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Research Article

Treatment of Cheese Whey and Bioelectricity Generation in MFCs as a Substitute Source of Energy in Wastewater

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Abstract. Renewable energy is a primary energy source that naturally replenishes over time. It is derived from various large-scale sources, including ocean tides, sunlight, rainfall, wind, biomass, and geothermal heat generated deep within the Earth. In 2008, about 19% of global energy consumption came from renewable sources, with approximately 13% from biomass and 3.2% from hydroelectric power. A microbial fuel cell (MFC) is a reliable technology that generates electricity while removing contaminants from wastewater. The bacteria in the MFC's anode facilitate the breakdown of the substrate, producing electrons and protons through anaerobic respiration of the substrate. This research is based on finding a more effective means and technique for high production of electricity using MFCs as well as to ascertain the efficiency of MFCs in the treatment of Whey as wastewater. The APHA, photometric method, turbidometry and ascorbic acid methods were used to determine components like BOD, COD, TSS, phosphorus and sulphate. Cheese whey shows promise for electricity generation in microbial fuel cells (MFCs) compared to other biomass sources. The highest voltage and current achieved with cheese whey were 56.8 mV and 5.68 mA, while the maximum current and power densities for MFC I were 0.339 mA/cm^2 and 19.2 mW/cm^2. In MFC II, peak voltage and current reached 73.7 mV and 7.37 mA, with a maximum current density of 0.44 mA/cm^2 and power density of 32.4 mW/m^2. This experiment showed efficient COD removal rates of 83.97% and 92.85% for MFC I and MFC II, respectively, and BOD$_5$ values of 61.95 and 73.95, indicating good biodegradability of the substrate, with BOD/COD ratios of 0.65 and 0.60.

Keywords: Cheese Whey, BOD, COD, Wastewater, Electricity, MFCs and *Saccharomyces cerevisiae*.

1. Introduction

Renewable energy is a natural energy source that replenishes itself frequently. It is harnessed on a large scale from sources like ocean tides, sunlight, rainfall, wind, biomass, and geothermal heat generated deep within the Earth. In 2008, renewable energy contributed about 19% of global energy consumption, with biomass accounting for approximately 13% and hydroelectricity for 3.2%. The choice of energy source significantly impacts greenhouse gas emissions, water resource allocation, mineral use, and equipment production and transport. Renewable energies tend to be more sustainable than many traditional energy forms. However, assessing the sustainability of renewable energy requires careful evaluation of resource efficiency, techno-economic feasibility, cost analysis, life cycle impacts, environmental externalities, economic assessments, production costs, research and development objectives, and reliability of water demand and distribution, among other factors (Sadiq et al., 2023). In general, renewable energy solutions may not suit every community due to several factors: i) The distribution of organic matter varies by geography. ii) Energy usage depends on cultural practices within different communities. iii) Other limiting factors include growth rates and available infrastructure.

The sustainable use of renewable energy requires analyzing its impact across three main aspects: environmental effects, financial viability, and external costs. Therefore, before implementing renewable energy solutions, thorough research is necessary to ensure no adverse social, environmental, or economic impacts occur. Microbial fuel cells (MFCs) represent a promising yet challenging technology. In MFCs, microorganisms connect to electrodes by transferring electrons, which are either accepted or released through an electrical circuit (Rabaey et al., 2007).

Microbial fuel cells (MFCs) are a central type of bioelectrochemical system (BES) that directly convert organic material into electricity through the metabolic processes of microbes. MFCs are seen as a promising technology for meeting rising energy needs, particularly by utilizing wastewater as a substrate. This approach not only generates electricity but also treats wastewater, potentially reducing operating costs in wastewater treatment plants (Lu et al., 2009). The idea that bacteria could produce electricity was first demonstrated by Potter in 1911. MFCs efficiently generate power and remove contaminants from wastewater, where bacteria in the anode chamber aid in breaking down the substrate, releasing electrons and protons through anaerobic respiration. Electrons move from the anode to the cathode via an external circuit, while protons pass through a proton exchange membrane (PEM) to the cathode chamber, where they combine with a mediator (Venkat et al., 2008).

MFCs offer distinct advantages over traditional technologies, such as lower operating costs due to passive oxygen diffusion to the cathode (eliminating the need for aeration), reduced sludge production, and electricity generation. Significant advancements have been made recently to improve power output through innovative reactor designs and new electrode materials. For instance, using cost-effective materials like activated carbon cathodes and graphite fibre brush anodes has significantly reduced electrode costs, paving the way for affordable, large-scale MFC systems (Zhang et al., 2014). In MFCs, microbes convert biochemical energy, produced during substrate metabolism, into electricity while simultaneously treating wastewater. The MFC setup consists of an anode and a cathode, separated by a PEM and connected externally. Within the anode chamber, microbes break down the substrate, releasing electrons and protons, which then travel to the cathode through the external circuit and PEM, respectively. Under anaerobic conditions, certain bacteria can transfer electrons to the anode, where these electrons then move through a conductor with specific resistance to the cathode. At the cathode, they combine with protons and oxygen to form water, generating current and voltage.

As microbes degrade the substrate in wastewater within the MFC, electricity is generated. Given the anticipated depletion of fossil fuels, renewable energy sources are increasingly valuable due to issues like greenhouse gas emissions. Currently, human activities are the primary cause of climate change through CO_2 emissions (Barat et al., 2008; Najafpour et al., 2011). Sourcing energy from sustainable sources, like biomass, is reliable, and sustainable, and contributes to reducing global CO_2 emissions (Jung and Regan, 2007; Greenman et al., 2009; Oh et al., 2009). While fuel cells represent an alternative energy method, many fuel cell technologies require hydrogen, which is typically derived from fossil fuels (Jafary et al., 2013).

Microbial fuel cells (MFCs) are an innovative approach used to generate green energy, such as hydrogen gas and electricity, directly from various organic and inorganic substances while also treating biodegradable pollutants in wastewater (Rahimnejad et al., 2012). The efficiency of MFCs can be enhanced through several key processes essential to their operation, including cell metabolism, proton exchange membrane (PEM) transport, bacterial electron transfer, cathode oxidation, and managing both external and internal resistance. These operational factors greatly impact electron transfer and energy production (Jafary et al., 2012). A unique feature of MFC technology is the microbes themselves. Certain electricity-producing bacterial strains can directly transfer electrons generated through metabolic processes across the membrane to an external circuit, without the need for added artificial mediators (Tardast et al., 2014).

The study goal is to use cheese whey, a byproduct of the dairy industry, as a target for microbial fuel cells (MFCs) to remediate wastewater and as a substrate for the production of bioelectricity. Although a variety of biomass sources have been investigated in the past for the production of bioelectricity, cheese whey has received little attention despite its high organic content and widespread availability. This study shows that cheese whey has a remarkable potential for attaining high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) removal rates, in addition to its better efficiency in producing bioelectricity when compared to other biomass sources. Through the integration of cutting-edge approaches including ascorbic acid methods, photometric techniques, and APHA standards, this study offers a thorough examination of the physicochemical factors affecting MFC performance. The results provide a scalable way to combine renewable energy technologies with industrial waste management by filling important gaps in the optimization of MFC systems for both efficient wastewater treatment and sustainable energy generation.

2. Method

Several methods were administered to determine the required parameters during this analysis. First, the Cheese Whey was collected from Pak Sut, located at Haspolat, Nicosia North-Cyprus, and the microbial fuel cell was operated over 32 days. The experiment was conducted in batch mode, with all essential parameters and characteristics of the cheese whey thoroughly prepared and adjusted as required for the study.

2.1. TOC and TN Analysis

Cheese whey wastewater was gathered in a beaker and filtered through a 0.45μm filter. Next, 1 ml of the filtered whey wastewater was added to a beaker with 99 ml of distilled water, resulting in a total volume of 100 ml. Analysis of total carbon (TC), total nitrogen (TN), and total organic carbon (TOC) was performed using a TC/TN analyzer. The machine used in this analysis undergoes the catalytic combustion principle.

2.2. BOD Analysis

APHA standard method was used to analyse BOD in water and wastewater. Biochemical Oxygen Demand degraded significantly on storage between the periods of collection and analysis primarily causing low BOD value.

2.3. COD Analysis

Forty grams of dried green tea leaves were mixed with 100 ml of distilled water in a 250 ml beaker glass. The solution was heated on a heater with a magnetic stirrer at 95-100℃ for 15 minutes. After mixing, the filtrate solution was separated with filter paper on a vacuum filtration. Calculation of Percentage COD Removal Effi- ciency: The percentage COD removal efficiency was determined using equation (1). Where, CODin is the in- fluent COD and CODeff is the effluent COD.

$$\%COD\ removal = \frac{(CODin - CODeff \times 100)}{CODin} \qquad (1)$$

2.4. Total Suspended Solid

White cheese whey wastewater was collected in a beaker, and 1 ml of it was added to a beaker containing 199 ml to reach a total volume of 200 ml. This 200 ml of whey wastewater was then blended at high speed for exactly two minutes. The blended sample was poured into a beaker, stirred, and immediately, 2 ml was transferred into a vial. Another vial was filled with 2 ml of deionized water, and used to calibrate the spectrophotometer, setting the display to 0 mg/L TSS. The sample vial was swirled to release any gas bubbles and ensure even suspension of any particles, then cleaned and placed into the spectrophotometer. Results were displayed as mg/L TSS at a wavelength of 810 nm.

2.5. Phosphorus Analysis

The ascorbic acid method was employed to determine the phosphorus content. 5mL of the sample was pipette and made up to 50 mL using distilled water and placed into an acid-cleaned, dry 250 mL Erlenmeyer flask. 1 drop of phenolphthalein indicator was added. 8.0mL of combined reagent was added and mixed thoroughly. It was allowed for 10 minutes for colour development. Absorbances were measured at 880nm.

2.6. Sulphate Analysis

The Turbidometry method was applied to determine the sulphate content in the Cheese Whey. Analyzing the sulphate content in white cheese whey wastewater is crucial, as it not only reveals the sulphate concentration in the sample but also highlights the potential issues that may arise from the reduction of sulphate to hydrogen sulfide.

2.7. Microbial Fuel Cell Setup

A two-chamber MFC system was assembled, labelled as MFC I and MFC II (Figure 1). Two plastic containers, each with a reactor volume of 1200 ml, were designated as the cathode and anode chambers. Each container lid had two holes drilled for inserting the salt bridge and electrode. In the anode chamber, 1000 ml of substrate (cheese whey) was added, and the pH was adjusted to 7.0 with a 0.5 M NaOH solution. The anode container was sealed tightly with tape. For the cathode chamber, a 0.5 M KOH solution served as the catholyte and an air sparger was included to ensure constant aeration. MFC II was inoculated with *Saccharomyces cerevisiae*, while MFC I was left uninoculated. Voltage and current were measured using a digital multimeter every 24 hours for 32 days, and the power and current densities were calculated using equation 2-4. Where I is the current intensity, P is the power production, V is the voltage, R is the resistance 10 ohms, and A is the total surface area.

$$Power\ density = \frac{VI}{A} \qquad (2)$$

$$Current\ density = \frac{I}{A} \tag{3}$$

$$Current = \frac{I}{A} \tag{4}$$

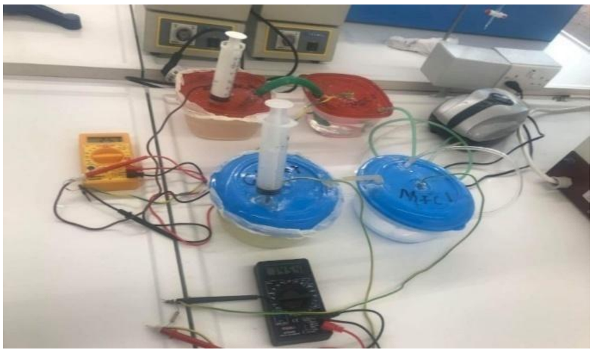

Figure 1. Double Chamber Microbial Fuel Cell

2.6. Saccharomyces cerevisiae Inoculation

Saccharomyces cerevisiae was used as a pure culture alongside other microorganisms naturally present in cheese whey wastewater. Commercially available active dry yeast was employed to break down the organic material. Four grams of *Saccharomyces cerevisiae*, in pellet form, was crushed using a mortar and pestle. The crushed yeast was then dissolved in 40 mL of warm distilled water by gently swirling. Once dissolved, it was transferred to a 250 mL sealed bottle containing wastewater and left at room temperature for 24 hours to allow for acclimation. After this period, 20 mL of the solution was added to the constructed model to proceed with the experiments.

3. Result and Discussion

The experiment was performed in batch mode and all necessary parameter as well as Cheese Whey Wastewater characteristics was demonstrated.

3.1. Characteristic content of Cheese Whey

As shown in Table 1, the experiment was conducted using two reactors, MFC I and MFC II, both with identical volumes and capacities. MFC II was inoculated with *Saccharomyces cerevisiae*, while MFC I was left uninoculated. Before operating the MFCs, tests for Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Suspended Solids (TSS), Total Organic Carbon (TOC), Total Carbon (TC), and Total Nitrogen (TN) were performed.

Table 1. Environmental parameters at three research stations

Station Research	Coordinate point	Environmental parameters				
		Temp (^{o}C)	Salinity (ppt)	pH	Brightness (m)	DO (mg / L)
1	6 ° 54'46.6 "S 110 ° 29'08.9 "E	30.5	32	7.4	3.28	5.52
2	6 ° 55'25.0 "S 110 ° 28'44.1 "E	31	28	7.4	3.44	5.41
3	6 ° 55'13.6 "S 110 ° 29'01.2 "E	30.6	30	7.8	3.52	5.78

As indicated in the Table 2, the experiment was conducted in two reactors, MFC I and MFC II, both with the same volume and capacity. *Saccharomyces cerevisiae* was added to MFC II, while MFC I remained uninoculated. After operating the MFCs, tests were conducted to measure Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Suspended Solids (TSS), Total Organic Carbon (TOC), Total Carbon (TC), and Total Nitrogen (TN).

Table 2. Final Characteristics of White Cheese Whey

Parameters	MFCI	MFCII
TOC	2873mg/L	3418mg/L
TN	247mg/L	396mg/L
TC	3018mg/L	3456mg/L
BODs	5250mg/L	3750mg/L
COD	3414mg/L	1711mg/L
TSS	1096mg/L	1213mg/L
Sulphate	214.6mg/L	265mg/L
Phosphate	31 mg/L	23 mg/L

Table 3. Power Production, COD Reduction, and TSS Levels in a Double-Chamber MFC I Supplied with White Cheese Whey in Batch Mode

Time Days	pH	Voltage mV	Current Ma	Power Density (mW/cm^2)	Currrent Density (mA/cm^2)	COD mg/L	TSS mg/L
0	7.00	5.7	0.57	0.19	0.034	21300	6112
1	6.71	7.9	0.79	0.37	0.047	21045	6094
2	6.94	10.5	1.05	0.66	0.063	20086	5870
3	6.68	13.8	1.38	1.13	0.082	20969	5567
4	6.76	17.6	1.76	1.84	0.105	19445	5278
5	6.83	21.4	2.14	2.73	0.128	19131	5005
6	6.75	28.5	2.85	4.84	0.169	19055	4721
7	6.69	34.7	3.47	7.18	0.207	18287	4903
8	6.76	36.9	3.69	8.11	0.220	18568	4454
9	6.97	39.8	3.98	9.44	0.237	18010	4109
10	6.66	41.5	4.15	10.3	0.247	17658	3870
11	6.78	47.6	4.76	13.5	0.284	17091	4187
12	6.83	51.2	5.12	15.6	0.305	16760	3908
13	6.89	56.8	5.68	19.2	0.339	16334	3867
14	6.75	55.9	5.59	18.6	0.333	15711	3440
15	6.81	53.1	5.31	16.8	0.316	14816	3102
16	6.65	50.7	5.07	15.3	0.302	14472	2870
17	6.68	51.2	5.12	15.6	0.305	13311	3169
18	6.72	49.6	4.96	14.7	0.296	12887	2715
19	6.88	50.2	5.02	15.0	0.299	12957	2450
20	6.81	48.4	4.84	13.9	0.288	11485	2213
21	6.61	49.1	4.91	14.3	0.293	9841	2016
22	6.69	46.7	4.67	13.0	0.278	9436	1914
23	6.74	47.2	4.72	13.3	0.281	8147	1882
24	6.81	44.0	4.40	11.5	0.262	7334	1653
25	6.82	45.3	4.53	12.2	0.270	7953	1732
26	6.62	43.3	4.33	11.2	0.259	6840	1433
27	6.70	41.1	4.11	10.1	0.245	5119	1165
28	6.69	41.8	4.18	10.4	0.249	4715	1143
29	6.63	38.8	3.88	8.97	0.231	4239	1126
30	6.87	36.5	3.65	7.94	0.218	3362	1119
31	6.81	37.2	3.72	8.25	0.222	3658	1115
32	6.76	36.2	3.62	7.81	0.216	3414	1096

As shown in Table 3, there was a steady increase in power generation from the initial setup day of the reactor, followed by a significant rise. Some fluctuations in voltage and current were observed, likely due to daily feeding of the reactor and pH adjustments. The highest voltage and current recorded were on day 13, with values of 56.8 mV and 5.68 mA, respectively, at an optimal pH of 6.89. COD levels steadily decreased from 21,300 mg/L to 3,414 mg/L. TSS, an indicator of water quality, also showed a consistent reduction, reflecting the breakdown of suspended particles in the substrate from 6,112 mg/L to 1,096 mg/L.

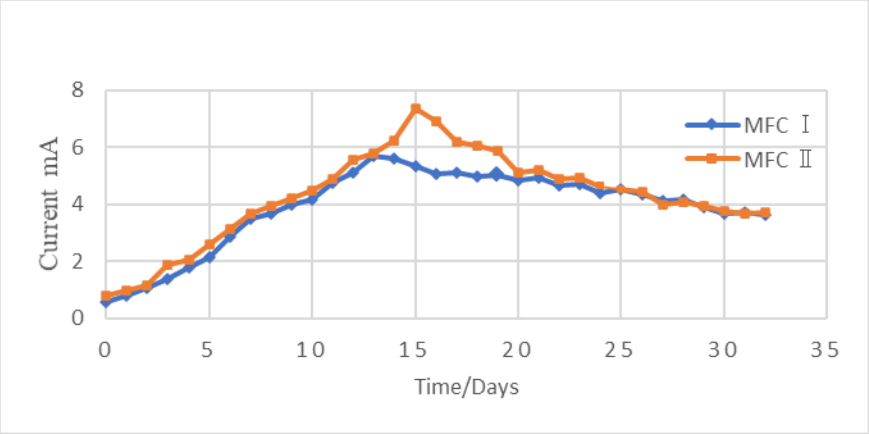

Figure 2. Generated Current recorded from MFC I and MFC II

The current experienced a gradual increase over two to three days before beginning to decline (Figure 2). The peak current recorded was 5.68 mA in MFC I, which is higher than the electricity generation results from cheese whey using an MFC reported by Antonopoulou et al. (2014). This increase in current reflects the growth of exoelectrogenic microorganisms within the substrate of MFC I. In MFC II, the lack of oxygen allows *Saccharomyces cerevisiae* to produce byproducts such as ethanol, acetic acid, and carbon dioxide, which release additional energy. The reduction of NAD+ to NADH during this process generates ATP, protons, and electrons (Nelson et al., 2005). The maximum current produced in MFC II reached 7.37 mA, exceeding the results found by Aswin et al. (2017). The MFC was effectively producing this current because its efficiency relies on the catalytic processes of the substrate. The current generation began to stabilize at pH levels of 6.89 and 6.86, as shown in Tables 3 and 4. The optimal pH for microbial activity in the absence of oxygen is between 6.5 and 7.0 (Venkata et al., 2008). The increase in current is attributed to a higher rate of substrate breakdown and the availability of nutrients such as sulfate and phosphate, which are essential for microorganisms' metabolism. This process facilitates the formation of a biofilm on the electrode surface. The utilization of total carbon (TC) and total nitrogen (TN) shown in Table 2 indicates that exoelectrogenic microorganisms in MFC I and *Saccharomyces cerevisiae* in MFC II can use carbon as an energy source and nitrogen for growth. This phenomenon impacts the rate of electric current generation by microorganisms (Mohan et al., 2007). Consequently, the rate of electric current produced decreases compared to the initial operation of the MFC, during which biofilm formation occurs.

Table 4. Power Production, COD Reduction, and TSS Levels in Double-Chamber MFC II Supplied with White Cheese Whey in Batch Mode

Time Days	pH	Voltage mV	Current Ma	Power Density (mW/cm^2)	Currrent Density (mA/cm^2)	COD mg/L	TSS mg/L
0	7.00	7.80	0.78	0.03	0.05	23934	6300
1	6.68	9.60	0.96	0.05	0.06	22345	6284
2	6.79	11.7	1.17	0.81	0.07	23086	6212
3	6.83	18.6	1.86	2.06	0.11	20867	6253
4	6.67	20.4	2.04	2.48	0.12	19745	6198
5	6.72	25.7	2.57	3.94	0.15	19432	6105
6	6.64	31.5	3.15	5.91	0.18	19657	5872
7	6.61	36.8	3.68	8.07	0.21	18981	5431
8	6.73	39.2	3.92	9.16	0.23	18664	5532
9	6.88	41.9	4.19	10.5	0.24	18047	5230
10	6.72	44.8	4.48	11.9	0.28	17854	5102
11	6.63	48.7	4.87	13.0	0.29	16775	4991
12	6.65	55.4	5.54	18.3	0.33	15962	4733
13	6.70	57.7	5.77	19.8	0.34	16135	4705
14	6.68	62.1	6.21	22.9	0.37	15449	4213
15	6.86	73.7	7.37	32.4	0.44	12784	4018
16	6.69	69.1	6.91	22.9	0.41	12273	3765
17	6.61	61.9	6.19	22.8	0.37	9757	3643
18	6.54	60.3	6.03	21.7	0.35	10788	3750
19	6.64	58.7	5.87	22.8	0.34	9757	3643
20	6.76	51.1	5.11	21.2	0.30	9685	3409
21	6.71	51.9	5.19	20.5	0.31	9211	3340
22	6.68	48.7	4.87	15.6	0.29	8434	3117
23	6.58	49.3	4.93	16.1	0.29	7549	3203
24	6.61	46.2	4.62	14.1	0.28	7132	3016
25	6.67	45.1	4.51	14.5	0.27	6753	2785
26	6.72	44.3	4.43	12.7	0.26	5170	2852
27	6.75	39.8	3.98	12.1	0.24	4514	2344
28	6.65	40.7	4.07	11.7	0.24	3016	1902
29	6.74	39.3	3.93	9.20	0.23	2569	1832
30	6.81	37.4	3.74	8.34	0.22	2204	1629
31	6.77	36.6	3.66	7.98	0.21	1736	1221
32	6.69	37.1	3.71	8.20	0.22	1711	1213

In MFC I, white cheese whey wastewater was used as the substrate without any inoculation. The analysis was conducted with KOH serving as the catholyte, while in MFC II, *Saccharomyces cerevisiae* was employed as an inoculum to facilitate substrate breakdown. In the absence of oxygen, *Saccharomyces cerevisiae* enters a fermentation state, producing pyruvate. This pyruvate is then converted to acetaldehyde by the enzyme pyruvate decarboxylase. Subsequently, acetaldehyde is transformed into ethanol through the action of alcohol dehydrogenase, which requires NADH. Without oxygen, the regeneration of NADH to NAD+ is essential for maintaining the glycolytic process (Nelson and Feldmann 2005). With high concentrations of Total Carbon (TC) at 12,836 mg/L and Total Nitrogen (TN) at 3,780 mg/L in MFC I, and 13,820 mg/L and 4,027 mg/L in MFC II, the microorganisms utilize carbon as an energy source and nitrogen for cellular structure (Igoni et al., 2008). As ethanol is produced, electrons are released in the process. The voltage generated in MFC II reached 73.7 mV, aligning with findings by Liu et al. (2004). MFC, I achieved a maximum voltage of 56.8 mV, which is higher than the results reported by Aswin et al. (2017). The generated voltage illustrates the growth curve of exoelectrogenic microorganisms, as the voltage increases during the exponential phase, as seen in Figure 2, but eventually stabilizes and declines as the MFC enters the decline phase due to microbial death, indicating nutrient depletion in the anodic chamber (Nair et al., 2013).

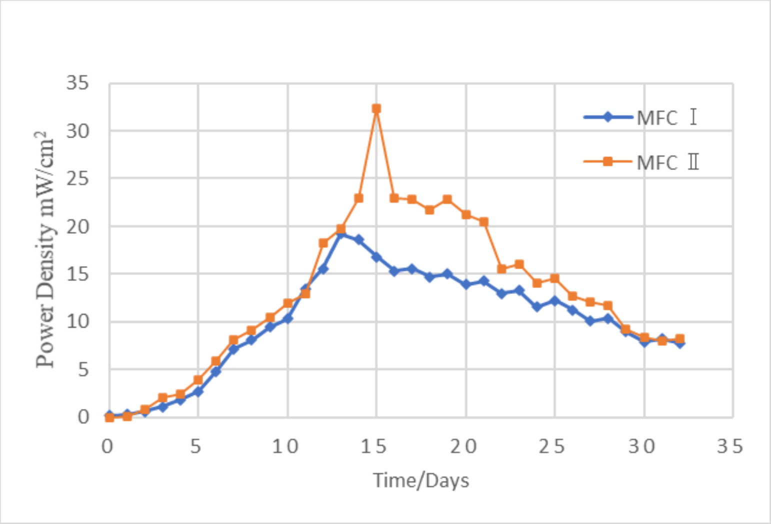

Figure 3. Power density recorded from MFC I and MFC II

The presence of exoelectrogenic microorganisms in white cheese whey wastewater consists of various types of microorganisms, with different species growing at varying rates depending on their metabolic activities. In MFC I, the highest power density recorded was 19.2 mW/cm² on day 13. As illustrated in Figure 3, the continuous increase in power density throughout the experiment can be attributed to the microbial degradation of the substrate, where they utilize organic carbon for energy and nitrogen for building their cellular structure. This finding is greater than the results reported by Mishra et al. (2017) and Nimje et al. (2011). However, by day 14, power density began to decline, likely due to the microorganisms entering the stationary phase, where the number of viable cells equals the number of dead cells. This phase adversely affects the overall condition of the anode for power generation (Logan et al., 2009). Achieving high power density in a short time frame is challenging, as complete degradation of the substrate requires a significant amount of time. In MFC II, the power density reached 32.4 mW/cm² on day 15. *Saccharomyces cerevisiae* utilizes the energy derived from the NADH/NAD+ redox cycle. These results are higher than those reported by Aswin et al. (2017) and Nimje et al. (2011).

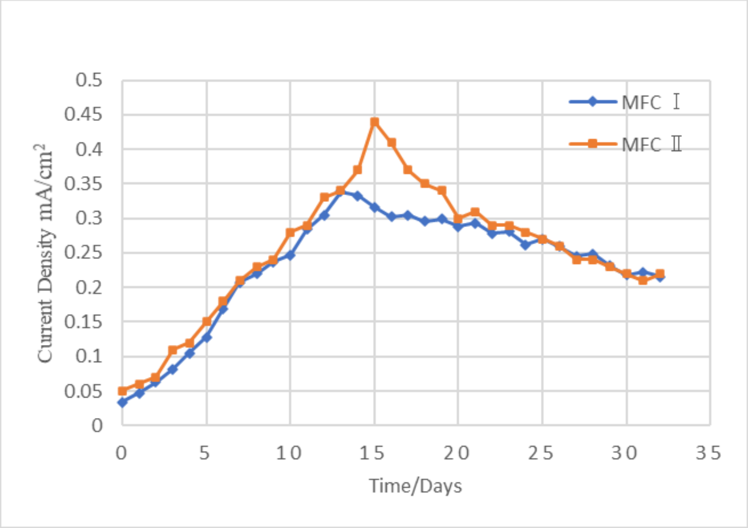

Figure 4. Current density recorded from MFC I and MFC II

The highest current density achieved in MFC I was 0.34 mA/cm², as shown in Figure 4. The increase in current density occurred in a staged manner, reflecting the flow of electrons generated by the metabolic activities of exoelectrogenic microorganisms within the substrate. The peak current density was observed at pH 6.89 but began to decline after a few days, indicating a decreased availability of biodegradable organic matter in the cheese whey wastewater. This finding aligns with results from Mishra et al. (2017) and Nimje et al. (2011). Following the peak current density, the power output stabilized. The electrical energy diminished due to the death of bacterial cells and the consumption of carbon (TC) and nitrogen (TN) sources, as indicated in Tables 1 and 2. The degradation of this wastewater illustrates the potential of exoelectrogenic bacteria to utilize available substrates, such as phosphorus, a macronutrient, converting organic phosphorus in the wastewater to orthophosphate (Behera et al., 2010), and sulfate, which is reduced to sulfide by sulfate-reducing bacteria. This sulfide then combines with hydrogen gas, a byproduct of bacterial metabolism, to form H2S, leading to a decrease in the electrical energy generated (Trinh et al., 2009). In MFC II, the maximum current density reached was 0.44 mA/cm² at pH 6.86. The optimal pH for microbial activity in the absence of O_2 is between 6.5 and 7.0, consistent with the findings of Venkat et al. (2008).

3.2. COD Concentration

The primary objective of the experiment was to evaluate the efficiency of microbial fuel cells (MFCs) as waste treatment systems, and throughout the process, the MFCs were continuously monitored for waste removal, measured as chemical oxygen demand (COD). Both reactors demonstrated their effectiveness in removing COD, showcasing the ability of microorganisms to decompose waste in the wastewater while simultaneously generating electricity. Over 32 days, the microorganisms in the MFC effectively eliminated organic pollutants, with MFC II achieving a higher and more consistent COD removal efficiency of 92.85%. This indicates that Saccharomyces cerevisiae effectively contributed to reducing COD levels. The efficiency observed in this study surpasses the 65% removal rate reported in a previous study by Aswin et al. (2017).

It was noted that COD concentrations were higher over shorter periods compared to longer ones, as volatile fatty acids (VFAs) and alcohols remained present and had not yet been fully decomposed. COD is commonly used as a measure of substrate quality, so efforts to reduce COD levels are crucial for environmental protection (Yadvika et al., 2007). MFC I, exhibited a lower COD removal efficiency of 83.97% compared to MFC II, as illustrated in Figure 5.

The levels of biodegradable organic matter in the substrate are directly related to the efficiency of removal, which in turn correlates with the performance outcomes of the process. This study indicates that the efficiency of COD removal in the anaerobic treatment method increases with a longer hydraulic retention time (HRT), as demonstrated in Figure

6. This improvement may be attributed to the extended contact time between the substrate and microorganisms, aligning with findings from Mishra et al. (2017).

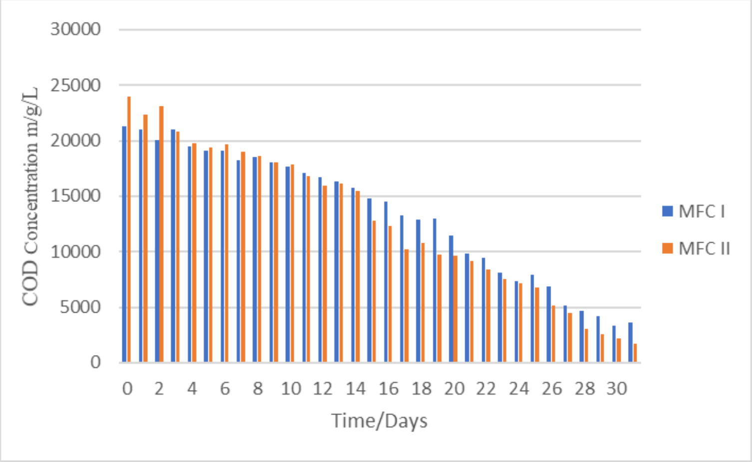

Figure 5. MFC I and MFC II COD Concentration

3.3. *Cheese Whey Wastewater Phosphate Analysis*

Phosphate (PO_4^{3-}) plays a crucial role in wastewater treatment as it is a macronutrient that microbes require in relatively large quantities for their metabolic processes (Figure 6). In MFC I, the concentration of PO_4^{3-} was measured at 94.6 mg/L, while MFC II recorded a concentration of 89.98 mg/L, as shown in Table 1. By the conclusion of the experiment, phosphorus levels decreased, with values dropping to 31.8 mg/L in MFC I and 23 mg/L in MFC II, resulting in removal efficiencies of 66.4% and 73.3%, respectively. MFC I, exhibited a lower phosphorus removal rate compared to MFC II, suggesting that *Saccharomyces cerevisiae* effectively utilized phosphorus in its environment. This enhanced removal in MFC II may also be attributed to a low redox potential, which encourages microorganisms to either release PO_4^{3-} or convert organic phosphorus in the wastewater into orthophosphate. Orthophosphates represent the most prevalent form of PO_4^{3-} compounds (Behera et al., 2010).

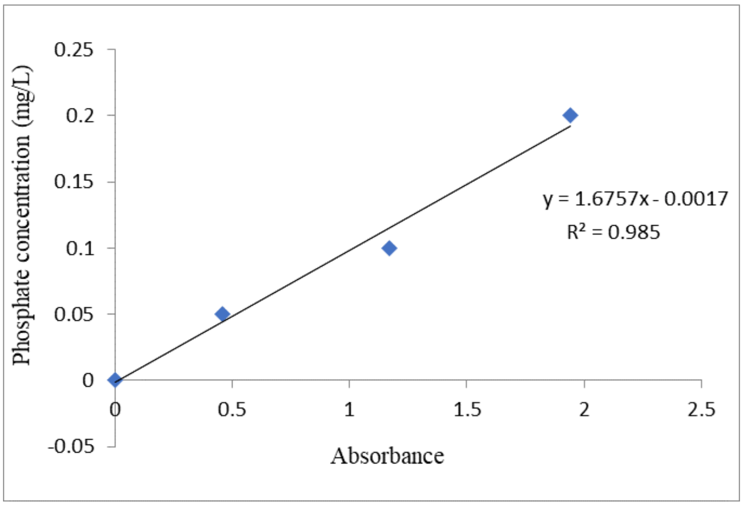

Figure 6. Phosphate Concentration Standard Curve for MFC I MFC II

3.4. *Cheese Whey Wastewater Sulphate Analysis*

The concentration of sulfate (SO_4^{2-}) in the MFCs was measured, and the results after 32 days of operation indicated a significant presence of sulfate in both reactors (Figure 7). In MFC I, the initial concentration of SO_4^{2-} was 397.3 mg/L, which decreased to 214.6 mg/L by the end of the experiment, yielding a removal efficiency of 45.9%. This relatively low removal efficiency suggests that the microorganisms present in the cheese whey wastewater can utilize SO_4^{2-} as their primary electron acceptor, subsequently reducing it to sulfide (Wei et al., 2013). Certain microorganisms are selective in the types of acceptors they can use, while others can utilize a variety of them. In MFC II, the initial concentration of SO_4^{2-} was 463.7 mg/L, which dropped to 265 mg/L after the experiment, resulting in a removal efficiency of 42.85%. The presence of *Saccharomyces cerevisiae* in the medium enabled the uptake of a significant amount of sulfate as a nutrient source. These findings are consistent with those reported by Khanal and Huang (2003). The biological processes involving sulfates primarily involve anaerobic reduction to sulfides and hydrogen sulfide. Sulfate-reducing bacteria (SRB), such as *Desulfovibrio* or *Desulfobacterium*, utilize hydrogen and organic matter as electron donors while employing sulfates as acceptors (Lu et al., 2013).

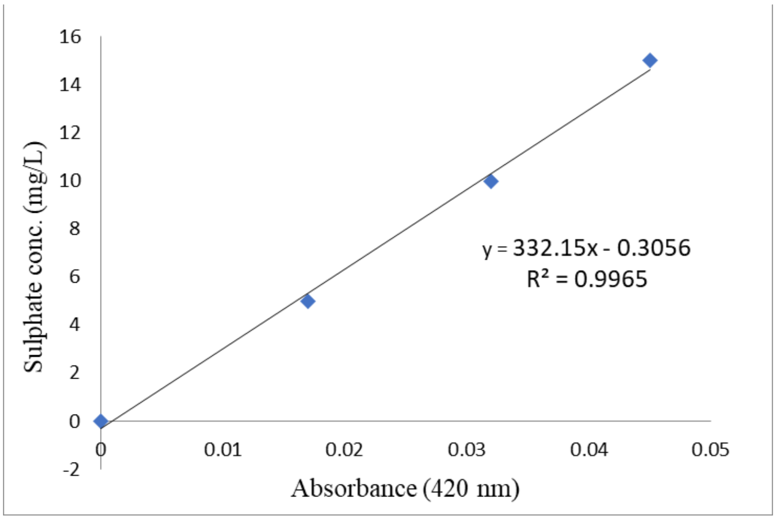

Figure 7. Sulphate Concentration Standard Curve for MFC I and MFC II.

3.5. Microbial Fuel Cell pH

pH plays a crucial role in MFC systems, affecting both power generation and the treatment rate of wastewater entering the anode chamber (Figure 8). It is influenced by the pH of the wastewater flowing into the anode and the buffer solution in the cathode chamber (Behera and Ghangrekar, 2009). In the anode chamber, which is devoid of oxygen, microorganisms transfer electrons from the degradation of organic matter to the anode electrode. Despite the lack of oxygen, the high electronegativity ensures that most of the produced electrons are captured and reduced (Patil et al., 2009). As shown in figure 9, the optimal pH for microbial activity in anaerobic conditions is between 6.5 and 7.0 (Venkata et al., 2008). The pH of the cheese whey wastewater used in this study ranged from 6.5 to 7.0, with the highest energy output from the MFCs observed at pH levels of 6.89 and 6.86 in MFC I and MFC II, respectively, as shown in Figure 9. These findings are consistent with studies conducted by Li et al. (2008) and Jadhav et al. (2009). The maximum efficiency of the MFC was recorded at pH 7.0, and the results indicate that lower pH levels corresponded to reduced energy output. Other studies have similarly shown that microbial activity tends to be slower at pH levels below the optimal range compared to the ideal pH.

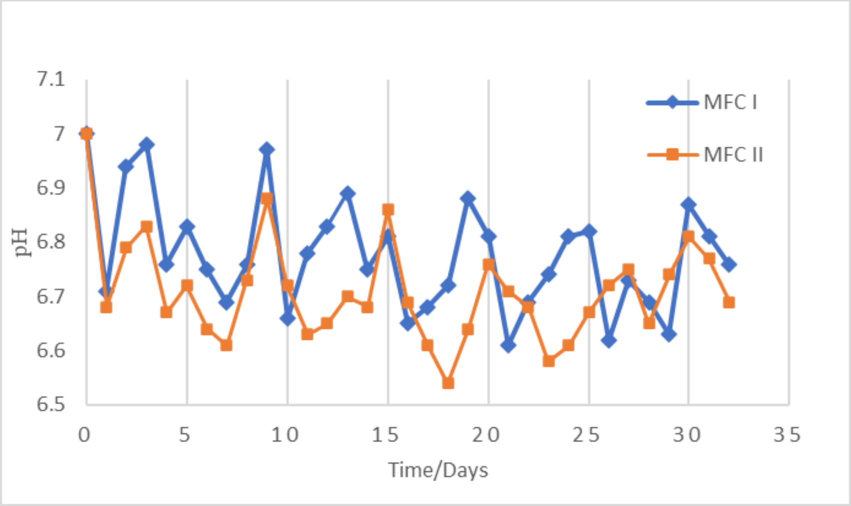

Figure 8. MFC I and MFC II pH

3.6. *Cheese Whey Wastewater Suspended Solid Analysis*

Total suspended solids (TSS) are recognized as significant pollutants that negatively affect water quality and its overall characteristics (Bilotta and Brazier, 2008). As illustrated in Figure 9, MFC I demonstrated a gradual reduction in TSS from 6112 mg/L to 1096 mg/L, consistently declining from the start of the treatment process. As the concentration of suspended particulate matter increases, the substrate becomes clearer, leading to reduced turbidity, which is also reflected in Figure 2, showing a marked decrease throughout the treatment. In MFC II, the TSS concentration dropped from 6300 mg/L to 1213 mg/L. These findings align with the results reported by Bilotta and Brazier (2008). TSS is a crucial parameter, as high levels can lead to a depletion of dissolved oxygen (DO) in the effluent water.

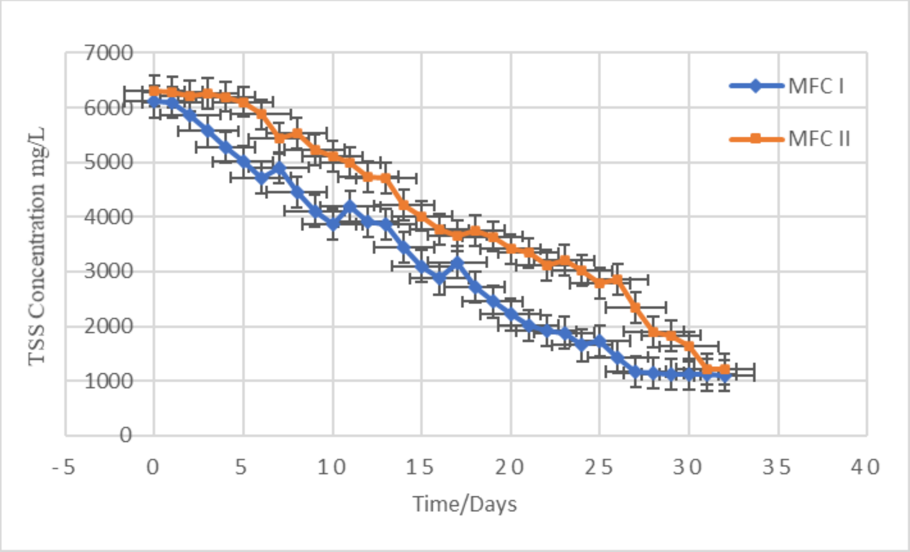

Figure 9. MFC I and MFC II Total Suspended Solid Concentration

3.7. *Proficiency in Wastewater Treatment*

The microbial fuel cells (MFCs) utilized in this study effectively produced electricity continuously while simultaneously treating the wastewater used as fuel. The various characteristics of the white cheese whey wastewater employed in this research are summarized in Table 1. Daily measurements of the effluent wastewater concentration revealed significant changes in the parameters presented in Tables 1 and 2. The biodegradability of wastewater can be assessed using the BOD/COD ratio; wastewater with a BOD/COD ratio greater than 0.5 is considered biodegradable and suitable for biological treatment. If the BOD/COD ratio falls between 0.3 and 0.5, biological treatment requires the introduction of seed bacteria, as the process will be slower due to the time needed for bacterial adaptation to facilitate degradation. A BOD/COD ratio below 0.3 indicates that biodegradation is unlikely, as the wastewater can inhibit the metabolic activities of the bacterial seed due to its toxic or refractory properties (Ganjar et al., 2010). In this study, the BOD/COD ratios were 0.65 for MFC I and 0.60 for MFC II. The results indicated that removal efficiency increased with retention time, achieving 83.97% in MFC I 92.85% in MFC II for COD, and 61.95% in MFC I and 73.95% in MFC II for BOD5. This demonstrates the effectiveness and potential of the MFC system as an alternative to traditional biological wastewater treatment methods (Durruty et al., 2012). The significant removal rates of COD and BOD5 observed in this study are likely attributed to the extended retention time. This suggests that the utilization of organic matter in cheese whey wastewater may not solely align with electricity generation, as other factors such as biomass production and fermentation could also be involved. Despite the low efficiency of converting organic matter into electricity, the organic content in cheese whey wastewater is abundant, allowing for the dual goals of wastewater treatment and electricity generation to be achieved simultaneously (Ghangrekar and Shinde, 2007). Sulfate removal efficiencies were 45.9% for MFC I and 42.85% for MFC II, which may be low due to the formation of hydrogen sulfide (H2S) with sulfate (SO_4^{2}-) acting as the final electron acceptor. Phosphate removal efficiencies were recorded at 66.4% for MFC I and 73.3% for MFC II, likely due to low Redox potential encouraging microorganisms to release PO43- or convert organic phosphorus in the wastewater to orthophosphate (Behera et al., 2010). Total suspended solids (TSS) also serve as an important indicator of water quality, with removal rates of 82.68% in MFCI and 80.74% in MFC II.

4. Conclusion

Cheese whey demonstrates significant potential for electricity generation through microbial fuel cells compared to other biomass resources. The highest electricity output recorded from cheese whey was 56.8 mV and 5.68 mA. For MFC I, the peak current and power density were 0.339 mA/cm^2 and 19.2 mW/cm^2, respectively. In MFC II, the maximum voltage and current achieved were 73.7 mV and 7.37 mA, with a peak current and power density of 0.44 mA/cm^2 and 32.4 mW/m^2. The experiment yielded a substantial COD removal efficiency of 83.97% in MFC I and 92.85% in MFC II, alongside BOD5 removal efficiencies of 61.95% and 73.95%, respectively. This indicates that the substrate is biodegradable, reflected in BOD/COD ratios of 0.65 and 0.60 for the two cells. Overall, this microbial fuel cell experiment, which harnesses microbial activity in cheese whey for electricity generation, has been identified as a promising green solution due to its environmentally friendly and sustainable energy production potential. Implementing this technology can help alleviate environmental issues associated with wastewater and minimize waste. Microbial fuel cells can also address energy needs, potentially helping to mitigate energy crises while offering commercialization opportunities to meet energy demands. To improve the performance and efficiency of these systems, additional enhancements such as inoculation and the introduction of carbon sources can be beneficial. Additionally, maintaining proper buffering can help stabilize pH levels within the system, thereby supporting bacterial longevity and enhancing the overall performance of microbial fuel cells.

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