	31.82	27.8
$\Delta H_{CH4}(\frac{V_{MEC}-V_{AD}}{22.4\ L/mol})$ (kJ)	7.94	19.6	18.35	-
$(W_{CH4})_e/W_V$	200	188	60.68	-
W_S	30.60	33.63	31.76	27.75
$W_{CH4}\ /W_S$	1.23	1.47	1.51	1.07

ΔH_S =3900 kcal/kg or 16302 kJ/kg for 97.2 % dry manure -TS [165]
22.4 kJ/ g VS= 16302kJ/kg/0.972/1000/0.75

Thereby contribution of hydrogen to methane formation may have resulted in high energy recovery. Also direct conversion of CO_2 into methane by methanogens which can capture electrons from electrogens on cathode biofilm could have acted as an essential factor in energy recovery. On the other hand high ratio of methane production to input electrical

energy can also be attributed acetolastic methanogens on the anode. It is stated by Cai et al. [129] that methanogens were leaning to take electrons from anodophilic bacteria (*Geobacter*) on the anode rather than acetate oxidation by theirself. Electron flow from electrogens to methanogens on the anode may have resulted in low current production in MEC+AD reactors. It is known that acetoclastic methanogenesis is difficult to occur at low concentrations of organics due to the free Gibbs energy [169]. However in the present study cattle manure with concentration of minimum 30 g VS/L were used for feeding the reactors. Therefore acetoclastic methanogenesis would gradually increase and even replace anodic oxidation (electrogens) to become the dominant pathway to degrade organics [169]. Finally growth of acetoclastic methanogens on anode may have suppressed electrogen growth on anode and as a result of this current production decreased [148, 169]. Even though the current production was low in MEC+AD reactors, methane production rates were higher than AD reactor in the range of 27-66 %.

Energy efficiencies obtained in the present study were convenient with those studies conducted at single chamber MECs operated on continuous mode [48, 60, 118, 131, 135]. An UASB MEC reactor succeeded energy efficiency of more than 1200% with synthetic wastewater at 7 g COD/L concentration at HRT of 6 days and applied voltage of 1.0 V [60]. Hussain et al. [118] treated acetate based synthetic WW with high NH_4-N concentration at flowrate of 1.6 L/d and influent COD of 1-1.2 g COD/L in an upflow MEC unit equipped with stacked layered electrodes. They reported energy efficiencies of between 300-450 %. They also claimed that efficiencies decreased when domestic wastewater was used. Cusick et al. [48] installed a single chamber pilot scale (1100 L) MEC to treat winery wastewater at 31^{o}C and voltage supplementation of 0.9 V. 144 stacked type electrode pairs, pH adjustment and acetate amendment were used to enhance the performance of the MEC. Finally energy content obtained from the total biogas generated in MEC, was 16 times higher than the energy content of the electrical input to the reactor which shows that the process is economically profitable under those operational terms [48].

It is shown in Table 4.15 that approximately 60-78 % of methane produced in MECs could have been originated from the bulk sludge or biofilm on the electrodes due to the commonly suggested pathways of acetotrophic and hydrogenotrophic methanogenesis without the effect of applied power. With the effect of power supply, MEC+AD reactors

surpassed methane production of AD reactor in the range of 27-66 %. It is estimated that roughly 30 % of methane produced in anaerobic process originates from hydrogentrophic methanogenesis [63, 177, 178]. However addition of power to MEC reactors can enhance the growth of hydrogenotrophic methanogens faster than acetotrophic methanogens especially at low HRTs [55, 60, 144]. Thereby contribution of hydrogen to methane formation can exceed 30 %. In this study electrical energy efficiency of MEC+AD reactors were over 100 % owing to the direct conversion of CO_2 into methane via electron capturing and occurence of hydrogenotrophic methanogenesis in the biofilm or suspended sludge. It is also thought that at the anode acetoclastic methanogens captured the electrons from electrogens and dominated the anode biofilm leading to low current productions [135, 153].

Energy efficiency relative to energy content of removed subsrate in MEC+AD reactors was appropriate with the some of the reference studies [137, 146] and it was rather higher than the others [113, 126, 127, 135]. A pilot scale single chamber MEC operated semi continuously at 0.3 V voltage supplementation and mesophilic condition succeeded overall efficiency of 90 % and more at OLRs between 1.44 to 5.76 kg VS/m3/d. with mixed sewage sludge as the substrate [147]. In another study, Yin et al. [137] reported 75 % of overall energy efficiency with a single AD–MEC co-cultivating *Geobacter* with *Methanosarcina* which achieved 24% more CH_4 production at 25^{o}C compared to same MEC without bacteria selection. They used acetate as the substrate and the applied voltage was 1.0 V.

It can be seen from Table 4.15 that energy recovery was very promosing due of the additional methane production originated from voltage application to MEC+AD reactors. Substrate removal was higher in MEC+AD reactors. Also voltage input increased the energy efficiency due to organic degradation compared to AD reactor.

5. CONCLUSION

Microbial electrolysis cell (MEC) is a new technology that is expected to be an alternative method for waste/wastewater treatment. Beside with the refinement of the wastes, valuable end products such as hydrogen, methane, ethanol, hydrogen peroxide, acetate, ethanol, and and etc. can be produced with this tehnology. In MECs, an external voltage around 0.2 - 1.2 V is needed to be applied to the electrodes to drive the process and overcome the thermodynamic barriers for product formation. Methane generation by MECs is called as electromethanogenesis (bioelectromethanogenesis) in which methane is produced biologically through direct reduction of carbon dioxide by electrons or through indirect reduction of CO_2 by H_2 that is formed by the combination of electrons and protons.

Many studies have been practiced for methane generation in MECs and some of those studies have focused on combination of MEC with anaerobic digestion to enhance methane generation from organic materials. Also effects of integration of MEC and AD on the obstacles of anaerobic digestion such as VFA accumulation, abrupt pH changes, temperature sensitivity, the need for high hydraulic retention times and etc., were investigated in those works mostly at batch mode. Therefore in this study, it is aimed to enhance methane production from cattle manure in combined MEC+AD reactors operated continuously at high OLR and short HRT conditions with different voltage supplementations of 0.3, 0.6 and 1.0 V. To our knowledge cattle manure has not been used before as a sole waste in combined MEC+AD reactors. Also, there are not many studies focused on the effects of high OLRs such as 15-30 g VS/L/d and low HRTs such as 1-2 days on methane production in MEC+AD reactors. Effects of different power applications on methane production were investigated along with the other parameters. The results obtained in terms of biogas production and methane content, organic removal rates and current production are presented below.

5.1. Evaluation of Biogas Production and Methane Yield Results

Biogas productions increased consistently in MEC+AD reactors from the lowest OLR of 5 g VS/L/d (HRT: 6 days) to highest OLR of 30 g VS/L/d (HRT:1 and 2 days) indicating that reactors were not inhibited due to any distortion (VFA accumulation) which can originate from high OLR or short HRT. Biogas productions in MEC+AD reactors

changed between 1.23 L/L/d (HRT:6 day, OLR:5 g VS/L/d, V_{ap}: 0.3 V) and 5.11 L/L/d (HRT:2 day, OLR:30 g VS/L/d, V_{ap}:1.0 V) depending on HRT and OLR. MEC+AD reactors were operated at OLR of 30 g VS/L/d in two modes; one of them was at HRT of 1 day with manure content of 30 g VS/L, the other one was at HRT of 2 days with manure content of 60 g VS/L. Biogas productions at HRT of 1 day and 2 days at the same OLR were 3.56 and 5.11 L/L/d respectively. The results indicate that HRT of 1 day was not appropriate for an efficient biogas production at those OLRs due to insufficient time for methanogens in order to operate fully active.

Methane yield of the MEC+AD reactors varied between 0.09 and 0.24 L CH_4/g VS, decreasing by the increase in OLR. The highest specific methane generation of 0.24 L CH_4/g VS was obtained at organic load and hydraulic retention time of 5 g VS/L/d and 6 days respectively. Biogas productions and methane yields of this study were superior to anaerobic digestion process at short HRTs such as 6 days and lower. Also the results obtained at short HRTs (1-6 days) and high OLRs (5-30 g VS/L/d) were better than most of the studies conducted with two chamber MECs owing to combined effect of MEC and anaerobic process. Methane yields obtained in the present work, were also similar or even superior to other anaerobic digestion studies conducted at different reactor designs (hybrid reactor, two stage reactors, biotrickling filter) fed with cattle manure or co-digested cattle manure at longer HRTs (>15 days) and lower HRTs (<10 g VS/L/d).

Although biogas production was naturally related to OLR and HRT, it seemed that biogas production was affected from the applied voltage amounts during a major part of the study. At the last 3 sets, when the OLR was increased further to 22.5 g VS/L/d and more, biogas productions of $(MEC+AD)_{0.6V}$ and $(MEC+AD)_{1.0V}$ became higher compared to $(MEC+AD)_{0.3V}$. So it can be concluded that, applied voltages of 0.6 and 1.0 V were significantly effective on biogas production at OLRs of 20-30 g VSL/d. It is thought that biogas production of $(MEC+AD)_{0.3V}$ reactor was lower than the other two MEC+AD reactors due to the lower voltage supplementation. It was shown that electromethanogenesis could take place in high and low strength wastewaters. In the present study, cattle manure at TS and VS content of 8.7 % and 6 % respectively was treated efficiently in MEC+AD reactors. Methane rate of the biogas produced from MEC+AD reactors at all HRTs and OLRs were in the range of 75-80 %. The percentage

of methane in the biogas was totally independent of the applied voltage and the applied HRTs and OLRs in this study.

During the entire study biogas productions and methane yields of MEC+AD reactors were superior to control reactors at all HRTs (6, 4, 3 days). The differences in biogas production and methane yield of MEC+AD reactors and control reactor can be attributed to the hydrogenotrophic methanogenesis and electromethanogenesis that take place in MEC+AD reactors as a result of voltage application. The biogas production and methane yield of contol reactor even at the best times were lower at least 22% and 26 % respectively compared to MEC+AD reactors. Voltage application to MECs can enable direct CO_2 reduction with electrons that are given to the MEC by electrical circuit. Also in suitable conditions, electrons in MECs combine with protons and form H_2. Later on H_2 is used by hydrogenotrophic methanogens. Also acetoclastic methanogenesis could have become the dominant pathway to degrade organics at high VS concentrations of 30 g VS/L as it was in the present study and have contributed to methane generation as well.

Energy assesments of the reactors showed that $(MEC+AD)_{0.3V}$ reactor exhibited the highest energy efficiency in terms of input energy. Methane obtained from $(MEC+AD)_{0.3V}$ reactor were 200 folds of the energy supplied to the reactor. Energy efficiency of $(MEC+AD)_{0.6V}$ reactor was very similar to that of obtained from $(MEC+AD)_{0.3V}$ reactor. However when the applied voltage was increased to 1.0 V, energy efficiency of the reactor decreased sharply. The energies obtained from MEC+AD reactors at all power applications were higher than the energies that were given to the reactors. In the case of energy recovery from the removed substrate, MEC+AD reactors applied with voltages of 0.6 and 1.0 V exhibited 140-150 % energy recovery from the substrate. This indicated that energy obtained from the total methane production from the reactors were higher than the energy content of the removed substrate.

5.2. Evaluation of Organic Removal Results

Organic substrate removal efficiencies of MEC+AD reactors varied during the study due to different OLRs and HRTs applied. Highest COD, TS and VS removal efficiencies of MEC+AD reactors were obtained at HRT of 6 days and OLR of 5 g VS/L/d. The highest removal efficiencies were between 41.4 and 44.9 % for COD, 26.1 and 29.5 % for TS and 34.3 and 37.7 % for VS respectively. On the other hand, the lowest COD, TS and VS

removal efficiencies of MEC+AD reactors were obtained at HRT and OLR of 1 day and 30 g VS/L/d respectively. It was clear that organic removal efficiencies were in parallel with methane yields obtained from MEC+AD reactors. The efficiencies decreased when the HRT was decreased as well. Organic removals of MEC+AD reactors at higher HRTs (4, 6 days) were higher compared to shorter HRTs such as 1 and 2 days. Nonetheless, organic removal efficiencies were higher compared to control reactors due to voltage application. It is thought that voltage application to MEC+AD reactors enhanced the degradation of organic substances due to hydrogenetrophic methanogenesis and/or electromethanogenesis and acetoclastic methanogenesis process on the electrodes. This proposal can be supported by biogas production and methane content of the MEC+AD reactors compared to anaerobic control reactor.

Considering the organic removal efficiencies in MEC+AD reactors applied with different voltages, it can be concluded that power application was slightly effective on the organic removal rates. At high OLRs such as 20 g VS/L/d and more, voltage applications of 0.6 and 1.0 V were slightly more effective on organic removal compared to applied voltage of 0.3 V. At lower OLRs (<10 g VS/L/d) or at HRTs of 3 to 6 days, voltage application of 0.6 V was slightly more effective compared to other voltages. Even though the effect of applied voltage amount on organic removal rate was little, the results were in parallel with the biogas productions at related MEC+AD reactors. The higher the removal rate was in a particular MEC+AD, the higher the biogas production was in the same MEC+AD reactor. At the end, we can sum up that considering the short HRTs and high OLRs applied to the MEC+AD reactors, organic removal rates obtained in MEC+AD reactors were appropriate with other studies that were conducted with real wastewater/waste or manure.

5.3. Evaluation of Current Production

Microbial electrolysis cells are operated with an external power supplementation to enhance the generation of end products. As a result of voltage application, various amount of current is produced in MECs depending on the electrode types, reactor design, substrate and scale of the applied voltage. MEC+AD reactors presented various current productions as a result of different voltage applications. The highest current productions were exhibited at applied voltage of 1.0 V during the entire study. The current productions at power supplementation of 1.0 V varied mostly between 4 and 6 mA/L. On the other hand

the lowest current productions in MEC+AD reactors were obtained at the lowest power applicaiton of 0.3 V. The current productions obained at supplied power of 0.3 V were between 1 and 2.5 mA/L generally. Current productions in MEC+AD reactors were strictly depended on the applied voltages. At the last two sets of the study, biogas production of $(MEC+AD)_{0.3V}$ was lower than the other two MEC+AD reactors operated at applied voltages of 0.6 and 1.0 V. The reason of this can be attributed to variation in microbial community at high OLR and short HRT as well as insufficient voltage application to keep the process continue. As a result, the decrease in current production was followed by a decrease in biogas production in $(MEC+AD)_{0.3V}$. It is thought that methanogens and electrogens in the biofilm of the electrodes competed over the electrons and substrate. This competition caused to lower current productions in the $(MEC+AD)_{0.3V}$ reactors. Interestingly the decrease in current production and biogas production in $(MEC+AD)_{0.3V}$ were not observed in other MEC+AD reactors. At the most part of the study, biogas production in MEC+AD reactors were generally close to each other at different applied voltages. This indicates that applied voltage and current production were not the main driver of the biogas production in the reactors. Instead, it is suggested that biofilm formed on cathode even on anode electrodes was the main reason in biogas production. Although the current productions obtained in the present study were mostly lower compared to most other studies, methane production not being affected from this may indicate the dominance of hydrogenetrophic and acetoclastic methanogens on the electrodes.

5.4. Future Work

Being a new, sustainable, and alternative green energy source, Microbial Electrolysis Cell tecnology has a great potential on biogas production, biogas upgrading, wastewater treatment, value-added chemical production and etc. Since the MEC technology can be used at both low concentrated and high concentrated wastewaters, it can be an oppurtunity for replacing the conventional aerobic and anaerobic treatment methods if investigated properly. It has a potential of energy recovery more than energy input. Due to the potential of MEC technology in biogas production, a part of the investigations directed to the combination/integration of MEC technology with anaerobic digestion. Although promosing results were obtained from those studies, there are still some issues that must be clarified.

In the future works, the behaviour of the MEC+AD reactors should be determined in the case of higher organic loading rates as much as 40 g VS/L/d and more needed to be applied to the reactors. These studies should be conducted at pilot scale reactors in order to estimate the actual effects that can be occured on the inductrial scale MEC+AD reactors. The research for optimum applied voltage amount in MEC+AD reactors is crucial due to energy recovery concerns and due to contradictory claims in the studies conducted so far. The effects of different applied voltages, OLRs and HRTs on the microorganism cummunities should be implemented clearly. The interaction of electrogens and methanogens can be paid more attention due to detailed electron transfer mechanisms. Beside the suggestions remarked above, the oppurtunities of two stage anaerobic systems consisting of MEC+AD reactors, pretreatment methods in MEC+AD reactors can also be studied in the future works.

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