Bull. Chem. Soc. Ethiop. **2024**, 38(5), 1479-1491.
© 2024 Chemical Society of Ethiopia and The Authors
DOI: https://dx.doi.org/10.4314/bcse.v38i5.22

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

INVESTIGATION OF THE ELECTRICAL POTENTIAL OF WHEY USING A RIVER SEDIMENT MICROBIAL FUEL CELL

Esra Ateş[1] and Selim Latif Sanin[2]

[1]Department of Environmental Engineering, Faculty of Engineering, Artvin Çoruh University, Artvin, Türkiye
[2]Department of Environmental Engineering, Faculty of Engineering, Hacettepe University, Ankara, Türkiye

(Received April 2, 2024; Revised May 24, 2024; Accepted July 3, 2024)

ABSTRACT. This study aimed to use whey and river sediment microbial fuel cells (SMFCs) to produce electrical potential, which has been investigated rarely in previous studies. In this study, the majority of the microorganisms in the river SMFC mixed culture were *Bacteroides* and *Clostridium*. After the voltage and internal resistance were measured, the current and power density were calculated. The power density ($279*10^{-3}$ mW/cm^2) and maximum current density ($1100*10^{-6}$ mA/cm^2) were determined through computations. Bacteria present in river SMFCs showed the potential to generate electricity without any external mediators. By utilizing organic materials, bioelectricity can be produced affordably and sustainably.

KEY WORDS: Electrical potential, SMFC, Treatment, Whey, River sediment

INTRODUCTION

With the increase in population worldwide, the number of industries, such as food processing, textiles, pulp, and paper, continues to increase. As a result, the amount of waste produced and discharged into receiving water bodies by these industries is also increasing. Among food processing industries, the dairy industry is very important because between 0.2 and 10 liters of wastewater per liter of processed milk are produced [1]. One of the most important wastewaters produced from dairy products is cheese whey because, according to the Food and Agriculture Organization (FAO), more than 18 million tons are produced annually [2]. Cheese whey results in a chemical oxygen demand (COD) in the range of 50–102 g/L [3]; thus, it is suitable for biological treatment. There are different treatment methods, and the microbial fuel cell (MFC) is one of them. An MFC is a device for treating wastewater while bioenergy is recovered [4]. In other words, MFCs use bacteria to catalyze the conversion of organic matter into electrical power. Whey is abundant in waste streams from food manufacturing facilities. Whey may be efficiently used as a feedstock item for bioenergy. 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 an MFC to produce bioelectricity. Electrons produced by the microbial digestion of whey are transported to an electrode to create an electric current [5].

The goal of this study was to use sediment MFCs for power generation in order to treat cheese whey, which is typically left untreated (particularly in small firms) and leads to environmental issues such as eutrophication and pollution of surface and groundwater [1]. A sediment microbial fuel cell (SMFC) is a type of microbial fuel cell that can generate electrical current from the organic matter content of sediment through bacterial metabolism. SMFCs have been developed to provide a renewable power source and remove organic matter [6]. The use of these materials for the harvest of energy from natural sediment has been intensively investigated, and their

*Corresponding authors. E-mail: esra_ates@artvin.edu.tr

application in wastewater treatment has been studied in recent years. SMFCs have simple structures and can generate electrical energy while decontaminating wastewater. They can extract electrical energy from organic compounds in natural sediments [7]. SMFCs can be constructed from different types of sediment, which can be marine, river, or lake [8]. In our study, river SMFCs were used to determine what happens in a river when cheese whey is discharged into it.

River sediment microbial fuel cells can be used for the bioremediation of sediments but can also be utilized for the treatment of wastewater. Such microbial fuel cells are generally operated in a single compartment. In these systems, organic matter removal occurs in the sediment, and the sediment represents the anode compartment. The electrode placed at the anode is buried in the sediment. The cathode represents the liquid part of the system (wastewater or river water), and the electrode is suspended in the liquid. Because of the complexity of electromicrobiology in river SMFCs, studies have been conducted by changing various parameters to understand how they work. In the system, oxygen uptake in the cathode part is an important parameter. Generally, the cathode electrode is far from the surface of the liquid part, so obtaining the necessary oxygen from the air can become difficult for the cathode part. In a study in 2007, oxygen availability in a cathode was increased by using a rotating cathode, and the power density was raised to the maximum power density (49 mW/m^2) from a non-rotating cathode system (29 mW/m^2) [9]. In another study conducted in 2012, to prevent oxygen deficiency in the cathode, a floating cathode was used. Additionally, a microbial attachment process (which is called the biocathode) was applied to the cathode to increase the performance of the system. A power density of 1.00 mW/m^3 was obtained from this system [10]. The type of electrode affects the performance of SMFC systems. Different types of electrodes, such as platinum mesh, graphite disks, stainless steel, carbon brushes, carbon cloths, and graphite felt, have been used as electrodes [11]. Graphite felt (GF) and activated carbon fiber felt (ACFF) were used to understand the effect of these electrodes. A 33.5 ± 1.5 mW/m^2 power density was obtained with ACFF [11]. By using a sheet iron anode with GF, a maximum power density of 170 mW/m^2 was determined, which is 4.8 times the power density with GF (35 mW/m^2) [12]. In one study, conductive metal brushes were utilized as a cathode electrode. Additionally, in this study, marine and river SMFCs were compared, and it was determined that river sediment generates the highest power density, approximately 121 $\mu W/cm^2$ [13]. The external resistance load is also one of the parameters studied. The 100, 510, and 1100-ohm external resistance loads were studied, and the highest power density was 1.2 ± 0.2 mW/m^2 with a 510-ohm load [14]. Increasing the conductivity can positively affect the power density obtained from river SMFC systems. To do that, the SMFC system was enhanced with biochar, which was produced from coconut shells. Compared to those without modification, the SMFC power generation performance was enhanced by a factor of two to ten. Additionally, this modification increased the TOC removal rate by 1.7 to four times compared to the absence of modification [15]. The degradation of different organic compounds by using river SMFCs was investigated. For example, the removal of cellulose [16], acid-volatile sulfide (AVS), and polycyclic aromatic hydrocarbons (PAHs) [17] from sediments was studied. The highest cellulose removal efficiency was $72.7 \pm 2.1\%$ [16], and the removal efficiencies of PAHs and AVS were 71.56% and 89.76%, respectively [17]. It is known that decreasing the distance between the cathode and anode compartments decreases the internal resistance of the MFC system. Therefore, reducing the water layer between the cathode and sediment can give the same results. In a study conducted in 2023, the power density was enhanced by 72–134% in SMFCs without a water layer between the sediment and the cathode [18].

In this study, aluminum foil paper was used to investigate the performance of this electrode in river SMFC systems. COD, temperature, pH, conductivity, and TDS were measured before and after operating the system, and their changes were analyzed. While the system was being built, organic removal and power generation in the system were examined by leaving them as much as possible on their own without any external intervention. In other words, the system was operated by applying a process similar to the natural attenuation process, which relies on natural processes to clean up or attenuate pollution in soil and groundwater [19].

Unlike other studies with whey, sediment MFC is used in this study. With this approach, essential isolation of bacteria on the anode from oxygen is provided. In other words, the anaerobic sediment, or mud, stays anaerobic throughout the study, so obligate anaerobic bacteria is kept separate from the aerobic ecosystem [20]. Therefore, the pollution reduction and energy production performance of MFC can increase.

EXPERIMENTAL

Origin of the wastewater and inoculation studies

Whey was obtained from a factory producing dairy products in Trabzon, Türkiye. The pH of the whey supplied was approximately 6 at 25 °C, and the fat ratio was 0. It is also known that the number of solids in whey, which is the Brix value, is 6 percent on average. The effluent had an initial chemical oxygen demand (COD) of 51 g/L, while its conductivity was 7.76 mS/cm.

To homogenize the whey, the wastewater was passed through a mixer. Bacteria in the whey consortium have the potential to influence the bioelectrochemical processes that take place in MFCs [21]. Therefore, the waste sample was exposed to heat in a drying oven at 100 °C for one hour in order to minimize the existence of these bacteria in whey. The temperature of 100 °C was selected to hinder the growth of microorganisms in the system, prevent the liquid from evaporating out of the whey structure, and keep the organic load from changing. Whey was used without dilution. The temperature, pH, total dissolved solids (TDS), electrical conductivity, and COD parameters of the wastewater were measured before and after the systems were operated.

The whey was first diluted by 1/25 and then 1/5 to allow the microorganisms, which are bottom mud taken from the river, to slowly acclimate to the whey environment, and these microorganisms were subsequently introduced into the system.

Sediment sampling

The sand taken from the Eastern Black Sea coast was first sieved through an 850-micrometer sieve to obtain homogeneous sediments. The resulting sediment was sterilized at 150 °C for 1 hour [22]. The particle density of the sand is 2.54 g/cm^{3}, and the density of the sand is 1.32 g/mL. The air space in it was 60%.

Microcosm configuration and operation

A single-chamber MFC was formed to treat cheese whey and produce electricity. The organic content of cheese whey consists of lactose, and the biochemical oxidation and reduction reactions and general reactions are described as follows:

Half reactions:

Anode reaction = $C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e^-$

Cathode reaction = $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$

The general reaction of organic decomposition is as follows:

$C_{12}H_{22}O_{11} + 12O_2 \rightarrow 12CO_2 + 11H_2O$

In the system, a 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 multistranded copper cables were used. One of the electrodes was placed under the sand (anode), and the other was placed on top of the sand (cathode). In other words, a distance of 5 cm was left between the anode and cathode. Electrical tape was used to adhere the cables to the aluminum foil paper. Additionally, epoxy adhesive was used to seal the holes in the electrode that had been positioned beneath the sand surface. To include microorganisms in the system, a 15-mL falcon tube was filled with isotonic water and 7 mL of the particles taken from the medium, which were contacted with 1/5 diluted whey. After a minute in an ultrasonic cleaner for the removal from the surface, two milliliters were removed from the tube's top and added to the system as an inoculant. The whey sample, which had been frozen in the refrigerator, was defrosted, passed through a precleaned mixer, and filtered. After heating the whey sample to 100 °C and cooling, 50 mL of undiluted whey were added to the sediment microcosm.

Electrochemical measurements

The voltage (V) was recorded for the system using a multimeter (UNI-T UT61C). Since the recording time of the multimeter was adjustable, the data were recorded every minute. The resistance (R) of the system was also recorded to determine its internal resistance. The current of the system was also calculated from the following equation:

Voltage (V) = Current (I) × Resistance (R), where I is the ampere and V is the voltage in mV.

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

Power (W) = IV

The power density and current density were calculated by dividing the obtained power and current by the surface area of the anode [23].

Wastewater characterization

A spectrophotometric method was used to determine the chemical oxygen demand. First, 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. Then, 4.9 g of potassium dichromate was added to 150 mL of H_2SO_4. Added 6 g of $HgSO_4$ to the mixture and waited for it to dissolve. Afterward, 6 g of Ag_2SO_4 was added, and 350 mL of H_2SO_4 was added slowly. The mixture was stirred for three days. Three milliliters of this solution were added to the HACH kit bottle, after which 2 mL of the sample to be measured was added to the COD heater. The mixture was heated at 148 °C for 2 hours, after which the absorbance was read at a wavelength of 600 nm through a spectrophotometer. Parameters other than COD, which included 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.

Sediment characterization

X-Ray fluorescence (XRF) analytical techniques were used to characterize the sediments. This method is used for elemental and chemical analysis. The energy of these fluorescent X-rays is characteristic of the atoms present in the sample, allowing for the identification of elements [24]. Briefly, 0.5 kg of unground sand was sent to the Mineral Research and Exploration Laboratory for XRF analysis to determine the presence of substances such as SiO_2, Al_2O_3, Fe_2O_3, CaO, MgO, Na_2O, CuO, and ZnO, etc. are present in the content.

Identification of bacteria

Metagenomic analysis is a technique used to study genetic material from environmental or biological samples. This approach allows us to understand the diversity, abundance, and interactions of microbes in any system [25]. The mixed bacterial cultures put into the system were subsequently sent to BM Software Consultancy and Laboratory Systems Limited Company for analysis. Scanning electron microscopy (SEM) is a remarkable technique used for imaging and analyzing the surface of various materials at high resolution [26]. Images of the sediment that interacted with the microorganisms were obtained through SEM analysis. The images were captured at a voltage of 10 kV and a magnification of 10000×.

RESULTS AND DISCUSSION

The results of the paper are evaluated based on (i) the chemical composition of the river sediment, (ii) the identification of microorganisms in the sediment, and (iii) the electrical potential developed in the microcosm.

Evaluation of chemical composition of the river sediment

Generally, the chemical composition of sand consists of silica (SiO_2). The XRF results show that the sand contains 73.9% silica, as shown in Table 1. After silica, the sand contains the most Al_2O_3 (alumina), and it can be found in sea sand because it is a major component of many minerals. Additionally, the sand contains 4.6% Fe_2O_3, which is naturally present in the sand. The organic matter content of the sand is 2.3% of its chemical structure. In addition, the sand included 2.7% Na_2O and 2.2% CaO.

Table 1. Chemical composition of the river sediment.

Analysis	Percentage (%)	Analysis	Percentage (%)
Loss on Ignition	2.30	NiO	<0.01
Al_2O_3	10.5	P_2O_5	0.1
BaO	<0.01	PbO	<0.01
Bi_2O_3	<0.01	Rb_2O	<0.01
CaO	2.2	S(S)	-
CdO	<0.01	SO_3	0.06
Co_3O_4	0.01	SiO_2	73.9
Cr_2O_3	0.03	SnO_2	<0.01
CuO	<0.01	SrO	0.01
F	<0.01	TiO_2	0.4
Fe_2O_3	4.6	V_2O_5	0.02
K_2O	1.2	Y_2O_3	<0.01
MgO	1.8	ZnO	0.01
MnO	0.1	ZrO_2	0.01
Na_2O	2.7		

Identification of microorganisms in the sediment

The microorganisms were mixed in culture, as shown in Figure 1. However, most of the bacteria were *Bacteroides* (19.91%) or *Clostridium* (18.72%).

Species of Bacteroides are obligatory anaerobic bacteria that are gram-negative. Depending on the species, these non-endospore-forming bacilli can be either motile or nonmotile [27]. Owing to being obligate anaerobes, this approach is beneficial for MFCs because the anodic reactions in MFCs are typically anaerobic. Additionally, they are capable of degrading complex organic materials, such as those present in wastewater [28]. *Bacteroides* can generate biofilms, which are microbial communities that adhere to surfaces [29]. By expanding the electron transfer surface area, biofilms can improve MFC performance [28].

Clostridium species are typically gram-positive bacteria with rod-like structures, and their size varies. The genus *Clostridium* contains microorganisms that can be found in the soil, water, and animal and human digestive systems. The majority of species are obligatory anaerobes because they can flourish only under conditions of total oxygen deprivation. *Clostridium species* are extremely resilient to heat, desiccation, harmful substances, and detergents [30]. Since clostridium has the same properties as *Bacteroides*, it can be used as a microbial fuel cell [31, 32].

These results of metagenomic analysis are supported by the results of SEM analysis. Images of *Bacteroides* and *Clostridium* are shown in Figure 2.

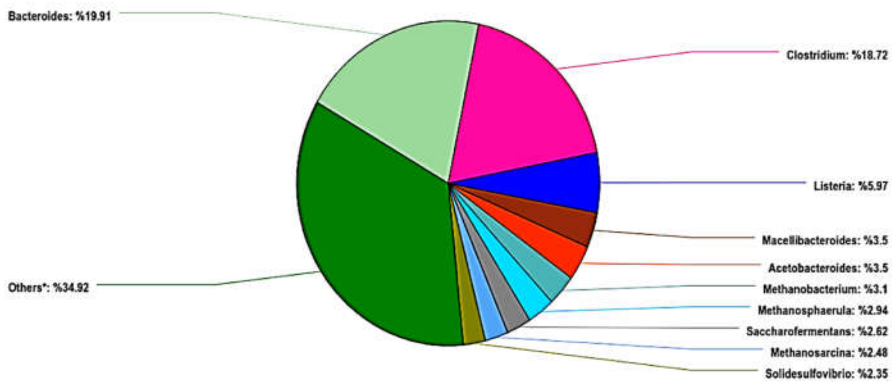

Figure 1. Circle chart for the species level.

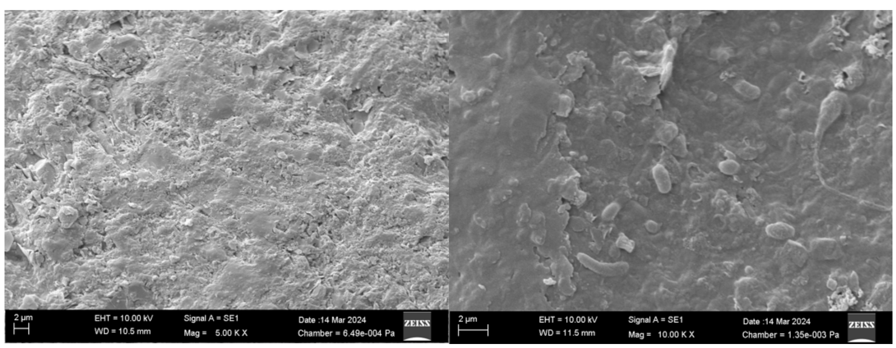

Figure 2. SEM analysis of the sediment without (left one) and with (right one) native microorganisms.

Upon closer inspection of the bacterial species reported in the literature, it becomes evident that microbial fuel cells derived from *Proteobacteria* [33], *Cytophaga*, *Flexibacter*, and *Bacteroides* [34]. Despite having the potential to function as a microbial fuel cell, *Clostridium* has not been the subject of many investigations. Nonetheless, this kind of microbe was used in a 2019 study to examine the bioelectricity-generating potential of a by-product known as sago hampas, which is a by-product generated from the extraction of sago starch from the pith of the sago palm [35, 36]. *Bacteroides* and *Clostridium species* can be employed as microbial fuel cells for the production of bioelectricity, as is known from experiments reported in the literature.

Evaluation of electrical potential developed in the microcosm

Between the 2nd and the 9th hours of operation, the system's resistance averaged 9 kΩ, as shown in Figure 3. From the 9th to the 35th hours, there were sharp fluctuations in the system's resistance, which increased to a maximum resistance of 1050 kΩ. From 35 to 62 days, the average resistance of the system was 8 kΩ. At hour 62, the battery ran out, so the process until hour 71 was considered zero. Depending on the quality of the 9-watt batteries used for the system, it was determined that the batteries ran out between 5 and 7 days. In addition, at hour 71, the electrode at the cathode was punctured and replaced. After the 71st hour, the system's resistance was measured as 2 kΩ on average. At the 230th hour, the resistance of the system was approximately 100 kΩ. At hour 231, the multimeter battery died again, and a data gap of 4 hours occurred. On average, the resistance was set at 2 kΩ until the end of the system's operating time. However, there was a data gap between the 333rd and 353rd hours, again due to battery depletion.

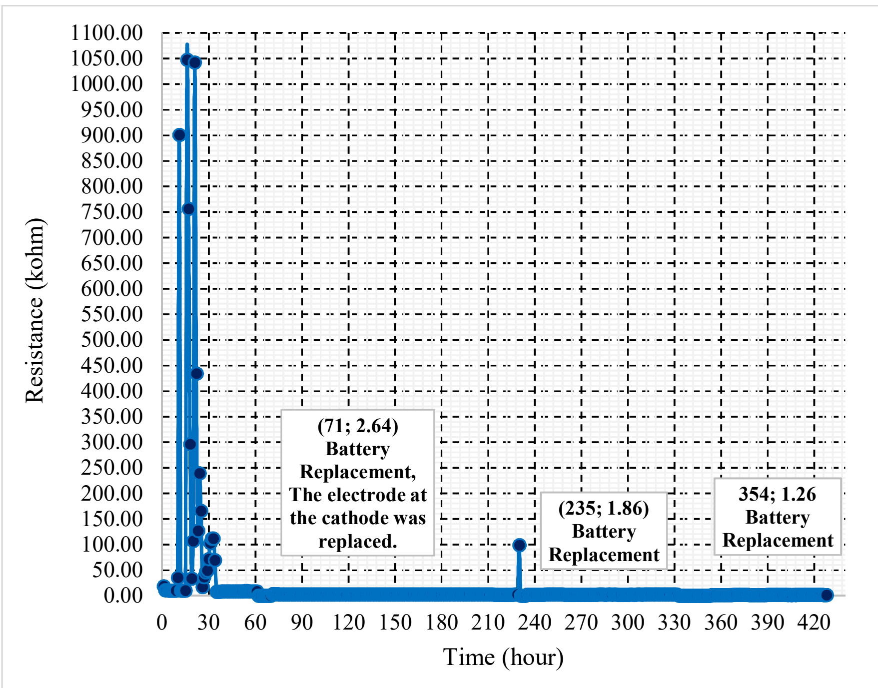

Figure 3. System resistance measurements.

The data could not be received between 8 and 26 hours and between 212 and 217 hours due to device problems (values are accepted as 0). In the system, positive values were taken until hour 155. A maximum electrical potential of 264 mV was obtained in this range, as shown in Figure 4. From 27 to 114 hours, more stable data were obtained, and the average of these data was calculated as 232 mV. At the 288th hour, the battery of the multimeter used in the system ran out because it lasted for an average of two weeks. It was also observed that the whey liquid in the system decreased over time and became depleted. Therefore, the operation of the system was stopped at the 410th hour (day 17).

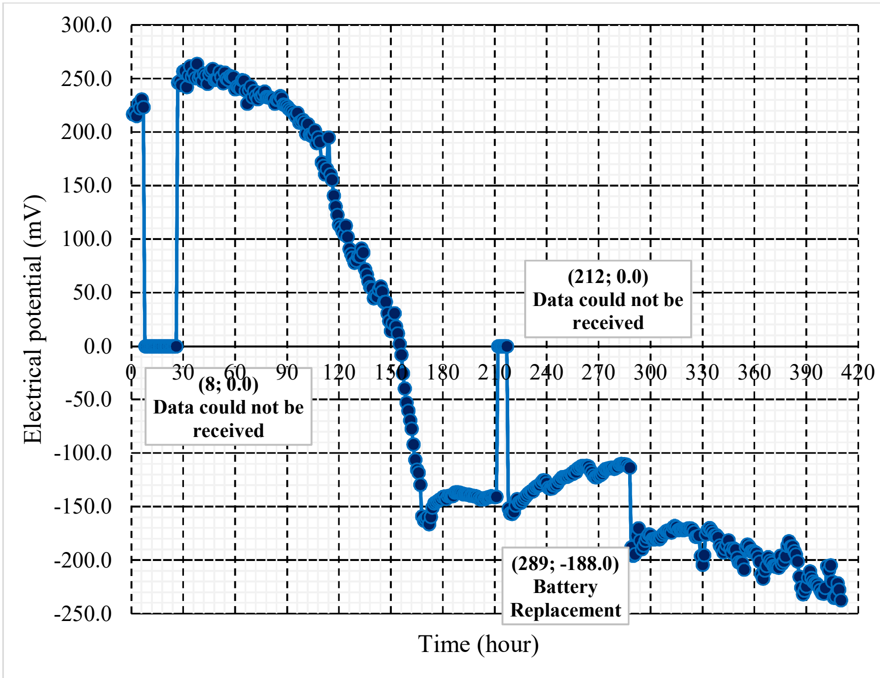

Figure 4. Electrical potential measurements of the system.

The maximum and minimum densities and power densities were calculated using the maximum and minimum resistance values obtained from the resistance measurements of the system. When the calculated current density was examined, it was found that the best maximum value was $1100*10^{-6}$ mA/cm^2, which is understood from Figure 5. A comparison of these results with those of a study performed by SMFC for dairy wastewater treatment showed that the best maximum value was greater than the current density ($67.85*10^{-6}$ mA/cm^2) [23]. However, if the resistance of the system reaches a maximum, the value obtained ($1.52*10^{-6}$ mA/cm^2), as shown in Figure 6, is considerably lower than the value in the mentioned study.

The best maximum was $279*10^{-3}$ mW/cm^2 (as shown in Figure 7), and the minimum was $0.38*10^{-3}$ mW/cm^2 (as shown in Figure 8). These values exceeded the value ($52.92*10^{-6}$ mW/cm^2) in the study conducted in 2010 [23]. Because of the sharp fluctuations in the resistances, there is a significant difference between the maximum and minimum current and power densities of the systems. For this reason, a resistance equal to or higher than the calculated resistance should be connected to the system to increase the current passing through the system and to increase the stability of the system's operation.

Studying the removal of whey with sediment microbial fuel cells is not extremely common. Comparing the electrical potential value (264 mV) to the electrical potentials (453 mV [37] and 925 mV [38]) in the literature, it was discovered that, when previous studies involving the removal of whey by MFC were investigated, the electrical potential value was at a level that needs improvement. It is possible to conclude that the power density value ($279*10^{-3}$ mW/cm^2) value discovered significantly advances the research reported in the literature.

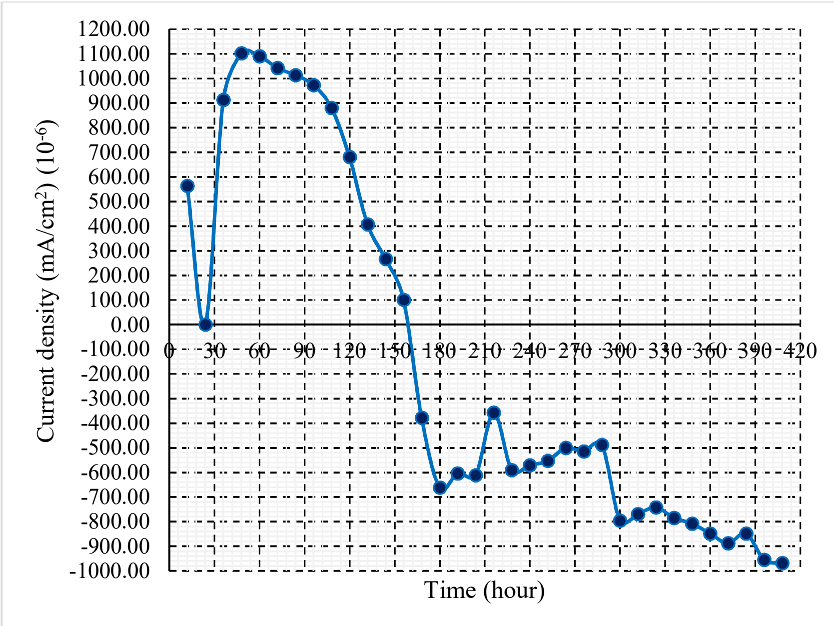

Figure 5. Maximum current densities of the system (from minimum internal resistance).

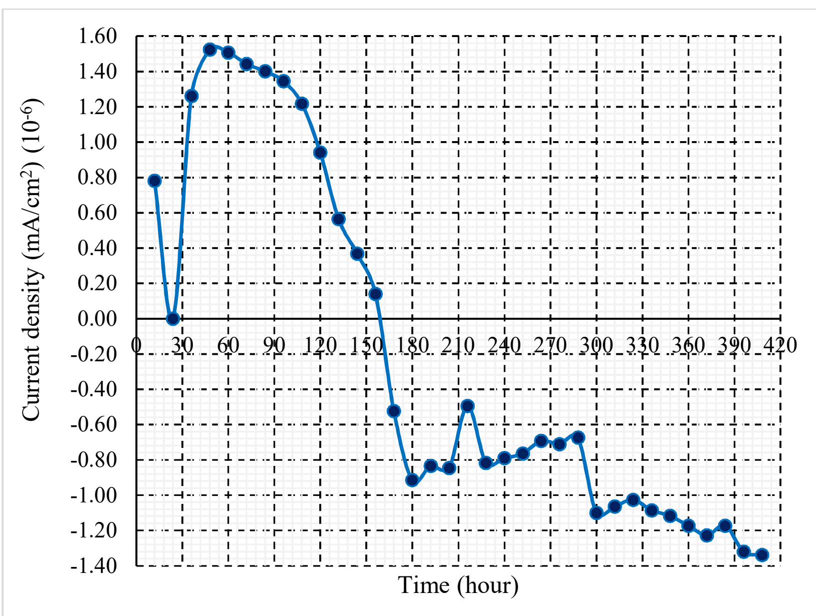

Figure 6. Minimum current density of the system (from minimum internal resistance).

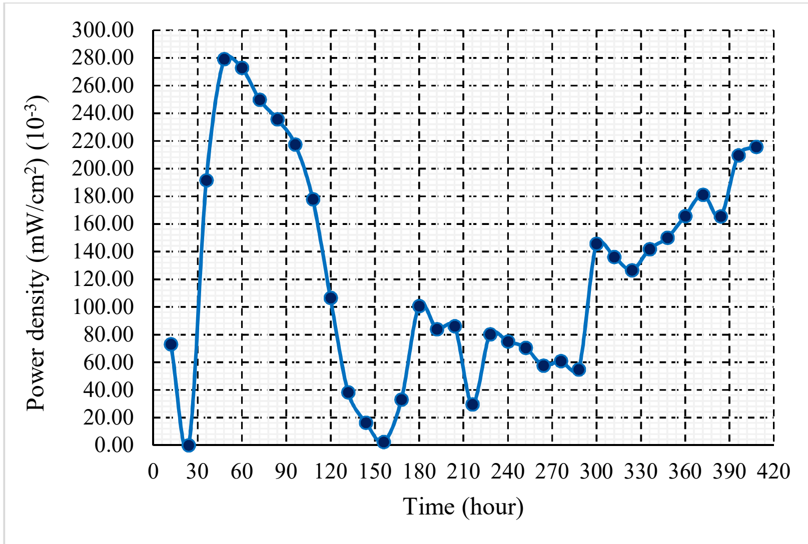

Figure 7. Maximum power densities of the system (from minimum internal resistance).

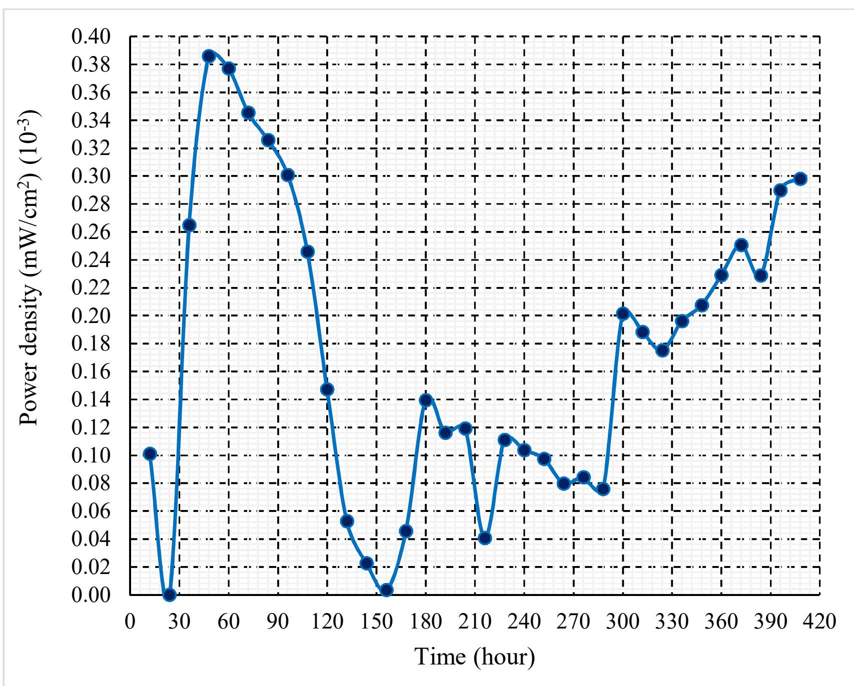

Figure 8. Minimum power density of the system (from minimum internal resistance).

Evaluation of wastewater treatment performance

The removal rates were 94% and 76%, respectively. In a previous study, cellulose removal was performed using river SMFCs, and an approximately 73% removal rate was obtained [16]. This study proves the accuracy of the removal rate we achieved.

The conductivity, pH, TDS, and temperature were determined, and the results are shown in Table 2. As understood from the table, the system and ambient temperature do not change much. However, the conductivity and TDS increase in all the cases, which means that the amount of ions in the system increases. In addition, the pH of the whey increased. This could be attributed to the elevated pH of the water in the SMFC, as protons are consumed by cathodic processes [40].

Table 2. Parameter values of the whey before and after putting it in the system.

Parameters	Before treatment	After treatment
pH	4.3	5.7
Conductivity (µS/cm)	5840	12000
TDS (ppm)	2190	3710
System Temperature (°C)	16	14
Ambient Temperature (°C)	17	16

CONCLUSION

In this study, an attempt was made to generate electricity utilizing cheese whey with river SMFCs. The microorganisms in the river SMFC were mixed in culture, and it was found that most of the microorganisms consisted of *Bacteroides* and *Clostridium*. Volt and internal resistance measurements were made for three weeks, and the current and power density were calculated from the results obtained. As a result of the calculations, the maximum current ($1100*10^{-6}$ mA/cm^2) and power density ($279*10^{-3}$ mW/cm^2) were obtained. These measurements were performed without using constant external resistance. Therefore, for more stable results, the voltage, current density, and power density can be recalculated with external resistance by determining the value close to the internal resistance of the system.

REFERENCES

1. Akansha, J.; Nidheesh, P.V.; Gopinath, A.; Anupama, K.V.; Suresh Kumar, M. Treatment of dairy industry wastewater by combined aerated electrocoagulation and phytoremediation process. *Chemosphere* **2020**, 253, 126652.
2. Cruz-Salomón, A.; Ríos-Valdovinos, E.; Pola-Albores, F.; Lagunas-Rivera, S.; Cruz-Rodríguez, R.I.; Cruz-Salomón, K. del C.; Hernández-Méndez, J.M.E.; Domínguez-Espinosa, M.E. Treatment of cheese whey wastewater using an expanded granular sludge bed (EGSB) bioreactor with biomethane production. *Processes* **2020**, 8, 931.
3. Ribera-Pi, J.; Badia-Fabregat, M.; Calderer, M.; Polášková, M.; Svojitka, J.; Rovira, M.; Jubany, I.; Martínez-Lladó, X. Anaerobic membrane bioreactor (AnMBR) for the treatment of cheese whey for the potential recovery of water and energy. *Waste Biomass Valori.* **2020**, 11, 1821–1835.
4. Vilajeliu-Pons, A.; Puig, S.; Salcedo-Dávila, I.; Balaguer, M.D.; Colprim, J. Long-term assessment of six-stacked scaled-up MFCs treating swine manure with different electrode materials. *Environ. Sci. (Camb.)* **2017**, 3, 947–959.
5. Nasirahmadi, S.; Safekordi, A.A. Whey as a substrate for generation of bioelectricity in microbial fuel cell using *E. coli*. *Int. J. Environ. Sci. Technol.* **2011**, 8, 823–830.

6. Zabihallahpoor, A.; Rahimnejad, M.; Talebnia, F. Sediment microbial fuel cells as a new source of renewable and sustainable energy: Present status and future prospects. *RSC Adv.* **2015**, 5, 94171–94183.
7. Xu, B.; Ge, Z.; He, Z. Sediment microbial fuel cells for wastewater treatment: Challenges and opportunities. *Environ. Sci. (Camb.)* **2015**, 1, 279–284.
8. Danhassan, U.A.; Lin, H.; Lawan, I.; Zhang, X.; Ali, M.H.; Muhammad, A.I.; Sheng, K. Critical insight into sediment microbial fuel cell: Fundamentals, challenges, and perspectives as a barrier to black-odor water formation. *J. Environ. Chem. Eng.* **2023**, 11, 109098.
9. He, Z.; Shao, H.; Angenent, L.T. Increased power production from a sediment microbial fuel cell with a rotating cathode. *Biosens. Bioelectron*. **2007**, 22, 3252–3255.
10. Wang, A.; Cheng, H.; Ren, N.; Cui, D.; Lin, N.; Wu, W. Sediment microbial fuel cell with floating biocathode for organic removal and energy recovery. *Front. Environ. Sci. Engi. China*, **2012**, 6, 569–574.
11. Song, T.S.; Tan, W.M.; Wu, X.Y.; Zhou, C.C. Effect of graphite felt and activated carbon fiber felt on performance of freshwater sediment microbial fuel cell. *J. Chem. Technol. Biotechnol.* **2012**, 87, 1436–1440.
12. Zhang, H.; Zhu, D.; Song, T.S.; Ouyang, P.; Xie, J. Effects of the presence of sheet iron in freshwater sediment on the performance of a sediment microbial fuel cell. *Int. J. Hydrogen Energy* **2015**, 40, 16566–16571.
13. Alipanahi, R.; Rahimnejad, M. Effect of different ecosystems on generated power in sediment microbial fuel cell. *Int. J. Energy Res.* **2018**, 42, 4891–4897.
14. Mitov, M.; Bardarov, I.; Mandjukov, P.; Hubenova, Y. Chemometrical assessment of the electrical parameters obtained by long-term operating freshwater sediment microbial fuel cells. *Bioelectrochemistry* **2015**, 106, 105–114.
15. Chen, S.; Tang, J.; Fu, L.; Yuan, Y.; Zhou, S. Biochar improves sediment microbial fuel cell performance in low conductivity freshwater sediment. *J. Soils Sediments* **2016**, 16, 2326–2334.
16. Zhu, D.; Song, D.W.T. Enhancement of cellulose degradation in freshwater sediments by a sediment microbial fuel cell. *Biotechnol. Lett*. **2016**, 38, 271–277.
17. Chen, L.; Zheng, X.; Zhang, K.; Wu, B.; Pei, X.; Chen, W.; Wei, X.; Luo, Z.; Li, Y.; Zhang, Z. Sustained-release nitrate combined with microbial fuel cell: A novel strategy for PAHs and odor removal from sediment. *J. Hazard. Mater.* **2023**, 455, 131610.
18. Kim, A.; Simson, A. Rapid optimization of 3D printed sediment microbial fuel cells. *Int. J. Energy Environ. Eng.* **2023**, 14, 243–255.
19. Surampalli, R.Y. *Natural Attenuation of Hazardous Wastes*, American Society of Civil Engineers, USA; **2004**; p. 2.
20. Logan, B.E. *Microbial Fuel Cells*, A John Wiley & Sons, Inc.: New Jersey; **2007**; p. 25.
21. Kassongo, J.; Togo, C.A. The potential of whey in driving microbial fuel cells: A dual prospect of energy recovery and remediation. *Afr. J. Biotechnol.* **2010**, 9, 7885–7890.
22. Spahn, I.; Hall, K.; Bell, S.; Heiderscheit, C.; Kertsman, T.; Churchill, T. *How to Clean Beach Sand*, USA, May **2024**. Available at: https://www.wikihow.com/Clean-Beach-Sand.
23. Saravanan, R.; Arun, A.; Venkatamohan, S.; Jegadeesan, Kandavelu, T.; Veeramanikandan, T. Membraneless dairy wastewater-sediment interface for bioelectricity generation employing sediment microbial fuel cell (SMFC). *Afr. J. Microbiol. Res.* **2010**, 4, 2640–2646.
24. Montana State University. *X-Ray Fluorescence (XRF),* USA, February **2024**. Available at: https://serc.carleton.edu/research_education/geochemsheets/techniques/XRF.html.
25. Inc, G.E. *What is Metagenomics? -Definition, Steps, Process and Applications*, India, February **2024**. Available at: https://geneticeducation.co.in/what-is-metagenomics-definition-steps-process-and-applications/.
26. Swapp, S. *Scanning Electron Microscopy (SEM)*, USA, March **2024**. Available at: https://serc.carleton.edu/research_education/geochemsheets/techniques/SEM.html.

27. Rosenberg, E.; F. DeLong, E.; Lory, S.; Stackebrandt, E.; Thompson, F. *The Prokaryotes - Springer*; **2014**.
28. Vishwanathan, A.S. Microbial fuel cells: A comprehensive review for beginners. *3 Biotech*, **2021**, 11, 1–14.
29. Mahmoud, R.H.; Gomaa, O.M.; Hassan, R.Y.A. Bio-electrochemical frameworks governing microbial fuel cell performance: Technical bottlenecks and proposed solutions. *RSC Adv.* **2022**, 12, 5749–5764.
30. Rogers, *K. Clostridium: Bacteria, Characteristics, & Infection*, England, March **2024**. Available at: https://www.britannica.com/science/Clostridium.
31. Han, S.; Gao, X.Y.; Ying, H.J.; Zhou, C.C. NADH gene manipulation for advancing bioelectricity in clostridium ljungdahlii microbial fuel cells. *Green Chem.* **2016**, 18, 2473–2478.
32. Cao, Y.; Mu, H.; Liu, W.; Zhang, R.; Guo, J.; Xian, M.; Liu, H. Electricigens in the anode of microbial fuel cells: pure cultures versus mixed communities. *Microb. Cell Fact* **2019**, 18, 1–14.
33. Drendel, G.; Mathews, E.R.; Semence, L.; Franks, A.E. Microbial fuel cells, related technologies, and their applications. *Appl. Sci.* **2018**, 8, 2384.
34. Gezginci, M.; Uysal, Y. The effect of different substrate sources used in microbial fuel cells on microbial community. *JSM Environ. Sci. Ecol*. **2016**, 4, 1035.
35. Jenol, M.A.; Ibrahim, M.F.; Bahrin, E.K.; Kim, S.W.; Abd-Aziz, S. Direct bioelectricity generation from sago hampas by *Clostridium beijerinckii* SR1 using microbial fuel cell. *Molecules* **2019**, 24, 2397.
36. Awg-Adeni, D.S.; Bujang, K.B.; Hassan, M.A.; Abd-Aziz, S. Recovery of glucose from residual starch of sago hampas for bioethanol production. *Biomed. Res. Int.* **2013**, 2013, 935852.
37. Dalvi, A.D.; Mohandas, N.; Shinde, O.A.; Kininge, P.T. Microbial fuel cell for production of bioelectricity from whey and biological waste treatment. *Int. J. Adv. Biotechnol. Res.* **2011**, 2, 263–268.
38. Mahato, J.; Miah, M.; Shovon, M.S.; Roy, N.; Easmin, M.S.; Sharma, S.C.D. Electricity production by microbial fuel cell using cheese whey wastewater of the dairy industry in Rajshahi, Bangladesh. *Chem. Biochem. Eng. Q.* **2022**, 35, 421–430.
39. Zhao, Q; Min, J.; Hongmei, C.; Yanli, L. An overview of recent advances in sediment microbial fuel cells recent advances in sediment microbial fuel cells. *IOP Conference Series Earth and Environmental Science* **2021**, 922, 012002.