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ANALYSIS AND STUDY OF THE BIOELECTRIC PRODUCTION POTENTIAL OF ACTINOMYCETE AND MICROBIAL ISOLATES IN INDUSTRIAL GLASS FACTORY WASTEWATER USING A MICROBIAL FUEL CELL

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Abstract

A microbial fuel cell (MFC), a novel technology, is a biochemical catalyzer system that can convert the chemical energy of materials to bioelectric energy. This system can serve as a unique device for the treatment of wastewater. Based on this knowledge, we decided to study the bioenergy production ability of Actinomycete and microbial isolates in industrial glass factory wastewater as the decomposers of organic materials in this wastewater and the generation of Voltage and current in two batches and fed-batch conditions. At the most favorable condition maximum of 1.08 V (current 3.66 mA and power density 2.88 mW/m^2), 81.2% chemical oxygen demand was obtained for a fed-batch system. Also, the outcomes of MFC's essential parameters, for example, pH and TDS, were studied before and after the performance of MFC. The results showed a significant decrease after the operation of the MFC. To realize which Actinomycete were the most powerful bioelectric microorganism, the growth curve and electricity performance of three kinds of Actinomycete was selected. Results showed that the C2 would be more potent because its Voltage of 0.224 V and current of 1.187 mA possessed by it would result in an excellent power density of 141.42 mW/m^2.

Keywords: Actinomycete. Bioelectric. Industrial. Microbial fuel cell. Wastewater.

1. Introduction

The extensive energy demands of people are the most common concern all over the world. However, fossil fuels as a primary source of energy can meet the energy demands of people. Still, they may contribute to air pollution, global warming, environmental degradation, and unexpected health problems among the people (Guo et al. 2013; Kaushik and Jadhav 2017; Sahu 2019). Interestingly, the development of Microbial Fuel Cells (MFC) with the ability to simultaneously generate bioenergy and treat the wastewater of industries seems to be a good vehicle to overcome the mentioned drawbacks. MFC, as a biological electrochemical system, has mainly consisted of several parts, such as an organic source as a substrate, a microorganism as a biocatalyst, a proton exchange membrane, and an electrochemical chamber for collecting generated electrons.

In MFC, bacteria, for instance, operate as catalysts to break down organic or inorganic materials under anaerobic circumstances (without oxygen) and transfer the chemical energy of these materials directly into electrical energy. In the MFC system, bioenergy is produced in two steps: in the first,

microorganisms in the anode chamber oxidize the substrate and release their stored electrons. The electrons go to the electrode of the electron acceptor (cathode) in the second stage, where they produce electricity. These oxidation and reduction processes in the MFC lead to the formation of current, voltage, and ultimately power density (Guo et al. 2013; Shankar et al. 2014; Kaushik and Jadhav 2017; Yakar et al. 2018; Sahu 2019).

It is stated that some of the bacteria are natively electroactive. It means that they can directly oxidize fuels (substrate) and transfer the electrons to the electrode (anode) for producing bioenergy in MFCs (Sydow et al. 2014; Huarachi-Olivera et al. 2018). Actinomycete, as a gram-positive and rod-like anaerobic bacteria, is abundantly found in soil and wastewater industries (Lacey 2008; Hall and Copsey 2015). Actinomycete can also produce different bioactive compounds that are needed for antifungal and antibiotic drug production. Still, they can also produce various types of enzymes that can degrade the organic materials in the wastewater and soil to stimulate the generation of current, voltage, and power (Liu and Cheng 2014; Ng et al. 2017). The main objective of the present study is the isolation of microbial and Actinomycete Maragheh glass factory wastewater in Iran, and the investigation of the electrical bioenergy production capability via double chamber MFCs. In this study, we used a carbon plate as an anode and cathode catalyst and a salt bridge as a proton exchanger. In addition, we measured the average pH, chemical oxygen demand (COD), and total dissolved solids (TDS) value of glass factory wastewater before and after the MFC operation and investigated their effect on the current, Voltage, and power generation in MFC.

2. Material and Methods

Sample collection

The unique sampler (Van vein grab, 0.2 m2) was used to gather wastewater fragments containing Actinomycete from depths of 1 and 2 meters of the glass plant in order to build the MFC system. The samples were then promptly sent to Maragheh University of Medical Sciences' microbiological lab for additional research. In order to minimize the vegetative forms of Actinomycete and other bacteria and isolate the Actinomycete spores, the samples were first heated in a hot water bath for one hour at a temperature of 55 degrees. To get rid of Actinomycete spores and other germs, the samples were heated in a hot water bath for an hour at 55°C. In this study, particular Actinomycete culture mediums, such as SCA, ISP2, and Kuster's agar, were employed for Actinomycete cultivation. Actinomycete was isolated using the serial dilution technique.

One gram of each sample was dissolved in 9 ml of physiological serum with 0.9% NaCl. Then dilutions were prepared from the samples. To study Actinomycete, colony morphology was first examined using a Nikon TN PSE 30 stereo microscope. The color of the back and top of the colony, the firmness of the colony, and other morphological characteristics of the colonies were investigated. The cross-streak method was used for the primary screening of active Actinomycete. Colonies with a morphological appearance similar to Actinomycete were selected. To prepare a pure culture of bacteria, Actinomycete colonies were isolated in powdered and dry form on the surface of agar and cultured in SCA culture medium.

For molecular identification of Actinomycete isolates, the 16S rDNA gene was amplified by chain reaction (PCR). The correctness of the polymerase chain reaction of the 16S rDNA gene was confirmed by 0.8% agarose gel electrophoresis stained with ethidium bromide. For a purification, PCR product purification kit was used.

Fuel Cell Construction and Experiment

In this study, the bioreactor used for the generation of bioenergy was typically composed of two chambers: an anode chamber and a cathode chamber with equal volume (1 L volume). These chambers were constructed of glass materials. Carbon plates were used as an anode and cathode with a 5 cm size.

A wastewater sample with microorganisms was present in the anaerobic anode chamber. These

microorganisms might boost the oxidation capacity of electrogenic bacteria since they were cultivated in the growth medium required for their growth. In the same proportion as the anode chamber, the cathode chamber contained distilled water and 0.1 M phosphate buffer. The copper wires used to link the anode and cathode electrodes have a 100 kΩ external resistance. Instead of using a proton exchange membrane, the sodium chloride salt bridge was employed as a proton exchange mechanism to transfer the solute between the cathode and anode. To stop the anode and cathode solutions from combining, cotton was used to plug the salt bridge's aperture. For 10 days, the MFC was operated in batch and fed-batch modes, with 1 ml of 50% glucose supplied to the fed-batch system each day. Both batch and fed-batch ways of measuring electrochemical parameters were used. The cathode compartment was left open before the experiment to allow contact with the air, which allowed O_2 to mix with the transferred protons to generate H2O. One input port and one output port were added to the anode chamber, and they were silicone and paraffin-sealed to create an anaerobic environment.

Physicochemical properties

The physicochemical properties of wastewater: average pH, chemical oxygen demand (COD), and total dissolved solids (TDS) were determined according to standard methods. A pH meter (HM TDS-3, USA) was used to measure the pH at the beginning and end of the batch experiment. The chemical oxygen demand (COD) was measured by the spectrophotometer (Optima SP-300, Japan) following the colorimetric method. The average pH, COD, and TDS were measured and recorded before and after the operation of the MFC.

Analysis: Calculation and measurement

The bioelectric activity of the MFCs was related to the voltage and current of the system. The cell voltage of the system was measured with a multimeter (SUNWA YX-1000A). The current of the system was calculated according to the equilibrium below, with the resistance set around 16 KΩ:

$$V = IR$$

Where (I) was current (A), (V) was voltage (V), and (R) was resistance (Ω).

All data was manually recorded on a daily basis. Then, power density was measured with the below equilibrium:

$$P = IV$$

Where (P) was power, (I) was current, and (V) was voltage (V).

The chemical oxygen demand of wastewater was determined by the standard dichromate open reflux method. The percentage of removals was calculated according to equilibrium.

$$\text{Removal (\%)} = \frac{(Ci - Cf)}{Ci} \times 100$$

Ci was the Initial concentration (mg/l), and Cf was the finial concentration (after treatment; mg/l). The Polarization curve of the MFC was obtained to evaluate the relationship between voltage and current by measuring voltages at external resistances (100 Ω).

Microbial growth curve

For studying the growth curve, at the first step, Actinomycetes were inoculated in nutrient broth. Data of the growth curves were recorded by taking 100 ml samples every hour for 72 hours from the

anodic chamber, and the absorbance was measured at 600 nm via a spectrophotometer. Noted, nutrient broth without Actinomycete was used as a control experiment.

Growth kinetics of Microbial isolates

A sterile syringe was used to withdraw 1 ml of the anode compartment's broth medium for this purpose, and after recording the electrical data, the absorbance of the liquid was measured with a spectrophotometer at 600 nm starting from the moment the system began operating every hour for each bacteria. In order to calibrate the device and serve as a control, the basic nutritional broth medium (without bacterial inoculation) was utilized. At the conclusion, the growth curve of electrogenic bacteria was generated. Standard bacteriological and biochemical tests were used to identify and differentiate the indicator colonies after the initial growth of bacteria in the plate and the purification of the indicator colonies in terms of morphology and non-contamination. The colonies were first cultured on blood agar and EMB media, and then warm staining was carried out for them. The nature of the isolates was then established using experiments that included measurements of the consumption of sugar, gas generation, H2S production, indole, sulfate, and citrate, as well as aerobic or anaerobic metabolism, fermentation pathways, squalene hydrolysis, catalase, and movement tests.

Selection of potential Bacteria through monoculture

To search for the potential hyperbioelectric strain in the wastewater sample, a double-chamber MFC was designed. Samples were then cultured in nutrient agar media using a serial dilution technique and incubated at 37°C for 24-48 hours. After the incubation period, the OD of each pure culture and electricity generation were measured. The bacterial isolate that was taken for the study in MFC was tested in various biochemical tests to identify the species of the bacteria. (Urease test, Citrate utilization, SIM test, Tipple sugar iron (TSI) test, Methyl red test (MR), Voges Proskauer test.

Electrochemical activity of Actinomycete

To demonstrate the electrogenic capability of Actinomycete, all the chambers were sterilized at first. Then, 100 ml of sterile nutrient broth and 100 ml of deionized water, respectively, were added to the anode and cathode chambers. Isolated bacterial pure (from anodes) cultures were inoculated in 100 ml of sterile nutrient broth for 24-48 hours at 37°C. After the incubation period, 1 ml of incubated bacteria was added to the anode chamber. After the operation of MFC, electricity generation was measured as equilibriums stated.

3. Results

Electricity generation by double chamber MFC

In the present study, the MFC system was composed of two anode and cathode chambers. The salt bridge as a proton exchanger was used to separate these chambers from others (Fang and Achal 2019; Saha et al. 2019). The microorganisms in the anodic chamber could utilize the organic and inorganic materials of wastewater as substrates, oxidase them in the absence of oxygen, and remove their electrons.

Then, these electrons were transferred to the cathode chamber through the salt bridge and involved in the bioelectric generation (Fang et al. 2013; Ebadinezhad et al. 2019; Fang and Achal 2019; Saha et al. 2019).

In the present study, the bioelectric generation of MFC in batch and fed-batch modes was analyzed for ten days. The generation of bioelectricity was observed from the beginning of the run. The voltage of this system was measured via a multimeter (Samsudeen et al. 2015).

The maximum voltage of the batch system was produced on the first day, and the maximum voltage of the fed-batch system was produced on the second day and was 0.98 Volts and 1.08 Volts,

respectively. The maximum current of this system was made in the mid-day and reported at about 2.34 mA, respectively, for the batch system. Finally, the power density was calculated by two equilibriums: P = V × I and V = I × R, where P was the power density (mW), V was the potential (V), and, I was the current (mA) (Fang et al. 2013; Rahmani et al. 2020). According to these experiment data, the maximum power density of the batch and the fed-batch systems was produced after five days of 1.19 mW in the batch system. After five days, the amount of substrate decreased, which led to a decrease in electric generation (Sun et al. 2016; Bejjanki et al. 2021). Sahu et al., in the study, obtained similar results for sugar industry wastewaters (1.42 V open voltage, current 23.66 mA, and power density 5.1 mW) (Sahu et al. 2019). Also, we observed a significant difference between the two batch and fed-batch systems (Figures 1 and 2). Adding the glucose to the wastewater in the fed-batch system increased the generation of electricity (Sahu et al. 2019). It means that microorganisms in the wastewater were able to consume glucose as substrate and synthesize enzymes to oxidase glucose as a result of the oxidation of glucose by microorganisms, increasing the electron transfer for power production. Compared to the batch system, current and power density were increased by about 1% and voltage was increased by about 5% in the fed-batch system (Figure 3).

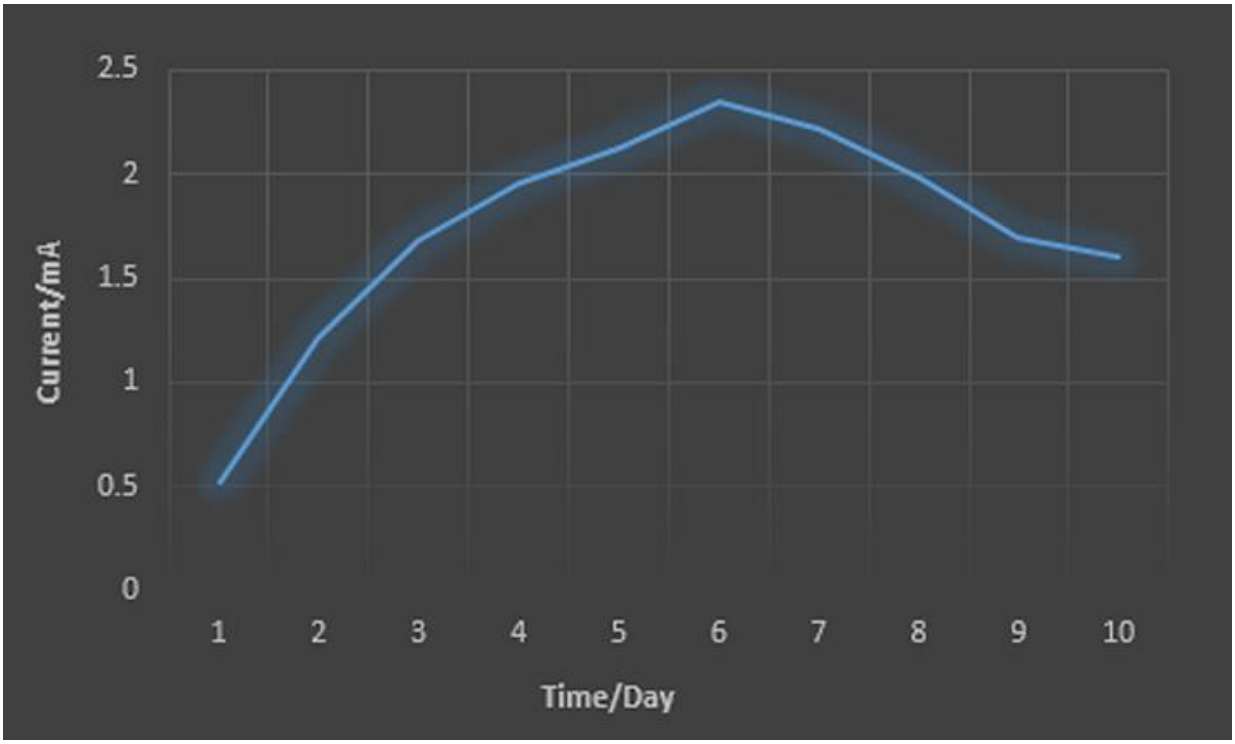

Figure 1. Generation of Current by glass factory wastewater in batch.

Characterization of wastewater

The production of electricity can be influenced by wastewater characteristics (Christwardana et al. 2016; Kaushik and Jadhav 2017). The electrochemical parameters of wastewater were studied for ten days in the batch and fed-batch modes of the reactor (Wang et al. 2014). Some parameters of wastewater, such as chemical oxygen demand (COD), total dissolved solids (TDS), and pH, were studied before and after the MFC performance (Wang et al. 2014). The result of the analysis is shown in Supplementary Table 1. The bacterium that was present in the anode chamber synthesized the special enzymes, which were able to oxidize the organic material in wastewater and decrease the concentration of COD. So the bacteria in the anode chamber could decrease the amount of COD and subsequently reduce the electricity generation (Wang et al. 2014; Pannell et al. 2016).

The elimination effectiveness of COD was estimated at around 81.2% (reduction from 500 mg/l to 90 mg/l) in accordance with the equilibrium that was specified. The absence of substrate at the process' conclusion indicates that organic degradation took place. Actinomycete's capacity to lower COD was comparable to the rumen microorganisms' activity in the Prabowo et al. investigation, which decreased

COD by up to 67.9% (from 10815 ppm to 3472 ppm) (Christwardana, Prabowo, Tiarasukma and Ariyanti 2016).

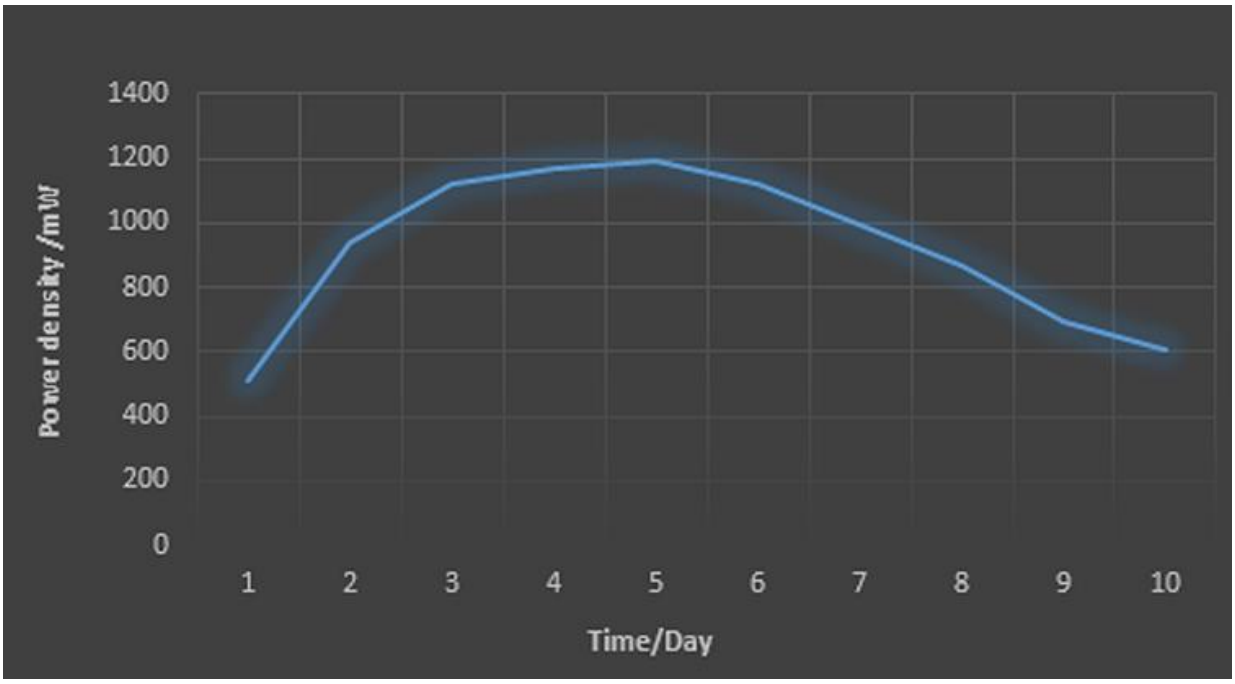

Figure 2. Generation of Power density by glass factory wastewater in batch.

Table 1. Summarizes the Bioelectrical properties of C_1, C_2, and D_2 Bacterium.

Characterization	C_1	C_2	D_2
GRAM	Negative	Negative	Positive
Morphology	Bacillus	Rod	Cocci
Urease	+	-	+
Citrate	-	+	+
TSI	-	-	+
SIM	+	-	-
MR. Methyl red test	+	-	+
VP. Voges Proskauer	-	-	-

Examining the effluent's visual traits after electricity generation and contrasting them with the original sample revealed that during the battery's operation, the effluent had been significantly freed of smelly and dark-colored compounds, which suggests that sulfur compounds and other organic substances may have been decomposing. Microbial populations had been living in the effluent. By absorbing the protons generated during wastewater degradation, the water in the cathode chamber, also known as the catholyte, changed color, and its pH fell from 7.4 to 6.

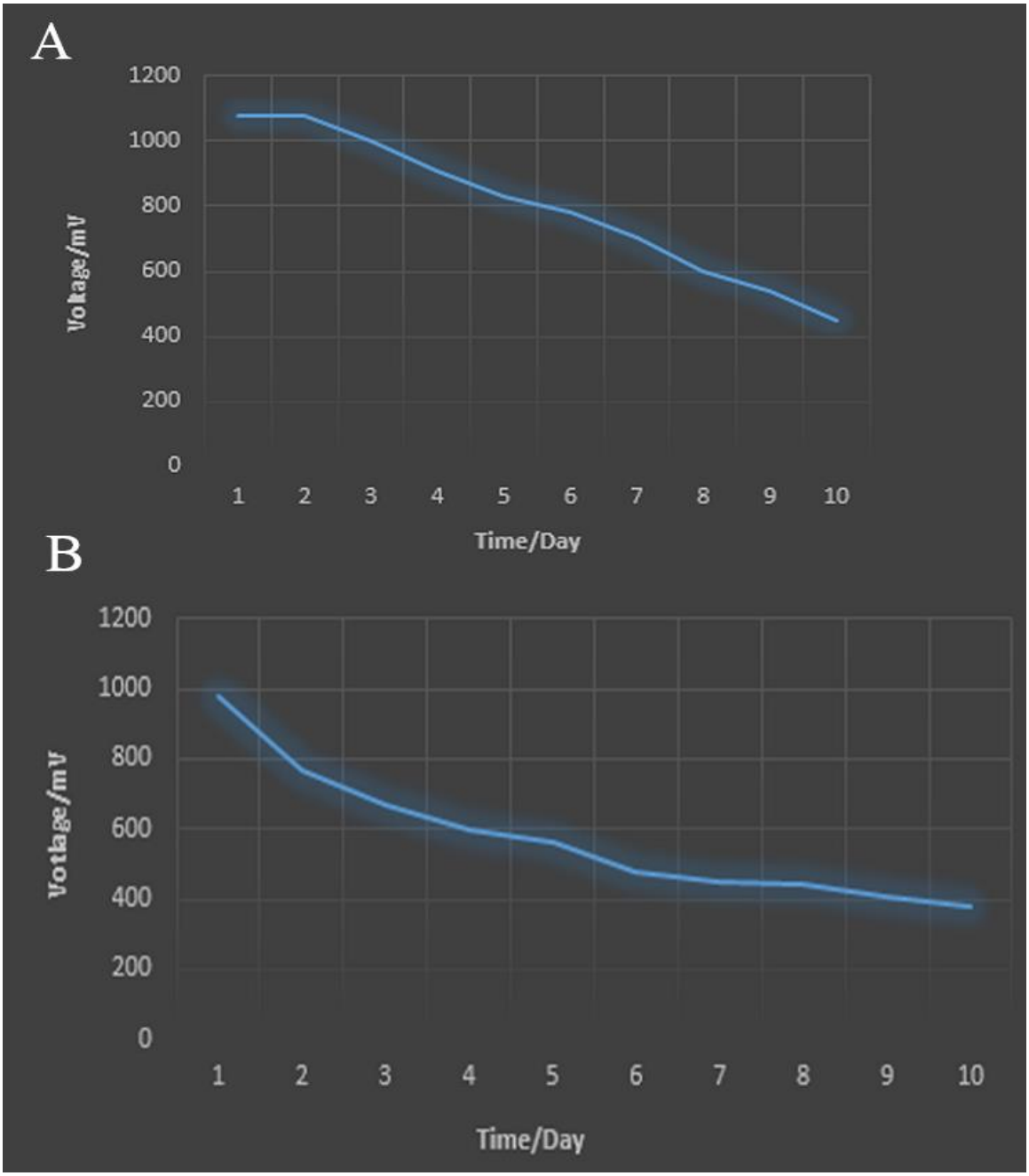

Figure 3. Comprises the voltage generation by glass factory wastewater in A - batch and B - fed-batch mode.

Bacteria growth curves and Identification of electrogenic bacteria

The bacterial growth curve was measured during the 72-hour period. After extracting electricity from wastewater by designing two separate systems in the microbial fuel cell, the bacteria in the reactor associated with the Batch system were evaluated by cultivating the wastewater on the culture medium. With proper incubation of the samples, 3 bacteria were isolated and identified with the help of various biochemical and bacteriological tests (Table 1). These 3 bacteria, named C1, C2, and D2, were first cultured on blood agar and EMB media. According to the results, it is clear that over time, the growth of all the Actinomycete bacteria increased. The results of this study showed that C2 bacteria had the highest affinity for the substrate and grew very quickly.

So, the C2 bacteria, or Citrobacter freundii, was able to produce a high bioelectric energy value (224.79 mV) and a high power density (141.42 mW) in comparison to the D2 bacteria, which was able to produce the lowest potential value (109.59 mV) and power density (36.21 mW). Also, we observed a noticeable relationship between the growth of bacteria and the generation of power density (Table 2). On the other hand, the high number of bacteria in the anode chamber could produce numerous enzymes that

can oxidize a large amount of carbonic material in the wastewater. Oxidation of this material leads to the release of electrons .These electrons were then transferred to the cathode chamber, where they made the current and finally produced high power density (Shankar et al. 2014; Sharma et al. 2015; Bejjanki et al. 2021).

This effect was similar to the result of Huarachi-Olivera et al. studies about the generation of bioelectric from microalga Chlorella Vulgaris and bacterial communities (Huarachi-Olivera et al. 2018).

Table 2. Comprises the Bioelectrical properties of C_1, C_2, and D_2 Bacterium.

Bacteria	Power density	Current	Voltage	Growth of bacteria
C_1	86.99	0.557	178.22	0.174
C_2	141.42	1.187	224.79	0.246
D_2	36.21	0.572	109.59	0.195

According to the biochemical tests, isolate C1 was identified as Pseudomonas aeruginosa, isolate C2 was identified as Citrobacter freundii, and isolate D2 was identified as group D streptococci. Biochemical studies in the Saha et al. study revealed that among the several types of microorganisms that were present in the three locations of Dhaka city of Bangladesh, Bacillus Siamensis and Bacillus Tequilensis were more potential electro-genic bacteria and produced a high level of voltage and power density (Sahaet al. 2019). In addition, Islam et al. identified two potential isolates, Bacillus Stratosphericus and Bacillus, in the Tannery wastewater by using biochemical tests. These bacteria were able to produce 1.21 V and 1.15 V (Islam et al. 2020). These results are similar to our study and confirm the results we obtained.

Amplification of 16S rDNA gene of active isolates

The molecular identification of the isolates was investigated using 16S rDNA gene sequencing. For this purpose, the polymerase chain reaction of the 16S rDNA gene was performed. The length of the PCR product resulting from the amplification of the 16S rDNA gene is 1500 nucleotides (Figure 4).

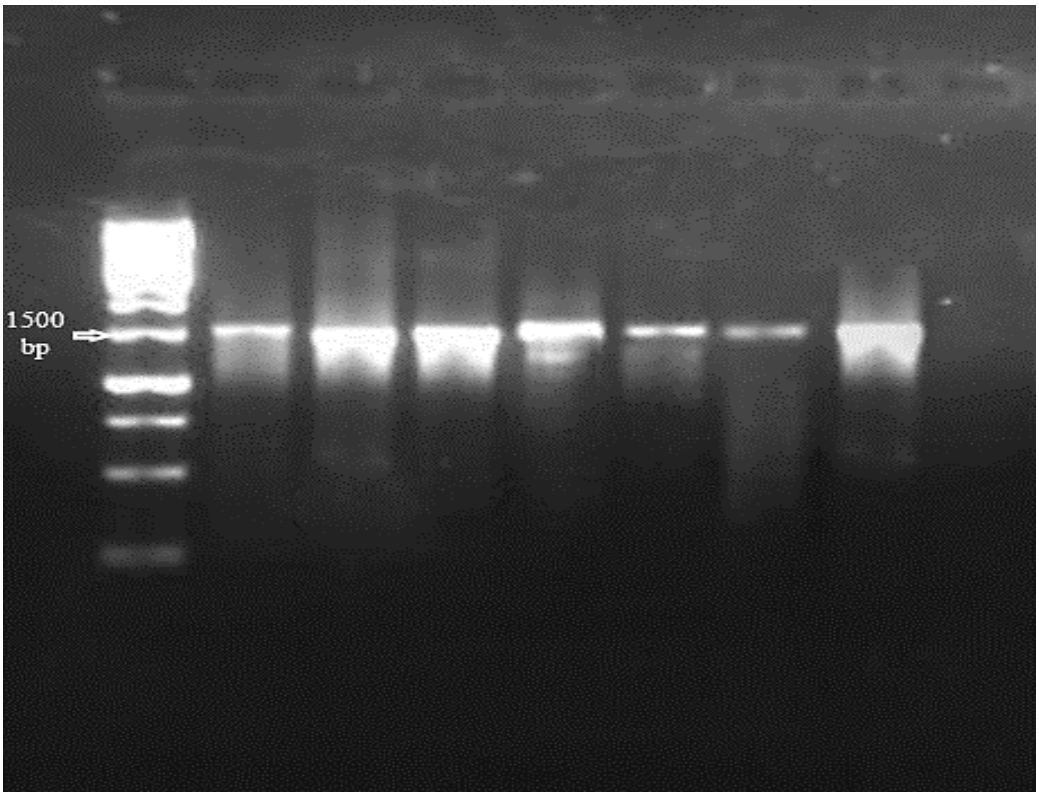

Figure 4. Electrophoresis of 16S rDNA gene amplification using agarose gel.

Statistical analysis of research data

The evaluation of the available data in each step was done with the help of SPSS version 21 software and by drawing appropriate tables and graphs in Excel.

4. Discussion

Similar results were obtained from the Hao et al. and Fang et al. studies (Fang et al. 2013; Hao et al. 2015). According to the stated result, the polarization curve for both systems is shown in the curve. The polarization curve was plotted as a function of current density, voltage, and power density measurement at 100 Ω. When the current increased, the cell voltage decreased. The polarization curve of the MFC system can also determine the maximum power density of the system. Power density measures the bioelectric production ability of the MFC system.

According to the results, there was no significant difference between the functions of the two Batch and Fed-batch systems. Also, Kaushik et al. reported that the COD value of pulp industry wastewater was reduced by about 67.5% (Kaushik and Jadhav 2017). Rahmani et al. also stated that decreasing the COD leads to redacting the bioelectric generation (Rahmani et al. 2020). Another feature of MFC, TDS (Total dissolved solids), was investigated using the gravimetric method. During the MFC operation, TDS was reduced from 5333 mg/l to 1300 mg/l. During MFC operation, microbes were able to decompose the organic or sulfuric material in the wastewater and remove the pungent and stinky odor and dark-colored compounds. Interestingly, Kaushik et al.'s study confirms our results (Kaushik and Jadhav 2017). Besides COD and TDS, the pH of the system before and after the process was also investigated. The acidity of the mixture solution in the cathode chamber decreased from 7.4 to 6.8, while the pH of the anode chamber increased from 8.1 to 8.9. Kaushik et al. reported that low pH inhibited the production of bioelectric by Pseudomonas fluorescent bacteria (Kaushik and Jadhav 2017).

Paez et al. reported that, during the electron exchange, hydrogen ions are pumped toward the exterior of the membrane, causing a drop in pH values. E. coli uses anaerobic fermentation as a metabolic route for the production of ATP as a source of energy, simultaneously producing mixed acids that could generate a rise in acidity and decrease pH values (Páez et al. 2019).

5. Conclusions

In recent years, several researchers have suggested using photosynthesis cultures as the anode and cathode in biofuel cells because of the high energy of solar radiation and the great potential for electron harvesting from photosynthesis. Research has also been conducted in this area. Recent years have seen a sharp rise in interest in microbial fuel cells, and there has been a great deal of worldwide study in this area. Each of these studies will in some way contribute to the development of our understanding of how microorganisms might be used for energy generation and other purposes. The bioelectric production capability of the MFC system from wastewater was tested in the current study under batch and fed-batch settings. Actinomycete, an oxidizing agent in the wastewater, increased voltage and wastewater material oxidation in the anode chamber. The batch and fed-batch systems' maximum voltages (0.98 mV and 1.08 mV, respectively) were attained on the first and second days, respectively. The maximum power density for both the batch and fed-batch systems was noted on the fifth day, according to the polarization turn. High power density (141.42 mW) and bioelectric energy value (224.79 mV) might be produced by C2 bacteria or Citrobacter freundii.

So we concluded that Citrobacter freundii was selected as the potent strain. The observations showed that instruction in MFC and the usage of microorganisms as catalyzers would help us improve the bioelectric generation from wastewater while simultaneously treating wastewater. During this study, the topic of microbial fuel cells was explained in detail, and simple experiments were conducted with not very expensive materials and equipment to investigate the electron-generating activity of bacteria in industrial wastewater. In addition, processes related to wastewater treatment were tested, and interesting results were obtained. Based on the observations, it was determined that electricity can be produced from

bacteria, but answering the question of how much electricity can be extracted from a bacteria and for how long will require much more extensive and long-term studies in this field. The simultaneous purification activity of bacteria living in the wastewater was another observation made during this project. According to the existing standards, various industries are required to reduce COD by at least 90% by using suitable purification systems. With the brief investigations carried out in this study, it was found that the efficiency of removing COD in the microbial fuel cell is more than 80 %, which clearly shows the proper performance of this device. In addition to removing dissolved solids to a great extent. Therefore, the dual and successful role of the reactor designed in this project in the production of bioelectricity from wastewater can be well explained. Also, in a general summary, it can be stated that microbial fuel cells are new technologies that are used to produce electricity from organic materials and waste. Currently, their usage is limited because the power efficiency of these systems is low. Therefore, before achieving more progress in increasing the power and optimizing this system, it is necessary to carefully study the microbiology of flow generation.

Therefore, technologies related to genetic changes and gene rearrangements should be carefully tested and investigated in the not-too-distant future in microbial fuel cell systems and on electrogenic microorganisms so that a community of microbes can be optimum provided to obtain the highest electron transfer potential and performance in MFCs in any condition. Therefore, the continuous improvement of microbial fuel cells and extensive studies in this field will increase the power and reduce the costs associated with power generation in these systems. In addition to that, it may have other double or multiple outputs, such as the simultaneous treatment of wastewater or acting as a biosensor.

However, there are several issues and constraints in this area, and in light of the aforementioned situations, additional study is required to improve the effectiveness and range of application of these biological reactors.

Authors' Contributions: ABDOLHOSSEIN ZADEH, A.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; SHOKRI, R.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; MOADDAB, S.R.: acquisition of data, analysis and interpretation of data, and critical review of important intellectual content; RAHNEMA, M.: acquisition of data, analysis and interpretation of data, and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

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Ethics Approval: Not applicable.

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