

**INVESTIGATING ENERGY GENERATION POTENTIAL
OF WHEY WASTEWATER USING A SEDIMENT
MICROBIAL FUEL CELL**

**PEYNİR ALTI SUYUNUN ENERJİ ÜRETİMİ
POTANSİYELİNİN SEDİMENT MİKROBİYAL YAKIT
HÜCRESİ KULLANARAK ARAŞTIRILMASI**

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To my family

ABSTRACT

INVESTIGATING ENERGY GENERATION POTENTIAL OF WHEY WASTEWATER USING A SEDIMENT MICROBIAL FUEL CELL

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This study intends to employ sediment microbial fuel cells (SMFCs) for energy production to process cheese whey, a frequently untreated material in small businesses that can result in environmental issues. To understand the energy potential of whey, this study examines the influence of whey concentration and sediment composition on the microbial community and the relationship between electrical potential-power density-removal percentage of organic for whey removal. Microbial study indicated that different whey dilution ratios (1/25 vs. 1/5) influenced microbial populations: *Pseudonocardia* and *Ralstonia* species were predominant in the 1/25 system, but *Clostridium* and *Bacteroides* species were more prevalent in the 1/5 system. The maximum electrical potential recorded was 592 mV. The highest power density value was 140 mW/m² and the maximum energy production was 8.9 W·sec. An investigation of chemical oxygen demand (COD) removal revealed that the best removal rates obtained were between 95% and 70%. pH, temperature, total dissolved solids (TDS) and conductivity values were measured before and after the systems were operated. Increased conductivity and TDS measurements across all systems indicate elevated ion

concentrations. Moreover, the pH fluctuations differed between the systems. These findings highlight the significance of whey content, sediment properties, and MFC design in enhancing energy generation and organic contaminant elimination in wastewater treatment processes.

Key Words: Energy Potential, River sediment, SMFC, Treatment, Whey



ÖZET

PEYNİR ALTI SUYUNUN ENERJİ ÜRETİMİ POTANSİYELİNİN SEDİMENT MİKROBİYAL YAKIT HÜCRESİ KULLANARAK ARAŞTIRILMASI

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Bu çalışmayla, küçük işletmelerde sıklıkla işlenmeyen ve çevresel sorunlara yol açabilen bir malzeme olan peynir altı suyunun işlenmesinde enerji üretimi için sediman mikrobiyal yakıt hücrelerinin (SMYH) kullanılması amaçlanmaktadır. Peynir altı suyunun enerji potansiyelini anlamak için bu çalışma, peynir altı suyu konsantrasyonunun ve sediman kompozisyonunun mikrobiyal topluluk üzerindeki etkisini ve peynir altı suyu giderimi için elektrik potansiyeli-güç yoğunluğu-organik giderim yüzdesi arasındaki ilişkiyi incelemektedir. Mikrobiyal çalışma, farklı peynir altı suyu seyreltme oranlarının (1/25 ve 1/5) mikrobiyal popülasyonları etkilediğini göstermiştir: *Pseudonocardia* ve *Ralstonia* türleri 1/25 sisteminde baskınken, *Clostridium* ve *Bacteroides* türleri 1/5 sisteminde daha yaygındı. Kaydedilen maksimum elektrik potansiyeli 592 mV olmuştur. En yüksek güç yoğunluğu değeri 140 mW/m² ve maksimum enerji üretimi 8,9 W.sn olmuştur. Kimyasal oksijen ihtiyacı (KOİ) gideriminin incelenmesi, elde edilen en iyi giderim oranlarının %95 ile %70 arasında olduğunu ortaya koymuştur. pH, sıcaklık, toplam çözünmüş katı madde (TDS) ve iletkenlik değerleri sistemler çalıştırılmadan önce ve çalıştırıldıktan sonra ölçülmüştür. Tüm sistemlerde artan

iletkenlik ve TDS ölçümleri yüksek iyon konsantrasyonlarına işaret etmektedir. Ayrıca, pH dalgalanmaları sistemler arasında farklılık göstermiştir. Bu bulgular, atık su arıtma süreçlerinde enerji üretimini ve organik kirletici giderimini artırmada peynir altı suyu içeriğinin, tortu özelliklerinin ve MFC tasarımının önemini vurgulamaktadır.

Anahtar Kelimeler: Enerji Potansiyeli, Nehir sedimanı, SMYH, Arıtma, Peynir altı suyu



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SYMBOLS AND ABBREVIATIONS

Symbols

V	Voltage
R	Resistance
I	Current
P	Power
E	Energy
MΩ	Megaohm
kΩ	Kiloohm

Abbreviations

EJ	Exajoules
FAO	Food and Agriculture Organization of the United Nations
COD	Chemical Oxygen Demand
MFC	Microbial Fuel Cell
SMFC	Sediment Microbial Fuel Cell
PEM	Proton Exchange Membrane
mW	Milliwatt
μW	Microwatt
GAC	Granule Activated Carbon
GF	Graphite Felt
ACFF	Activated Carbon Fiber Felt
TOC	Total Organic Carbon
PAHs	Polycyclic Aromatic Hydrocarbons
AVS	Acid Volatile Sulfide

ARB	Anode-Respiring Bacteria
LED	Light-emitting Diode
XRD	X-ray Diffraction
XRF	X-ray Fluorescence
SEM	Scanning Electron Microscopy
TSS	Total Suspended Solids
TS	Total Solids
TP	Total Phosphorous



1. INTRODUCTION

The amount of energy consumed worldwide is enormous and still rising. The total final energy consumed worldwide in 2019 was 418 exajoules (EJ) [1]. Except for the early 1980s and 2009, after the monetary crisis, the amount of energy consumed has risen almost every year for more than 50 years. Globally, as populations rise and people become wealthier, there is an increasing energy demand. It will be harder to shift our energy systems from fossil fuels [2]. New energy must satisfy this increased demand and aim to replace current fossil fuels in the energy mix. Clean energy techniques are employed, and their application is growing, to meet this demand.

Clean energy techniques, also known as renewable or green energy, derive their energy from continuously renewing naturally occurring processes or sources [3]. Some of the most common clean energy methods include solar energy, wind power, geothermal energy, hydropower, bioenergy, etc. [3,4]. Among the clean energy techniques mentioned, bioenergy, a form of renewable energy that is derived from living organic materials [5], has gained importance in recent years. Because the amount of waste from sectors such as food processing, paper, and textile pulp, etc. is increasing in line with the increase in production in these sectors due to global population growth.

The dairy industry is one of the most significant in the food processing sector since it produces 0.2–10 L of effluent for every liter of processed milk [6]. Cheese whey is one of the most significant wastewaters from dairy products, with over 18 million tons produced annually, according to the Food and Agriculture Organization of the United Nations (FAO) [7]. Whey can be converted into bioenergy and used as a valuable waste product since it has a chemical oxygen demand (COD) of 50–102 g/L [8]. This strategy lessens the influence on the environment and is consistent with sustainable practices. As a byproduct of making cheese, whey has many chemical components, including carbohydrates. These substances can be used by microorganisms in a microbial fuel cell (MFC) system to produce bioelectricity. In this system, electrons produced by the microbial digestion of whey are transported to an electrode to create an electric current [9]. The following subsections provide general information about MFCs.

1.1. History of Discovery of MFC

In 1791, a study began to discover energy production from microbial activities. In 1791, Galvani (an Italian physician and physicist) investigated the nature and effects of electricity in animal tissue by twitching an isolated frog leg upon passing a brief electrical discharge through it, which revealed the bioelectric phenomenon. In 1910, Potter (a professor of botany at the University of Durham, UK) demonstrated that organisms could deliver current and produce voltage by researching how microorganisms degrade organic compounds. In 1911, Potter discovered electrical energy from *Saccharomyces* and *Escherichia coli* cell cultures using platinum electrodes, and he built a basic MFC. In 1931, Cohen in Cambridge, UK, revived Potter's idea by describing how a batch of biological fuel cells generated over 35 V. Rohrbach, Scott, and Canfield designed the first biological fuel cell in 1962, using *Clostridium butyricum* as a biomaterial to generate hydrogen through glucose fermentation. In 1966, Williams demonstrated that rice husk is a potential source of lignocellulose because it can produce many useful enzymes and biofuels upon fermentation, resulting in 40 mA at 6 V using biological fuel cells. In 1993, Allen and Bennetto at King's College in London, UK, demonstrated improved biological fuel cells using various microbes to increase both the reaction rate and the efficiency of electron transfer using mediator systems. In 1999, Kim, Hyung, and Chang at the Korean Institute of Science and Technology, South Korea, discovered first that certain electrochemically active bacterial species use no mediator molecules [4]. Following these studies, using different microbes and more complex electrodes significantly improved MFC performance [5]. The chronological development of MFC is shown in Figure 1.1.

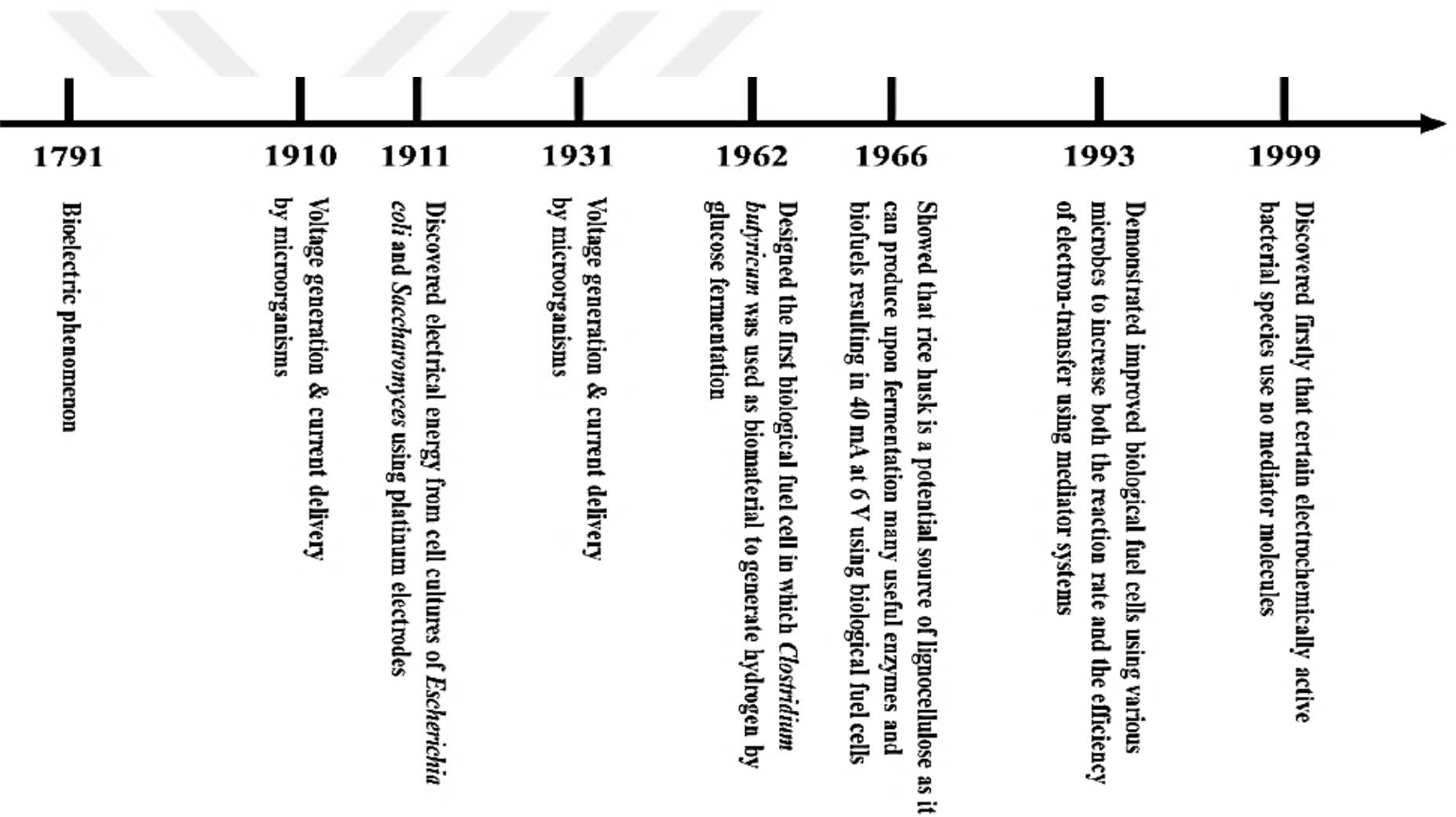


Figure 1.1: History of MFC systems [10]

1.2. Definition of MFC

MFCs are devices that treat wastewater and recover bioenergy[11]. In other words, MFC uses bacteria to catalyze the conversion of organic matter into electrical power.

In general, the parts that make up MFCs are as follows:

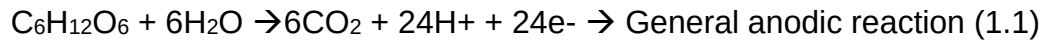
1. Anode: Microbial metabolism facilitates metabolic processes in the anodic compartment, generating electrons and protons.
2. External Circuit: The external circuit conveys electrons from the anode to the cathode.
3. Proton Exchange Membrane (PEM): Protons diffuse through an electrolytic solution to the cathode.
4. Cathode: Electrons, protons, and oxygen interact to produce water molecules.

[12]

5. Bacteria: Microorganisms that utilize their metabolic processes to transform organic substances into electricity [13].
6. Substrate (Fuel): Substrate is biomass which serves as the nutrition for microorganisms [14].
7. Electron Shuttle (Mediators): These compounds enhance electron transfer from the bacterium to the anode when direct electron transfer is inefficient [13].
8. Internal resistance: It encompasses resistances from the electrodes, electrolytes, membrane, and many internal components.
9. External resistance: Any electrical load or apparatus connected between the anode and cathode is classified as external resistance.

The general configuration of MFCs is shown in Figure 1.2.

The general biochemical oxidation and reduction reactions are described as follows:



[15]

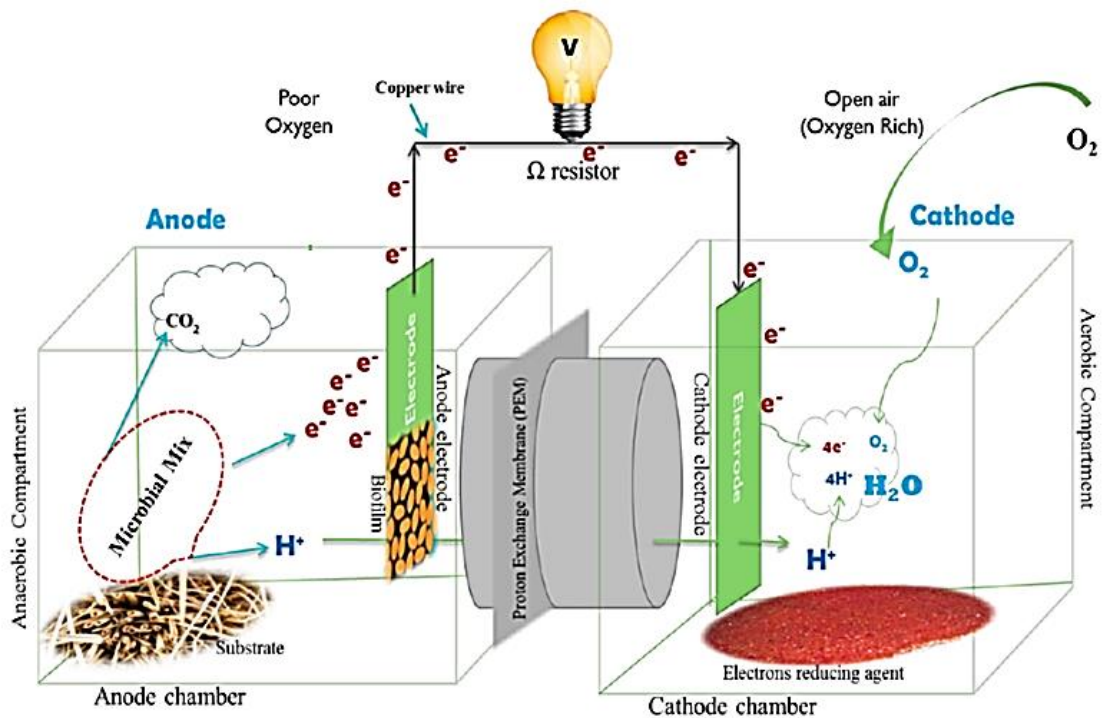


Figure 1.2: MFC configuration [10]

1.3. MFC for Whey Treatment

MFC treats cheese whey and uses it to produce electric power. Generally, cheese whey undergoes MFC treatment using two-chamber MFC configurations [9,16–30] as illustrated in Table 1-1. Besides, cylindrical graphite configurations [31], cube-shaped two chambers [32], single chambers [33,34] and tubular [35] MFC are used. To evaluate the performance of different configurations, single-chamber, and dual-chamber MFC are used at the same time [36,37]. There is no specific process for managing cheese whey. However, MFC treatment also uses cheese whey that has undergone heat treatment [17,34] filter sterilized [22], and pretreated-acidified diluted [34]. Microorganisms like *Enterobacter cloacae* [17], *Klebsiella pneumoniae* [18], *Escherichia coli* [9,21,25], *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus casei* [16] are used as MFC. The

MFC treatment method is applied by using a mediator like riboflavin and humic acid [21,25] or without a mediator. The application of the MFC treatment method to cheese whey led to varying levels of COD degradation and electricity generation. 89% COD removal in a single chamber, 94% COD removal in the dual chamber [37], 95% COD removal for cheese whey, and 86% COD removal for concentrated cheese whey were obtained [32]. Cheese whey treated with MFC generated electrical potential at 751.5 mV [9], 23 mV [16], 453 mV [18], 97.1 mV [21], 529.3 mV [24], 925 mV [27], 545 mV and 643 mV [37], and 345 and 383 mV [30] .

Table 1.1: MFC configurations in literature

Two chamber configurations	Single chamber configuration
Nasirahmadi & Safekordi, 2011 [9]	Colombo et al., 2017 [33]
Antonopoulou et al., 2010 [16]	Antonopoulou et al., 2020 [34]
Kassongo & Togo, 2010 [17]	Barber et al., 2015 [36]
Dalvi et al., 2011 [18]	Kondaveeti et al., 2019 [37]
Kassongo & Togo, 2011 [19]	
Stamatelatou et al., 2011 [20]	
Nasirahmadi & Safekordi, 2012 [21]	
Tremouli et al, 2013 [22]	
Michalopoulos et al., 2017 [23]	
Darmawan et al., 2018 [24]	
Nasirahmadi & Akbari-adergani, 2018 [25]	
Mohanakrishna et al., 2020 [26]	
Mahato et al., 2021 [27]	
Shanmuganathan et al., 2017 [28]	
Prado et al.2019, [29]	
Bampos et. al, 2024 [30]	
Ghasemi et al., 2017 [32]	
Kondaveeti et al., 2019 [37]	

1.4. Objective and Scope of the Study

This study aims to utilize sediment MFCs (SMFCs) for power generation to treat cheese whey, a commonly untreated substance in small enterprises that can lead to environmental problems like eutrophication and surface and groundwater pollution [6]. This study investigates the impact of whey content and sediment composition on the microbial community, as well as the correlation between electrical potential, power density, and the percentage of organic elimination for whey treatment.

SMFCs, a specific type of MFC, utilize bacterial metabolism to generate electrical current from the organic matter content of sediment [38]. Recent research has thoroughly investigated their application for wastewater treatment and the extraction of energy from natural sediment. SMFCs are easy to build and can clean wastewater in addition to producing electricity. They can harvest electrical energy from the natural sediment's organic molecules [39]. There are various kinds of SMFC such as marine, freshwater, soil, etc. SMFCs which are taken from rivers and lakes are called freshwater SMFCs.

SMFCs provide a simpler, more sustainable method for sediment remediation. These systems use microbial communities in sediments to metabolize pollutants and generate electricity as a byproduct. The electrical connection between the anaerobic sediment and the aerobic water column enhances microbial metabolic rates, accelerating pollutant degradation [40].

Sediment MFC will be employed in this investigation as opposed to previous whey studies since it will enable the necessary separation of bacteria on the anode from oxygen. To put it another way, obligatory bacteria will be kept apart from oxygen because the anaerobic sediment, or mud, remains anaerobic for the duration of the investigation [41]. As a result, MFC's ability to produce energy and reduce pollutants may improve.

In this study, river SMFC, one of the freshwater SMFCs, was used to investigate the energy potential of whey, which is generally known to be discharged into the river, in a laboratory setting. During the system's construction, organic removal and power generation were investigated by letting it function primarily on its own, without outside assistance.

1.5. SMFC

SMFC can be employed not only for wastewater treatment but also for bioremediation of sediment typically run in a single compartment. The sediment portion of these systems serves as the anode compartment and is where organic matter removal occurs. Buried in the sediment is the electrode that is used at the anode. The electrode is suspended in the liquid, which is represented by the cathode, which is the system's liquid component (wastewater or river water).

Several factors affect SMFC performance such as electrode material, distance between the electrodes, depth of water, the height of anode embedded in the sediment, cathode configuration, type of water, etc. [42]. There are studies about the parameters affecting the SMFC systems. In the next section, literature studies with freshwater SMFCs will be discussed.

1.5.1. Literature Review

Researchers have varied several parameters in freshwater SMFC to understand its intricate electromicrobiology. One of these parameters is the type of sediment used. According to a study conducted in 2004 using different sediments, which are marine, saltmarsh, and freshwater sediments, to understand the effect of sediment type on microbial diversity, it was understood that *Geobacter* and *Geothrix fermentans* bacteria are dominant for freshwater SMFC (river) [43]. In 2008, a battery-less and wireless sensor was built, powered by a river SMFC. A maximum power density of 12 mW/m² was achieved from the SMFC [44]. In another study, SMFC systems were installed both in the laboratory and in the river and power densities were determined. For the laboratory scale, the power density recorded was around 9 mW/m². For river scale, during the open circuit phase, the power density was 1.9 mW/m², which increased to 8.8 mW/m² when the circuit was closed [45]. In a 2013 study, SMFC systems were created with sediment taken from soil and river. After several months of operation, power generation continued to improve, with the river sediment SMFC producing a maximum power of 410 µW. After 18 months, in the systems, the voltage reached 900 mV for the river sediment SMFC and 650 mV for the soil SMFC. As a result, it was found that the river sediment SMFC showed better performance due to its higher organic content and microbial activity [46].

Same SMFCs taken from separate locations have effects on the performance of SMFC. For example, a study made by using lake SMFC in 2012 has shown that the MFC inoculated with sediment from the site with the highest Fe(III) concentration produced the highest voltage of 580 mV, while the SMFC with the lowest Fe(III) concentration produced less than 30 mV [47]. Like this study, sediment was taken from different points for the same SMFC and the effect of temperature on SMFC was investigated in 2013. The study concludes that temperature has a significant influence on organic matter decomposition in lake sediments, with higher temperatures accelerating the process. However, the extent of decomposition is also influenced by sediment type and the presence of specific microbial communities [48]. A 2014 study investigated the power generation capacity of SMFCs utilizing tidal river sludge. This sediment was compared with other freshwater sediments, which come from lakes and rivers. As a result of the study, the maximum power density reached 7.5 mW/m², higher than that of freshwater sediment MFCs [49]. In another study in 2015, SMFC inoculations were made with soil samples taken from the estuary and the river. Inoculation resulted in *Geobacter psychrophilus* being the predominant species. In the same study, two different surface modifications were evaluated on the anode: carboxylate (-COO-) and sulfonamide (-SO₂NH₂). The study found that surface modifications did not change the dominant bacteria but did influence the electron transfer efficiency and biofilm growth dynamics [50].

Another crucial parameter in the system is the oxygen uptake in the cathode portion. Since the cathode electrode is typically located far from the liquid component's surface, it may be challenging for the cathode part to get the essential oxygen from the air. In a 2007 study with river SMFC, the use of a rotating cathode increased the oxygen availability of the cathode and increased the power density to the maximum power density (49 mW/m²) compared to a nonrotating cathode system (29 mW/m²) [51]. In additional 2012 investigation, a floating cathode was employed to avoid cathode oxygen shortage. To improve the system's performance, a microbial attachment procedure was also used for the cathode, also known as the biocathode. This system produced a power density of 1.00 mW/m³ [52].

The physical structure of the electrode used also affects the performance of freshwater SMFCs. In a 2009 study using lake sediment, porous and non-porous graphite electrodes were used. As a result, it was found that the maximum power density ($2.00 \pm 0.11 \text{ mW/m}^2$) generated was higher for the case where porous electrodes were used. Because they have larger surface area, which supported more extensive bacterial colonization and biofilm formation [53].

External resistance is one of the parameters studied in literature. In a study conducted in 2010, five different external resistances (10, 100, 400, 800, and 1000 Ω) were used for lake SMFC. The study found that the highest power density (3.15 mW/m^2) was observed at 1000 Ω , showing that external resistance optimizes the balance between current production and voltage [54]. In another study in 2015, loads with an external resistance of 100, 510, and 1100 Ω were used for river SMFC and it was determined that the highest power density value with a load of 510 Ω was $1.2 \pm 0.2 \text{ mW/m}^2$ [55].

The performance of freshwater SMFC systems is affected by the types of electrodes. Various materials have been employed as electrodes, including platinum mesh, graphite disk, stainless steel, carbon brush, carbon cloth, and graphite felt. To study the impact of electrodes, in 2011, Song et al. compared stainless steel cathode with granule activated carbon (GAC) cathode for lake SMFC. As per the research, the maximum power density achieved with the GAC cathode was 3.5 mW/m^2 , which was higher than that with the stainless-steel cathode [56]. Song et al. employed graphite felt (GF) and activated carbon fiber felt (ACFF) in 2012. For river SMFC, he discovered that using ACFF produced a power density of $33.5 \pm 1.5 \text{ mW/m}^2$ [57]. When Zhang et al. compared the sheet iron anode with GF, they found a maximum power density of 170 mW/m^2 , which is 4.8 times greater than the power density of 35 mW/m^2 with GF for river SMFC [58]. As a cathode electrode, Alipanahi and Rahimnejad used conductive metal brushes. Additionally, when river and marine SMFC were compared in the study, it was found that river sediment produced the maximum power density, at roughly $121 \mu\text{W/cm}^2$ [59]. In 2014, a study was conducted on this topic with lake SMFC and the effect of these electrodes on electricity generation using metallic and non-metallic anodic electrodes was investigated. The study concluded that the highest power density recorded was 913 mW/m^2 for zinc, 646 mW/m^2 for iron, 387.8

mW/m² for copper, 266 mW/m² for aluminum, and 127 mW/m² for graphite felt. The findings demonstrate that Zn exhibited superior performance in energy generation, succeeded by Fe [60]. In 2018, Prasad and Tripathi created SMFC systems using copper anode-zinc cathode and graphite anode-zinc cathode by using river SMFC. A power density of 2.36 mW/m² was obtained for the first case and 2.07 mW/m² for the second case. This shows that copper and zinc were identified as optimal electrode materials for SMFCs owing to their advantageous standard electrode potentials [61]. In a 2021 study, stainless steel electrodes were tested for their ability to immobilize phosphate by utilizing lake SMFC. The research involved both laboratory microcosms and field-scale installations for lake SMFC. Lab tests showed an 85% reduction in phosphate concentrations in water using the electrodes, while field tests achieved up to 94% reduction. In the study, beyond phosphate immobilization, SMFCs also contribute to the degradation of organic matter, reduce methane, and sulphur emissions, and generate a small amount of electricity (10 mW/m²) [62].

Investigations were conducted on the degradation of several organic compounds using freshwater SMFC. For instance, studies were conducted on the removal of polycyclic aromatic hydrocarbons (PAHs) [63,64], acid volatile sulfide (AVS) [64], and cellulose from sediments [65]. For river SMFC, Zhu et al. discovered that the maximum cellulose removal efficiency was $72.7 \pm 2.1\%$ [65], whereas Chen et al. [64] observed that the removal efficiencies of PAHs and AVS were 71.56% and 89.76%, respectively. In another study, the removal of phenanthrene and pyrene, which belong to the PAH family, was studied with SMFC obtained from a lake. As a result of the study, 99.47% removal of phenanthrene and 94.79% removal of pyrene were achieved in the SMFC system to which ferric hydroxide was added [63]. Furthermore, the behavior of SMFCs in environments containing organic matter has been examined when additional organic chemicals are introduced. In a 2014 study by using aquaculture pond sediment, 2% and 4% cellulose was added to the medium and it was found that COD removal was higher when 2% cellulose was added. SMFC with 2% cellulose exhibited the best COD removal efficiency (88.2%), while SMFC with 4% cellulose showed a decrease in efficiency (83.1%) due to fatty acid accumulation inhibiting microbial activity. According to the study, SMFC with 2% cellulose generated the highest open

circuit voltage (976 mV) [66]. In another study in 2015, river SMFCs were employed to improve the degradation of submerged macrophyte litter in shallow lake sediments. It was found that the presence of SMFCs led to significant removal of total organic carbon (TOC) at various sediment depths, with an increase in TOC removal efficiency by 18.9%, 20.9%, and 20.8% at sediment depths of 0–8 cm, 8–18 cm, and 18–28 cm, respectively. The research demonstrates that SMFCs can promote carbon sequestration and mitigate the adverse effects of eutrophication and terrestrialization in shallow freshwater lakes [67].

Contaminant concentration is one of the parameters studied. In a 2017 study, sediment from a tofu wastewater outlet (which is river) served as the medium, with electrodes placed at varying depths. Tofu wastewater concentrations were adjusted to 25%, 50%, 75%, and 100%. Anode positions were set at 2 cm and 4 cm below the sediment surface. The highest power density was achieved with a 25% wastewater concentration, producing 217.14 mW/m² at 2 cm anode depth and 215.95 mW/m² at 4 cm [68].

Mixing and aerating are other factors which affect the performance of freshwater SMFCs. In a 2016 study, by mixing the sediment and aerating the water, SMFC systems were run. It was revealed that stirring the sediment and aerating the water increased voltage temporarily, suggesting that mass transfer limitations at the anode and cathode surfaces were impacting the overall performance. In addition, measurements were made by doubling the surface area of the anode electrode (using two anodes in series) and as a result, it was understood that doubling the anodic surface area did not result in improved performance [69].

The power density that is obtained from freshwater SMFC systems can be positively impacted by increasing conductivity. To achieve that, biochar made from coconut shells was added to the SMFC system to improve it. The performance of the SMFC power generation was improved by a factor of two to ten when compared to those without the change. In addition, compared to no alteration, this modification enhanced the TOC removal rate by 1.7 to four times [70].

The performance of SMFCs can be improved by adding external chemicals to the environment. Zhou et al. tested the effects of different iron oxide amendments, including ferric oxyhydroxide, goethite, and magnetite. These amendments did not significantly improve SMFC performance. However, amending the sediments with a mixed solution containing soluble ferric citrate and colloidal iron oxyhydroxide resulted in a threefold increase in voltage production compared to unamended sediments [71].

The bacterial nature of the biofilms formed by the SMFCs used is a crucial factor. The methods used to form biofilms can change the structure and performance of biofilms. In the study conducted in 2017 by using freshwater sediment taken from creek, two different enrichment methods were used to cultivate microbial biofilms—general enrichment and ferric citrate (FeC) enrichment. These methods produced different microbial communities. General-enriched bioanodes performed better, with a maximum power density of 79 mW/m², while FeC-enriched bioanodes had lower performance, with a maximum power density of 38 mW/m² [72]. A 2019 study found that ferric citrate enrichment, while selective, did not enhance the proliferation of anode-respiring bacteria (ARB) as expected and instead led to a decrease in microbial diversity, ultimately affecting MFC performance [73].

It is well known that reducing the gap between the MFC system's cathode and anode compartments lowers its internal resistance. Thus, the same outcomes may be obtained by decreasing the water layer between the cathode and the sediment. In the 2023 study, SMFCs without a water layer between the sediment and the cathode had power density improvements of 72-134% [74].

When the studies with freshwater SMFCs were examined, it has been observed that there are very few studies on whey. In this study, the extent to which the organic load of whey can be removed using freshwater SMFCs and the efficiency of the electrical energy generation potential were investigated. Thus, the gap in the literature on this subject has been tried to be filled.

2. MATERIALS AND METHODS

This chapter provides a detailed explanation of the construction of the systems used for whey removal and energy generation.

2.1. Wastewater and Inoculation

Whey was obtained from a factory producing dairy products in Trabzon, Türkiye. It has been received from the factory that the pH value of the whey supplied is approximately 6 at 25 °C and the fat ratio is zero. It is also known that the amount of solids in whey, which is the brix value, is 6% on average.

To homogenize the whey, the wastewater was passed through coarse filter paper, and the precipitate was removed. It is possible for whey bacteria to affect the bioelectrochemical reactions that occur in MFCs [17]. To decrease the effect of microorganism of the whey, the sample was pasteurized. In other words, the vegetative bacteria were eliminated. The raw material was first heated to 100 °C; however, even at this temperature, precipitate development was seen. Other temperatures that did not result in precipitate formation were used because the sample was part of the system without precipitation. After 100 °C, 90, 80, and 72 °C were applied, respectively. However, at temperatures other than 72 °C, there was little precipitation in the whey. For this reason, the wastewater was kept at 72 °C for 1 hour. The study used whey with dilution (1/5) in the first system and without it in the second. To compare the bacteria performance, sucrose solution with a similar organic structure and similar organic load was added to the systems prior to the addition of whey. Temperature, pH, Total Dissolved Solids (TDS), electrical conductivity, and COD parameters of wastewater were measured before and after the systems were operated.

To give the river bottom mud-derived microorganisms time to adjust to the whey environment, the whey was first diluted by 1/25 and then by 1/5 before the bacteria were added to the systems. These microorganisms were used for the first and second systems.

2.2. Sediment Sampling

Two types of sand with similar chemical and physical structures were used in the systems as sediments. Although the sands were similar, their chemical structures were not the same. Therefore, by using these two sand types, the effects of differences in sediment on system performance were tried to be examined.

The sands taken from the Eastern Black Sea coast were first sieved through 850-micrometer sieves to obtain homogeneous size sediments. The sediments were sterilized at 150 °C for 1 hour [75].

To find density and air-space ratio of the sand, the following steps were taken

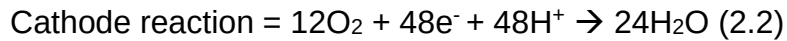
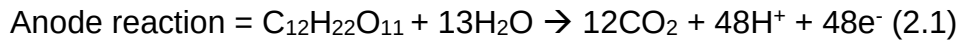
- ✓ The sand dried at 103 °C for 24 hours.
- ✓ A 10 ml graduated cylinder was taken and measured when empty.
- ✓ The graduated cylinder was weighed again by adding 5-6 ml of sand.
- ✓ The weight of the empty cylinder was subtracted to obtain the mass of the sand sample itself, and the density of the sand was found by dividing by the measured volume of the sand.
- ✓ The sand sample was poured from a 10 mL graduated cylinder into a 50 mL graduated cylinder.
- ✓ 7-8 mL of distilled water was measured in a 10 mL graduated cylinder.
- ✓ Distilled water was added to a 50 mL graduated cylinder containing the sand. It was thoroughly mixed so that the water could completely penetrate the sand.
- ✓ The total volume of the sand/water mixture was measured.
- ✓ The difference in the amount of water between the required volume and the measured volume was found. The percentage of sand was calculated [76].

As a result, it was found that the density of the sand-1 (sediment-1) is 1.32 g/mL and the moisture content in it is 60%. For sand-2(sediment-2), it has a density of 1.52 g/mL and 67% moisture content.

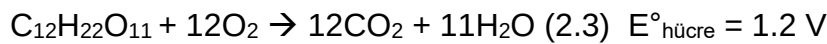
2.3. Reactor configuration and operation

Single chamber type MFC is formed to treat cheese whey and produce electricity. The organic content of whey consists of lactose and so, the biochemical oxidation and reduction reactions and general reaction are described as follows:

Half reactions:



The general reaction of organic decomposition:



$E^\circ_{\text{hücre}}$ represents the theoretical electric potential of the overall reaction.

Before the systems were installed, the durability of varied materials as electrodes was examined. These materials were carbon paper, steel mesh and aluminum paper-tape. As a result of the experiments, it was found that the carbon paper deformed in about a month and the steel mesh in about two weeks. Against these, the aluminum foil paper could be used in the system for about two months. The resulting systems were created with aluminum foil paper and aluminum paper-tape.

Two different configurations were created. In the first one (Figure 2.1), 5 cm thick sand was placed in a 5-liter beaker. Aluminum foil paper, which has a 15-cm diameter, was used as an electrode at the anode, and it was buried 2.5 cm deep in the sand. Multi-stranded copper cable was used for the anode part, and the cable was glued to aluminum foil paper (Figure 2.3). The gaps formed in the electrode placed under the sand surface were closed using epoxy adhesive. For the cathode, a 19.5-cm-long aluminum foil tape was used as an electrode. To complete the system, crocodile clip lead was utilized in the cathode part. The diluted whey was placed 15 cm (2500 mL by volume) from the bottom, and it was in contact with the bottom part of the electrode at the cathode.

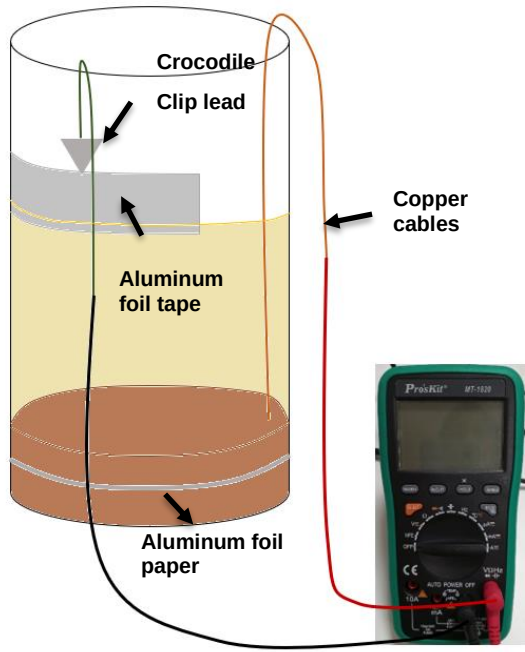


Figure 2.1: View of system-1

In the second system (Figure 2.2), another 5-cm-thick layer of sand was placed on the bottom of a 5-liter beaker. Electrodes with a diameter of 15 cm for the anode and cathode were formed from aluminum foil paper, and multi-stranded copper cable was used (Figure 2.3). One of the electrodes was placed under the sand (anode) and the other on top of the sand (cathode). In other words, 5 cm was left between the anode and cathode parts. Electrical tape was used to adhere the cables to the aluminum foil paper. Also, epoxy adhesive was utilized to seal the holes in the electrode that had been positioned beneath the sand surface. 50 ml of undiluted whey was added to the system.

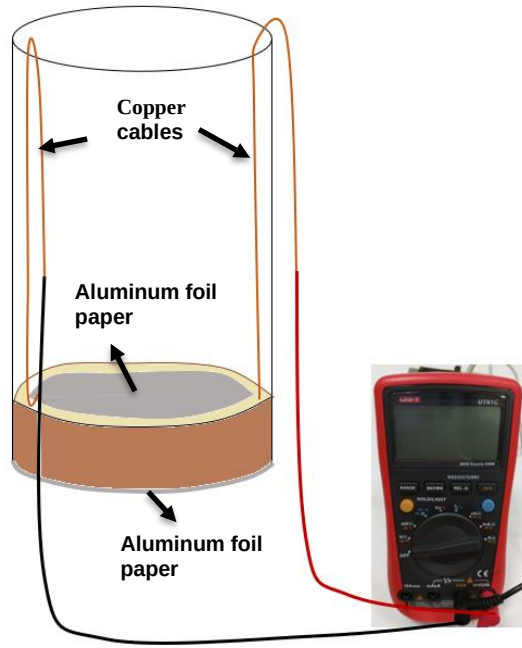


Figure 2.2: View of system-2



Figure 2.3: Top (left) and bottom (right) view of copper cable glued aluminum foil paper

The outsides of the systems were enveloped in aluminum foil to shield them from light exposure. Furthermore, glass coverings with a diameter of 20 cm, which were enveloped in aluminum foil, were positioned on each system (Figure 2.4).

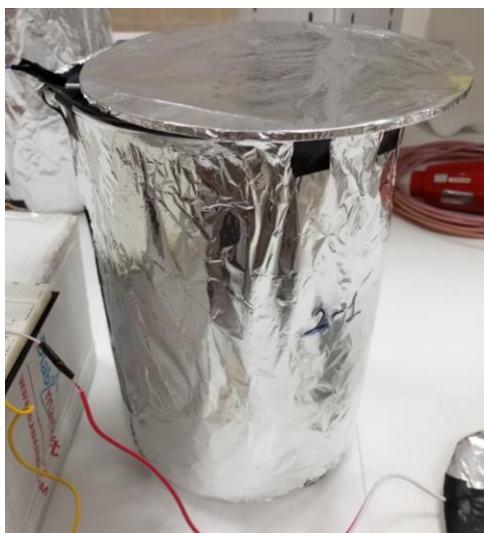


Figure 2.4: External view of the systems

To inject microorganisms into the system, isotonic water and 7 mL of the medium-extracted particles were placed in a 15-mL falcon tube and exposed to a 1/5 diluted whey solution. Two milliliters were taken from the top of the tube and put into the system as an inoculant after being cleaned for a minute in an ultrasonic cleaner.

After being frozen in the fridge, the whey sample was thawed, run through a mixer, and filtered. The systems were then filled with diluted and undiluted whey after cooling to a temperature of 72 °C.

The two systems were run for two types of sand used as sediment. First, system-1 and -2 were run without resistance for sediment-1. The systems were run with only pure water with sediment in the medium, then with the microorganisms inoculated into the sediment in the presence of pure water. The pure water was removed and replaced with sucrose solution with an organic load like whey. Finally, the sucrose solution was removed from the system and replaced with whey and measurements were taken. Each measurement took over 24 hours, and when a state was created in the measurements, its resistance was measured first and then its electrical potential. Measurements were conducted on both systems-1 and -2 for sediment-1 and -2. Consequently, measurements of resistance and electrical potential were conducted on four systems constructed using two diverse types of sediments. The average resistance values for these four systems were calculated over a 24-hour period. From these measurements, the sizes of the resistors to be placed in the systems were determined. After that,

electrical potential measurements were recorded for these four systems over a period of 3 days each by using sucrose solution and whey, initially without any resistor and then with resistor. Breadboard was used to connect the resistors to the systems. A breadboard is a rectangular plastic board with numerous little holes as shown in Figure 2.5. These apparatuses facilitate the effortless insertion of electrical components for the purpose of prototyping, which involves constructing and assessing a preliminary version of an electronic circuit. For instance, one can assemble a circuit with a battery, switch, resistor, and an LED (light-emitting diode) using these apparatus [77]. An example of a resistor connected system is shown in Figure 2.6.

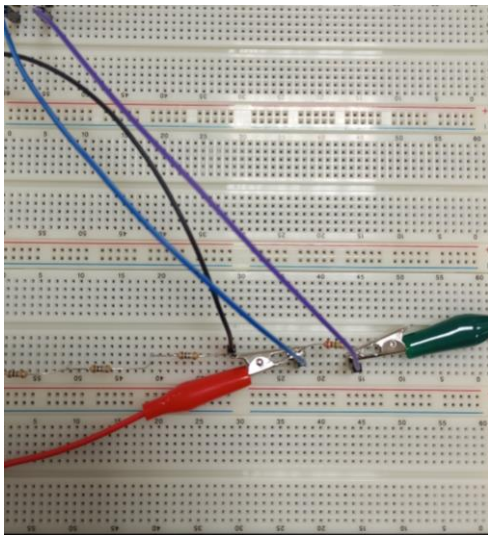


Figure 2.5: Breadboard



Figure 2.6: An example of a resistor connected system

2.4. Electrochemical measurements

Voltages (V) and resistances were recorded to computers for the systems with two different multimeters. One of the multimeters (the PrósKit MT-1820) records two thousand data points for 20 minutes and takes this data second by second. The other multimeter (UNI-T UT61C), with its adjustable recording time, recorded data every minute. In addition, the resistance (R) of the system was recorded to understand the internal resistance of the system. Therefore, the magnitude of the resistors to be connected to the system was determined. The current of the system was calculated from the following equation:

$$\text{Voltage (V)} = \text{Current (I)} \times \text{Resistance (R)} \quad (2.1)$$

where I is the ampere and V is the voltage in mV.

The power of the system was calculated with the following equation.

$$\text{Power (P)} = I \times V, P = \text{Watt (W)} \quad (2.2)$$

$$\text{Energy (E)} = P \times t, t = \text{Second (s)} \quad (2.3)$$

Power density was calculated by dividing the obtained power by the surface area of the anode [78].

2.5. Characterization

2.5.1. Wastewater Characterization

The spectrophotometric method was used to determine the COD. In the method, firstly, potassium dichromate was dried at 150 °C for 2 hours. After being cooled in a desiccator and brought to a constant weight, 4.9 g was weighed. 4.9 g of potassium dichromate was added to 150 ml of H₂SO₄. Add 6 g of HgSO₄ to the mixture and wait for it to dissolve. Afterwards, 6 g of Ag₂SO₄ was added and 350 ml of H₂SO₄ was added slowly. The mixture was stirred for 3 days. 3 ml of this solution was filled into the kit bottles of HACH, and then 2 ml of the sample to be measured was added to the COD heater. It was heated at 148 °C for 2 hours and the absorbance value was read at 600 nm wavelength in the spectrophotometer.

The following calibration curve as shown in Figure 2.7 was created using this method and this calibration curve was used in COD measurements. Parameters other than COD, which are temperature (AMT-300), pH (pH -009(I)A Pen Type

pH Meter), conductivity (C65 Waterproof EC Pens), and TDS (TDS-3 TDS/TEMP Meter) were measured with portable meters.

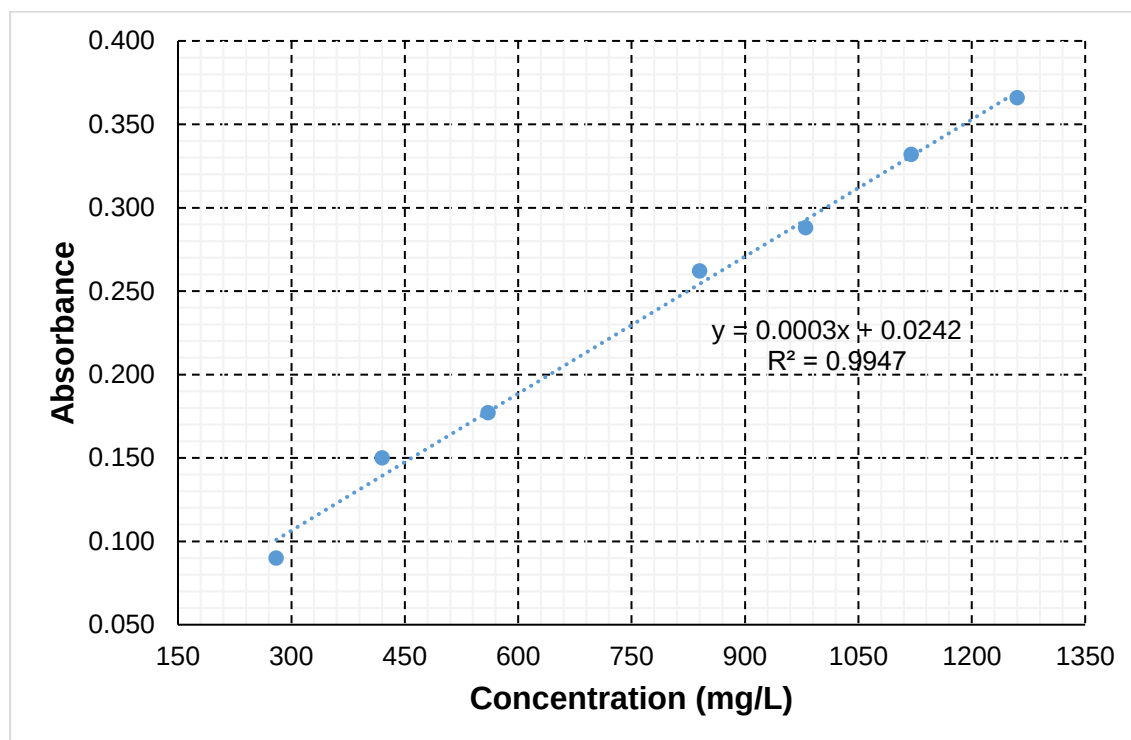


Figure 2.7: Calibration curves for COD measurements [79]

Whey samples were sent to Çınar Environmental Laboratory for comprehensive characterization. COD, pH, temperature, conductivity, ammonia, nitrite, nitrate, phosphorus, potassium, sulfate, total solids, suspended solids, total organic carbon, and iron parameters were measured for the characterization.

2.5.2. Sediment Characterization

X-ray diffraction (XRD) and X-ray fluorescence (XRF) analytical techniques were used to characterize the sediment. XRD is a technique that determines a sample's composition or crystalline structure. The atoms in the sample interact with the X-rays, which diffract at different angles. This diffraction pattern can be used to determine the sample's composition, crystallinity, and phase purity [80]. In this study, the sediments used for sediments were pulverized and sent to the Black Sea Technical University laboratory for standard XRD measurement between 2 and 70 °. XRF is a method used for elemental and chemical analysis. These fluorescent X-rays' energy is characteristic of the atoms present in the sample, allowing for element identification [81]. To find out if materials such as SiO_2 ,

Al_2O_3 , Fe_2O_3 , CaO , MgO , Na_2O , CuO , ZnO , etc. are present in the content, 0.5 kg of unground sediments were submitted to the Mineral Research and Exploration Laboratory for XRF analysis.

2.5.3. Identification of Bacteria

The river bottom sediment, which is assumed to contain microorganisms, was first contacted with whey. The following procedures were then applied to the sediment with and without contact with the whey to verify whether bacterial culture was present in the sediment:

Physiological water was prepared by adding 9 grams of NaCl to one liter of pure water. 4 ml of physiological water was poured into 100-ml flasks, and stones weighing five grams were placed in them. It was kept in a shaking incubator at 30 °C for 15 minutes. Each of the two sets of four tubes held nine milliliters of physiological water. 1 ml of samples were placed in the first samples of the sets, and 1 ml of the first samples were added to the second samples; 1 ml of the second sample was added to the third samples; and finally, 1 ml of the third sample was added to the fourth samples. Thus, the samples were diluted, respectively, by 10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4} ratios. Then, 1 ml was taken from the main samples and inoculated on the nutrient agar. 100 μl were taken from the diluted samples and inoculated. It was kept in an incubator at 30 °C for 4 days for the microorganisms to grow, and Figures 2.8 and 2.9 were obtained. It was determined that there were 5 types of bacteria colonies in non-contact stones and 8 types of bacteria colonies in contact stones. After the bacterial colonies were detected, the bacteria species were tried to be determined.

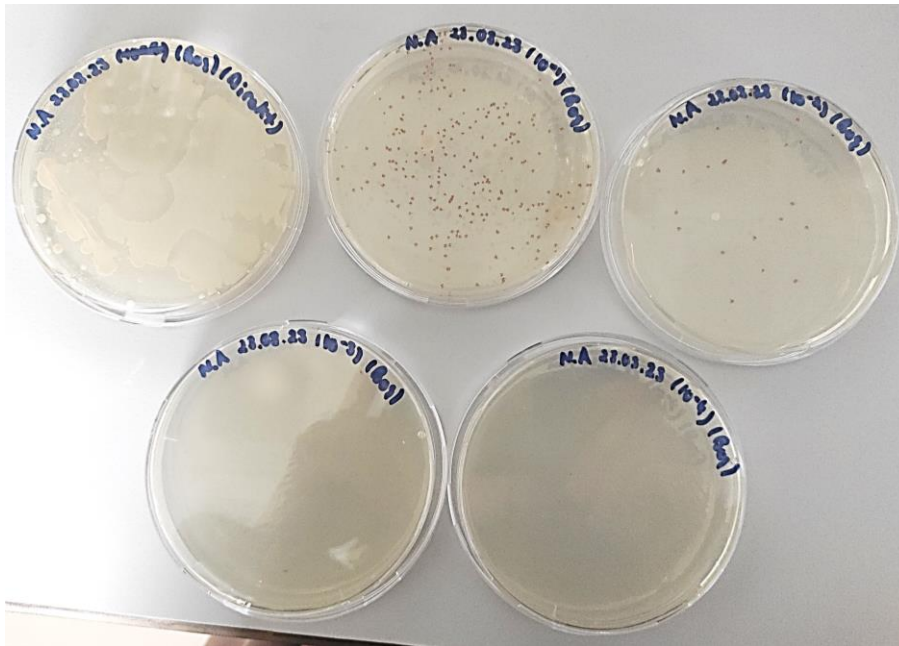


Figure 2.8: Bacteria culture analysis from stones that are not in contact with whey

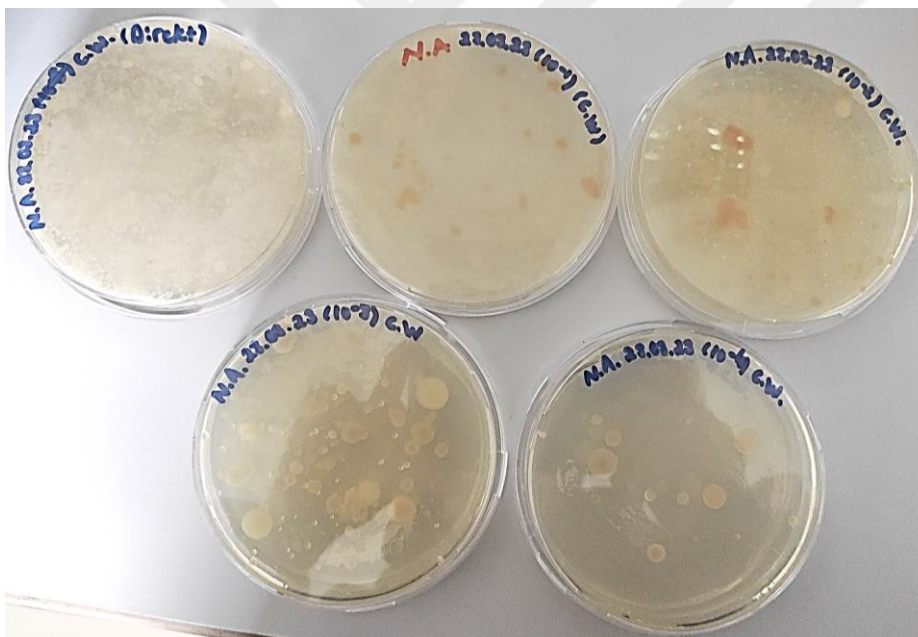


Figure 2.9: Bacteria culture analysis from stones that are in contact with whey

Metagenomic analysis is a technique used to study genetic material from environmental or biological samples. It allows us to understand the diversity, abundance, and interaction of microbes in any system [82]. The mixed bacterial cultures put into the system were sent to BM Software Consultancy and Laboratory Systems Limited Company for this analysis. Microorganism cultures were subjected to the following procedures before being sent to the laboratory:

7 mL of the particles, which were exposed to whey solution diluted 1/25 and 1/5, extracted from the medium and isotonic water were placed in a 15 mL falcon tubes. 0.5 milliliters were taken from the top of the tubes after cleaning in an ultrasonic cleaner for one minute. They were placed in a 2-milliliter screw cap tube containing 0.5 milliliter of 50% glycerol solution [83]. These tubes were then stored in the freezer of the refrigerator until they were sent to the laboratory.

To visualize the bacteria placed in the system, images of the sediment with which it interacted were analyzed by Scanning Electron Microscopy (SEM) analysis. Images were acquired at 10000 magnification and a 10 kV voltage.



3. RESULTS

The experiment findings are evaluated based on the following factors: (i) whey characteristics; (ii) sediment chemical composition; (iii) identification of microorganisms in the sediment; (iv) electrical potential and resistance comparison; (v) electrical potential and powers under resistive and non-resistive situations; and (vi) wastewater treatment performance.

3.1. Evaluation of Whey Characterization

Whey wastewater is an alkaline, highly organic discharge. The composition of whey wastewater is very changeable due to variables such as the type of raw milk used, the percentage of non-valued whey, and the volume of cleaning water employed. The amount of wastewater generated by cheese whey is approximately four times that of processed milk [84]. As a result, it is a noteworthy byproduct of the dairy business that needs to be managed carefully.

Approximately 90% of the organic load is made up of the lactose that is left in the cheese whey after milk is processed [85]. The lactose content can be regarded as the primary cause of COD. The COD levels of cheese effluents range from 0.8 to 102 g/L. Basic pH values have been reported ranging from 3.3 to 9.0. TS content of whey can vary between 50 and 70 g/L [86]. The COD of the whey used in this study is within the specified range and is 70 g/L. According to the results obtained from Çınar Laboratory, the pH of this sample is 4.37 and the TS is 41 g/L.

The range of the total phosphorus level is 10–2200 mg/l. According to whey total organic carbon concentration values in different studies, the concentration of this parameter can vary from 55 to 3500 mg/l. The maximum conductivity of whey can be around 8 mS/cm depending on the type of whey produced in the process and the addition of NaCl during cheese production [84]. In this study, the measured total phosphorus value of whey is 189 mg/L. Total organic carbon value is 17 g/L. In addition, the conductivity of the wastewater is approximately 5.5 mS/cm.

Other parameters in the whey structure were analyzed and determined to be 124 mg/L sulphate, 2.73 mg/L nitrite, 3.78 mg/L nitrate, 149 mg/L ammonia, 0.286 mg/L iron, and 689 mg/L potassium, as shown in Table 3.1.

Table 3.1: Properties of whey

Parameter	Unit	Test Result
pH	-	4.37
Temperature	° C	23.2
Conductivity	µS/cm	5,480
Total Suspended Solids (TSS)	mg/L	1,263
Chemical Oxygen Demand (COD)	mg/L	70,148
Total Solids (TS)	mg/L	41,140
Sulphate (SO ₄ ²⁻)	mg/L	124
Nitrite (NO ₂ ⁻)	mg/L	2.73
Nitrate (NO ₃ ⁻)	mg/L	3.78
Ammonia (NH ₃)	mg/L	149
Iron (Fe)	mg/L	0.286
Total phosphorous (TP)	mg/L	189
Potassium (K)	mg/L	689
Total organic carbon (TOC)	mg/L	17,390

3.2. Evaluation of Chemical Composition of the Sediments

In general, SiO₂ (silica) makes up the chemical composition of sediment-1. Table 3.2 illustrates that the XRF result shows a silica content of 73.9%. Sediment-1 contains Al₂O₃ (alumina), an essential component of numerous minerals [87], making it the second most abundant mineral after silica. Furthermore, the sediment has a naturally occurring 4.6% Fe₂O₃ content [88]. Organic materials contained in the sediment itself make up 2.3% of the sediment's chemical structure. The sediment also included 2.2% CaO and 2.7% Na₂O.

The findings from XRF for sediment-1 are corroborated by the results of the XRD analytical technique. Figure 3.1 shows the significant concentrations of silica, and albite, which is a mineral that belongs to the feldspar family and makes up over half of the Earth's crust [89].

Table 3.2: Chemical composition of the sediment-1 [79]

Analysis	Percentage (%)	Analysis	Percentage (%)
Loss on Ignition	2.30	NiO	<0.01
Al ₂ O ₃	10.5	P ₂ O ₅	0.1
BaO	<0.01	PbO	<0.01
Bi ₂ O ₃	<0.01	Rb ₂ O	<0.01
CaO	2.2	S(S)	-
CdO	<0.01	SO ₃	0.06
Co ₃ O ₄	0.01	SiO ₂	73.9
Cr ₂ O ₃	0.03	SnO ₂	<0.01
CuO	<0.01	SrO	0.01
F	<0.01	TiO ₂	0.4
Fe ₂ O ₃	4.6	V ₂ O ₅	0.02
K ₂ O	1.2	Y ₂ O ₃	<0.01
MgO	1.8	ZnO	0.01
MnO	0.1	ZrO ₂	0.01
Na ₂ O	2.7		

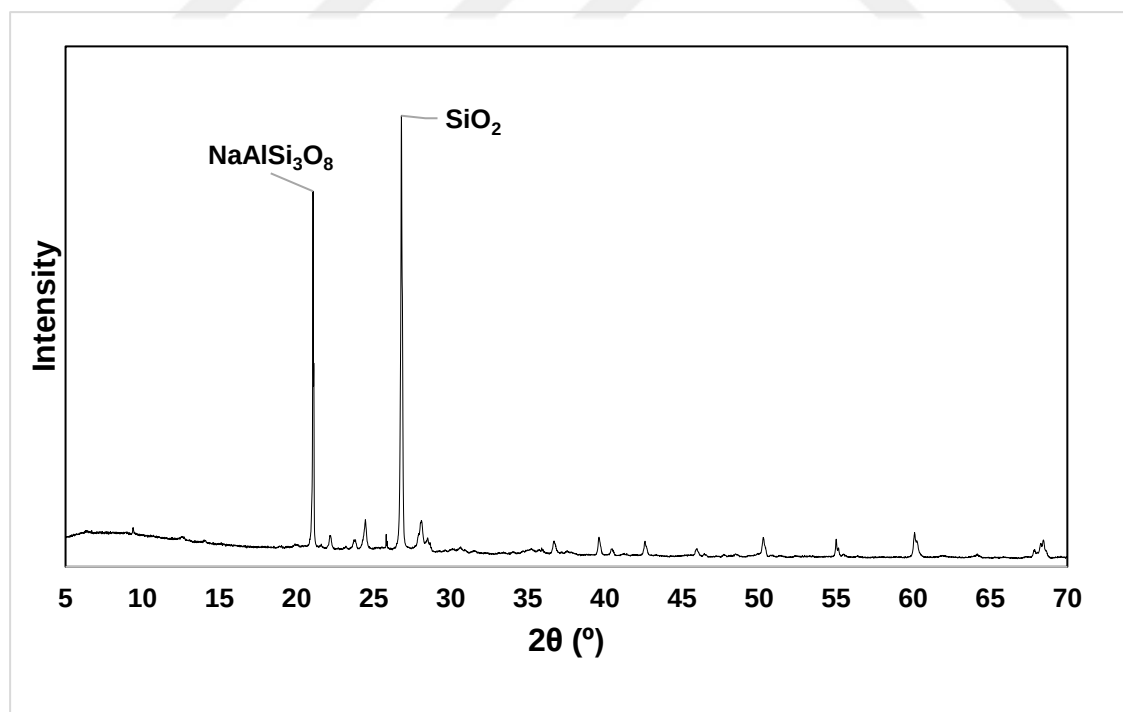


Figure 3.1: XRD of sediment-1

For sediment-2, the chemical composition of sediment consists of mostly silica (SiO_2), again (as shown in Table 3.3). Like sediment-1, the XRF result indicates that alumina predominates in this sample, following silica. Furthermore, in this sediment, Fe_2O_3 ranks third in terms of percentage. However, sediment-2 contains twice as much of this compound as the sediment-1 does. The sediment's organic matter content contained in sediment-2 itself is 2.2% of the chemical structure, and it also contains 2.7% Na_2O and 3.2% CaO . Unlike the sediment-1, this one contains more than 2% of the MgO compound.

XRD data of sediment-2 corroborates the XRF findings. The most common minerals in this sediment, like in the first one, are albite and silica as shown in Figure 3.2.

Table 3.3: Chemical composition of the sediment-2

Analysis	Percentage (%)	Analysis	Percentage (%)
Loss on Ignition	2.20	NiO	<0.01
Al_2O_3	9.5	P_2O_5	0.1
BaO	0.01	PbO	<0.01
Bi_2O_3	<0.01	Rb_2O	<0.01
CaO	3.2	S(S)	0.06
CdO	<0.01	SO_3	-
Co_3O_4	0.01	SiO_2	69.1
Cr_2O_3	0.05	SnO_2	<0.01
CuO	<0.01	SrO	0.01
F	<0.01	TiO_2	0.8
Fe_2O_3	9.2	V_2O_5	<0.01
K_2O	0.9	Y_2O_3	<0.01
MgO	2.3	ZnO	0.01
MnO	0.2	ZrO_2	0.01
Na_2O	2.2		

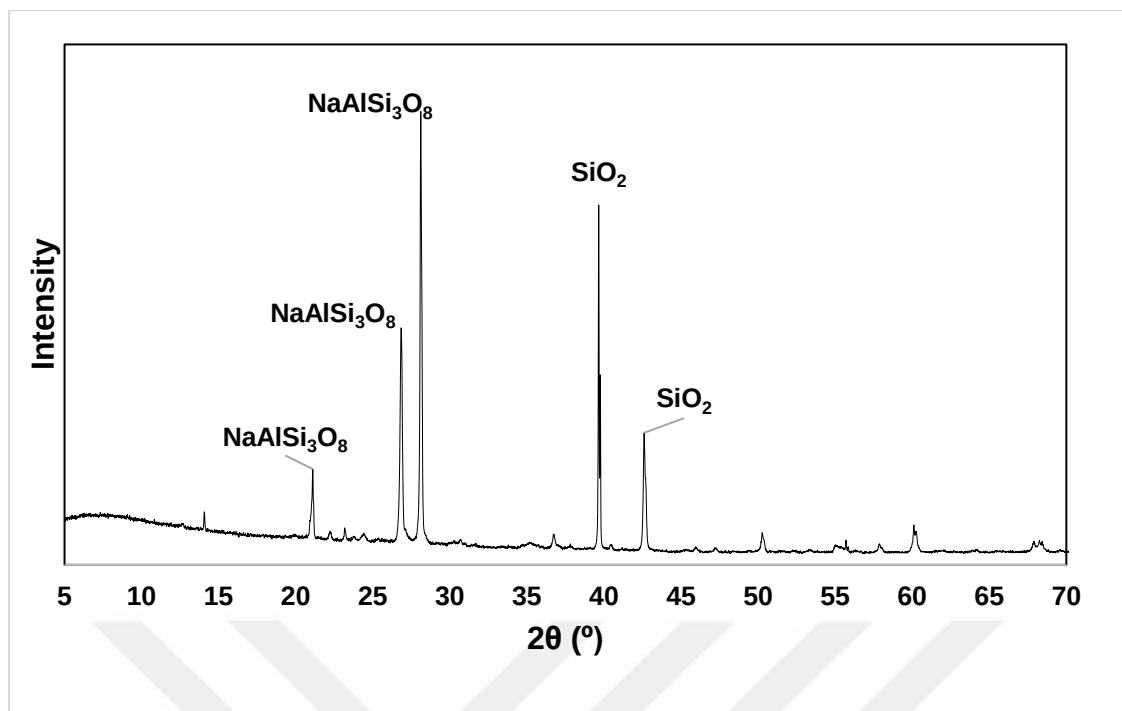


Figure 3.2: XRD of sediment-2

3.3. Identification of Microorganisms in the Sediments

Whey was added to the system-1 with sediment (building sand) in two different concentrations to study microorganisms. In one of these concentrations, whey was diluted 1/25 and in the other, whey was diluted 1/5 and placed in the system. By metagenomic analysis, 248 microorganism genera were analyzed, shown in APPENDIX 1. While 186 genera were identified for the first concentration, 111 genera were identified for the second concentration.

The bacterial species found were analyzed according to their capacity to become MFCs.

For the first concentration, Figure 3.3 depicts the dispersion of microorganisms in the mixed culture. *Pseudonocardia* (28.43%) and *Ralstonia* (11.31%) constituted the bulk of microorganisms. A genus of actinomycetes called *Pseudonocardia* produces secondary metabolites that are important to biotechnology. Some of these metabolites have anti-bacterial, anti-fungal, and anti-tumor properties, despite their limited clinical importance. Interestingly, some *Pseudonocardia* species can form symbiotic partnerships with leaf-cutter ants that feed on fungi [90]. However, there is currently no concrete evidence to support the frequent use of *Pseudonocardia* as an MFC. Typically, MFCs are

based on other microorganisms that can produce electricity through their metabolic processes, such as certain bacteria or archaea. The potential of these fuel cells to solve wastewater management, global warming, and energy security problems is enormous [91]. In conclusion, even though *Pseudonocardia* has a long history of advancements in biotechnology, nothing is known about how specifically it might be used in MFCs at this time. A well-researched *Knallgas* bacteria called *Ralstonia eutropha* has been modified to produce n-butanol, isobutanol, and terpene molecules in chemolithoautotrophic environments, among other things. Despite not being frequently linked to MFCs, its potential for CO₂-based fuel molecule production makes it a compelling subject for further study in this area [92,93]. Other microbes are usually employed in MFCs to produce energy through metabolic activities. On the other hand, *Ralstonia eutropha*'s capacity to use CO₂ and its adaptability to various substrates may provide new opportunities for the generation of sustainable energy. To fully understand its unique function in MFCs and how well it functions as an anodic substrate, more research is required [92,94].

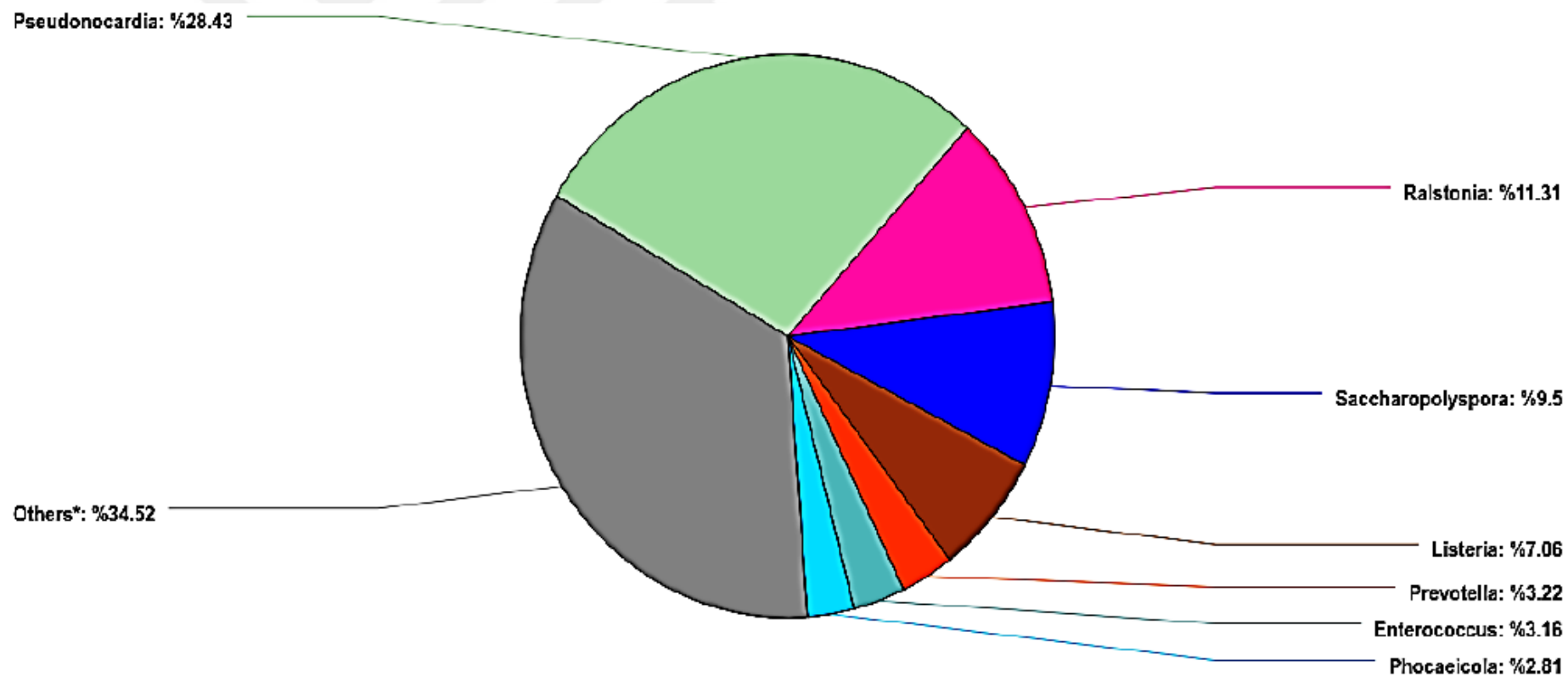


Figure 3.3: Circle chart for the species level for first concentration (1/25 dilution)

The distribution of microorganisms in the mixed culture for the second concentration is as shown in Figure 3.4. But most of the bacteria were *Clostridium* (18.72%) and *Bacteroides* (19.91%). Gram-negative, obligate anaerobic bacteria belong to the *Bacteroides species*. These non-endospore-forming bacilli can be motile or nonmotile, depending on the species [95]. This method works well for MFCs since their anodic responses are usually anaerobic due to their status as obligatory anaerobes. They may also break down complex organic compounds, such the ones found in wastewater [91]. Microbial colonies known as biofilms, which stick to surfaces, can be produced by *Bacteroides* [96]. Biofilms can enhance MFC performance by increasing the electron transfer surface area [91]. The majority of *Clostridium* species are rod-like, gram-positive bacteria that come in varied sizes. Microorganisms belonging to the genus *Clostridium* are present in soil, water, and the digestive tracts of humans and animals. Most organisms are forced to be anaerobes because they can only survive in conditions of complete oxygen deprivation. *Clostridium* cells that are dormant are incredibly resistant to heat, desiccation, dangerous chemicals, and detergents [97]. *Clostridium* and *Bacteroides* can be utilized as MFCs since they share the same characteristics [98,99]. The outcomes of the SEM analysis corroborate these metagenomic analysis findings. Figure 3.5 displays pictures of *Bacteroides* and *Clostridium*.

A more thorough examination of the bacterial species described in the literature reveals that *Proteobacteria* [100], *Cytophaga*, *Flexibacter*, and *Bacteroides* [101] are the sources of MFCs. Despite its potential as MFC, *Clostridium* has not received much attention. In 2019, however, this kind of microbe was used to investigate the bioelectricity-making potential of sago hampas, a byproduct that is made when sago starch is extracted from the pith of the sago palm [102,103]. According to published research, *Bacteroides* and *Clostridium species* can be used as MFCs in the production of bioelectricity.

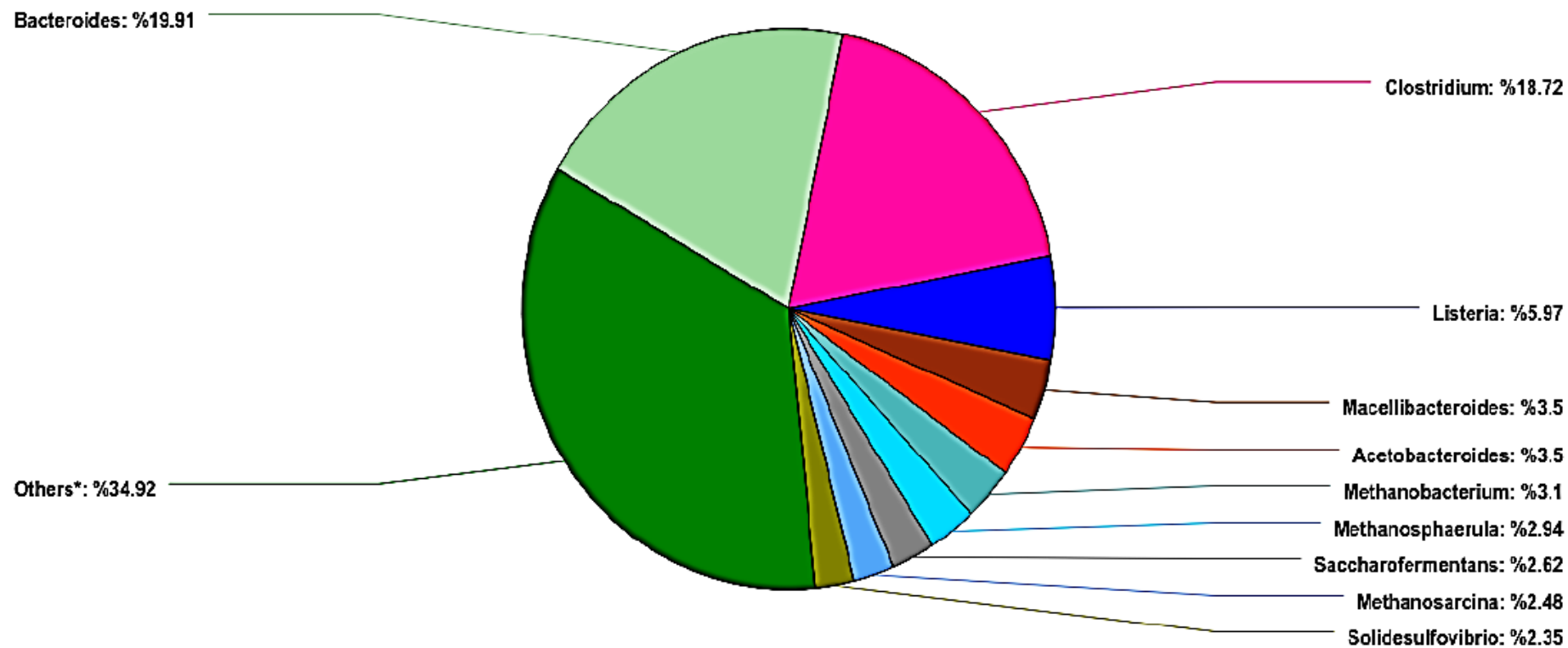


Figure 3.4: Circle chart for the species level for second concentration (1/5 dilution) [79]

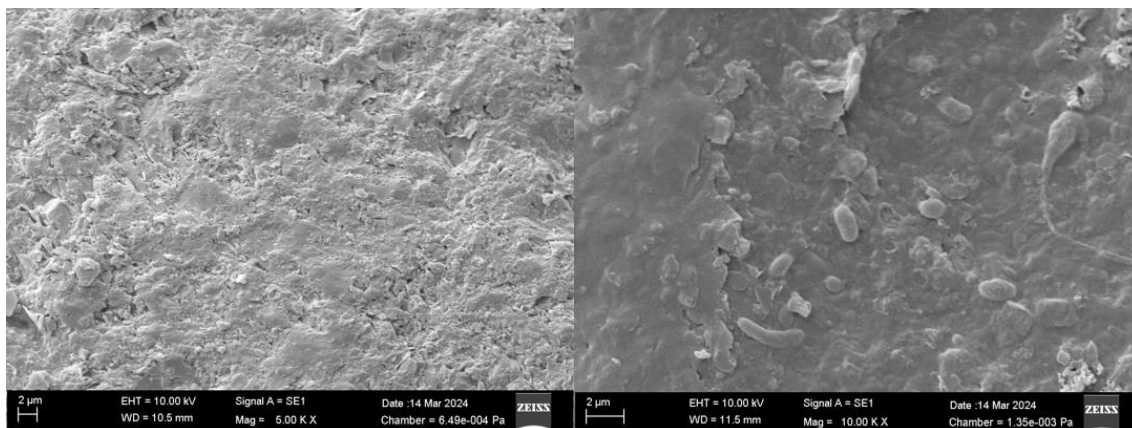


Figure 3.5: SEM analysis of the sediment without (left one) and with (right one) native microorganisms [79].

Apart from these evaluations, microorganisms were evaluated according to their ability to use nitrogen, sulfur, and iron as electron acceptors. Because when the characterization of whey was examined, it was found that it contains nitrite, nitrate, ammonium, sulphate, and iron (as seen in Table 3.1). With respect to APPENDIX 1, the following results were obtained.

Microorganisms that recognize nitrogen as an electron acceptor: *Enterobacter*, *Erwinia*, *Serratia*, *Citrobacter*, *Salmonella*, *Bacillus*, *Lactobacillus*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Achromobacter*, *Hyphomicrobium*, *Paracoccus*, *Rhizobium*, *Bradyrhizobium*, *Moraxella*, *Bradyrhizobium*, *Thiobacillus*, *Rhizobium*, *Clostridium* and *Veillonella* [104].

Microorganisms that recognize sulfur as an electron acceptor: *Desulfobulbus*, *Desulfovibrio*, *Clostridium* and *Pseudomonas* [105–108].

Microorganisms that recognize iron as an electron acceptor: *Vibrio*, *Pseudomonas*, *Paracoccus*, *Thiobacillus*, *Bacteroides*, *Clostridium*, *Desulfovibrio*, *Bacillus* and *Thermoanaerobacterium* [109,110].

Since the systems used microorganisms obtained after contact with 1/5 whey, those present in these groups but with zero percentage of presence were excluded for further evaluation. These microorganisms are *Enterobacter*, *Serratia*, *Salmonella*, *Bacillus*, *Alcaligenes*, *Achromobacter*, *Paracoccus*, *Moraxella*, *Veillonella*, *Desulfobulbus*, *Vibrio* and *Thermoanaerobacterium*.

3.4. Preliminary Study Results

A preliminary study was carried out before the main systems were created. In this study, unlike the above conditions for whey, whey was heated at 100 °C and used without any filtration process. System-2 was operated for 17 days using only sediment-1, and resistance and volt measurements were taken. The measurements taken and the power density calculations made from these measurements are described in the following chapters.

3.4.1. Evaluation of Electrical Potential Developed in the Microcosm

During the 2nd to 9th hours of operation, the system's resistance averaged 9 kΩ, as illustrated in Figure 3-6. Between the 9th and 35th hours, the system's resistance exhibited significant oscillations, peaking at 1050 kΩ. The average resistance of the system was 8 kΩ from day 35 to day 62. At hour 62, the battery depleted, rendering the process from hour 62 to hour 71 as null. The lifespan of the 9-watt batteries utilized in the system was assessed to be between 5 and 7 days, contingent upon their quality. Furthermore, at hour 71, the electrode at the cathode was perforated and substituted. Following the 71st hour, the system's average resistance was recorded at 2 kΩ. At the 230th hour, the system's resistance was roughly 100 kΩ. At hour 231, the multimeter battery depleted once more, resulting in a 4-hour data gap. The resistance was consistently maintained at 2 kΩ during the system's operational duration. Nonetheless, a data gap occurred between the 333rd and 353rd hours, attributable to battery depletion.

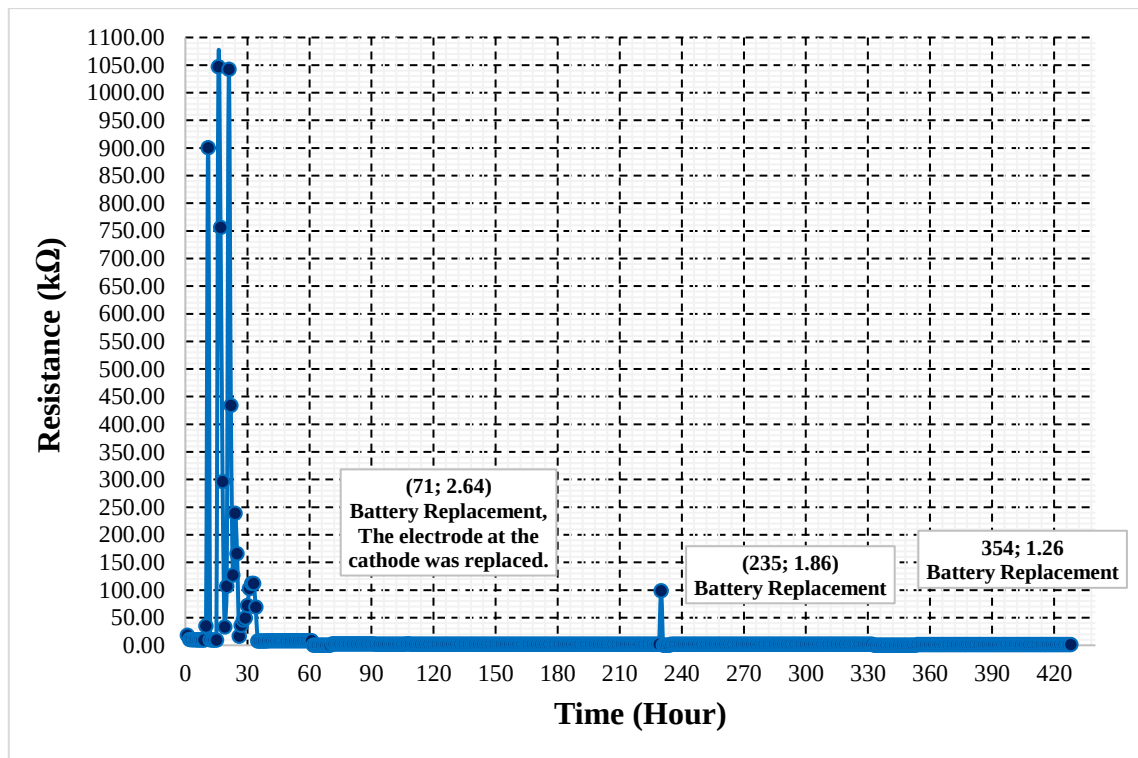


Figure 3.6: System-2 Resistance Measurements [79]

The data was not obtained between 8 and 26 hours and between 212 and 217 hours due to equipment malfunctions (values are recorded as 0.). Positive data were recorded until hour 155 in the system. A peak electrical potential of 264 mV was achieved within this range, as illustrated in Figure 3.7. Data ranging from 27 to 114 hours yielded more steady results, with an average computed at 232 mV. At the 288th hour, the multimeter's battery in the system depleted, having lasted an average of two weeks. The whey liquid in the system was seen to diminish with time and become exhausted. Consequently, the system's operation was halted around the 410th hour (day 17).

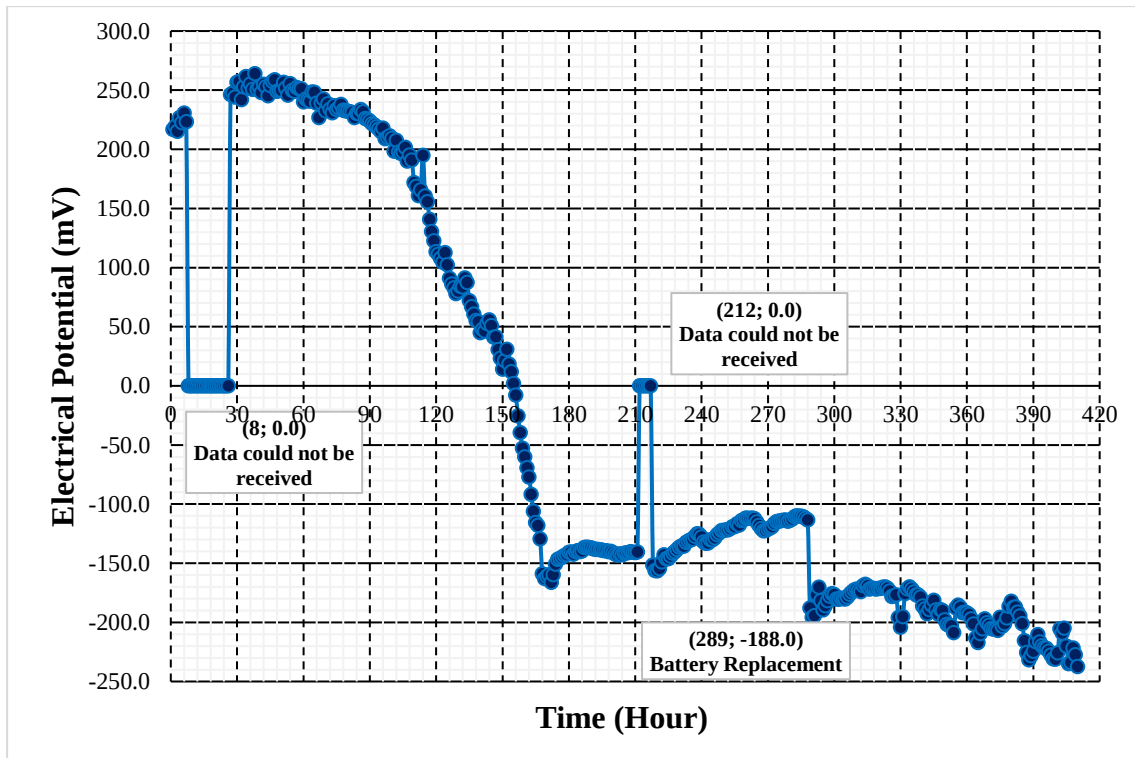


Figure 3.7: Electrical potential measurements of the system-2 [79]

The maximum and minimum power densities were determined using the corresponding maximum and minimum resistance values derived from the system's resistance measurements. The maximum value recorded was $279 \times 10^{-3} \text{ mW/cm}^2$ (shown in Figure 3.8), while the minimum value was $0.38 \times 10^{-3} \text{ mW/cm}^2$ (depicted in Figure 3.9).

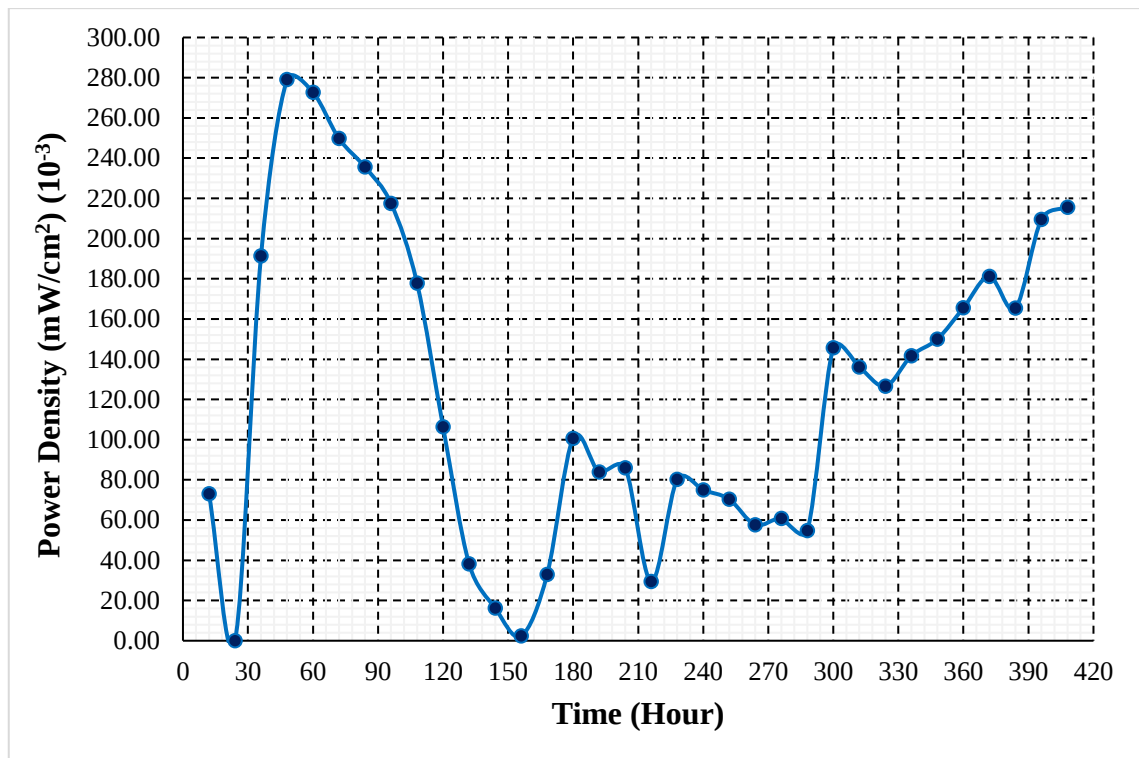


Figure 3.8: Maximum power densities of the system-2 [79]

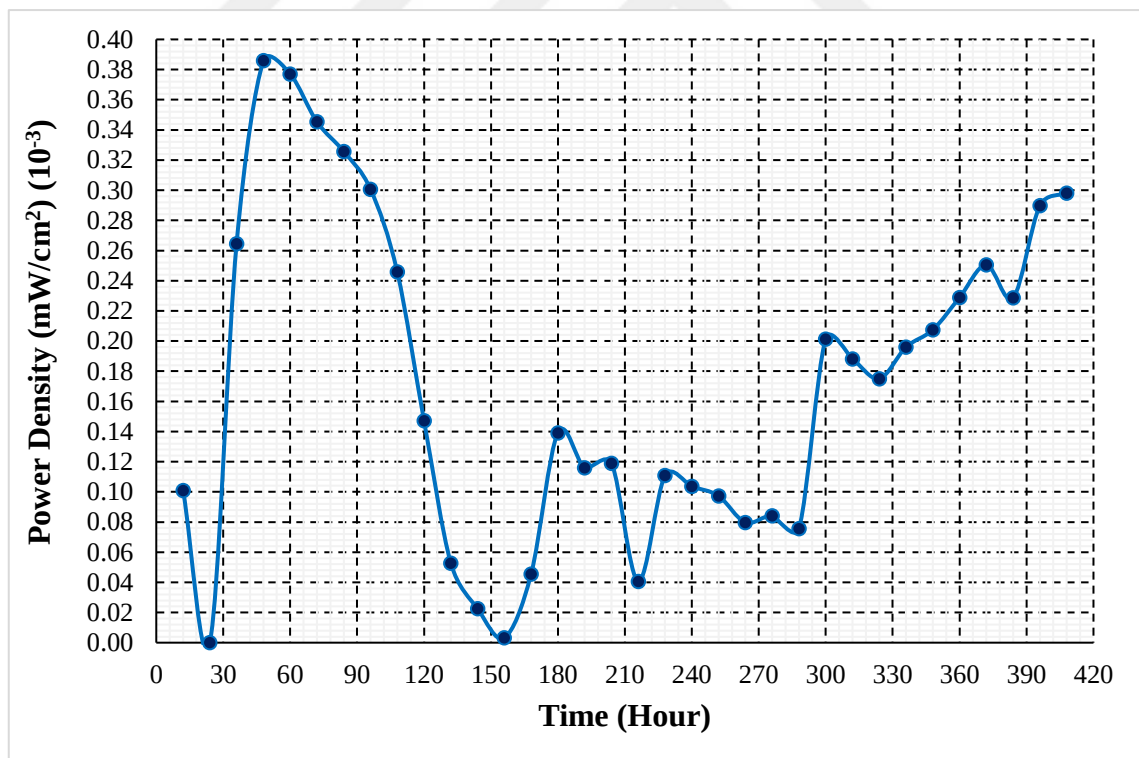


Figure 3.9: Minimum power density of the system-2 [79]

3.4.2. Evaluation of Wastewater Treatment Performance

The removal rates were 94% and 76%, respectively. The conductivity, pH, TDS, and temperature were measured, and the findings are presented in Table 3.4. The table indicates that the system and ambient temperatures remain relatively constant. Nonetheless, the conductivity and total dissolved solids (TDS) rise in all instances, indicating an increase in the concentration of ions inside the system. Furthermore, the pH of the whey elevated.

Table 3-4: Parameter values of the whey before and after putting it in the system [79]

Parameters	Before treatment	After treatment
pH	4.3	5.7
Conductivity ($\mu\text{S}/\text{cm}$)	5840	12000
TDS (ppm)	2190	3710
System Temperature ($^{\circ}\text{C}$)	16	14
Ambient Temperature ($^{\circ}\text{C}$)	17	16

3.4.3. Conclusion of Preliminary Study

As a result of the preliminary study, it was found that the electrical potential obtained in about six days fell below zero. It was determined that insufficient samples remained in the system to assess characteristics such as pH, conductivity, and TDS. For this reason, it was decided to keep the duration of the measurements as 3 days.

The measurements were conducted without the application of constant external resistance. Consequently, to achieve more reliable results, the voltage and power density can be recalibrated with external resistance by ascertaining a value approximating the internal resistance of the system.

From the results obtained, it was determined that organic matter was removed in the system and a maximum power density of $279 \times 10^{-3} \text{ mW}/\text{cm}^2$ was obtained. The results showed that significant power potential could be realized, and the decision was made to continue operating the system for even better results. Furthermore, high removal rates (94% and 76%) were obtained, thus demonstrating the feasibility of using this configuration.

Temperature was found to have no effect on the measured parameters, while pH, conductivity, and TDS all rose. The decision was made to ascertain the potential causes of this circumstance.

3.5. Evaluation of Electrical Potential and Resistance in the Microcosm

To further understand the operating principles of the systems in various conditions, the systems were tested with pure water without microorganisms, pure water with microorganisms, sucrose solution with microorganisms, and whey with microorganisms. In every measurement situation, sediments with specific chemical compositions as outlined in section 3.2 were used. During these operations, the electrical potential and resistance of the systems were recorded throughout a 24-hour period. The daily average power of the systems was calculated with the measurements obtained. In addition, 24-hour averages of the measured resistances were taken so that these systems, which were operated without resistor, could be operated with resistor in the next step. Using these estimated resistors, the systems were run for three days each using sucrose solution and whey in both resistance- and resistance-free conditions.

The acquired results are described in the subsections below.

3.5.1. Comparison of system-1 according to different sediment contents

Whey yielded the highest resistance measurement (average 29 MΩ) when the resistance results for the system-1 were compared. Upon analyzing the data presented in Figure 3.10, it can be inferred that sucrose solution exhibits the least resistance, at roughly 2 MΩ. Throughout the measuring process, it was noted that the resistance of pure water increased continuously, and variations were seen in the resistance value found in whey. The resistance of pure water was determined to be 18.5 MΩ, whereas the resistance of pure water with microorganisms was found to be 10 MΩ on average.

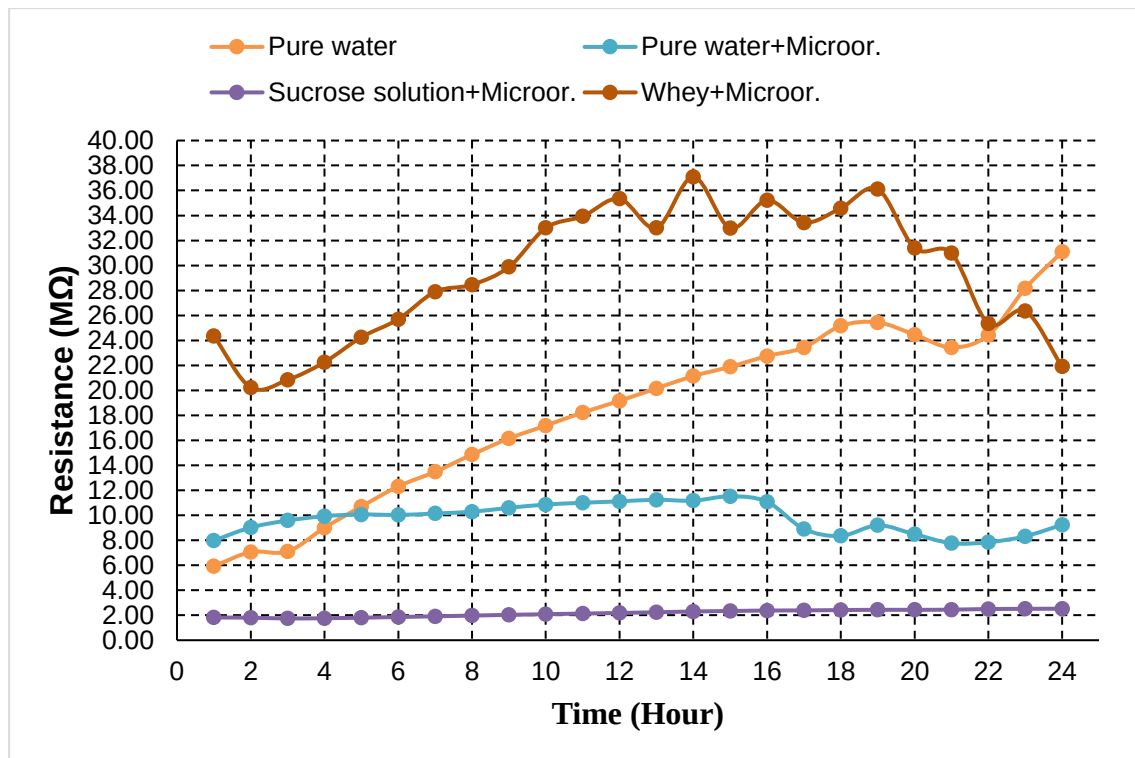


Figure 3.10: Resistance comparison of system-1 according to sediment-1

With sediment-2 which has a slightly different chemical structure, the system with the same characteristics was run, yielding the measurement findings shown in Figure 3.11. Unlike the previous measurement, the maximum resistance in this measurement was obtained when pure water was used, and the average value was 14 MΩ. The resistances of the system measurements in which pure water + microorganism, sucrose solution + microorganism, and whey + microorganism are added are similar and switch to a steady state at 2 MΩ. The lowest resistance was found to be 1.7 MΩ for the whey condition, 2 MΩ for the pure water microorganism condition, and 2.4 MΩ for the sucrose solution + microbe condition when the resistance averages were analyzed.

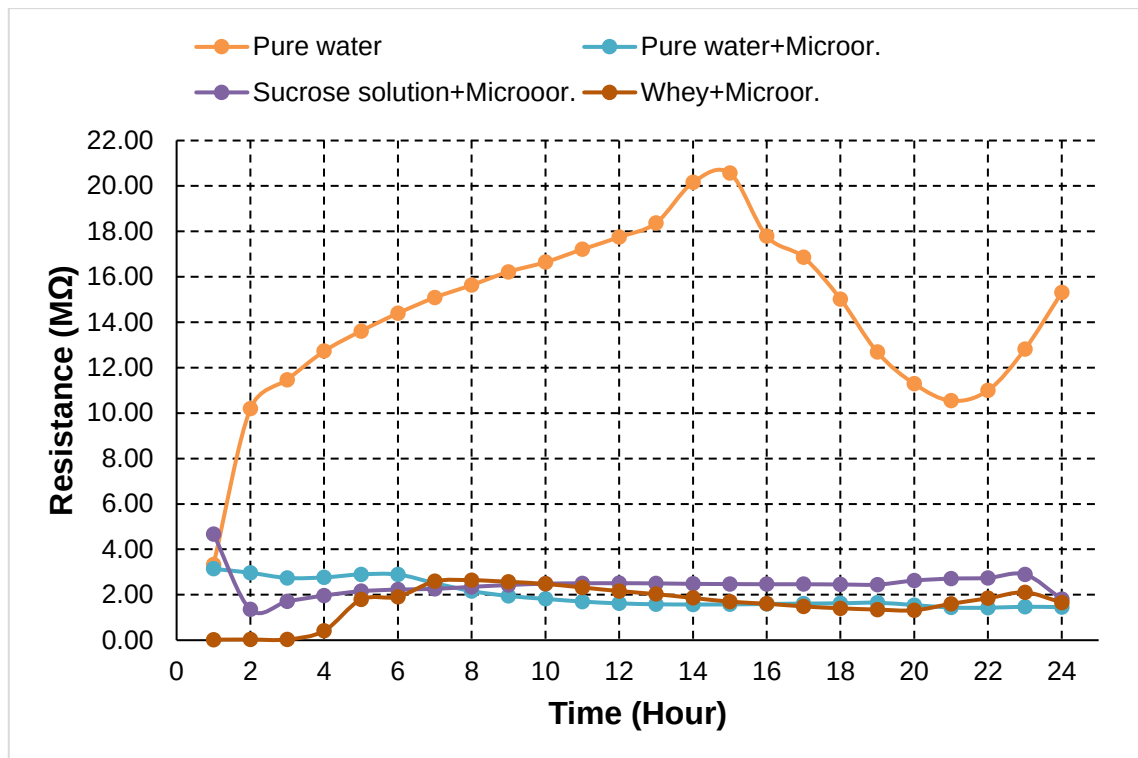


Figure 3.11: Resistance comparison of system-1 according to sediment-2

According to the electrical potential measurement data obtained using the sediment-1, the highest average electrical potential was achieved from whey, with an average of 343 mV. Upon examining the other possibilities, it was concluded that the averages were quite like each other. Pure water with microorganisms, pure water, and sucrose solution with microorganisms produced potentials of 242, 264, and 266 mV, respectively. Except for the pure water + microbe condition, the other three cases stabilized between 280 mV and 290 mV after 24 hours, given in Figure 3.12.

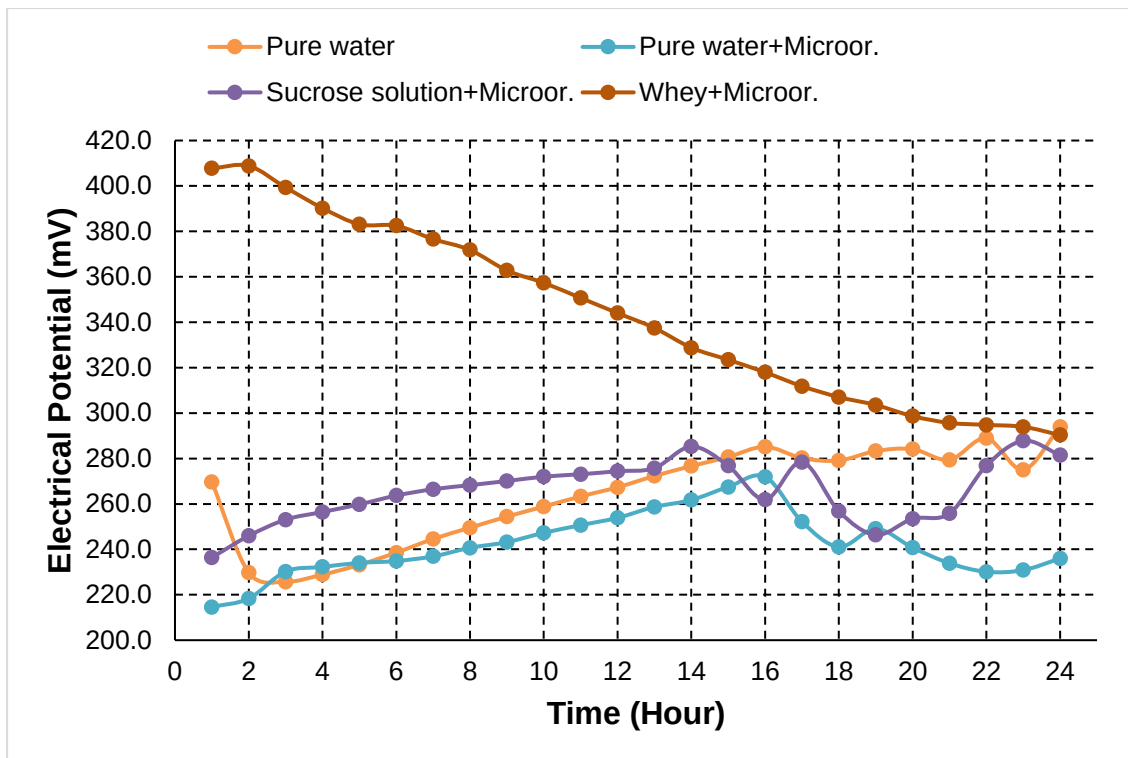


Figure 3.12: Electrical potential comparison of system-1 according to sediment-1

As illustrated in Figure 3.13, in the presence of sediment-2, sucrose solution (380 mV on average) had the highest electrical potential, followed by whey (301 mV on average). The average electrical potential of pure water was 257 mV, but the addition of microbes to the medium caused this potential to decrease to 228 mV.

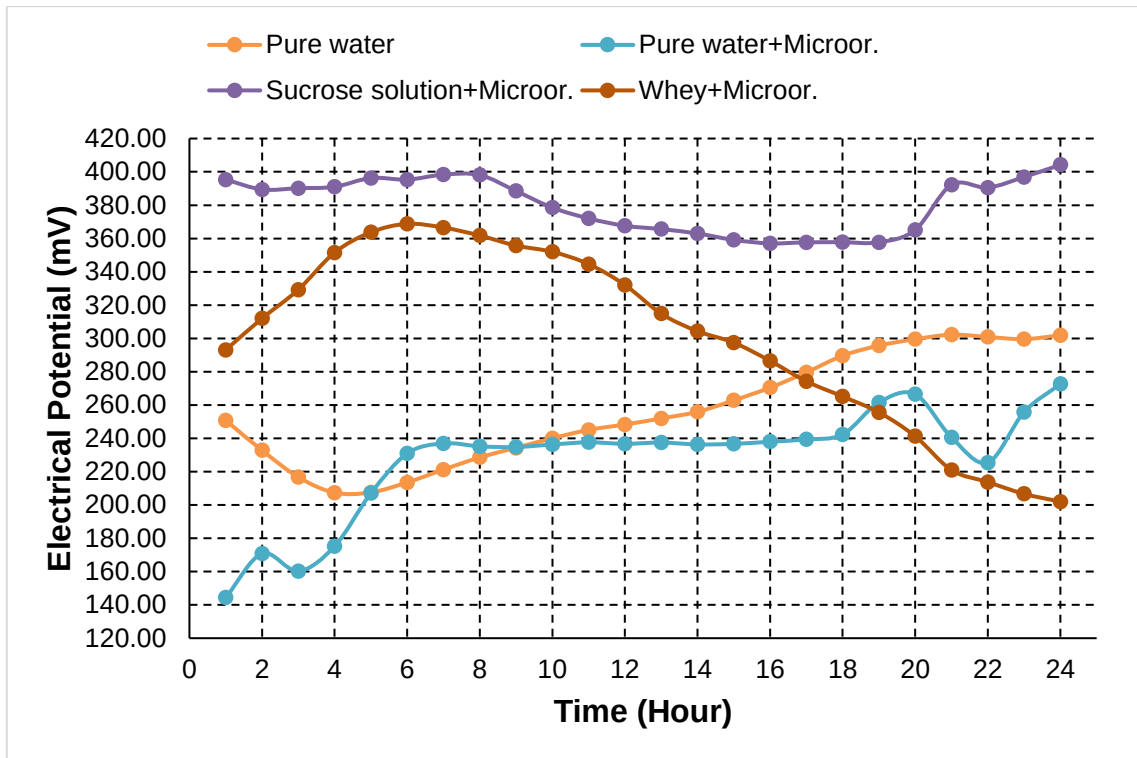


Figure 3.13: Electrical potential comparison of system-1 according to sediment-2

3.5.2. Comparison of system-2 according to different sediment contents

The average resistance of system-2, when assessed in accordance with sediment-1, is often about 30 k Ω (as shown in Figure 3.14), except for situations in which whey is present. The resistance scale is seen to rise from k Ω to M Ω upon the addition of whey to the system. It is evident from Figure 3.15 that at the end of the 24-hour period, the resistance value stabilized at 8 M Ω . In addition, the average of the collected data indicated that the resistance was 8 M Ω on average.

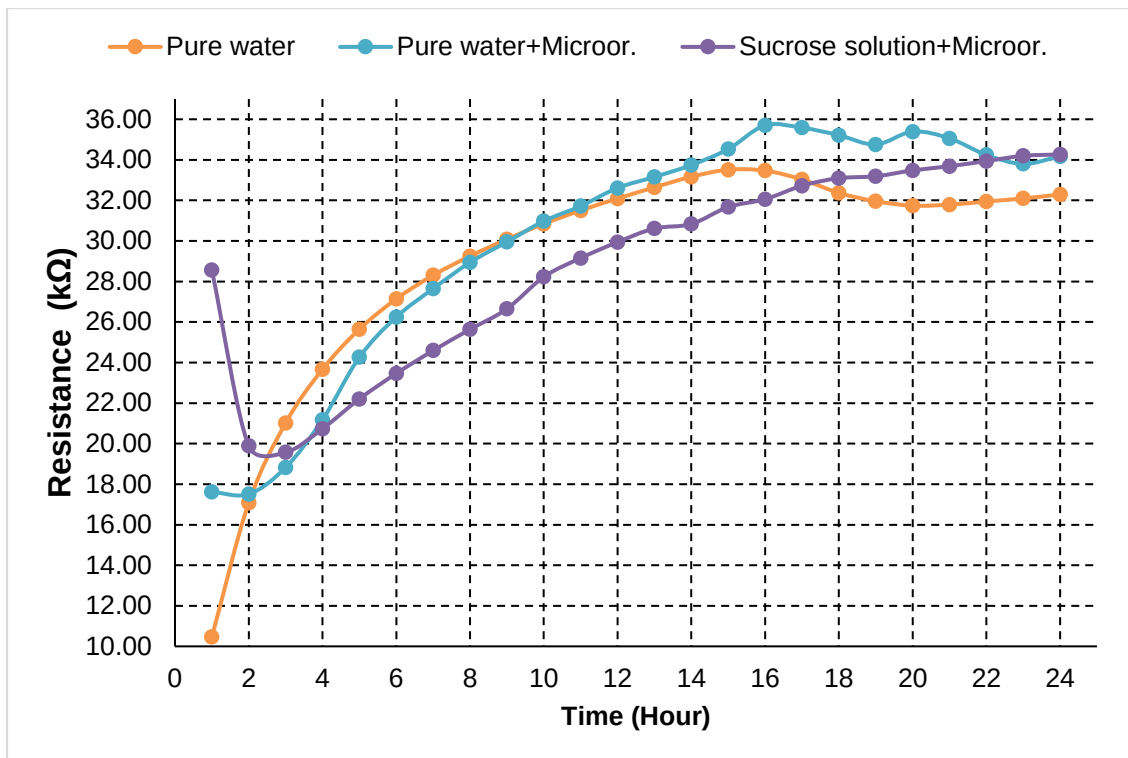


Figure 3.14: Resistance comparison of system-2 according to sediment-1

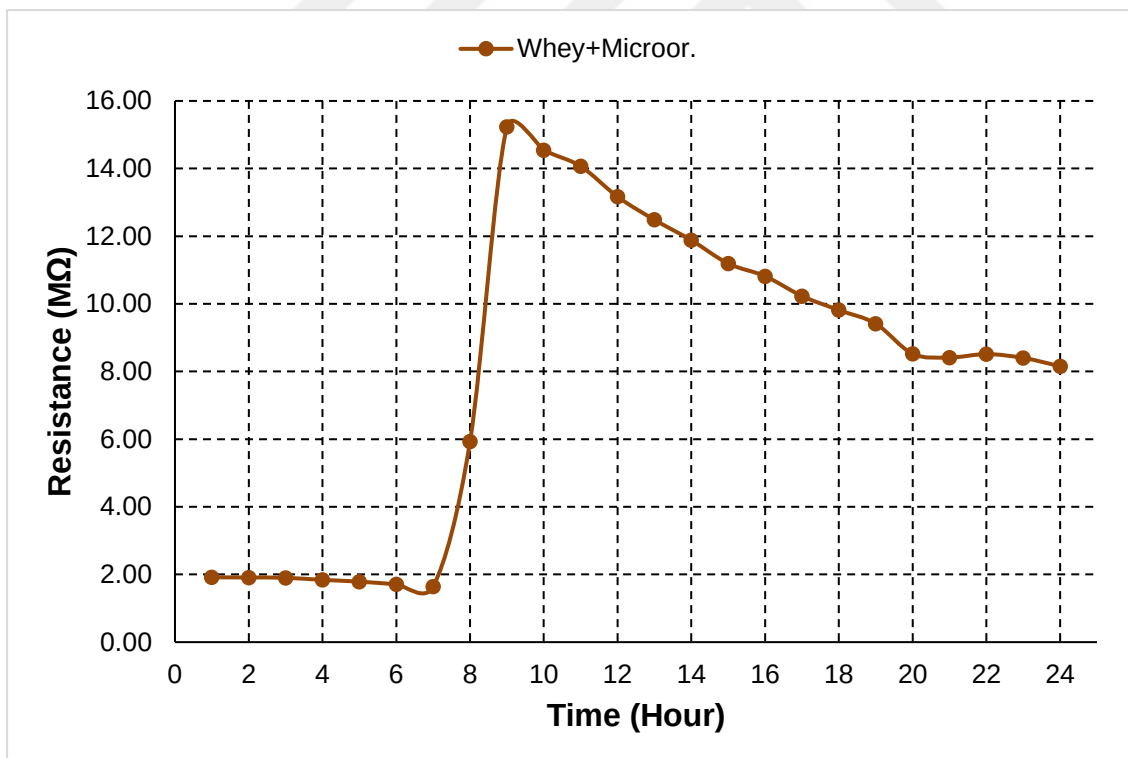


Figure 3.15: Resistance of system-2 with whey according to sediment-1

It is found that all conditions stabilize after 24 hours when sediment-2 is added to system-2. As shown in Figure 3.16, the stabilized resistances are 41 kΩ for pure

water, 31 k Ω for pure water + microorganism, 22 k Ω for sucrose solution + microorganism and approximately 30 k Ω for whey + microorganism. The mean resistances were 63 k Ω for whey + microorganism, 41 k Ω for pure water, 27 k Ω for pure water + microorganism, and 16 k Ω for sucrose solution + microorganism.

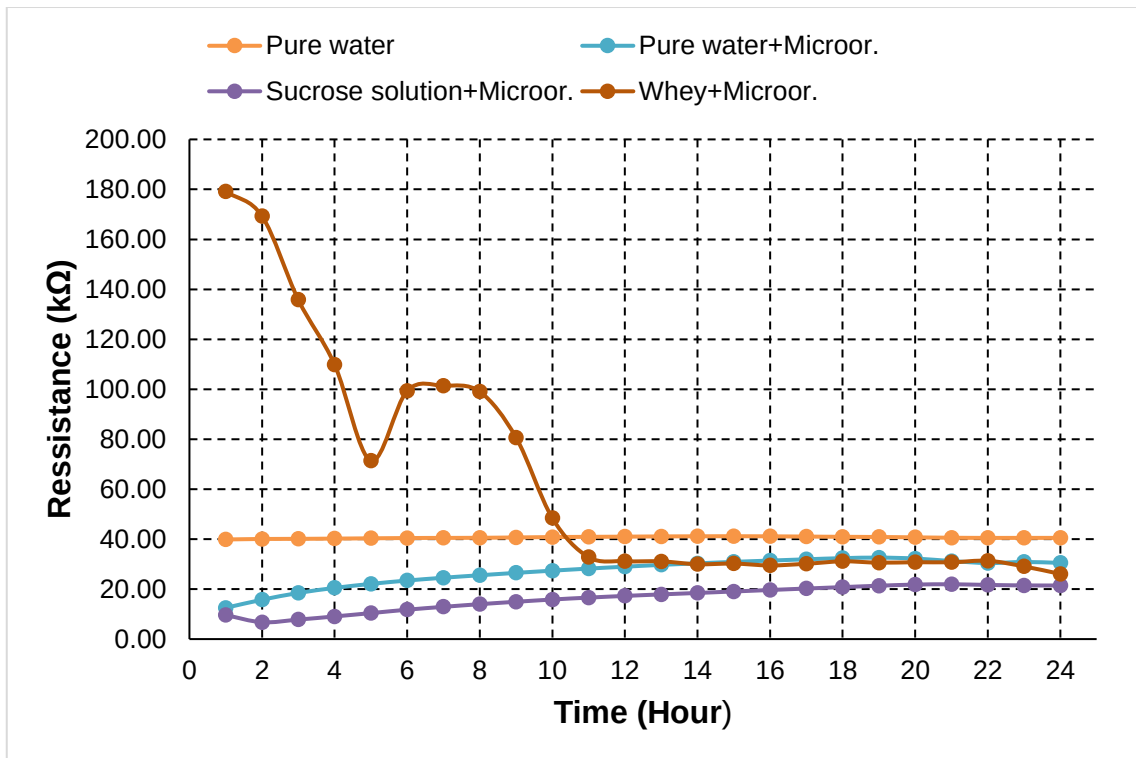


Figure 3.16: Resistance comparison of system-2 according to sediment-2

When the electrical potential of system-2 is assessed for sediment-1, it is observed that, except for whey, negative volts are produced. Every scenario yielded stable values, as shown in Figure 3.17. The values for pure water are -47 mV, pure water + microorganism is -46 mV, sucrose solution + microorganism is -8 mV, and whey + microorganism is 240 mV. The average values of -45 mV, -51 mV, -7 mV, and 253 mV were found by measurements taken in the same order.

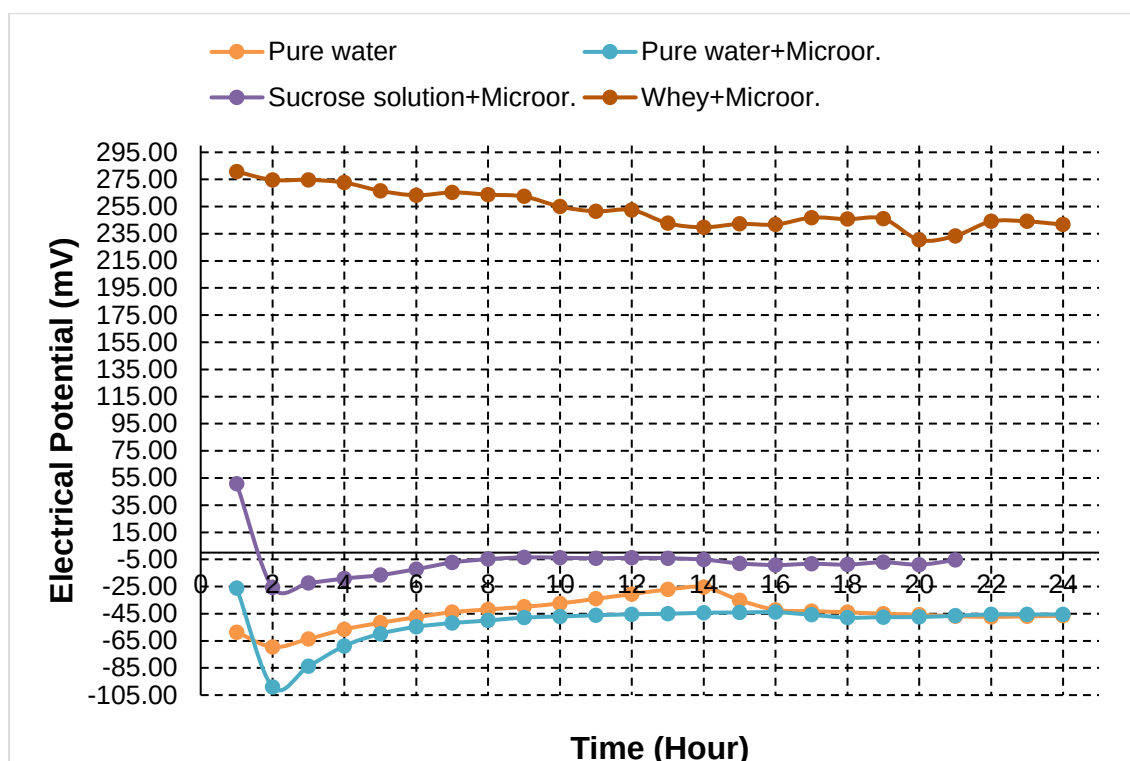


Figure 3.17: Electrical potential comparison of system-2 according to sediment-1

In system-2, no positive electrical potential value could be detected as Figure 3.18 shows. The mean electrical potential values of -37 mV for pure water, -42 mV for pure water + microorganism, -30 mV for sucrose solution + microorganism, and -158 mV for whey + microorganism were discovered, based on average values made from the results collected.

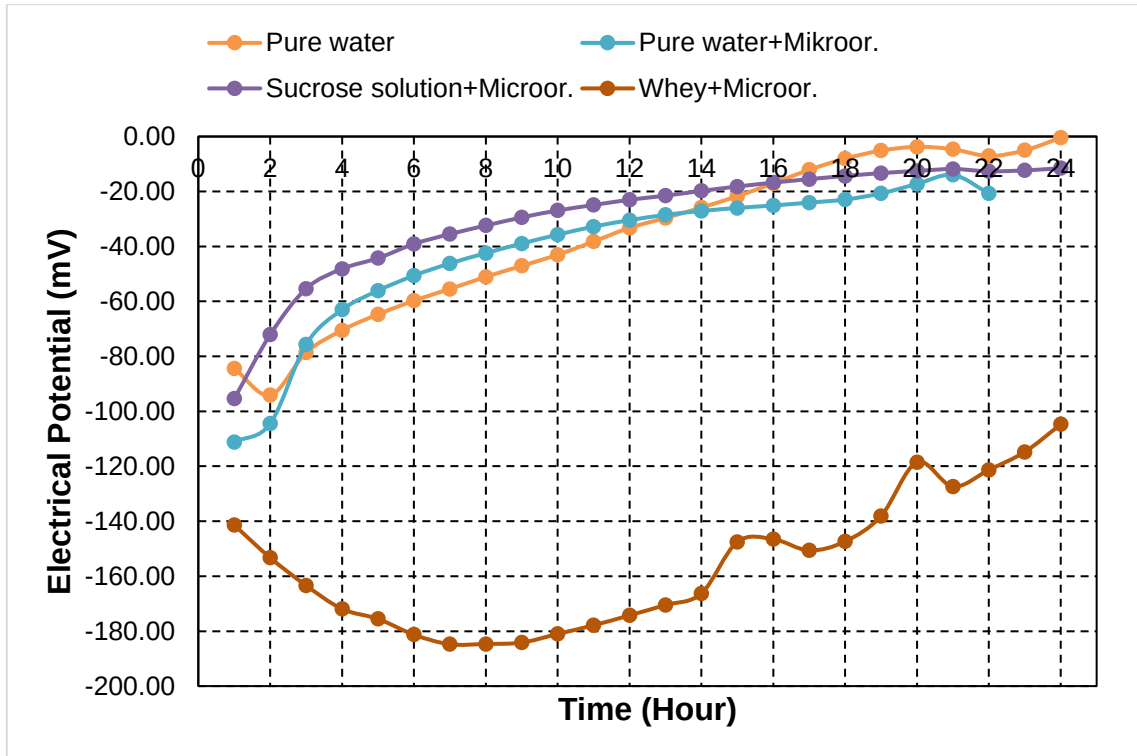


Figure 3.18: Electrical potential comparison of system-2 according to sediment-2

3.5.3. Evaluation of Whey and Sucrose Solution Results

After organizing the data obtained in sections 3.5.1. and 3.5.2. for pure water, whey and sucrose solution, Table 3.5 was generated.

In general, the resistance values obtained from system-1 are larger than system-2 under all conditions. It can be said that the main reason for this is that the distance between the anode and cathode is greater in system-1 than in system-2. Another reason may be that the conductivity of system-1 is lower than system-2 due to the dilution of whey.

When pure water conditions for sediment-1 are compared in both systems, it is seen that the resistance values obtained are higher in the presence of sucrose solution but lower in the presence of whey. The reason the resistance of pure water is less than that of sucrose solution is that pure water has low ionic content, which means rendering it an inadequate conductor of electricity. Conductivity significantly influences SMFC efficiency, with low-conductivity settings like pure water resulting in increased resistance in MFCs [111]. Although whey has higher conductivity than pure water and sucrose solution, its resistance is higher. This is because sucrose in the structure of sucrose solution is a simpler carbon source

that can be easily used by microorganisms. However, the structure of whey is more complex, and it is more difficult for microorganisms to access the carbon source.

The resistance values obtained for sediment-1 are higher than those for sediment-2 in both systems when examined.

From the results, it was observed that the electrical potential measurements made only with pure water were higher than the measurements taken after the microorganism entered the system. The main reason for this may be that in the presence of microorganisms, metabolic activities take place, and these activities change the chemistry of pure water.

Examining the electrical potential measurements for system-1 and -2, it became clear that sediment-1 had the greatest value for whey.

Following examination of Table 3.5, in general, the electrical potential measurements showed that negative values were obtained when sediment-2 was used. This is evidence that the nitrogen, sulfur and iron compounds in whey, which can be electrical acceptors, are utilized by the microorganisms in the system.

It was determined that the electrical potential derived from system-1 was greater than those acquired from system-2 for both sediments except under pure water conditions for sediment-2. It can be concluded that the configuration of system-1 is preferable in terms of electrical potential. The main reason for this situation is the use of diluted whey in system-1 and undiluted whey in system-2. Because microorganisms can work more easily in diluted media than in undiluted media. The reason for this is since the density of organic matter in undiluted media may adversely affect the working performance of microorganisms.

All electrical potential values obtained from sucrose solution and whey are lower than the calculated theoretical electrical potential value (1.2 V). This is an indication that external factors affect the electrical potential value measurements.

Table 3.5. Comparison of mean electrical potential and resistance values of sucrose solution and whey for 24 hours

System-1								
Sediment	Sediment-1				Sediment-2			
Type of liquid	Pure water	Pure water+ Microor.	Sucrose solution + Microor.	Whey+ Microor.	Pure water	Pure water+ Microor.	Sucrose solution + Microor.	Whey+ Microor.
Resistance	18.5 MΩ	9.7 MΩ	2 MΩ	29 MΩ	29 kΩ	30 kΩ	2.4 MΩ	1.7 MΩ
Electrical Potential	264 mV	242 mV	266 mV	343 mV	-44.6 mV	-51 mV	380 mV	301 mV

System-2								
Sediment	Sediment-1				Sediment-2			
Type of liquid	Pure water	Pure water+ Microor.	Sucrose solution + Microor.	Whey+ Microor.	Pure water	Pure water+ Microor.	Sucrose solution + Microor.	Whey+ Microor.
Resistance	14.4 MΩ	2 MΩ	30 kΩ	8 MΩ	40.6 kΩ	27 kΩ	16 kΩ	63 kΩ
Electrical Potential	256.5 mV	227.5 mV	-8 mV	240 mV	-37.40 mV	-41.5 mV	-30 mV	-158 mv

To study the operation of the systems in more detail, the electrical potential of each system was measured again. The next section describes these measurements, and the power densities are calculated from them.

3.6. Assessment of Electrical Potentials and Power Density under resistive and non-resistive situations

Volt measurements were obtained from systems-1 and -2 for 3 days each to assess their electrical potential and power densities. Furthermore, the two systems were run on both sediments to investigate how the chemical makeup of the sediments affected the volt measurement values. First, measurements were taken without resistor, and then, using the average resistance values given in Table 3.5, measurements with resistor were also made.

In the subsections that follow, the measurement findings are assessed.

3.6.1. Measurement of resistor-free Electrical Potentials

Upon analyzing system-1 for both sediments, it was seen that the electrical potential derived from whey was consistently higher than those acquired from sucrose solution. Nevertheless, the electrical potential derived from sediment-1 surpasses that obtained from sediment-2. The maximum electrical potential obtained from sediment-1 for whey is 592 mV and for sucrose solution, this value is 31 mV, as shown in Figure 3.19. The highest electrical potential value obtained from sediment-2 was 445 mV for whey and 408 mV for sucrose solution, which is understood from Figure 3.20.

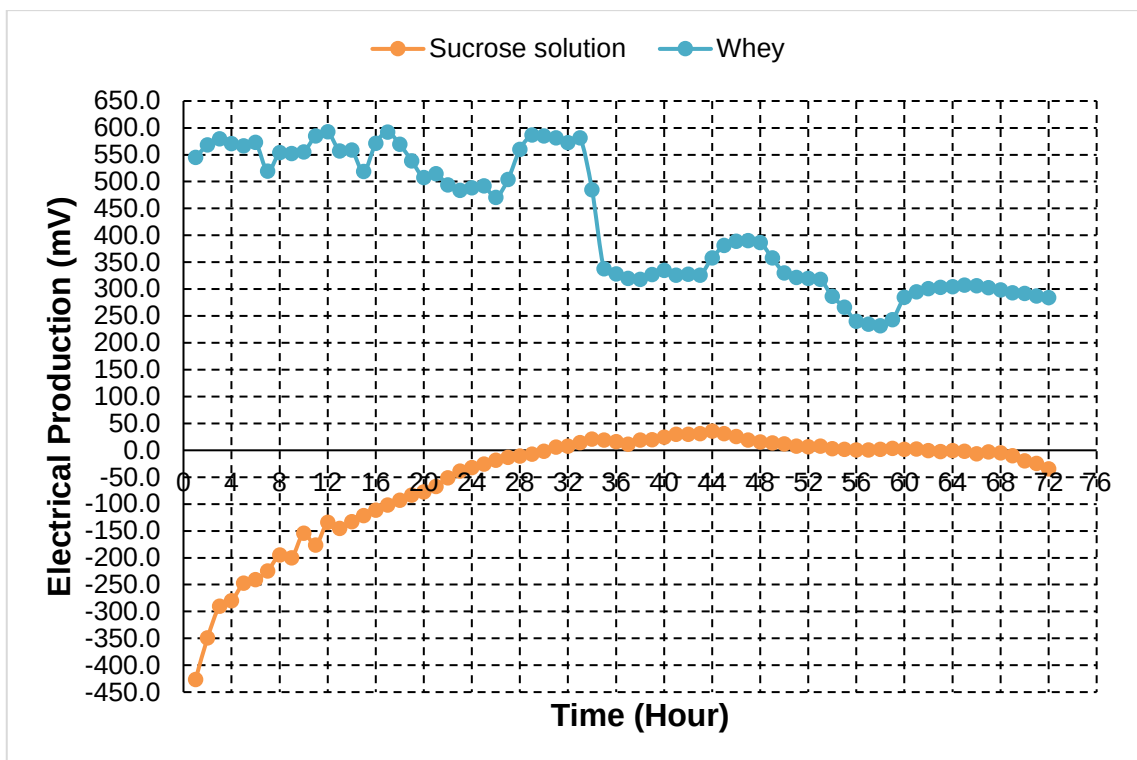


Figure 3.19: Comparison of electrical potential of system-1 based on sediment-1 without resistor

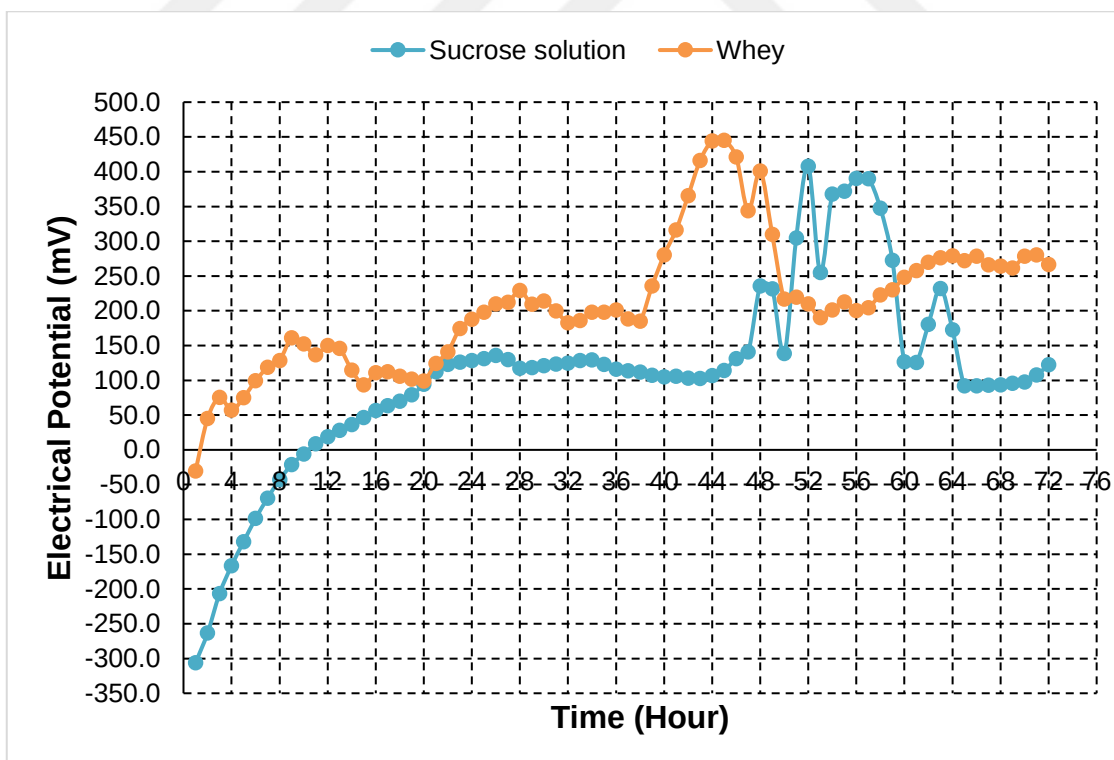


Figure 3.20: Comparison of electrical potential of system-1 based on sediment-2 without resistor

When evaluated according to the sediments in system-2, it was determined that whey gave a high electrical potential value. Among the sediments, higher electrical potential was obtained for whey in sediment-2. As sediment-1 was used, a maximum of 165 mV (Figure 3.21) was obtained for whey, but no positive value was measured for sucrose solution. In sediment-2, these values are 395 mV for whey and 12 mV for sucrose solution (Figure 3.22).

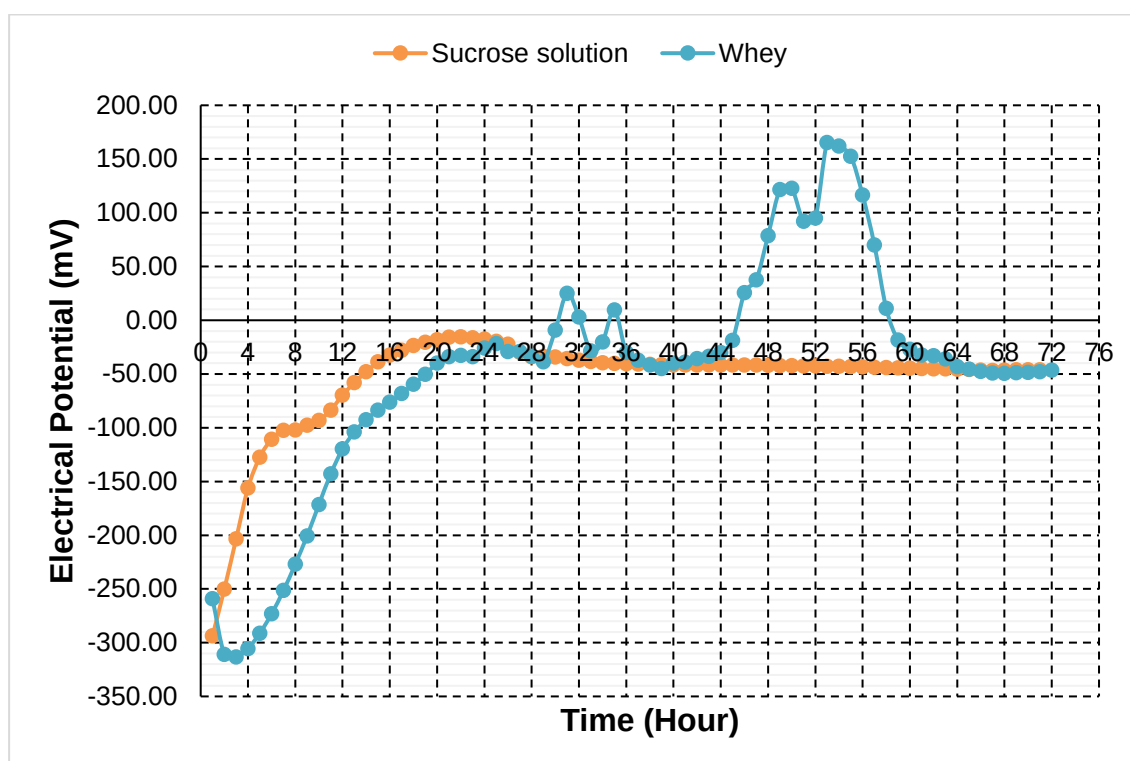


Figure 3.21: Comparison of electrical potential of system-2 based on sediment-1 without resistor

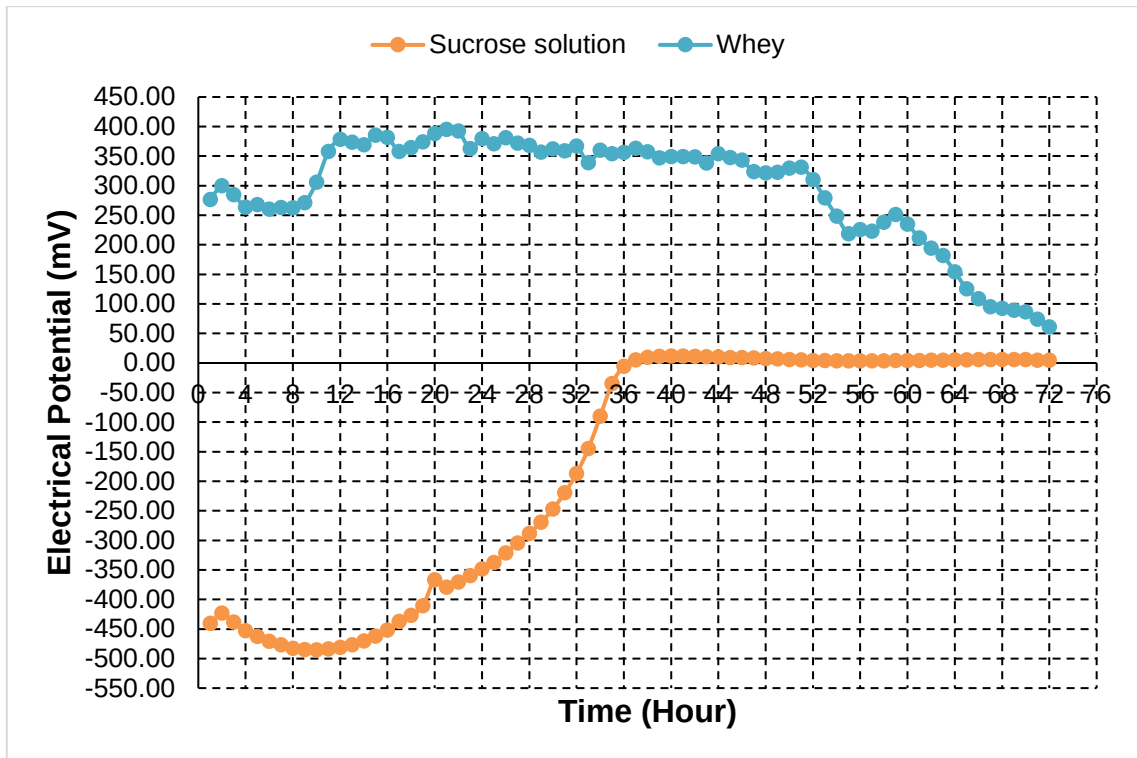


Figure 3.22: Comparison of electrical potential of system-2 based on sediment-2 without resistor

3.6.2. Measurement of Electrical Potentials in the presence of resistor

In the presence of resistor, the potential values obtained are better than in the case where sediment-1 is used, and whey is present. System-1 with sediment-1 yielded maximum electrical potential values of 207 mV and 170 mV for whey and sucrose solution, respectively (Figure 3.23). Maximum electrical potentials of 109 mV for whey and 9 mV for sucrose solution were obtained from sediment-2, given in Figure 3.24. When the values obtained with sediment-1 were analyzed, although positive values were measured for sucrose solution, no positive values were obtained for whey after the 58th hour. For sediment-2, no positive values were measured after the 10th hour for both sucrose solution and whey.

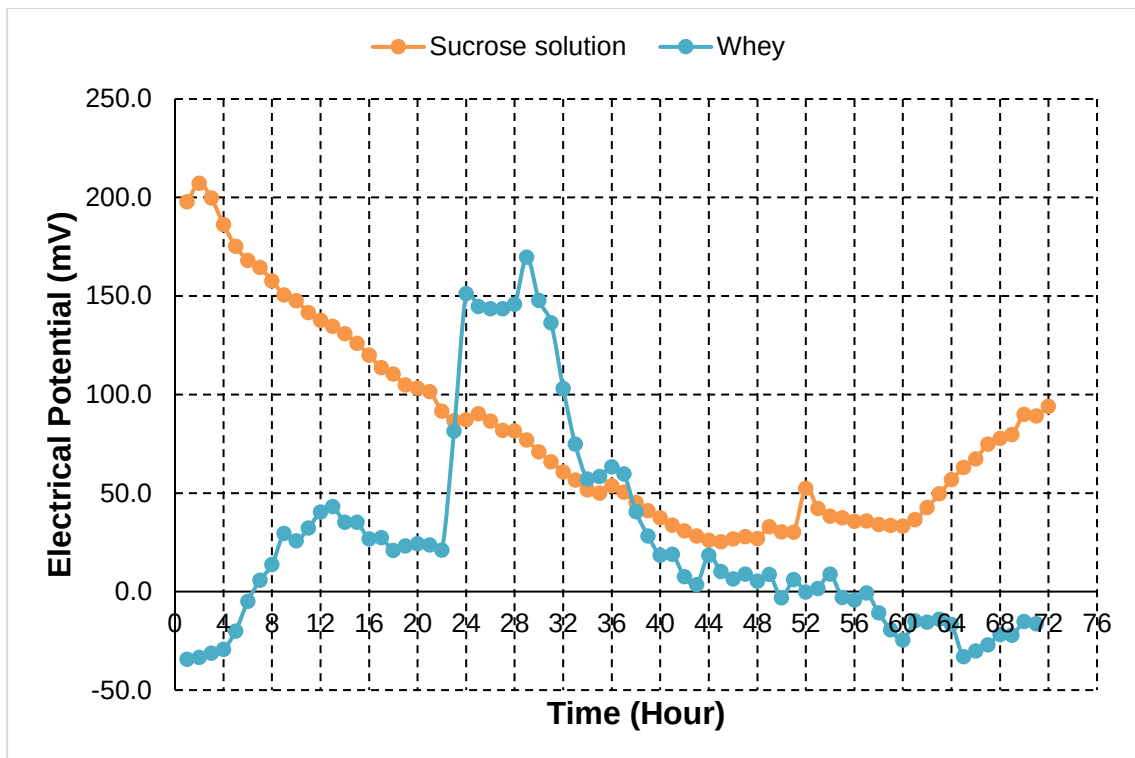


Figure 3.23: Comparison of electrical potential of system-1 based on sediment-1 with resistor

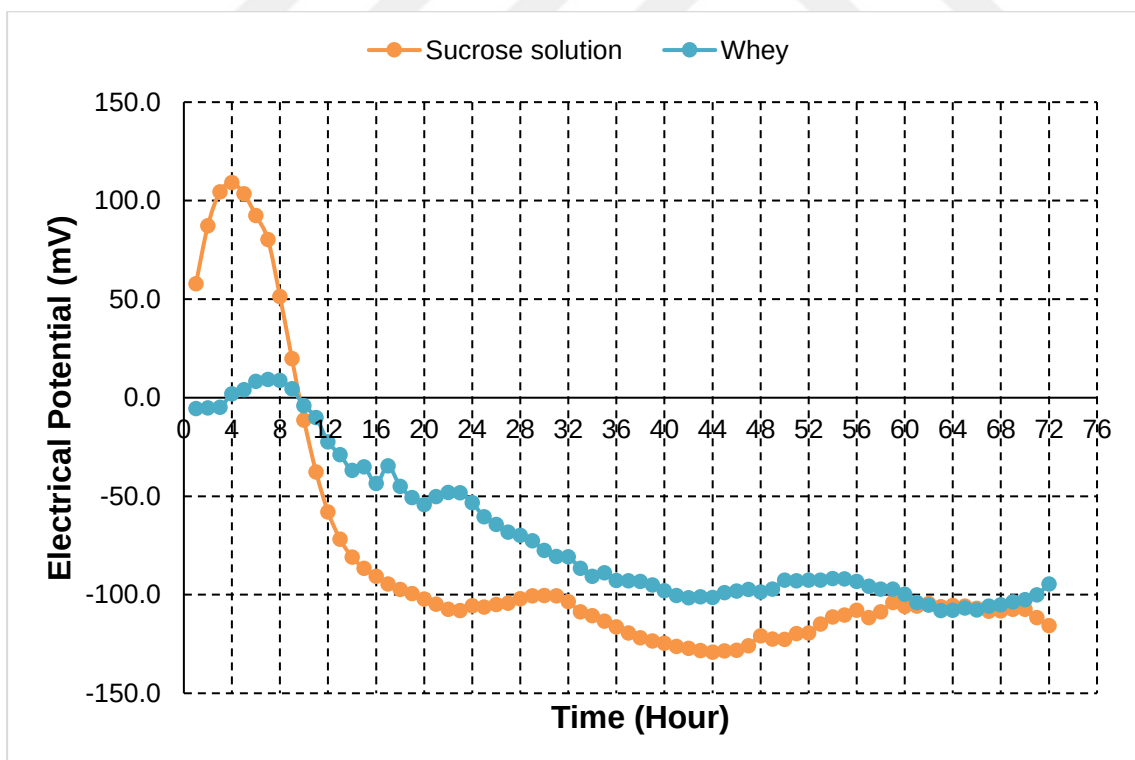


Figure 3.24: Comparison of electrical potential of system-1 based on sediment-2 with resistor

The measurements for sediment-1 in system-2 for sucrose solution and whey are broadly similar up to 59th hour. However, the maximum electrical potential of whey (357 mV) surpasses that of sucrose solution (288 mV), which is given in Figure 3.25. After evaluating the measurements collected from sediment-2, it was found that no positive value was produced from the sucrose solution. Although most of the values obtained using sediment-2 were also negative, a maximum electrical potential of 33 mV was measured for whey as shown Figure 3.26 until 8th hour.

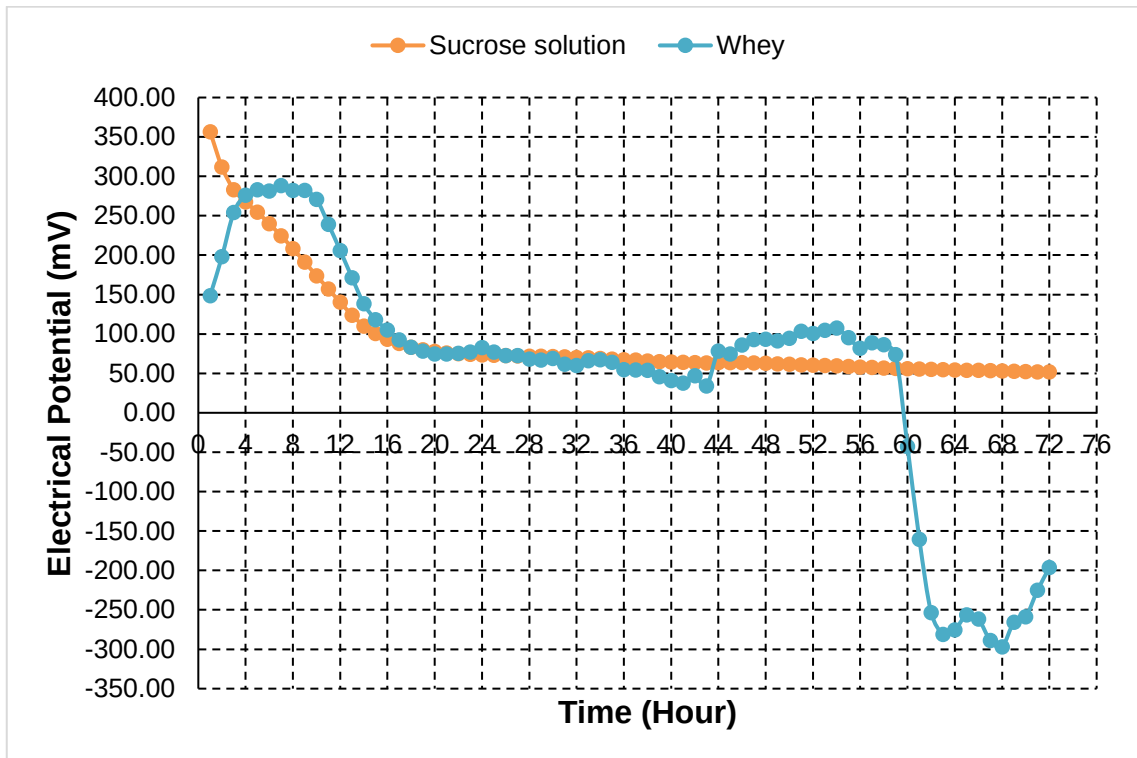


Figure 3.25: Comparison of electrical potential of system-2 based on sediment-1 with resistor

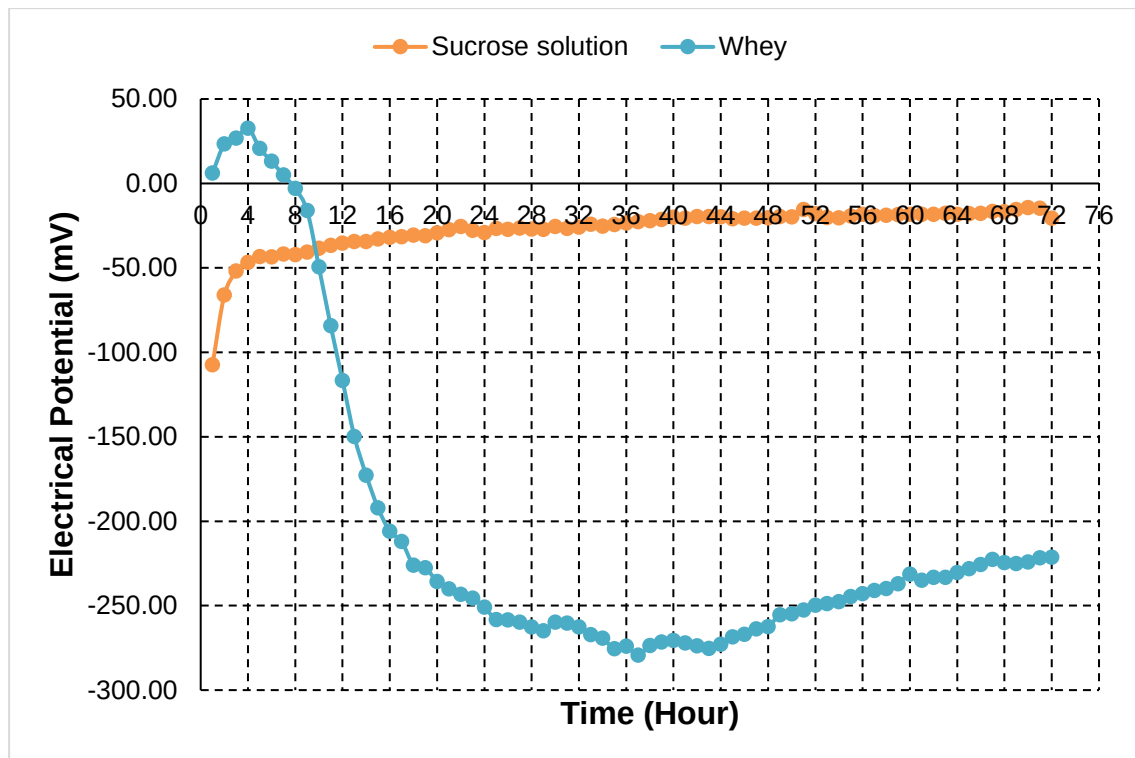


Figure 3.26: Comparison of electrical potential of system-2 based on sediment-2 with resistor

3.6.3. Evaluation of Electrical Potential Results and Calculation of Power Density

Table 3.6 was constructed by grouping the data acquired in sections 3.6.1. and 3.6.2. for whey and sucrose solution.

When all the measurements which were determined without resistor between maximum values were analyzed, it was understood that the highest electrical potentials were obtained from the whey placed in system-1. It is seen that system-1 with sediment-1 gives the highest electrical potential (592 mV) among the sediments. The maximum values obtained from system-2 indicate that a higher electrical potential (395 mV) is released in the presence of sediment-2.

Comparing the systems where resistors were connected, it is evident that system-2 provides greater value (307) for whey in sediment-1. Measurements results with resistors for system-1 by using whey from show that the value (207) obtained from sediment-1 is better.

Generally, the highest electrical potential values derived from whey are greater than those obtained from sucrose solution. Upon evaluating the data received

from the operation of system-1, it can be concluded that sediment-1 is the most suitable option for system-1.

Table 3.6. Comparison of maximum electrical potential values of sucrose solution and whey for 3 days

	System-1				System-2			
Sediment	Sediment-1		Sediment-2		Sediment-1		Sediment-2	
Type of liquid	Sucrose solution	Whey	Sucrose solution	Whey	Sucrose solution	Whey	Sucrose solution	Whey
Electrical Potential (without R)	31 mV	592 mV	408 mV	445 mV	-16 mV	165 mV	12 mV	395 mV
Electrical Potential (with R)	170 mV	207 mV	9 mV	109 mV	288 mV	307 mV	-14 mV	33 mv

The power densities of the systems during the 3-day period were determined by analyzing the electric potential generated from the studies. Calculations were made ignoring the negative electric potential in the graphs. The values used in the graphs are given in Appendix 2. The values in red in the table in APPENDIX 2 represent the values obtained from the positive electrical potential and the values in black represent the values obtained from the negative electrical potential.

After evaluating Figure 3.27, it was found that the most optimal outcomes were obtained when whey was placed in sediment-2. The maximum value for this situation is 66×10^{-5} mW/cm². The addition of sucrose solution to sediment-2 produced the second-highest value (39×10^{-5} mW/cm²). The maximum value of the electric potential obtained for the reaction in which whey was added to sediment-1 was 6.85×10^{-5} mW/cm².

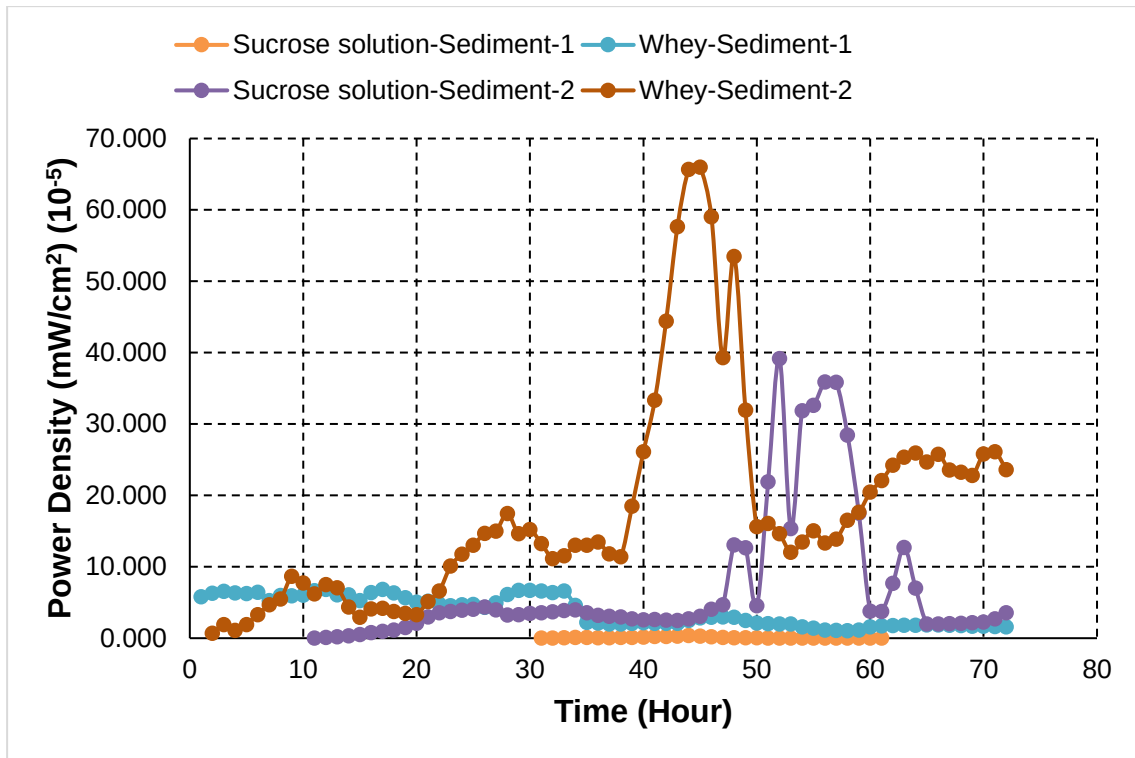


Figure 3.27: Comparison of power density of system-1 based on sediment-1 and -2 without resistor

According to Figure 3.28, the highest power density values were obtained in the presence of sucrose solution. The greatest of these values belongs to the case with sediment-1 with $22 \times 10^{-5} \text{ mW/cm}^2$. Looking at the other conditions, the second highest value was obtained from sediment-2 with sucrose solution ($6.2 \times 10^{-5} \text{ mW/cm}^2$).

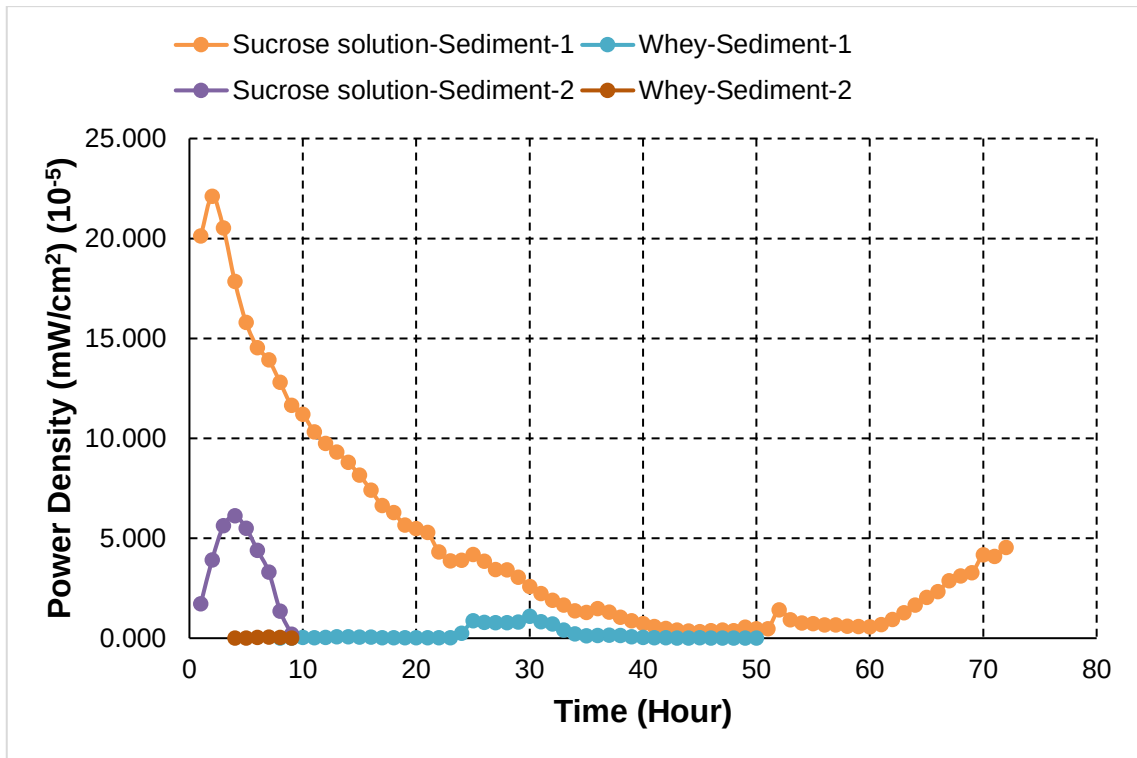


Figure 3.28: Comparison of power density of system-1 based on sediment-1 and -2 with resistor

The electrical potential values obtained from the sucrose solution system added to sediment-1 were all negative and were not evaluated. The positive value interval in which whey was added to sediment-1 (between hours 46 and 58) and the positive value interval in which sucrose solution was added to sediment-2 (between hours 37 and 72) overlapped and cannot be displayed on the graph.

Based on the potential results obtained when system-2 is not linked to any resistor, the measured values indicate that sediment-2 gives better power density result whey is introduced in it. The maximum value achieved was 1401×10^{-5} mW/cm² as shown in Figure 3.29. A maximum power density of 4.7×10^{-5} mW/cm² was achieved when sucrose solution was added to sediment-2.

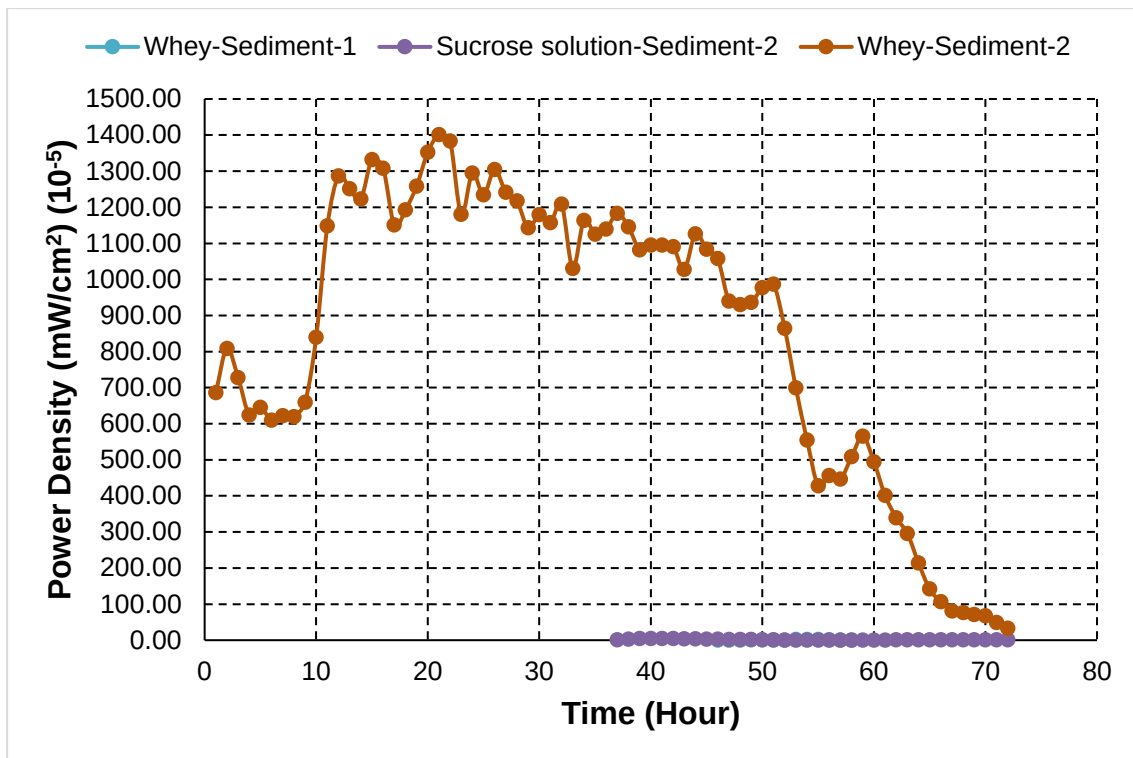


Figure 3.29: Comparison of power density of system-2 based on sediment-1 and -2 without resistor

The electrical potential values derived from the whey system incorporated into sediment-1 were negative and were not assessed. The range of positive values when whey was added to sediment-1 (between hours 1 and 59) and the range of positive values when whey was added to sediment-2 (between hours 1 and 7) are shown in Figure 3.30.

Following evaluating system-2 for resistance conditions, it was found that the circumstance including sucrose solution produced the highest power density ($4795 \times 10^{-5} \text{ mW/cm}^2$). The maximum power densities obtained when whey was added to sediment-1 and sediment-2 were 9.4×10^{-5} and $19.6 \times 10^{-5} \text{ mW/cm}^2$, respectively.

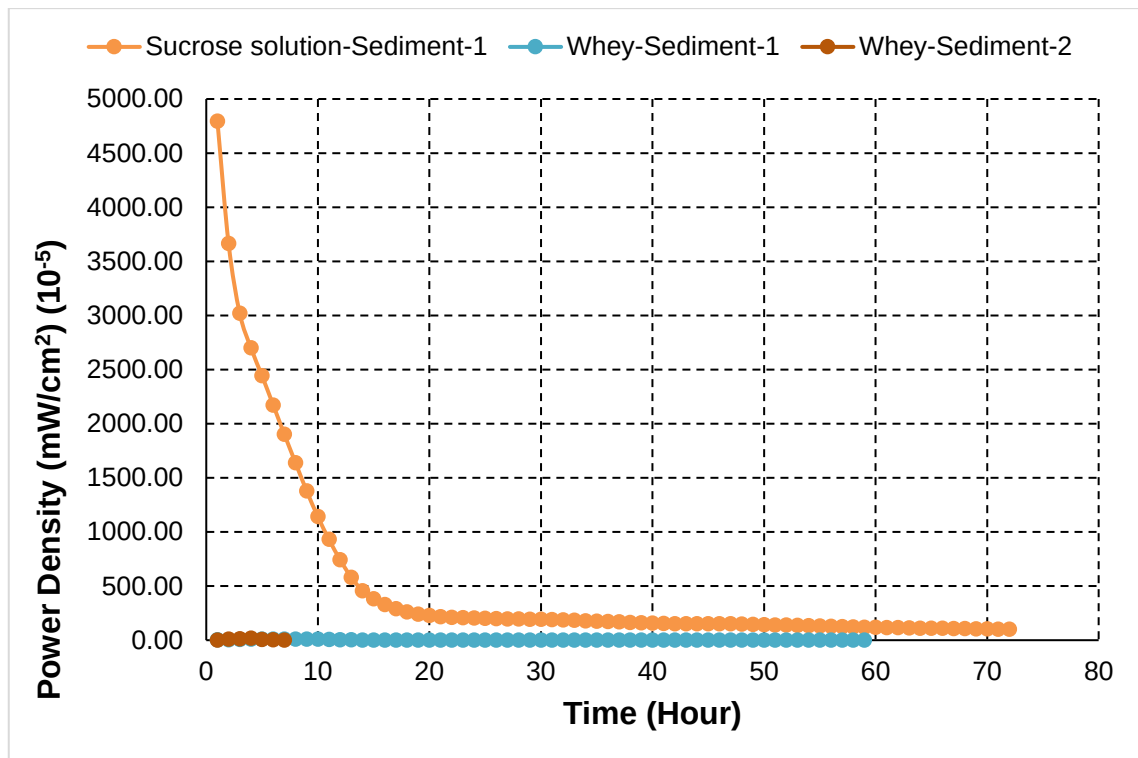


Figure 3.30: Comparison of power density of system-2 based on sediment-1 and -2 with resistor

When all the cases generated from the two different system configurations are analyzed, it is observed that the highest power density ($4795 \times 10^{-5} \text{ mW/cm}^2$) was obtained when sucrose solution was added to system-2 with sediment-1 and resistor. The second highest power density ($1401 \times 10^{-5} \text{ mW/cm}^2$) was obtained when whey was added to system-2 without resistor and with sediment-2. Based on these results, it was revealed that using sucrose solution may give better results than whey due to its simple chemical structure.

The following table (Table 3.7) was created to compare the power values obtained from the studies conducted with river SMFCs in the literature with the power values obtained from whey in this study.

By examination of the table, it is evident that the most significant power density resulting from whey removal in the study occurs when sediment-2 is placed in resistor-free system-2. For whey, although the electrical potential value is high for system-1 without resistor, this is not the case for the power density. This is because the measured resistances for sediment-1 and -2 of system-1 are in the order of $\text{M}\Omega$, whereas in the case of system-2 with sediment-2, the resistance is

in the order of $k\Omega$ (Table 3.5). In cases where whey is present, it can be said that generally, the best results are obtained from the conditions where sediment-2 is in the environment.

In studies with this type of MFCs, organic matter in the sediment is usually given to the microorganism as a substrate. This study distinguishes itself from other studies in this regard.

When compared with the studies in the literature, dairy wastewater and tofu wastewater were used in the studies that performed removal similar to the present study. In this context, no proton exchange membrane was put in the system in the study where dairy wastewater was used, similar to our study. The configuration of the system in the study is similar to system-1. However, it is also similar to system-2 in terms of the position of the electrodes in the system. In the study, a graphite electrode was used and an external resistance of $10\ \Omega$ was used. According to the study, the power density was $0.53\ \text{mW/m}^2$ [78]. The maximum power density value ($140\ \text{mW/m}^2$) obtained in this study is much higher than this value. However, when compared with the results ($217.14\ \text{mW/m}^2$ and $215.95\ \text{mW/m}^2$) obtained from the study using Tofu wastewater, it is understood that better results can be obtained from the study. In the study using tofu wastewater, the system configuration used is similar to the system-1 configuration in the present study. The main difference between this study and the present study is the use of stainless-steel wire mesh as the electrode [68].

When Table 3.7 is examined, the values found in general are similar and close to the values in literature. However, the same assessment does not apply to the resistant state of system-1 with sediment-1 and -2. Likewise, the value obtained from the non-resistant state of system-2 with sediment-1 is lower than the values obtained in the literature.

Table 3.7. Comparison of the maximum power density values with the values in the literature on river SMFCs studies

Literature	12 mW/m ² [44]	Types of liquids put into the system	River
	9 mW/m ²		
	1.9 mW/m ²		River
	8.8 mW/m ² [45]		
	7.5 mW/m ² [49]		Tidel river
	49 mW/m ²		
	29 mW/m ² [51]		River
	1.2 mW/m ² [55]		River
	33.5 mW/m ² [57]		River
	170 mW/m ²		
	35 mW/m ² [58]		River
	121 μ W/cm ² [59]		River
	2.36 mW/m ²		
	2.07 mW/m ² [61]		River
	217.14 mW/m ²		
	215.95 mW/m ² [68]		Tofu wastewater
	79 mW/m ²		
	38 mW/m ² [73]		Creek
	0.53 mW/m ² [78] *		Dairy wastewater

In this study	Without resistance	Whey	0.685 mW/m ²	Systems and Sediment Types	System-1, Sediment-1
			6.6 mW/m²		System-1, Sediment-2
			0.19 mW/m ²		System-2, Sediment-1
			140 mW/m²		System-2, Sediment-2
		Sucrose solution	0.036 mW/m ²		System-1, Sediment-1
			3.9 mW/m ²		System-1, Sediment-2
			0.47 mW/m ²		System-2, Sediment-2
					System-2, Sediment-2
	With resistance	Whey	0.11 mW/m ²		System-1, Sediment-1
			0.005 mW/m ²		System-1, Sediment-2
			0.94 mW/m ²		System-2, Sediment-1
			1.96 mW/m ²		System-2, Sediment-2
		Sucrose solution	2.2 mW/m ²		System-1, Sediment-1
			0.61 mW/m ²		System-1, Sediment-2
			479.5 mW/m²		System-2, Sediment-1

* This value was obtained using MFC and not SMFC. It is shown in the table because it is derived from dairy products.

When the energy values are calculated from the maximum power densities obtained, the following Table 3.8 is created. As a result of the study, it was calculated that a maximum of 8.9 W.sec. energy could be obtained from all the systems operated.

Table 3.8. Maximum energy generation of whey

Power Density (mW/m ²)	Energy (W.sec.)
6.6	0.42
140	8.9

3.7. Evaluation of Wastewater Treatment Performance

When assessing COD, it is generally seen that the systems utilizing sucrose solution achieve COD removal rates ranging from 95% to 99%. It was found that the lowest removal rate of 79% belonged to the second system where resistor was present, and sediment-2 was used (as shown in Table 3.10).

The calculation of COD removal percentages in relation to whey showed that the maximum removal (95%) was achieved in system-2, utilizing sediment-1 in a non-resistive medium. The removal percentage in system-2, which is formed by adding sediment-2 in the resistance-free environment, is 91% and is the second highest removal. In general, the removal rate for whey varies between 70 and 95%. The most inadequate removals were achieved in system-1 when sediment-1 was deposited in the absence and presence of resistance. The percentages of these removals are 60 and 20, respectively. In some studies done with the river SMFCs, the removal of organics such as PAHs and AVS was realized at around 72% and 90% [64], respectively. Moreover, the elimination of cellulose was carried out utilizing river SMFCs, resulting in an approximate removal rate of 73% [65]. Also, organic carbon removal rate of 20% [67] is available in literature. The literature studies provide evidence of the accuracy of the obtained removal percentages.

The calculated removal values show that reaching a maximum voltage or power density does not necessarily indicate an elevated level of removal in the systems. If the organic removal is poor, it indicates that a substantial portion of the organic matter present in the wastewater is not undergoing decomposition [112]. It is

important to mention that there is typically a trade-off between the generation of power and the treatment of wastewater in MFCs. Conditions that are ideal for power generation may not be ideal for wastewater treatment, and vice versa. Hence, achieving optimal performance in terms of both power output and organic removal might be a significant challenge for an MFC [112,113].

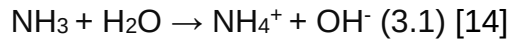
Throughout the operation of the systems, pH, temperature, TDS, and conductivity values were recorded both prior to and following the measurements. The data are displayed in Tables 3.9 and 3.10.

Upon examining the temperatures, it is noticeable that the internal temperatures range from 13 to 15 °C, while the ambient temperatures range from 15 to 17 °C. As understood, the internal and ambient temperature remain rather stable. Nevertheless, the conductivity and TDS exhibit a rise in all scenarios, indicating an augmentation in the concentration of ions inside the system.

After comprehending the pH values, it is observed that the values for system-1 exhibited a reduction, whereas the values for system-2 generally showed an increase. From examination of the literature, it is evident that pH decreases are caused by a variety of factors. The accumulation of hydrogen ions at the anode is one of these reasons. Protons (H^+) are generated during the microbial oxidation of organic materials at the anode. The transmission of these protons to the cathode chamber is necessary. Inefficient transfer results in proton accumulation in the anode chamber, causing a fall in pH [114]. Another possible factor could be the synthesis of organic acids. Specific microorganisms generate organic acids, such as acetic acid and lactic acid, as byproducts of their metabolism. The buildup of these acids in the anode chamber can greatly reduce the pH [14]. A further possibility could be the dissolution of carbon dioxide (CO_2). CO_2 is produced through the microbial oxidation of organic substrates. CO_2 dissolves in water and reacts to produce carbonic acid (H_2CO_3), which then breaks down into bicarbonate (HCO_3^-) and hydrogen ions (H^+), resulting in a decrease in pH [115].

On the other hand, there are also several reasons for pH increases in the system. One of the reasons for the increase in pH value is the reduction of oxygen at the cathode [114]. Another probable reason is the generation and buildup of ammonia. Nitrogenous organic molecules can undergo breakdown, resulting in

the production of ammonia (NH_3). The reaction between ammonia and water results in the formation of ammonium (NH_4^+) and hydroxide ions (OH^-), leading to an increase in pH:



In Tables 3.9 and 3.10, the presence of iron at measured pHs based on the Pourbaix diagram shown in Figure 3.31 is given. For the non-resistant case, iron in the sediment is expected to be present as Fe_2O_3 in the presence of sucrose solution. However, initial measurements show that iron is present in the systems as Fe_3O_4 . This is due to the presence of *Pseudomonas* microorganisms in the systems, which can make iron bioavailable. Likewise, when examined under resistant conditions, it is seen that iron in sucrose solution is in the form of Fe^{2+} . In this case, it can be said that microorganism makes iron available from the sediment. *Pseudomonas* species synthesize siderophores, specifically pyoverdine and pyochelin, which solubilize Fe(III) oxides by chelating iron and rendering it accessible [116]. It is not possible to see the effect of iron in sediments on these systems due to the presence of iron in the structure of whey as well as sulfur and nitrogenous compounds and the presence of microorganisms that can use these components as electron acceptors in the system.

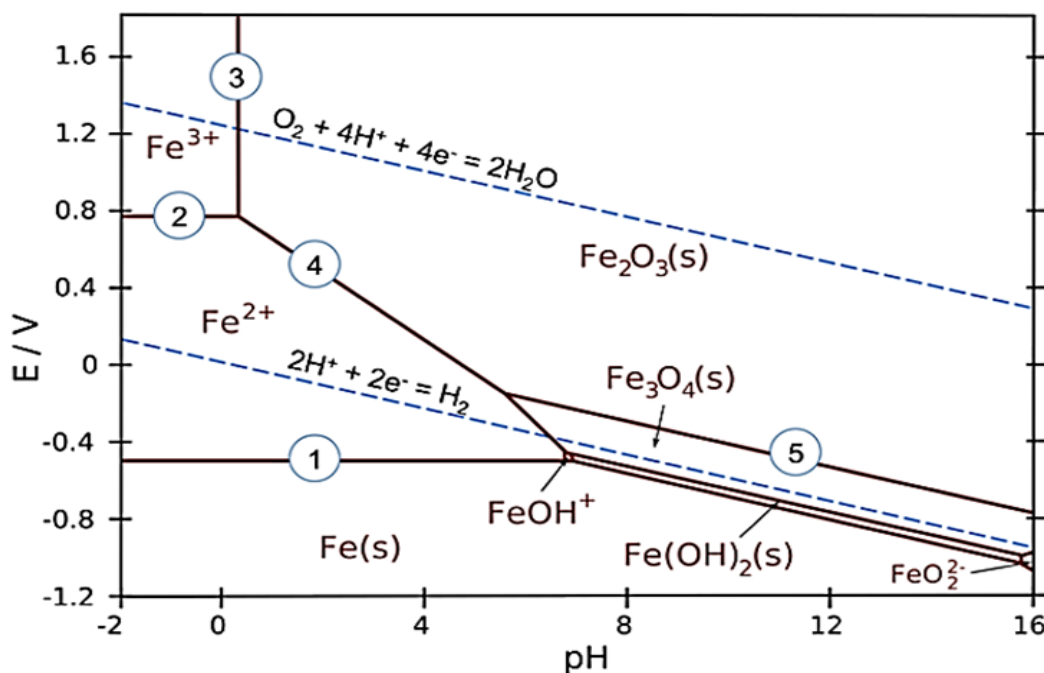


Figure 3.31: Pourbaix diagram for iron at ionic concentrations of 1.0 m [117]

Table 3.9. Parameter values of the sucrose solution and whey before and after putting it in the resistor-free systems

	Without Resistor								Without Resistor							
	System-1, Sediment-1				System-1, Sediment-2				System-2, Sediment-1				System-2, Sediment-2			
Parameters	Sucrose solution		Whey		Sucrose solution		Whey		Sucrose solution		Whey		Sucrose solution		Whey	
pH	7.5	6.2	5.2	4.7	6.6	6.4	5.8	4.5	7.1	8.0	5.0	7.4	7.3	7.6	5.1	7.5
Internal temperature (° C)	14	15	14	16	15	15	15	14	15	14	14	15	15	15	15	14
Iron	Fe ₃ O ₄ (S)	Fe ₂ O ₃ (S)	Fe ₂ O ₃ (S)	Fe ²⁺	Fe ₃ O ₄ (S)	Fe ₂ O ₃ (S)	Fe ²⁺	Fe ₂ O ₃ (S)	Fe ₃ O ₄ (S)	Fe ₂ O ₃ (S)	Fe ²⁺	Fe ₂ O ₃ (S)	Fe ₃ O ₄ (S)	Fe ₂ O ₃ (S)	Fe ₂ O ₃ (S)	Fe ₂ O ₃ (S)
Ambient temperature (° C)	16	16	16	17	17	16	17	15	16	16	16	17	17	17	16	15
TDS (ppm)	2	32	260	426	3	29	557	764	0	937	1890	1730	0	1160	2610	2240
Conductivity (µS/cm)	1	79	500	1230	3	89	1229	1661	7	1937	5600	5400	0	2800	7950	5280
COD removal (%)	95		60		98		-		98		95		98		91	

Table 3.10. Parameter values of the sucrose solution and whey before and after putting it in the systems with resistor

	With Resistor								With Resistor							
	System-1, Sediment-1				System-1, Sediment-2				System-2, Sediment-1				System-2, Sediment-2			
Parameters	Sucrose solution		Whey		Sucrose solution		Whey		Sucrose solution		Whey		Sucrose solution		Whey	
pH	7.6	6.2	5.2	4.3	6.8	5.5	5.3	4.4	7.9	7.3	5.0	6.6	6.8	6.6	5.1	6.6
Iron	Fe ₂ O ₃ (S)	Fe ₂ O ₃ (S)	Fe ²⁺	Fe ²⁺	Fe ²⁺	Fe ²⁺	Fe ²⁺	Fe ²⁺	Fe ₂ O ₃ (S)	Fe ₂ O ₃ (S)	Fe ²⁺	Fe ²⁺	Fe ²⁺	Fe ²⁺	Fe ²⁺	Fe ²⁺
Internal temperature (° C)	13	14	13	14	14	13	13	13	13	14	13	13	13	13	13	13
Ambient temperature (° C)	15	15	16	15	15	15	15	15	15	15	16	15	15	15	15	15
TDS (ppm)	3	28	211	705	3	32	165	377	2	1660	2670	2280	2	2170	1690	2500
Conductivity (µS/cm)	1	3	55	990	1477	2	65	387	1	3420	8280	5960	1	5400	3680	5600
COD removal (%)	99		20		95		70		95		80		79		86	

4. CONCLUSION

Based on the studies conducted, the thesis yielded the following results.

- ✓ For two different whey concentrations, *Pseudonocardia* (28.43%) - *Ralstonia* (11.31%) and *Clostridium* (18.72%) - *Bacteroides* (19.91%) species were obtained.
- ✓ In general, the resistance values measured from system-1 are higher than those measured from system-2. One of the reasons for this is that the distance between the anode and the cathode in system-1 is greater than in system-2. Another reason for this may be due to the conductivity differences between the two systems due to the dilution of whey.
- ✓ Typically, the resistance values for sediment-1 are higher compared to sediment-2 in both systems examined.
- ✓ The highest electrical potential value obtained from whey removal was 592 mV.
- ✓ The study found that the highest power density (140 mW/m^2) from whey removal was achieved with sediment-2 in the resistor-free system-2.
- ✓ The highest energy value obtained is 8.9 W.sec.
- ✓ It is commonly observed that systems employing sucrose solution typically achieve COD removal rates between 95% and 99%.
- ✓ The assessment of COD removal percentages concerning whey indicated that the highest removal rates obtained were between 95% and 70%.
- ✓ Conductivity and TDS show an increase across all situations.
- ✓ It was noted that the pH values of system-1 decreased while those of system-2 tended to rise.

5. FUTURE WORKS

In terms of real-world application of SMFCs, these systems can be combined with power management systems (which consists of capacitor, voltage comparator and charge pump) to effectively power wireless sensors for remote monitoring [44]. Minimal quantities of energy can be harvested from river sediments via SMFCs, achieving power densities of up to 8.8 mW/m². Field tests validate the feasibility of SMFCs for renewable energy extraction in aquatic habitats [45].

When the studies conducted with whey in recent years are examined, it is observed that the protein in whey is generally tried to be utilized. Because whey proteins exhibit several health benefits, including antioxidant, antimicrobial and anti-carcinogenic properties. They support immunity, bone health and brain development, among others. Therefore, membrane-based processes such as ultrafiltration (UF), microfiltration (MF) and nanofiltration (NF) are used to efficiently separate whey proteins while preserving their functional and nutritional properties [118].

In many cases, deriving protein from whey is more beneficial owing to the superior economic yield and established market for whey protein products. Nonetheless, if the disposal of whey or energy recovery is the principal issue, or if the whey possesses a low protein concentration, the utilization of SMFCs for energy generation may merit investigation. It is also possible to combine the two methods, in which the residual whey after protein extraction is used as a substrate for SMFCs.

According to the findings of this study, the following suggestions should be considered to address the lack of scientific research on the potential of employing SMFCs to generate energy from whey wastewater.

- ✓ A wider range of whey dilutions could be investigated to determine the optimal concentration for both microbial diversity and electrochemical performance.
- ✓ Different sediment compositions and configurations can be explored to understand their impact on resistivity and power density.
- ✓ By focusing on biochemical processes that contribute to hydrogen ion accumulation, organic acid synthesis, oxygen reduction, and ammonia

production, the mechanisms driving pH changes in different systems can be investigated.

- ✓ To increase the power density and energy potential of the systems without compromising the electrical potential, strategies can be developed to reduce the system resistance.



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APPENDIXES

APPENDIX 1 - Comparison of microorganisms

	Species	Microorganisms 1 Percentage (%)	Microorganisms 2 Percentage (%)
1	<i>Pseudonocardia</i>	28.427917185	0.000000000
2	<i>Ralstonia</i>	11.314184412	1.197471179
3	<i>Saccharopolyspora</i>	9.497077011	0.000000000
4	<i>Listeria</i>	7.059493911	5.972480476
5	<i>Clostridium</i>	1.382352320	18.720714020
6	<i>Enterococcus</i>	3.155140029	1.881740424
7	<i>Bacteroides</i>	1.257835089	19.910747490
8	<i>Lactococcus</i>	1.973281557	0.059501673
9	<i>Prevotella</i>	3.218453876	0.148754184
10	<i>Phocaeicola</i>	2.811134795	0.133878765
11	<i>Acetobacteroides</i>	0.008441846	3.495723317
12	<i>Methanosphaerula</i>	0.000000000	2.937895128
13	<i>Staphylococcus</i>	1.451997552	0.000000000
14	<i>Macellibacteroides</i>	0.000000000	3.495723317
15	<i>Saccharofermentans</i>	0.000000000	2.618073633
16	<i>Aquisediminimonas</i>	0.858957854	0.000000000
17	<i>Faecalibacterium</i>	1.281050166	0.000000000
18	<i>Chryseobacterium</i>	0.909608931	0.022313128
19	<i>Methanosarcina</i>	0.063313847	2.476757159
20	<i>Lacipirellula</i>	0.000000000	1.985868353
21	<i>Paenibacillus</i>	0.555051390	0.000000000
22	<i>Acinetobacter</i>	1.103771395	0.304946077
23	<i>Serratia</i>	0.877952008	0.000000000
24	<i>Sporichthya</i>	0.504400312	0.000000000
25	<i>Pseudomonas</i>	0.333452926	0.922275939
26	<i>Methanobacterium</i>	0.124517232	3.101524730
27	<i>Rummeliibacillus</i>	0.453749235	0.000000000
28	<i>Solidesulfovibrio</i>	0.000000000	2.350316103
29	<i>Methylibium</i>	0.394656311	0.081814801
30	<i>Erwinia</i>	0.768208007	0.074377092

	Species	Microorganisms 1 Percentage (%)	Microorganisms 2 Percentage (%)
31	<i>Shinella</i>	0.407319081	0.528077352
32	<i>Bacillus</i>	0.839963700	0.000000000
33	<i>Reyranella</i>	0.339784311	1.353663072
34	<i>Bifidobacterium</i>	0.143511386	1.323912235
35	<i>Sphingomonas</i>	0.517063082	0.654518408
36	<i>Pedomicrobium</i>	0.514952620	0.000000000
37	<i>Weissella</i>	0.365109850	0.037188546
38	<i>Rhizorhabdus</i>	0.337673849	0.000000000
39	<i>Curvibacter</i>	0.333452926	0.029750837
40	<i>Cutibacterium</i>	0.322900618	0.037188546
41	<i>Stenotrophomonas</i>	0.538167697	0.000000000
42	<i>Brevibacillus</i>	0.381993542	0.000000000
43	<i>Oleomonas</i>	0.000000000	1.123094087
44	<i>Aquabacterium</i>	0.046430154	1.108218669
45	<i>Paludibacter</i>	0.000000000	1.078467832
46	<i>Acidovorax</i>	0.002110462	1.695797694
47	<i>Exiguobacterium</i>	0.278580926	0.081814801
48	<i>Bradyrhizobium</i>	0.284912310	0.133878765
49	<i>Caulobacter</i>	0.297575080	0.044626255
50	<i>Propionispira</i>	0.000000000	0.892525102
51	<i>Gracilibacter</i>	0.329232003	0.000000000
52	<i>Aminobacter</i>	0.000000000	1.182595761
53	<i>Lactiplantibacillus</i>	0.240592618	0.000000000
54	<i>Akkermansia</i>	0.299685541	0.029750837
55	<i>Salmonella</i>	0.236371694	0.000000000
56	<i>Mesobacillus</i>	0.312348311	0.000000000
57	<i>Panacagrimonas</i>	0.417871388	0.000000000
58	<i>Dongia</i>	0.217377540	0.000000000
59	<i>Hyphomicrobium</i>	0.476964312	0.096690219
60	<i>Nordella</i>	0.240592618	0.148754184
61	<i>Sphingobacterium</i>	0.206825233	0.044626255
62	<i>Eubacterium</i>	0.217377540	0.000000000
63	<i>Blautia</i>	0.447417850	0.000000000

	Species	Microorganisms 1 Percentage (%)	Microorganisms 2 Percentage (%)
64	<i>Tahibacter</i>	0.000000000	0.684269245
65	<i>Succinivibrio</i>	0.301796003	0.029750837
66	<i>Devosia</i>	0.000000000	0.676831536
67	<i>Pantoea</i>	0.196272925	0.000000000
68	<i>Phascolarctobacterium</i>	0.198383386	0.000000000
69	<i>Hydrogenophaga</i>	0.000000000	0.639642990
70	<i>Nitrospira</i>	0.170947386	0.000000000
71	<i>Gemmiger</i>	0.166726463	0.000000000
72	<i>Mesorhizobium</i>	0.198383386	0.000000000
73	<i>Streptococcus</i>	0.341894772	0.066939383
74	<i>Prevotellamassilia</i>	0.170947386	0.000000000
75	<i>Tenebrionicola</i>	0.162505540	0.000000000
76	<i>Pseudescherichia</i>	0.234261233	0.000000000
77	<i>Advenella</i>	0.147732309	0.037188546
78	<i>Smithella</i>	0.000000000	0.617329862
79	<i>Miltoncostaea</i>	0.183610156	0.000000000
80	<i>Dyadobacter</i>	0.000000000	1.197471179
81	<i>Enterobacter</i>	0.375662157	0.000000000
82	<i>Sinanaerobacter</i>	0.257476310	0.505764225
83	<i>Azospira</i>	0.000000000	0.520639643
84	<i>Wautersiella</i>	0.145621847	0.000000000
85	<i>Phenylobacterium</i>	0.263807695	0.379323168
86	<i>Propionivibrio</i>	0.000000000	0.490888806
87	<i>Brevundimonas</i>	0.000000000	0.989215322
88	<i>Methanospirillum</i>	0.000000000	1.137969505
89	<i>Paludibaculum</i>	0.000000000	0.461137970
90	<i>Mycoplana</i>	0.000000000	0.416511714
91	<i>Lactobacillus</i>	0.346115696	0.022313128
92	<i>Limosilactobacillus</i>	0.156174155	0.000000000
93	<i>Peribacillus</i>	0.208935694	0.000000000
94	<i>Paracoccus</i>	0.179389232	0.000000000
95	<i>Lachnospira</i>	0.113964924	0.000000000
96	<i>Pseudoflavitalea</i>	0.000000000	0.401636296

	Species	Microorganisms 1 Percentage (%)	Microorganisms 2 Percentage (%)
97	<i>Phreatobacter</i>	0.000000000	0.401636296
98	<i>Sedimentibacter</i>	0.037988308	0.394198587
99	<i>Calidifontibacillus</i>	0.109744001	0.000000000
100	<i>Priestia</i>	0.109744001	0.000000000
101	<i>Parabacteroides</i>	0.149842771	0.000000000
102	<i>Fusicatenibacter</i>	0.143511386	0.000000000
103	<i>Treponema</i>	0.000000000	0.371885459
104	<i>Nocardioides</i>	0.103412616	0.000000000
105	<i>Roseburia</i>	0.170947386	0.000000000
106	<i>Methylobacterium</i>	0.132959078	0.000000000
107	<i>Methylophilus</i>	0.000000000	0.357010041
108	<i>Flexilinea</i>	0.000000000	0.357010041
109	<i>Waltera</i>	0.099191693	0.000000000
110	<i>Terrimonas</i>	0.067534770	0.349572332
111	<i>Sediminibacterium</i>	0.097081232	0.000000000
112	<i>Alistipes</i>	0.097081232	0.000000000
113	<i>Lactacaseibacillus</i>	0.082308001	0.052063964
114	<i>Mycolicibacterium</i>	0.094970770	0.014875418
115	<i>Bartonella</i>	0.094970770	0.000000000
116	<i>Dorea</i>	0.116075386	0.000000000
117	<i>Roseimicrobium</i>	0.000000000	0.319821495
118	<i>Dialister</i>	0.170947386	0.000000000
119	<i>Anaerostipes</i>	0.101302155	0.000000000
120	<i>Parasutterella</i>	0.082308001	0.000000000
121	<i>Lelliottia</i>	0.080197539	0.000000000
122	<i>Ensifer</i>	0.080197539	0.000000000
123	<i>Methanobrevibacter</i>	0.090749847	0.000000000
124	<i>Bosea</i>	0.056982462	0.431387133
125	<i>Mediterraneibacter</i>	0.078087078	0.000000000
126	<i>Thermohydrogenium</i>	0.078087078	0.000000000
127	<i>Simplicispira</i>	0.000000000	0.275195240
128	<i>Sutterella</i>	0.120296309	0.000000000
129	<i>Coprococcus</i>	0.130848617	0.000000000

	Species	Microorganisms 1 Percentage (%)	Microorganisms 2 Percentage (%)
130	<i>Ligilactobacillus</i>	0.080197539	0.000000000
131	<i>Helimonas</i>	0.071755693	0.000000000
132	<i>Romboutsia</i>	0.071755693	0.022313128
133	<i>Methanoregula</i>	0.000000000	0.252882112
134	<i>Magnetospirillum</i>	0.069645231	0.029750837
135	<i>Thermoanaerobacterium</i>	0.069645231	0.000000000
136	<i>Ruminococcus</i>	0.109744001	0.000000000
137	<i>Micrococcus</i>	0.065424308	0.000000000
138	<i>Methanosphaera</i>	0.065424308	0.000000000
139	<i>Paraconexibacter</i>	0.065424308	0.000000000
140	<i>Sphingopyxis</i>	0.000000000	0.230568985
141	<i>Haemophilus</i>	0.080197539	0.000000000
142	<i>Hallella</i>	0.084418462	0.000000000
143	<i>Pediococcus</i>	0.063313847	0.000000000
144	<i>Methylothermalis</i>	0.059092924	0.000000000
145	<i>Corynebacterium</i>	0.056982462	0.000000000
146	<i>Tenebrionibacter</i>	0.080197539	0.000000000
147	<i>Vescimonas</i>	0.056982462	0.000000000
148	<i>Rugosibacter</i>	0.054872001	0.000000000
149	<i>Barnesiella</i>	0.054872001	0.000000000
150	<i>Acetobacter</i>	0.054872001	0.066939383
151	<i>Rothia</i>	0.052761539	0.000000000
152	<i>Holdemanella</i>	0.050651077	0.000000000
153	<i>Alicyclobacillus</i>	0.050651077	0.000000000
154	<i>Leuconostoc</i>	0.050651077	0.089252510
155	<i>Bilophila</i>	0.050651077	0.000000000
156	<i>Odoribacter</i>	0.048540616	0.000000000
157	<i>Methylocystis</i>	0.048540616	0.000000000
158	<i>Ciceribacter</i>	0.000000000	0.171067311
159	<i>Zoogloea</i>	0.000000000	0.252882112
160	<i>Olsenella</i>	0.046430154	0.000000000
161	<i>Aliarcobacter</i>	0.046430154	0.000000000
162	<i>Anaerobutyricum</i>	0.084418462	0.000000000

	Species	Microorganisms 1 Percentage (%)	Microorganisms 2 Percentage (%)
163	<i>Propionicimonas</i>	0.000000000	0.163629602
164	<i>Thiobacillus</i>	0.000000000	0.163629602
165	<i>Chimaeribacter</i>	0.044319693	0.000000000
166	<i>Desulfobulbus</i>	0.080197539	0.000000000
167	<i>Massilia</i>	0.067534770	0.000000000
168	<i>Moraxella</i>	0.042209231	0.000000000
169	<i>Methanothrix</i>	0.042209231	0.000000000
170	<i>Alcaligenes</i>	0.042209231	0.000000000
171	<i>Pandoraea</i>	0.040098770	0.000000000
172	<i>Rhizobium</i>	0.016883692	0.141316475
173	<i>Dysosmobacter</i>	0.037988308	0.000000000
174	<i>Sporomusa</i>	0.073866155	0.000000000
175	<i>Kluyvera</i>	0.037988308	0.000000000
176	<i>Latilactobacillus</i>	0.037988308	0.000000000
177	<i>Ferrovibrio</i>	0.000000000	0.133878765
178	<i>Legionella</i>	0.000000000	0.133878765
179	<i>Wujia</i>	0.035877846	0.000000000
180	<i>Gaiella</i>	0.035877846	0.000000000
181	<i>Gemmata</i>	0.000000000	0.126441056
182	<i>Senegalimassilia</i>	0.033767385	0.000000000
183	<i>Eubacterium</i>	0.033767385	0.000000000
184	<i>Flavonifractor</i>	0.033767385	0.000000000
185	<i>Methylobacter</i>	0.000000000	0.119003347
186	<i>Salinibacterium</i>	0.000000000	0.119003347
187	<i>Stenotrophobacter</i>	0.052761539	0.000000000
188	<i>Eggerthella</i>	0.031656923	0.000000000
189	<i>Butyricimonas</i>	0.031656923	0.000000000
190	<i>Humidesulfobrevibrio</i>	0.000000000	0.171067311
191	<i>Citrobacter</i>	0.000000000	0.111565638
192	<i>Dechloromonas</i>	0.000000000	0.104127929
193	<i>Pelolinea</i>	0.000000000	0.104127929
194	<i>Pedobacter</i>	0.000000000	0.156191893
195	<i>Mycobacterium</i>	0.027436000	0.000000000

	Species	Microorganisms 1 Percentage (%)	Microorganisms 2 Percentage (%)
196	<i>Arcobacter</i>	0.027436000	0.000000000
197	<i>Thomasclavelia</i>	0.027436000	0.000000000
198	<i>Fusobacterium</i>	0.027436000	0.000000000
199	<i>Faecalibacillus</i>	0.027436000	0.000000000
200	<i>Novosphingobium</i>	0.025325539	0.000000000
201	<i>Komagataeibacter</i>	0.025325539	0.000000000
202	<i>Acidaminococcus</i>	0.031656923	0.000000000
203	<i>Inhella</i>	0.023215077	0.000000000
204	<i>Duodenibacillus</i>	0.023215077	0.000000000
205	<i>Lachnoanaerobaculum</i>	0.023215077	0.000000000
206	<i>Pseudoxanthomonas</i>	0.000000000	0.081814801
207	<i>Methanomassiliicoccus</i>	0.000000000	0.081814801
208	<i>Sulfurospirillum</i>	0.000000000	0.081814801
209	<i>Vibrio</i>	0.021104616	0.000000000
210	<i>Glaciecola</i>	0.000000000	0.074377092
211	<i>Asprobacter</i>	0.018994154	0.000000000
212	<i>Paeniclostridium</i>	0.018994154	0.000000000
213	<i>Ilumatobacter</i>	0.018994154	0.000000000
214	<i>Candidatus Pelagibacter</i>	0.018994154	0.000000000
215	<i>Sphingosinicella</i>	0.000000000	0.111565638
216	<i>Pelosinus</i>	0.016883692	0.000000000
217	<i>Atribacter</i>	0.016883692	0.000000000
218	<i>Anaerotaenia</i>	0.016883692	0.000000000
219	<i>Nitrolancea</i>	0.016883692	0.000000000
220	<i>Sphingorhabdus</i>	0.016883692	0.000000000
221	<i>Lentibacter</i>	0.000000000	0.059501673
222	<i>Kaistia</i>	0.000000000	0.059501673
223	<i>Eubacterium</i>	0.014773231	0.000000000
224	<i>Parvimonas</i>	0.014773231	0.000000000
225	<i>Anaerosporomusa</i>	0.014773231	0.000000000
226	<i>Methylomonas</i>	0.000000000	0.052063964
227	<i>Schlegelella</i>	0.000000000	0.052063964
228	<i>Desulfovibrio</i>	0.000000000	0.052063964

	Species	Microorganisms 1 Percentage (%)	Microorganisms 2 Percentage (%)
229	<i>Veillonella</i>	0.012662769	0.000000000
230	<i>Alkalihalobacillus</i>	0.012662769	0.000000000
231	<i>Oxalicibacterium</i>	0.012662769	0.000000000
232	<i>Solimonas</i>	0.000000000	0.044626255
233	<i>Flavobacterium</i>	0.000000000	0.044626255
234	<i>Eisenbergiella</i>	0.010552308	0.000000000
235	<i>Schlesneria</i>	0.010552308	0.000000000
236	<i>Pyramidobacter</i>	0.010552308	0.000000000
237	<i>Galactobacillus</i>	0.010552308	0.000000000
238	<i>Delftia</i>	0.000000000	0.037188546
239	<i>Paucibacter</i>	0.000000000	0.037188546
240	<i>Achromobacter</i>	0.008441846	0.000000000
241	<i>Raoultella</i>	0.008441846	0.000000000
242	<i>Labilithrix</i>	0.000000000	0.029750837
243	<i>Planktomarina</i>	0.000000000	0.029750837
244	<i>Rhabdothermincola</i>	0.000000000	0.029750837
245	<i>Xanthobacter</i>	0.000000000	0.029750837
246	<i>Epilithonimonas</i>	0.000000000	0.029750837
247	<i>Succiniclasticum</i>	0.006331385	0.000000000
248	<i>Victivallis</i>	0.006331385	0.000000000

APPENDIX 2 - Power Density Values in Graph in Section 3.6.3

The red color values obtained from the positive electrical potential and the black color values obtained from the negative electrical potential.

Values for without resistor conditions

	System-1				System-2			
	Power Density (mW/cm ²) (10 ⁻⁵)				Power Density (mW/cm ²) (10 ⁻⁵)			
	Sediment-1		Sediment-2		Sediment-1		Sediment-2	
Time (Hour)	Sucrose solution	Whey	Sucrose solution	Whey	Sucrose solution	Whey	Sucrose solution	Whey
1	51.539	0.045	22.13	0.31	1627.25	4.75	6868.53	685.88
2	34.496	0.044	16.37	0.67	1179.80	6.83	6338.70	808.84
3	23.807	0.042	10.09	1.89	780.13	6.94	6799.66	727.87
4	22.209	0.037	6.54	1.08	459.88	6.60	7251.90	624.28
5	17.316	0.032	4.12	1.88	306.35	6.00	7572.73	645.24
6	16.413	0.015	2.30	3.28	231.14	5.28	7830.80	609.62
7	14.264	0.001	1.14	4.69	198.40	4.47	8047.11	622.12
8	10.758	0.001	0.43	5.49	196.70	3.65	8239.44	619.96
9	11.338	0.007	0.11	8.65	180.09	2.85	8307.98	659.76
10	6.761	0.033	0.01	7.73	164.02	2.08	8342.36	839.60
11	8.816	0.025	0.02	6.22	132.03	1.45	8278.64	1148.02
12	5.108	0.039	0.09	7.49	91.73	1.01	8179.75	1286.72
13	5.994	0.062	0.19	7.07	63.75	0.76	8040.48	1251.23
14	5.017	0.070	0.31	4.37	43.53	0.60	7820.43	1223.31
15	4.199	0.046	0.51	2.91	28.44	0.50	7547.93	1332.42
16	3.508	0.047	0.76	4.10	19.89	0.41	7227.85	1308.49
17	2.938	0.027	0.95	4.18	14.68	0.33	6762.13	1151.00
18	2.452	0.028	1.15	3.74	10.54	0.25	6445.55	1192.47
19	1.970	0.016	1.47	3.45	7.90	0.18	5968.07	1258.22
20	1.690	0.020	2.09	3.26	6.23	0.11	4765.75	1352.08
21	1.277	0.022	2.97	5.15	4.69	0.08	5095.22	1400.83
22	0.750	0.021	3.55	6.61	4.51	0.08	4861.43	1383.77
23	0.423	0.017	3.76	10.12	5.00	0.08	4576.99	1180.43
24	0.295	0.251	3.90	11.74	5.81	0.05	4298.81	1294.59
25	0.189	0.863	4.05	13.03	7.23	0.03	4026.50	1234.47
26	0.096	0.790	4.35	14.67	9.30	0.06	3657.21	1304.16
27	0.048	0.777	3.97	14.99	16.59	0.06	3284.99	1241.48
28	0.032	0.778	3.25	17.47	21.59	0.08	2935.32	1217.54
29	0.016	0.802	3.29	14.61	21.51	0.11	2566.09	1142.77
30	0.001	1.086	3.45	15.22	22.14	0.01	2157.69	1179.33
31	0.010	0.823	3.57	13.26	24.18	0.04	1708.53	1157.40
32	0.017	0.702	3.67	11.12	26.20	0.00	1238.59	1208.36
33	0.055	0.400	3.87	11.53	28.02	0.06	740.54	1030.66
34	0.118	0.211	3.94	13.01	29.48	0.03	287.95	1163.76
35	0.104	0.123	3.56	13.02	30.35	0.01	43.57	1125.74
36	0.072	0.129	3.18	13.46	31.01	0.06	1.04	1139.14
37	0.037	0.151	3.05	11.79	31.48	0.10	1.02	1183.25
38	0.100	0.135	2.96	11.41	31.94	0.12	3.18	1145.50
39	0.109	0.062	2.70	18.48	32.41	0.14	4.27	1081.70
40	0.169	0.030	2.59	26.13	32.69	0.11	4.70	1094.76
41	0.245	0.013	2.63	33.32	32.79	0.11	4.68	1095.14
42	0.250	0.013	2.51	44.41	32.80	0.09	4.33	1090.35
43	0.271	0.002	2.48	57.63	32.70	0.08	3.94	1028.11
44	0.357	0.000	2.68	65.67	32.76	0.06	3.49	1126.22

	System-1				System-2			
	Power Density (mW/cm ²) (10 ⁻⁵)				Power Density (mW/cm ²) (10 ⁻⁵)			
	Sediment-1		Sediment-2		Sediment-1		Sediment-2	
Time (Hour)	Sucrose solution	Whey	Sucrose solution	Whey	Sucrose solution	Whey	Sucrose solution	Whey
45	0.269	0.013	3.06	65.97	32.78	0.02	3.04	1083.96
46	0.186	0.004	4.07	59.02	32.84	0.05	2.72	1057.68
47	0.100	0.002	4.67	39.32	32.87	0.10	2.42	939.91
48	0.067	0.003	13.08	53.49	33.30	0.44	1.97	929.75
49	0.053	0.001	12.64	31.96	33.66	1.04	1.61	936.07
50	0.038	0.003	4.53	15.64	33.81	1.07	1.19	976.74
51	0.017	0.000	21.91	16.05	33.98	0.60	0.87	986.79
52	0.013	0.001	39.17	14.65	34.23	0.64	0.65	864.64
53	0.016	0.000	15.34	12.02	34.43	1.94	0.54	699.39
54	0.003	0.000	31.86	13.45	34.76	1.85	0.49	555.11
55	0.001	0.003	32.60	15.05	35.16	1.65	0.47	428.33
56	0.000	0.000	35.90	13.34	35.61	0.96	0.44	456.35
57	0.000	0.001	35.83	13.88	35.99	0.35	0.44	446.25
58	0.001	0.000	28.44	16.50	36.56	0.01	0.48	508.68
59	0.004	0.004	17.49	17.63	37.28	0.02	0.53	565.19
60	0.001	0.014	3.77	20.47	37.74	0.05	0.61	494.75
61	0.002	0.023	3.72	22.08	38.05	0.07	0.66	401.14
62	0.000	0.008	7.67	24.23	38.39	0.08	0.70	339.11
63	0.001	0.009	12.69	25.36	38.75	0.09	0.78	295.91
64	0.000	0.007	7.02	25.92	39.24	0.13	0.89	213.64
65	0.001	0.011	2.00	24.66	39.81	0.15	1.02	141.80
66	0.013	0.041	2.00	25.77	40.49	0.16	1.11	106.31
67	0.002	0.034	2.03	23.54	40.97	0.17	1.18	80.83
68	0.007	0.028	2.05	23.25	40.70	0.17	1.20	76.55
69	0.029	0.018	2.16	22.77	40.18	0.17	1.21	71.56
70	0.112	0.019	2.25	25.79	39.97	0.17	1.18	67.51
71	0.173	0.009	2.72	26.13	40.28	0.16	0.86	49.43
72	0.343	0.010	3.54	23.61	40.62	0.15	0.78	33.36

Values for with resistor conditions

	System-1				System-2			
	Power Density (mW/cm ²) (10 ⁻⁵)				Power Density (mW/cm ²) (10 ⁻⁵)			
	Sediment-1		Sediment-2		Sediment-1		Sediment-2	
Time (Hour)	Sucrose solution	Whey	Sucrose solution	Whey	Sucrose solution	Whey	Sucrose solution	Whey
1	20.119	0.045	1.717	0.016	4794.890	2.503	869.124	0.702
2	22.104	0.044	3.920	0.014	3665.113	4.442	329.744	9.949
3	20.519	0.042	5.618	0.013	3019.891	7.323	203.505	13.194
4	17.852	0.037	6.129	0.002	2701.238	8.631	165.270	19.597
5	15.790	0.032	5.502	0.009	2442.431	9.074	141.840	7.810
6	14.538	0.015	4.398	0.036	2173.019	8.960	142.512	3.123
7	13.922	0.001	3.309	0.045	1901.576	9.396	131.263	0.448
8	12.794	0.001	1.355	0.039	1639.417	9.010	134.685	0.159
9	11.649	0.007	0.200	0.010	1378.965	9.009	125.000	4.659
10	11.206	0.033	0.066	0.008	1141.774	8.301	110.830	44.622
11	10.322	0.025	0.729	0.052	931.055	6.484	101.310	129.807
12	9.742	0.039	1.728	0.259	742.836	4.807	94.685	248.688
13	9.312	0.062	2.654	0.432	580.316	3.330	89.351	409.524
14	8.805	0.070	3.371	0.700	457.698	2.164	89.705	544.676
15	8.163	0.047	3.860	0.636	381.191	1.577	81.318	673.573
16	7.405	0.047	4.231	0.976	329.726	1.249	76.825	774.203
17	6.637	0.027	4.604	0.619	291.629	0.971	75.521	820.191

	System-1				System-2			
	Power Density (mW/cm ²) (10 ⁻⁵)				Power Density (mW/cm ²) (10 ⁻⁵)			
	Sediment-1		Sediment-2		Sediment-1		Sediment-2	
Time (Hour)	Sucrose solution	Whey	Sucrose solution	Whey	Sucrose solution	Whey	Sucrose solution	Whey
18	6.276	0.028	4.871	1.043	262.122	0.785	70.812	932.330
19	5.654	0.016	5.095	1.325	240.522	0.691	72.707	944.470
20	5.486	0.020	5.368	1.512	228.027	0.628	64.984	1013.907
21	5.289	0.022	5.659	1.296	218.030	0.629	56.622	1051.665
22	4.314	0.021	5.927	1.193	211.672	0.642	49.253	1079.568
23	3.862	0.017	6.008	1.199	207.613	0.675	58.519	1099.965
24	3.903	0.251	5.730	1.465	203.963	0.772	63.607	1148.983
25	4.183	0.863	5.802	1.879	200.768	0.670	53.826	1217.698
26	3.845	0.790	5.669	2.129	198.298	0.596	56.123	1218.767
27	3.436	0.777	5.605	2.395	196.089	0.599	53.079	1231.378
28	3.412	0.778	5.361	2.511	194.813	0.526	54.675	1259.461
29	3.047	0.802	5.207	2.717	194.163	0.508	55.603	1281.645
30	2.588	1.086	5.194	3.086	192.686	0.536	49.204	1230.998
31	2.237	0.823	5.211	3.341	190.223	0.429	53.548	1236.772
32	1.898	0.702	5.503	3.349	186.827	0.410	50.234	1258.294
33	1.648	0.400	6.075	3.862	183.251	0.498	43.915	1303.520
34	1.367	0.211	6.306	4.237	179.095	0.513	48.089	1324.219
35	1.286	0.123	6.623	4.065	175.526	0.468	44.611	1385.952
36	1.481	0.129	6.972	4.421	172.026	0.342	41.481	1370.220
37	1.307	0.151	7.337	4.447	168.327	0.336	39.042	1424.867
38	1.044	0.135	7.648	4.471	163.863	0.332	37.055	1367.338
39	0.866	0.062	7.842	4.653	159.713	0.238	34.094	1345.312
40	0.722	0.030	7.984	4.947	156.749	0.190	29.981	1336.637
41	0.587	0.013	8.196	5.192	154.487	0.161	32.138	1351.563
42	0.486	0.013	8.335	5.303	152.738	0.249	28.940	1369.153
43	0.408	0.002	8.485	5.253	151.827	0.132	28.950	1383.673
44	0.351	0.000	8.589	5.294	152.418	0.697	29.807	1359.323
45	0.327	0.013	8.497	5.040	152.794	0.633	33.260	1315.220
46	0.366	0.004	8.460	4.963	152.946	0.842	31.896	1301.082
47	0.398	0.002	8.159	4.880	152.122	0.978	31.676	1269.839
48	0.372	0.003	7.513	5.003	149.277	0.991	31.467	1257.639
49	0.556	0.001	7.713	4.863	145.872	0.945	29.097	1190.942
50	0.475	0.003	7.732	4.410	143.574	1.013	29.823	1185.416
51	0.469	0.000	7.384	4.449	140.403	1.211	18.163	1165.191
52	1.415	0.001	7.338	4.414	138.370	1.145	24.103	1139.142
53	0.911	0.000	6.794	4.416	136.087	1.243	30.858	1129.450
54	0.753	0.000	6.371	4.339	133.442	1.307	31.447	1119.137
55	0.722	0.003	6.261	4.356	130.430	1.028	27.815	1092.272
56	0.654	0.000	5.989	4.481	127.416	0.764	25.226	1077.187
57	0.661	0.001	6.410	4.712	124.705	0.894	27.228	1060.975
58	0.596	0.000	6.070	4.865	122.445	0.849	26.846	1050.439
59	0.581	0.004	5.570	4.856	120.334	0.615	25.951	1026.248
60	0.569	0.014	5.781	5.128	118.936	0.208	25.244	977.655
61	0.683	0.023	5.755	5.563	117.116	2.911	25.311	1007.721
62	0.936	0.008	5.614	5.707	114.955	7.283	25.464	993.802
63	1.263	0.009	5.790	6.010	113.026	8.932	23.719	992.979
64	1.654	0.007	5.727	5.993	111.430	8.584	25.276	969.252
65	2.036	0.011	5.756	5.861	110.459	7.444	24.098	949.973
66	2.334	0.041	5.874	5.972	109.663	7.757	23.590	930.062
67	2.877	0.034	6.035	5.755	108.489	9.444	20.534	904.515
68	3.106	0.028	6.027	5.688	106.949	9.976	21.165	919.995
69	3.268	0.018	5.939	5.534	105.168	7.991	17.969	924.179
70	4.165	0.019	5.938	5.406	103.767	7.577	15.398	916.910

	System-1				System-2			
	Power Density (mW/cm ²) (10 ⁻⁵)				Power Density (mW/cm ²) (10 ⁻⁵)			
	Sediment-1		Sediment-2		Sediment-1		Sediment-2	
Time (Hour)	Sucrose solution	Whey	Sucrose solution	Whey	Sucrose solution	Whey	Sucrose solution	Whey
71	4.084	0.009	6.404	5.148	102.512	5.739	16.100	897.729
72	4.536	0.010	6.876	4.605	101.052	4.363	31.684	894.345



APPENDIX 3 - Publications Derived from Thesis

Ateş, E.; Sanin, S., L. Investigation of the Electrical Potential of Whey Using a River Sediment Microbial Fuel Cell. Bull Chem Soc Ethiop, 2024, 38, 1479–1491. DOI: <https://dx.doi.org/10.4314/bcse.v38i5.22>.

