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Original Article

Biotechnological performance of a cyanobacteria based microbial fuel cell

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Abstract

Microbial Fuel Cell (MFC) is an environmentally friendly and sustainable technology where microbial degradation on organic substrates generates electricity, with potential application to wastewater treatment. This study mainly focused on investigating the effects of wastewater source on efficient electricity generation using a bio-cathode. Carbon cloth (2×4 cm^2) were used as anode and cathode electrodes with 0.33Ω external resistor. Anode medium was leachate (setup 01) and rice wash water (setup 02) and cathode medium was *Chroococcus* sp. culture. Control setup was maintained by adding distilled water in cathode. Landfill leachate and rice wash water were used as the wastewater sources, all other conditions were the same in both setups. Highest electricity generation and wastewater treatment were recorded in setup 02 with rice wash water. The maximum voltage was 1,111 mV with current 3,366.67 mA and 467.55 mW m^{-2} power density. The wastewater treatment efficiencies reported as reductions in COD, Nitrate, Nitrite and Orthophosphate were 61.94%, 61.01%, 55.14%, and 26.01% in setup 02. These study outcomes disclose that dual chamber MFC with rice wash water as the wastewater source has potential for simultaneous power generation and wastewater treatment.

Keywords: cyanobacteria, electricity generation, microbial fuel cell, wastewater treatment

1. Introduction

Today's world faces an energy crisis mainly due to the increasing global energy demand, with increase in the global population. According to the population statistics it is estimated that the world population will rise by 33% and reach around 9.7 billion by 2050 (Coelho, Sanches-Pereira, Tudeschini, & Goldemberg, 2018). Thus, increasing energy demand with a growing human population and developing industrial applications has become one of the major problems in the near future, which requires a sustainable solution. According to the IEA, in 2019, fossil fuels (coal, oil, and natural gas) accounted for 84% of the world's primary energy supply, while renewable energy sources (including hydropower) accounted for 11% and nuclear energy accounted for 5% (International Energy Agency (IEA) 2020, Global energy review).

Environmental issues associated with water sanitation are considered an emerging problem all over the world. Wastewater discharges from both point and non-point sources are the major cause of pollution in available water sources, both underground and on the surface (Mahagamage, Chinthaka, & Manage, 2015). However, wastewater is known as a potential resource for energy generation and plant fertilizing nutrients (Sevda, Sarma, Mohanty, Sreekrishnan, & Pant, 2018). A recent study found that conventional activated sludge processes consume a significant amount of energy, on an average 1.1 kWh/m^3. Advanced treatment processes such as membrane bioreactors and anaerobic digestion can reduce the energy consumption by up to 40% (Belhaj, Ben Amar, &

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Dhahbi, 2017). Biofuels can be found in solid, liquid or gaseous forms and they are mainly divided into three generations considering their origin, manufacturing method and availability. In recent times, scientists have been working on third generation biofuels, utilizing algae and cyanobacteria for energy production (Madusanka, & Manage, 2018). Compact photobioreactors, which have a smaller footprint and require less water and nutrients compared to traditional open pond systems, can effectively support the growth of cyanobacteria and produce high yields of biomass and biofuels. Subsequently this approach can significantly reduce the production costs (Kothari, Prasad, Kumar, Singh, & Negi, 2016). In cultivation of cyanobacteria in compact photobioreactors for sustainable production of biofuels and bio-products the cyanobacterial biomass can effectively serve as an electron acceptor in the bio-cathode compartment of a microbial fuel cell, improving the performance of the MFCs (Liu *et al.*, 2018). That study also demonstrated that the use of cyanobacterial biomass as a bio-cathode resulted in higher power output and COD removal efficiency. Subsequently, cyanobacterial biomass is able to perform photosynthesis, producing oxygen and enhancing the electrochemical performance of the MFCs (Deng, Xie, Lu, Li, & Zhu, 2019).

Microbial fuel cells apply microbial decomposing ability to a wide variety of organic matter for electricity generation with simultaneous wastewater treatment (Imanthi, Idroos, & Manage, 2019). A conventional fuel cell has anode, cathode and separation membrane that only allows proton transport. Organic substrates are loaded into the anode compartment and then subjected to anaerobic digestion by bacteria, and the produced electrons are transferred into the cathode through an external circuit. In the cathode O_2 acts as the electron acceptor, produced by cyanobacterial photosynthesis (Imanthi, Idroos, & Manage, 2019).

Microbial fuel cells have several advantages over other renewable energy technologies such as solar, wind, and hydropower. Specifically, microbial fuel cells can produce electricity continuously, independent of the weather conditions, and have a higher energy conversion efficiency compared to other renewable energy technologies (Zhang, Zhu, Li, & Zhao, 2020).

The organic substrate can be household waste, landfill leachate or industrial waste such as dyes. Compared to the municipal wastewater, landfill discharge of leachate has severe impacts on the environment as it is high concentration organic wastewater. A study found that domestic wastewater contains a substantial amount of energy, which can be utilized via energy recovery. It estimated that the energy content of domestic wastewater in China is approximately 14.9 times higher than the total energy consumption of wastewater treatment plants in the country. This suggests that there is a huge potential for energy recovery from domestic wastewater through the use of technologies such as microbial fuel cells (Liu, Zhang, Chen, Yang, & Chen, 2018). In the operational process more energy can be produced by assembling more MFCs in series or parallel, where wastewater can be added in the continuous batch mode operation. Microbial fuel cell stack is able to treat a larger volume of wastewater and produce a more stable and consistent power output compared to single-chamber microbial fuel cells (Zhang *et al.*, 2018). Therefore, the present study focused on generating power using rice wash water and landfill leachate with bio cathode, with simultaneous wastewater treatment using indigenous microorganisms. Further, the wastewater treatment efficiency of MFC in laboratory conditions was also evaluated with respect to electricity generation.

Substrate oxidation in anode

$$C_6H_{12}O_6 + 12H_2O \rightarrow 6HCO_3^- + 30H^+ + 24e^- \quad (1)$$

Oxygen reduction in cathode

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (2)$$

The first half-reaction (1) is the oxidation of the organic matter by the microorganisms, which releases electrons and protons. The electrons are transferred to the anode electrode. The protons remain in the solution and create a proton gradient, which drives the flow of electrons through the external circuit to the cathode electrode. The second half-reaction (2) is the reduction of the terminal electron acceptor, which in most microbial fuel cells is oxygen. The electrons from the anode electrode combine with oxygen and protons from the solution to form water.

In this study, the main objective was to provide new findings of MFC as an eco-friendly and low-cost alternative source of energy using cyanobacteria and wastewater.

2. Methodology

2.1 Sample collection

Cyanobacteria samples were collected from hyper eutrophic Beira Lake (6.918089N, 79.859791E) which is situated in the capital of Sri Lanka. Cyanobacteria were used as the cathode medium and wastewater was used as the anode medium. Fresh leachate samples were collected from Karadiyana Open dump site. Rice wash water samples were collected from university canteen.

2.2 Construction of a laboratory scale microbial fuel cell

A conventional MFC consists of anode and cathode electrodes, the separator (proton permeable membrane) and mediators. MFC container basically constructed with Poly Acrylic (PA) sheet contains two chambers each with approximately 1500 mL volume. The working volume consisting of the cathode and anode media was 1000mL. The remaining volume in the chambers allowed for a small headspace with a volume of approximately 500 mL. Once the MFC body was constructed, chambers were sealed using silicone, while the lids remained unsealed. Anode and cathode chambers were separated using a cation exchange membrane (CMI-7000S) that allows only protons to go through from anode to cathode. Electrodes were carbon cloth (2×4) cm^2 with the lowest available resistance. A permanent external resistor was fixed to the external circuit (0.33Ω) and the internal resistance of the MFC was considered to be too low to have a significant effect on the results.

2.3 Operation of the laboratory scale microbial fuel cell

Anodic chamber was filled with 1000ml of wastewater (setup 01 with leachate and setup 02 with rice wash water), whereas the cathode camber was filled with a similar volume of pure cyanobacteria sample. The cyanobacteria bloom from Beira lake consisted mainly of *Microcystis* spp., *Spirulina* sp. and *Chroococcus* sp., and after two months of laboratory culture through a dilution process pure *Chroococcus* sp. samples were collected. Optimization was done through addition of soluble redox mediators (neutral red) to anode and this improves electron transfer consistently. Neutral red was added as an electron transfer mediator to the anodic electrode. Anode chamber was sealed in order to enhance anaerobic digestion of organic matter by heterotrophic bacteria (Figure 1). The constructed MFC was operated for 6 hours and the generated voltages were measured at one hour time intervals using a Multimeter (ut-1 Multi scale). Generated current, power, current density, and power density through the MFC were calculated using standard equations. Chemical Oxygen Demand (COD), Nitrate (as NO_3^-), Nitrite (as NO_2^-), and Orthophosphate in the anode chamber were analyzed following standard spectrophotometric methods (APHA, 2012) and Optical Density (OD) of cathode was measured using a spectrophotometer (UVD-3500) at 595 nm wavelength at one-hour intervals for a period of 6 hours.

3. Results and Discussion

The efficiency of MFCs was assessed by means of electricity generation and substrate utilization throughout the operating cycle. In the present research, bio cathode with cyanobacteria, rice wash water or landfill leachate at anode, and carbon cloth electrodes were used to detect the substrate effects on electricity generation. Carbon electrodes were used as they have higher potential values when compared to other electrode materials. The most frequently used anodes are carbon materials and this affects the stability of microbial culture in the anode, with high electric potential and large surface area (Sevda, Sarma, Mohanty, Sreekrishnan, & Pant, 2018). Thus, in the MFC setup, carbon cloth can be the most suitable anode electrode choice with the characteristics discussed above.

In the run with setup 01, initial voltage generated at the beginning was 61.9 mV and then it gradually increased to a maximum of 693 mV. It took a long time to generate the maximum voltage (about 4 hours) (Figure 2). After that it started to decrease with time and at 360 minutes 670 mV was recorded (Figure 2). Then the voltage decreased continuously. This may be due to degradation of the carbon sources by bacteria in the MFC. No significant voltage generation was detected in the control setup. This may be due to having no electron acceptor in the cathode chamber to complete the electron flow, as distilled water was used as the cathode medium. In the beginning there was an increasing trend in voltage (from 61.9 to 693 mV). The results showed that the initial current from the experimental MFC was 187.58 mA and it increased gradually to a maximum of 2,100.00 mA at 4 hours and then decreased gradually recording 2,030.30 mA at the end of the run (Figure 3) with the maximum power density of 181.91 $mWcm^{-2}$. There was no significant current generation in the control setup during operation.

Initial voltage recorded in the setup 02 at the beginning was 234 mV and it gradually increased to a maximum of 1,111mV at 360 minutes (Figure 2). Compared to the setup 01 the initial voltage recorded was high, while no significant voltage at the same time was seen in the control setup. In setup 02 the current was 709.09 mA in the beginning of the experiment. Following the run, current generation increased gradually with time to a maximum of 3,366.37 mA at 360 minutes and then decreased with time (Figure 3). No detectable current was seen in the control setup during the run period. Subsequently the maximum current density and power density were 420.83$mAcm^{-2}$ and 467.55 $mWcm^{-2}$ respectively.

However, the voltage was not stable over time. In both setups deviations from a linear increase were observed, maybe due to Ohmic losses (OL), concentration losses (CL) and activation losses (AL) in the MFC during the run. Activation losses are due to the structural problems of the MFC and the nature of anode microbial community. Increasing the electrode surface area can improve the power output and stability of microbial fuel cells by reducing the internal resistance and activation loss (Wang *et al.*, 2018) and

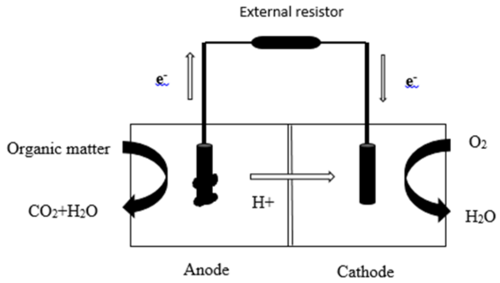

Figure 1. Structure of the constructed microbial fuel cell

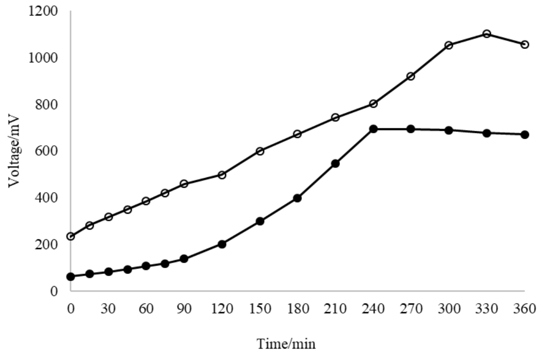

Figure 2. Time profile of voltage in the setup (closed circles for setup 01, open circles for setup 02)

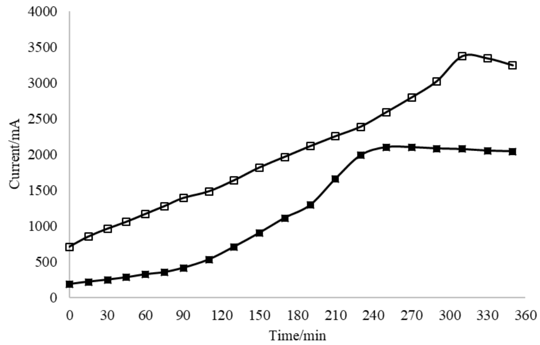

Figure 3. Time profile of current in the setup (closed squares for setup 01, open squares for setup 02)

therefore surface area of both electrodes was increased from the initial setup in this optimized setup to avoid frequent voltage variations deviating from the capacity of the cell.

Ohmic loss can be attributed to several factors including the internal resistance of electrodes, the resistance of the electrolyte solution, and the resistance of the membrane (Li, Hu, Yang, He, & Lu, 2018). Concentration losses arise with time due to substrate degradation by microorganisms. Therefore, organic matter concentrations decrease with time and are proportional to the electricity generated in the setup. This can be avoided using batch fed MFC which has a constant substrate concentration. While operating the cell the electrodes get covered, anode by microbes and cathode by cyanobacteria. Subsequently, the water molecules produced at the cathode can also reduce the efficiency of electron acceptors and affect the linear increase of generated voltage, which can be reduced by minimizing the electrode spacing, and using a membrane with a low resistivity such as CPM.

The study conducted by Cheng *et al.*, (2016) found that MFCs inoculated with naturally occurring microbial communities had a more diverse and stable microbial community compared to MFCs inoculated with external microbial inoculums. The study also demonstrated that MFCs inoculated with naturally occurring microbial communities had high power output and better treatment efficiency of organic matter. However, in the present study, rice wash water provided highly nutritional substrate for indigenous microorganisms to grow well, better than the landfill leachate, and the bacteria consortium oxidized the rice wash water as sole carbon substrate in higher rates under anaerobic conditions, which produced more electrons to the circuit enhancing the power generation while treating wastewater in the chamber. Cheng *et al.*, (2016) recorded that the MFCs operated using mixed bacterial cultures achieved substantially greater power densities than those with pure culture at anode. Indigenous bacteria in rice wash water are already adapted to the carbon source and they actively oxidize the substrate while producing electrons at higher rates. Power generation by the cell and the treatment efficiency of wastewater go together as proportional to each other (Sevda, Sarma, Mohanty, Sreekrishnan, & Pant, 2018). The results of the present study also show a positive relationship of the power generation with lowering concentrations of COD, nitrate, nitrite, and phosphate in the wastewater (Tables 1 and 2).

According to He *et al.*, (2016), the power generation performance of stacked microbial fuel cells (MFCs) with air-cathodes is higher than that of single cell MFC. The MFCs were stacked in a parallel configuration and the number of MFCs was varied from 2 to 10. Air-cathodes were used as electron acceptors instead of chemical cathodes. The results showed that the power density increased with the number of MFCs in the stack, reaching a maximum power density of 1.11 W/m^2 at 10 MFCs. This might be used as a solution addressing the low voltage values and power generation using a single cell MFC throughout the present study. Compared with the recorded information regarding the MFC, the present study encourages to further develop the setup in order to generate more power than achieved so far. For example, increasing the number of parallel MFCs and operating them simultaneously can be a good alternative providing higher power generation at the same time while treating different types of wastewater simultaneously.

COD is an indicator of the available organic substrate in the MFC, which can be converted into electricity throughout the process. In setup 01 COD was recorded at the start of the cycle as 301.2±3.31 mg L^{-1} and it decreased gradually to 230.8±3.31 mg L^{-1} at 6 hours of operation (Table 1). In the control setup a slight reduction of COD from 301.2 ±3.31 to 298.5 ±3.31 mg L^{-1} was recorded at 6 hours (Table 1). In setup 02 the initial COD measured was 234.62±6.62 ppm. Subsequently with time the COD decreased gradually and reached its minimum of 89.29±6.62 ppm after 6 hours. There was a somewhat rapid decline of COD in the experimental setup, while in the control setup there was a slight reduction of COD from 234.62 ±6.62 to 229.33±6.62 ppm after 6 hours, and then there was no reduction until end of the 6 hours (Table 2).

Anaerobic bacteria in wastewater degrade complex molecules into simpler molecules with time, and thus, the water treatment efficiency depends on the bacterial community in the substrate and in electrode biofilm. Cheng *et al.* in 2016 reported that some bacterial species can depend on different metabolic pathways such as fermentation or methanogenesis, and they do not contribute to electricity generation. Subsequently, they can reduce the waste treatment efficiency while providing a rich bacterial community. That might be the reason for not generating a high electricity output while using landfill leachate with a high microbial density, compared to use of rice washing wastewater.

Initial Nitrate concentration of setup 01 was recorded as 1.36 mg L^{-1} in anode chamber followed by a clear reduction to 0.53 mg L^{-1} after 6 hours. There was a rapid decline in nitrate concentration in the experimental setup compared to the control which had 1.36 to 1.32 mg L^{-1} in the same time period (Table 1). Initial Nitrate concentration in setup 02 was 4.43 ppm and gradually reduced to a minimum of 3.01 ppm after continuous operation for 6 hours. In the control setup there was a slight reduction of concentration from 4.43 to 4.39 ppm in the same time period; the reduction was insignificant in comparison to the experimental setup (Table 2).

Nitrite concentration recorded in the beginning in setup 01 was 1.36 mg L^{-1} and then the concentration decreased to 0.61 mg L^{-1} after 6 hours. In control setup there was a slight reduction of nitrate detected from 1.36 to 1.35 mg L^{-1} after 6 hours (Table 1). In setup 02 initial Nitrite concentration was recorded as 3.27 ppm. Following the operation of MFC, the concentration measured gradually decreased to 2.32 ppm after 6 hours. In the control setup there was no reduction in the same time period (Table 2). Anaerobic environment in the fuel cell reduces the efficiency of nitrifying bacteria, which results in a low efficiency with time. But in the initial stage they actively oxidize the substrate and give a high water treatment efficiency.

The setup 01 recorded an initial orthophosphate concentration of 635.86 µg L^{-1} and it declined to 508.25 mg L^{-1} at 6 hrs, with a large change compared to the control (635.86 to 631.46 µg L^{-1}) in the same time (Table 1). Subsequently, in setup 02 the initial orthophosphate concentration measured was 651.27 ppb. Subsequently there was gradual decline to the final concentration of 481.85 ppb. In the control setup it was slightly reduced from 651.27 to 640.26 ppb in the same time (Table 2). Phosphate removal is currently achieved largely by chemical precipitation, which is expensive and not

Table 1. Variation of chemical parameters in MFC setup 01

Time (Hours)	COD (mg/L)		N-Nitrate (mg/L)		N-Nitrite (mg/L)		Orthophosphate (µg/L)	
	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment
0	301.20±3.31	301.20±3.31	4.43	4.43	3.27	3.27	635.86	635.86
1	301.20±3.31	299.64±3.31	4.43	4.23	3.27	3.12	635.86	623.07
2	301.20±3.31	291.99±3.31	4.43	3.99	3.27	2.89	635.86	611.09
3	301.20±3.31	261.39±3.31	4.43	3.86	3.27	2.55	635.86	597.10
4	298.58±3.31	246.10±3.31	4.43	3.69	3.25	2.46	635.86	558.09
5	298.58±3.31	238.45±3.31	4.43	3.47	3.25	2.39	635.86	531.50
6	298.58±3.31	230.80±3.31	4.39	3.01	3.25	2.32	631.46	508.25

Table 2. Variation of chemical parameters in MFC setup 02

Time (Hours)	COD (mg/L)		N-Nitrate (mg/L)		N-Nitrite (mg/L)		Orthophosphate (µg/L)	
	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment
0	234.62±6.62	234.62±6.62	1.36	1.36	1.36	1.36	651.27	651.27
1	234.62±6.62	215.50±6.62	1.36	1.21	1.36	1.26	651.27	605.06
2	234.62±6.62	161.96±6.62	1.36	1.12	1.36	1.18	651.27	574.26
3	234.62±6.62	135.19±6.62	1.36	0.97	1.36	0.98	644.66	556.66
4	234.62±6.62	123.71±6.62	1.36	0.81	1.36	0.81	644.66	532.45
5	219.33±6.62	104.59±6.62	1.36	0.64	1.26	0.70	644.66	514.85
6	219.33±6.62	89.29±6.62	1.31	0.53	1.26	0.61	640.26	481.85

Table 3. Comparison of wastewater treatment percentages in setups 01 and 02

Set up No.	Parameter			
	COD removal %	N-Nitrite removal %	N-Nitrate removal %	Orthophosphate removal %
Setup 01	23.37	32.1	29.1	20.1
Setup 02	61.9	61.0	55.14	26.0

accessible in all parts of the world, and causes chemical pollution. Recent studies have shown that the fuel cell reduces 20% of orthophosphate in waste within 6 hours along with electricity generation, which is both cost-effective and environmentally friendly (Sevda, Sarma, Mohanty, Sreekrishnan, & Pant, 2018).

In the study it was found that the OD of the setup 01 cathode had increased by 11.4% at 595nm after 6 hours of operation, while in setup 02 it was increased by 14.3%. Subsequent increase in the oxygen production of the cell resulted from the cyanobacterial density increase. This shows that there is a positive relationship between the power generation by the cell and the growth of cyanobacteria at the cathode. Most of the fuel cells are operated with chemical compounds as electron acceptors. However, in this study cyanobacteria (*Chroococcus* sp.) collected from a hyper eutrophic water body were used. Cyanobacteria can perform photosynthesis efficiently under natural sunlight and increase oxygen concentration in the cathode compartment. These oxygen molecules act as electron acceptors and complete the circuit while producing H_2O using H^+ ions from anode reaction. Subsequently, with time the volume of the cathode increased due to production of H_2O molecules.

The major drawback with these laboratory scale MFCs is the high cost of materials, which have to compete with considerably low priced fossil fuels. However, in the environmental point of view MFC is an effective alternative to other renewable energy sources. Present MFC designs need improvements before a marketable product will be possible. Using cyanobacteria in cathode can reduce the cost of chemicals that have to be used as electron acceptors. But the construction and maintenance costs are still higher compared to the power output of the setup. Either substrate conversion rates have to be improved or the design has to be simplified to make MFCs a sustainable energy source and wastewater treatment method in the future.

According to the study results bacterial community in rice wash water was more effective in substrate oxidizing while producing more electrons to generate electricity. Degradation kinetics of indigenous bacteria is one of the major factors deciding the power output of a setup. Bacterial community in landfill leachate has shown less degradation ability compared to rice wash water. Subsequent difference in the electricity generation can be due to the complexity of the organic molecules in the wastewater. Rice wash water mainly contains carbohydrates that can be easily broken down into simpler molecules via degradation by bacteria. Landfill leachate is a complex mixture consisting of pollutants, including heavy metals, organic compounds, nitrogen, and phosphorus, which are barely broken down via simple degradation processes (Khan, Alvarez & Wei, 2018). However, the study suggests rice wash water as the most prominent wastewater source for MFC in terms of both electricity generation and wastewater treatment, and it is easily available at households.

4. Conclusions

The setups were operated with bio cathode (*Chroococcus* sp.) and wastewater (rice washed water or landfill leachate) at anode. Carbon cloth was used as the electrodes and in anode neutral red was used as an electron mediator. Cyanobacteria samples offered a high reduction capacity as electron acceptors. Rice wash water offered an enriched community of anaerobic microbes, which actively oxidized organic matter into simple molecules, more so compared to landfill leachate. The recorded maximum power density was 467.55 $mWcm^{-2}$, and considerable energy could be generated along with efficient wastewater treatment using rice wash water in anode and a cyanobacteria in cathode. Therefore, bioreactors based on electricity generation using MFCs may represent a completely new solution to treatment of domestic wastewater. Energy generation from household waste in a self-sustained way using limited space and time are the major advantages of MFC. Unstable, comparatively low power output and high initial cost can be considered drawbacks of these cells. If power output with regards to the operating costs and time can be increased using technological approaches, MFCs could be used in wastewater treatment plants, households, and industries as the generate electricity while treating wastewater, which is significant from both economic and ecological perspectives.

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