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Original Article

Microbial Fuel Cell for Bioremediation and Bioenergy Production Using Mixed Bacterial Culture Isolated from Municipal Waste

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Abstract

Introduction: Bioelectrical devices are being studied in a pilot project to treat wastewater and provide electricity, focusing on four bacterial strains and their impact on system parameters. **Objectives**: In this study, four electrogenic microorganisms tolerant to sewage discharge were isolated and evaluated and named AKS2, AKS14, BKS2 and CKW5. **Results**: Sewage wastewater was treated with these four bacterial strains consortia in different configurations of microbial fuel cells (MFC). Compared to BKS2, which produced a potential difference of 1.916±0.045 V and 7.222±0.051 mA, AKS2 had a higher potential to generate energy, measuring 1.943±0.064 V and 7.793±0.007 mA. On the other hand, CKW5 may produce a potential difference of 7.205±0.039 mA and 1.895±0.066 V, which is higher than the isolates but less than that of AKS2 and BKS2. In pH 7 and 35ºC temperature with 15% (v/v) bacterial inoculum, AKS13 showed higher potential than AKS14, which generated 1.875±0.039 V and 7.195±0.027 mA. Whereas AKS14 generates 1.871±0.006 V and 7.192±0.009 mA. These isolates have the following bioremediation capacities: AKS2 (89.88%) > CKW5 (89%), >BKS2 (88%) > AKS14 (85.48%). Graph Pad PRISM software, version 9.1.5, was used to statistically validate all of the bio remedial percentages using a two-way repeated measure (RM) ANOVA. With a R squared value of 0.1017, the results were determined to be statistically significant at $p < 0.01$. The study characterized four potent bacterial isolates using 16S rDNA sequencing of AKS2, AKS14, BKS2, and CKW5, revealing Escherichia coli, Salmonella enterica, Bacillus cereus, and Klebsiella pneumonia respectively. The novelty of the present study lies in the systematic isolation, comparative evaluation, and electrical stacking of indigenous electrogenic bacteria from municipal sewage wastewater for simultaneous bioremediation and bioelectricity generation in microbial fuel cells (MFC).

Keywords: *Bioelectricity, COD Removal, Electrogenic Microorganisms, Microbial Fuel Cell, Sewage Wastewater, Salt Bridge Enrichment*

Introduction

Every stage of a living thing's evolution, growth, and survival requires energy. The search for alternative energy technologies has been prompted by concerns about environmental harm and the depletion of fossil fuel supplies (Du *et al.*, 2007). Thermal, wind, and hydropower energy production are not reliable energy sources because our needs for energy are growing and cannot be fully met.

Pant and Adholeya (2007) investigated that industrial sectors are the main energy users, using energy for both production and treatment. In addition to raising energy consumption, industrial operations release effluent into the environment, which pollutes it. Water basins become eutrophic due to the massive amounts of wastewater released into aquatic systems (Saha *et al.*, 2005; Bikram *et al.*, 2023). The industrial-coloured effluent from distilleries blocks sunlight from penetrating and harms the aquatic ecosystem (Saha *et al.*, 2005; Tewari *et al.*, 2007). Wastewater from distilleries is now treated using physicochemical and biological (aerobic and anaerobic) techniques. The industries have an energy

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requirement due to these energy-intensive operations (Pant and Adholeya, 2007; Mohana *et al.*, 2009; Fida *et al.*, 2025).

Potential technology that removes pollutants from wastewater, such as chemical oxygen demand (COD), colour, and total dissolve solids (TDS), which simultaneously generating electricity is the microbial fuel cell, or MFC. (Wen *et al.*, 2010). It is a biological power source system in which organic matter is oxidised to create ATP, protons and electrons combine to convert chemical energy into electrical energy (Luo *et al.*, 2023).

Numerous wastewater types, such as dairy, molasses, recycling of paper, household, etc, have been used as MFC substrates (Pant *et al.*, 2010). MFC that use unconventional substrates like cattle dung, vegetable waste, can produce power outputs that are on par with or even better than those of conventional substrates like sugarcane effluent, raw distillery effluent etc. (Apollon *et al.*, 2025). Furthermore, MFC using petroleum sediment including hydrocarbons attained one of the highest recorded maximum power densities of 50,570 mW m^{-2}. MFC can support sustainable waste management techniques and carbon-neutral energy production by combining various organic waste sources.

The microbe is essential to the MFC ability to produce electrons and break down organic materials. Numerous exo-electrogenic bacteria have been identified as biocatalysts in MFC, including *Geobacter spp., Enterobacter spp., Shewanella oneidensis,* and *Bacillus spp.,* among others (Fida *et al.*, 2025; Nimje *et al.*, 2012). It is essential to extract a pure bacterial culture from wastewater in order to comprehend the exo-electrogenic activity of bacteria. Logan and Regan (2006) demonstrated the electrochemical activity of the pure culture they had extracted from the wastewater in a microbial fuel cell. According to recent literature, wastewater can now produce a greater current density in the MFC by using mixed microbial communities (Huang *et al.*, 2015; Venkata *et al.*, 2010). However, microbes are separated from sewage wastewater in order to better understand MFC efficiency under mixed microbial populations. Many factors frequently affect how well MFC perform and how efficiently they generate power. The three most important parameters that impact the MFC ability to produce electricity are the pH of the electrolyte, conductivity, and COD concentration in wastewater. Because it regulates bacterial growth and facilitates the effective passage of protons through the PEM, the anolyte pH has a major impact on overall performance (Raghavulu *et al.*, 2013). Therefore, to improve the MFC ability to produce power, the ideal electrolyte pH in the anode chamber must be determined. Enough nutrients are also needed for the isolated microbe to survive and produce electrons. Higher concentrations can occasionally prevent microbiological growth, which impairs performance. (Elakkiya and Matheswaran, 2013). The anode side of metabolic processes produces protons and electrons, which must flow through the membrane and be consumed at the cathode rate (Jadhav *et al.*, 2016).

The fundamental idea behind the operation of microbial fuel cells, or MFCs, is the production of bioelectricity through the breakdown of pollutants. Over time, the development of MFC architecture capable of supporting persistent microbial biofilms has increased logarithmically (Bose *et al.*, 2025). By increasing electron transfer rates, increasing surface areas, and refining the characteristics of anode and cathode materials, nanotechnology has greatly improved the performance and efficiency of MFCs (Dakal *et al.*, 2025). Substrate consumption is influenced by microbial fuel cell designs. Microbial fuel cells (MFC) have benefits for both producing green energy and managing waste (Sonawane *et al.*, 2024).

The study evaluates the efficacy of treating wastewater in an MFC using varied culture as a biocatalyst, accounting for membrane thickness, pH, conductivity, and BOD levels.

Materials and Methods

A. *Sample collection*

For validation of microbial strains to evaluate their efficacy in degradation of sewage pollutants via the usage of efficient bacterial fuel cells, the microbial samples were collected by collecting the sewage samples from sewage treatment plants situated in Jaipur city (Rajasthan). Different microbial samples were collected from five different sampling sites from the Jaipur sewage treatment plant. Out of these, wastewater samples were collected from three different tanks (Inlet/Grit Tank Water, Secondary Treatment Tank Water & Tertiary Tank water) and the remaining sludge samples were collected from two different tanks (Inlet/Grit Tank Water & Settling Tank). NOC was issued from Jaipur Development Authority (JDA/E.E./PHE-I/2021/d-22) for collection of sewage samples from I MLD STP Jawahar circle, Jaipur.

B. *Physical characteristics of Municipal Waste*

The effluent was obtained from the sewage treatment plant of Jawahar Circle situated in Jaipur, India. The significant features of the wastewater are pH: 6.8 to 7.23, BOD: 26.66 mg/L to 306.67 mg/L, COD: 1016 to 216 mg/L TDS: 805 to 101 mg/L, Conductivity: 715.33 to 803.33 µs/cm, correspondingly. Before usage, the wastewater was held at 40 °C and carried to room temperature. It contained analytical-grade reagents such as potassium ferricyanide, phosphate buffer, sodium hydroxide, and orthophosphoric acid.

C. *Bacterial culture isolation*

Samples for Bacteriological analysis were obtained from five sites of the selected treatment plant in Jaipur, Rajasthan, including wastewater and sludge samples, for physio-chemical analysis. Microbial cultures were isolated from waste using various plate techniques. The media was sterilised, diluted, and incubated overnight on an anaerobic agar plate. Colonies were collected, and biochemical verification was performed to purify the isolates.

D. *Assembly and process of Fuel Cells*

A two-chamber MFC was built utilising dual plastic bottles parted by a bridge made up of salt and agar-agar, with 350 mL in each chamber. An electrode consisted of a plain graphite plate. Isolated colonies were incubated for 12 hours. The circuit was connected to the anode and cathode using black and red copper wires, connecting them to the multimeter at their respective ends. The potential difference produced by the fuel cell was calculated via a multimeter. The MFC was operated at optimum temperature under static conditions for up to 18-20 days, with current recorded at 1-hour intervals.

E. *Optimization of MFC with various operating parameters*

The optimisation of MFC was influenced by various parameters such as salt bridge concentration, temperature, pH, and bacterial concentration, which significantly impact bacterial growth and bioelectricity production (Aghababaie *et al.*, 2015).

1. *Effect of Bacterial Concentration*

Bacterial concentration is a crucial factor influencing bioelectricity production, referring to the required amount of bacterial culture for optimal bioelectricity production. 24-hour old bacterial cultures were independently studied at various volumes from 5%-15% (volume/volume) bacterial culture broth to the substrate operating volume of 350 ml for all the bacterial cultures.

2. *Effect of pH*

The study optimised the substrate's pH, which affects bioelectricity production, by operating MFC at varying pH values from 5.0 to 9.0 and adjusting them before sterilisation using freshly prepared 1N HCl and NaOH solutions.

3. *Effect of temperature*

Temperature impacts bacteria's survival, growth, and metabolic activity, directly affecting bioelectricity production. The study examined temperature ranges from 25 °C to 45 °C at the equal interval of 5°C under identical conditions.

4. Determination of Percentage (%) change of Physicochemical Parameters

The physicochemical parameter's percentage change in relation of Biological Oxygen Demand earlier and afterward MFC process was calculated using the following formula.

$$Percentage\ (\%)\ Change = \frac{initial - final}{initial} \times 100$$

The change in percentage in physicochemical parameters indicates a rise or decline in Wastewater Treatment Efficiency (WWTE) after 18-21 days of MFC operation.

5. MFC Circuit Assembly: -

Four MFC were operated in parallel, series, and hybrid connections with all selected 4 potent electrogenic bacterial isolates consortia at selected pHs, temperatures, and inoculums to increase stack voltage or current.

Series Assembly

The route that currents follow as they pass through each component is the primary definition of a series circuit. These devices are linked together along the pathway and do not have any branches. The fact that currents move from one branch to another is the most crucial element in this situation. When all of the components in a circuit have the same current flow, the circuit is referred to as a series circuit (Figure 1A & 1B). Every component in this circuit is placed on a single line (Chaibi *et al.*, 2019).

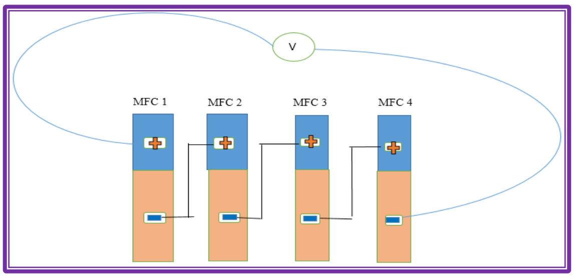

Figure 1A: *Schematic Diagram of Series Connection*

Figure 1B: *MFC setup of Series Connection*

Parallel Assembly

A parallel circuit (Figure 2A & 2B) is a circuit with two or more paths, where current flows through one or two branches before combining components. (Rodrigues *et al.,* 2021). It has no impact on the entire circuit. Productivity is increased, and the work process is not harmed if one component is removed (Bethoux, 2020).

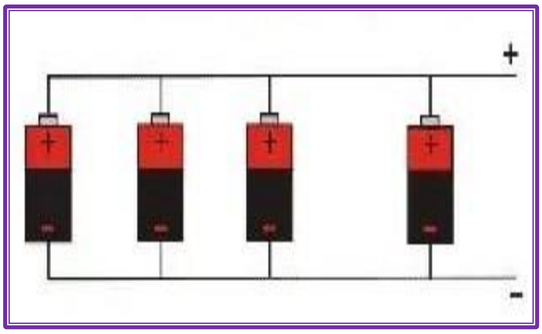

Figure 2A: *Schematic Diagram of Parallel* Connection

Figure 2B: *MFC setup of Parallel Connection*

Hybrid Assembly

Hybrid Assembly, MFC are in parallel as well as in series circuit (Figure 3A & 3B) additionally the fuel cell's electrochemical reactions produce power in the form of direct current (DC). For the majority of applications, a single fuel cell's output of less than 1 V is insufficient (Selmi *et al.*, 2022).

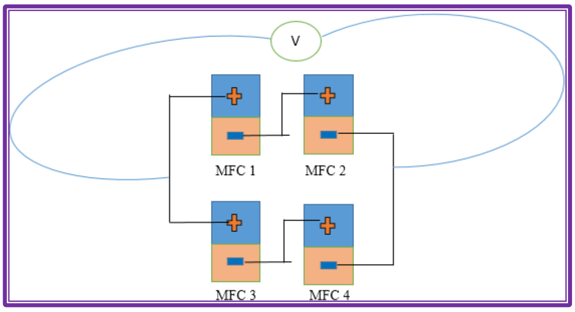

Figure 3A *Schematic Diagram of Hybrid Connection*

Figure 3B *MFC setup of Hybrid Connection*

6. 16S rDNA sequencing

The genomic DNA of three separated bacteria was extracted using phenol-chloroform, amplified for genotypic identification using universal 16S rDNA primers, sequenced, and uploaded to the NCBI database (Agrahari *et al.*, 2022). After examination, AKS2 was found to be *E. coli* with Accession No. OR144374, AKS14 was found to be *Salmonella enterica* with Accession No. OR146961, BKS2 was found to be *Bacillus cereus* with Accession No. OR16961, CKW5 was found to be *Klebsiella pneumoniae* with Accession No. OR146746.

This study was conducted in the period of 2020 to 2024.

Result

It was observed that among the all 30 bacterial strains used, *E. coli* (AKS2), *S. enterica* (AKS14), *B. cereus* (BKS2), and *K. pneumoniae* (CKW5) showed the maximum potential to generate energy at pH 7 and 35 °C with 15% (v/v) bacterial inoculum in relation of current (mA) and voltage (V) along with the bioremediation potential. The database shows that individual MFC voltage is too low to turn on devices, causing LEDs to glow lighter in series configurations and less brightly in hybrid configurations (Figure 4A & 4B).

In series connection (Table 1A), all on the zero-day maximum voltage of control showed 0.0950±0.0030 V and 0.09±0.0056 mA current, but on the 4th day microbial fuel cells showed the maximum voltage (V) and current (mA) 1.3067±0.0764 and 0.449±0.045, respectively. After the 4th day voltage and current dropped continuously, and on the 14th day they were minimum i.e., 0.2533±0.0208 (V) & 0.212±0.015 (mA), respectively (Chart 1).

In parallel connections (Table 1B), on zero-day, maximum voltage of control showed 0.0967±0.0503 (V) and 1.1933±0.0513 (mA), respectively which were maximum among the different configurations i.e., series and hybrid. MFC voltage (V) and current (mA) were increased with days up to 6th day. On 6th day, voltage (V) and current (mA) were maximum i.e., 0.4033±0.0503 and 2.2733±0.0643 respectively, which were gradually decreased. On the 14th day, MFC voltage and current were minimum i.e., 0.2367±0.0321 (V) and 1.2067±0.0208 (mA) respectively (Chart 2).

In the hybrid series (Table 1C), on zero-day maximum voltage of control showed 0.300±0.050 (V) and 0.260±0.003 (mA), respectively. Which was gradually increased on the 6th day all 4 Microbial fuel cells showed the maximum voltage and current 1.560±0.106 (V) and 0.629±0.0015 (mA), respectively. After 6th day voltage and current of MFC were dropped continuously and on the 14th day it was minimum i.e. 0.943±0.040 (V) and 0.297±0.032 (mA), respectively (Chart 3).

This study showed that the hybrid MFC configuration resulted with all four potent electrogenic bacterial isolates consortia, in an increase voltage as well as current followed by series circuit and then parallel circuit.

Table 1A: *The maximum voltage and current generated on different days with different configuration of MFC in Series connection*

Days	Control voltage (V) Mean±S.E.	MFC voltage (V) Mean±S.E.	Control current generated (mA) Mean±S.E.	MFC current generated (mA) Mean±S.E.
0th Day	0.0950±0.0030	0.6100±0.0361	0.09±0.0056	0.3233±0.0037
2nd Day	0.5233±0.0252	0.7167±0.0351	0.13±0.0030	0.331±0.0026
4th Day	*0.7367±0.0513	*1.3067±0.0764	*0.15±0.0030	*0.449±0.045
6th Day	0.6333±0.0416	0.9067±0.0513	0.13±0.0015	0.365±0.004
8th Day	0.3960±0.0142	0.8400±0.0458	0.12±0.0026	0.342±0.009
10th day	0.2040±0.0026	0.8067±0.0513	0.10±0.0010	0.331±0.003
12th Day	0.1040±0.0072	0.5533±0.0306	0.07±0.0035	0.295±0.005
14th Day	#0.0827±0.0086	#0.2533±0.0208	#0.05±0.0056	#0.212±0.015

*SE=Standard error, n(number of samples=3), * = maximum, #= minimum*

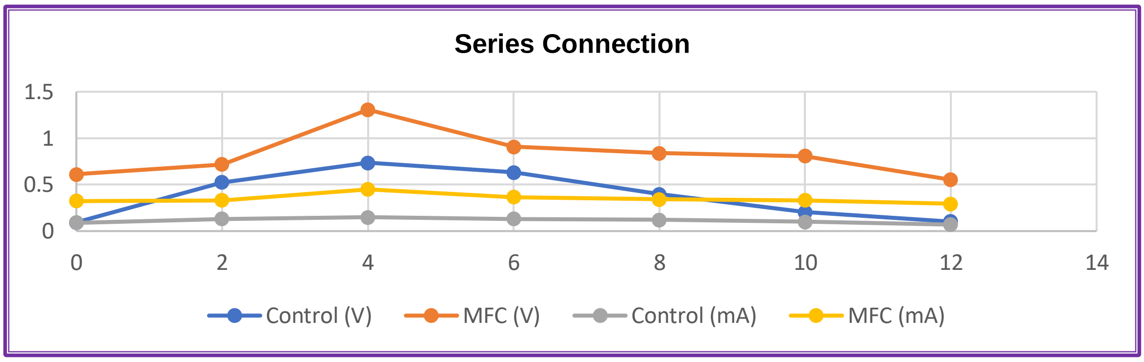

***Chart 1**: Voltage and current generation potential by MFCs in series configuration on 0th to 14th day*

Table 1B: *The maximum voltage and current generated on different days with different configuration of MFC in Parallel connection*

Days	Control voltage (V) Mean±S.E.	MFC voltage (V) Mean±S.E.	Control current generated (mA) Mean±S.E.	MFC current generated (mA) Mean±S.E.
0th Day	0.0803±0.0268	0.0967±0.0503	0.2007±0.0074	1.1933±0.0513
2nd Day	0.1667±0.0208	0.2047±0.0045	0.5967±0.0208	1.9367±2.0321
4th Day	0.1533±0.0451	0.3133±0.0208	1.9433±0.0764	2.0900±4.1217
6th Day	*0.2500±0.0400	*0.4033±0.0503	*2.1800±0.0265	*2.2733±0.0643
8th Day	0.1467±0.0351	0.3700±0.0436	1.87±0.0306	2.0033±0.0503
10th day	0.1367±0.0289	0.3387±0.0032	1.77±0.0200	1.9533±0.0551
12th Day	0.0903±0.0105	0.2820±0.0026	1.53±0.1305	1.7900±0.1493
14th Day	#0.0600±0.0100	#0.2367±0.0321	#1.03+0.0569	#1.2067±0.0208

*SE=Standard error, n(number of samples=3), * = maximum, #= minimum*

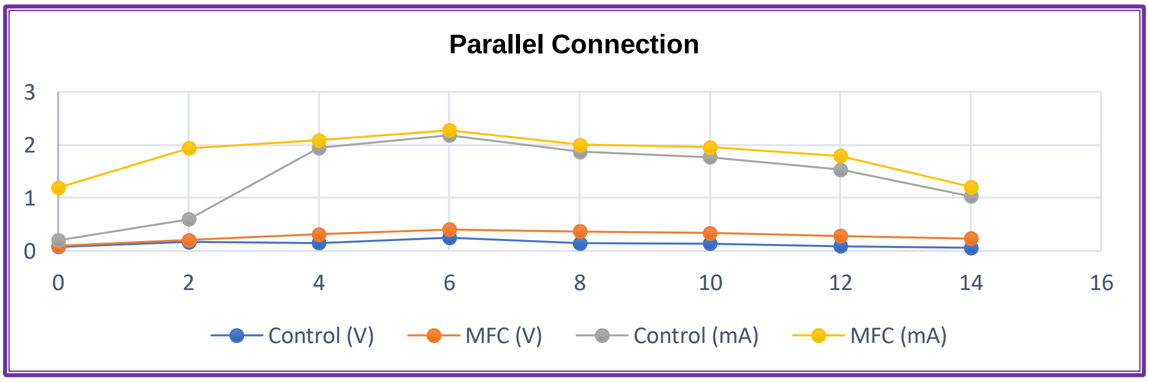

***Chart 2**: Voltage and current generation potential by MFCs in parallel configuration on 0th to 14thday*

Table 1C: *The maximum voltage and current generated on different days with different configuration of MFC in Hybrid connection*

Days	Control voltage (V) Mean±S.E.	MFC voltage (V) Mean±S.E.	Control current generated (mA) Mean±S.E.	MFC current generated (mA) Mean±S.E.
0th Day	0.300±0.050	0.590±0.066	0.260±0.003	0.290±0.009
2nd Day	0.733±0.042	0.923±0.025	0.331±0.003	0.400±0.016
4th Day	0.813±0.071	1.057±0.031	0.391±0.003	0.476±0.007
6th Day	*1.127±0.040	*1.560±0.106	*0.498±0.009	*0.629±0.0015
8th Day	1.1100±0.0265	1.477±0.025	0.39±0.0074	0.527±0.003
10th day	0.8867±0.0351	1.303±0.015	0.33±0.0055	0.423±0.006
12th Day	0.6400±0.0458	1.173±0.025	0.24±0.0057	0.360±0.009
14th Day	#0.5467±0.0643	#0.943±0.040	#0.19±0.0081	#0.297±0.032

*SE=Standard error, n(number of samples=3), * = maximum, #= minimum*

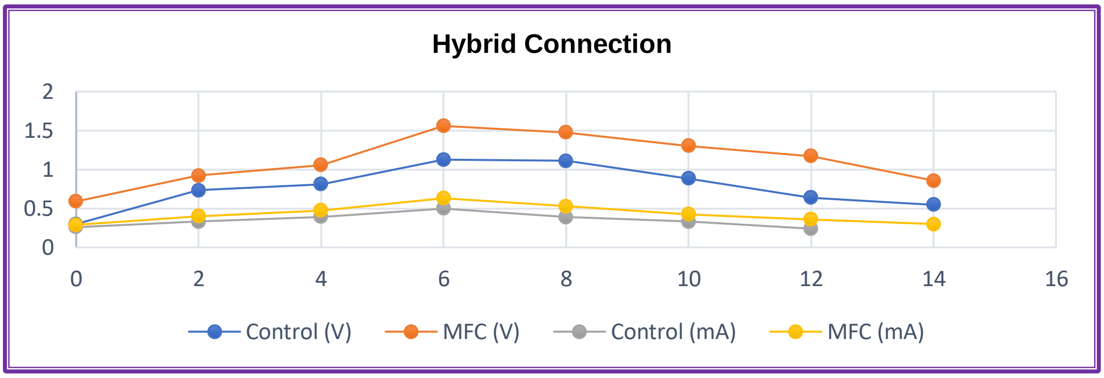

Chart 3: Voltage and current generation potential by MFCs in hybrid configuration on 0th to 14th day

Figure 4A*: Electricity Generation by Multiple Chamber Connected in Hybrid Connection*

Figure 4B*: LEDs to glow lighter in series configurations*

Discussion

The study estimated various physico-chemical parameters in wastewater and sludge samples, including colour, temprature, EC, BOD, TSS, TDS, hardness, COD, and total kjeldahl nitrogen. Poor waste disposal practices, human negligence, and greed lead to the unresolved or intermediate formation of toxic pollutants in industrial, agricultural, and residential sectors (Campo *et al.*, 2015).

The study analysed wastewater samples from different tanks to determine the presence of compounds like indole, hydrogen sulphide, and mercaptan, which cause foul dors. The samples showed varying colours, with dark yellow in tank 3, light yellow in tank 2, and dark blackish in tank 3. The pH values ranged from 6.8 to 7.233, and the temperature varied slightly from 25°C to 30°C. The electric conductivity ranged from 715.33–803.33 s/cm for wastewater to 750 s/cm for sludge samples (Saxena *et al.*, 2023).

For the benefit of society and the future, wastewater treatment is an important project that needs more attention (Ardakani *et al.,* 2020). The elimination rate of COD and MBBR reactor activity rate are inversely linked, even the MBBR reactor efficiency is influenced by the MBBR organic loading rates per carrier area.

The study analysed the biological oxygen demand (BOD) and COD values in wastewater samples, with tank 1 having the highest BOD value due to toxic metal deposition. The COD range was from 62.33–42.24 mg/L, with a acceptable edge of 50 mg/L. The urban wastewater utilised produced 1.67–2.33 kWh/m^3 of energy with a hydraulic holding period of 18–192 hours and a COD value of 600 mg/L (Saxena *et al.*, 2023).

The study found that wastewater samples had varying hardness values, with tank 1 having the highest hardness at 200 mg/L, while tanks 2 and 3 had the highest at 106 and 95 mg/L, respectively (Saxena *et al.*, 2023). Total Kjeldahl nitrogen (TKN) in sludge samples was 13.87–12.5 mg/L, while in wastewater samples it ranged from 1.503–32.82 mg/L. Ammonia is converted to ammonia by proton exchange membranes, nitrification, and ammonium incorporation in substrate biomass for microbial growth in the process of microbial-mediated remediation of nitrogenous waste (Saxena *et al.*, 2023).

The study analysed organic carbon levels in sludge samples, with varying values in tanks 1 and 2 samples. The total volatile solid values showed the possibility of bioremediation in wastewater, ranging from 56.35 to 60.61 mg/L. (Prakash *et al.*, 2018). In addition to producing bioelectricity, biological cells also help remove harmful contaminants and toxicants linked to nitrogen and sulphur from wastewater (Pal and Paul, 20008).

Consortium of these isolates in parallel or series or hybrid format within the microbial fuel cells showed efficient bio electricity generation with the most effective with Voltage and Current production. In a double-chambered microbial fuel cell, Larrosa-Guerrero *et al.* (2010) used a mixed waste water pattern that included both home and brewery wastewater and obtained improved results.Generally intended microorganisms act as a primary biological catalyst that speeds up the biological breakdown of a specific substrate, such as waste water that contains organic materials for the diffusion of electrons to the cathodic compartment (Obileke *et al.*, 2021).

The production of bioenergy was accompanied by the observation and calculation of waste bioremediation. *E. coli* AKS2 has the maximum bioremediation (89.88%) from the present database, monitored by BKS2 (88%), CKW5 (89%), and AKS14 (85%). All the bio remedial percentages were validated statistically through Graph Pad PRISM software, 9.1.5 taking two-way repeated measure (RM) ANOVA. The results were found to be statistically significant at $p < 0.01$ with R squared value at 0.1017. Bacteria at pH 9 and temperatures of 25 °C, 35 °C, and 45 °C showed lower bioremediation capacity and energy production. *E. coli* strain AKS2 displayed the highest potency in our investigation, followed by AKS14 (*S. enterica*), CKW5 (*K. pneumonia),* and BKS2 (*B. cereus)*. (Saxena *et al.*, 2024). Non-typhoidal *salmonella gastroenteritis* continues to spread globally, causing symptoms varying in severity based on risk factors (Sutar *et al.*, 2024).

The major novel contributions of this work include:

Four indigenous bacterial strains isolated from municipal wastewater were identified by 16S rDNA gene sequencing and proved to be effective bio electrochemical catalysts in mediator-free MFCs.

Out of the isolated bacteria, *E. coli* (AKS2) had the best results concerning electrochemical power production as well as bioremediation efficacy (COD removal efficiency ≈ 89.88%).

The optimum conditions for electricity generation and efficient wastewater treatment have been identified by this research as pH 7, 35 °C, and 15% inoculum for real sewage wastewater.

Different MFC series, parallel, and hybrid connections have been assessed, with the highest voltage and currents generated by hybrid connections, adequate for low power consumption devices such as LEDs.

The relationship between bioelectricity production and the reduction of important physicochemical parameters is established, which affirms the dual functionality of MFC as an application for environmental treatment and renewable energy.

Limitations

This study was conducted on a pilot scale and MFCs Electrochemical Performance Metrics and standard MFC performance indicators need to be further studied.

Future Scope

Upgradation of MFC/BFC technology on large scale (in terms of parallel, hybrid or series connection) can be done for practical applications, such as wastewater treatment or energy generation.

Conclusion

Traditional physicochemical methods are ineffective due to financial inefficiency and secondary pollutants. Bioremediation, using plants, microbes, and microbial products, is cost-effective and eco-friendly. In wastewater and sludge samples, microbial consortia are identified in this investigation. Microorganisms such as *Salmonella* species, *Escherichia* species, *Pseudomonas* species, *Staphylococcus* species, *Streptococcus* species, *Klebsiella* species, and *Bacillus* species were detected frequently in both wastewater and sludge samples, according to the study. Through the analysis of sewage samples from the treatment facility in Jaipur, the study demonstrated the effectiveness of MFC in lowering a range of physicochemical parameters. The study explores renewable energy sources, focusing on MFC power enhancement through pilot-scale optimization of microorganisms for optimal wastewater biodegradation and high energy production.

Conflict of Interests

There is no conflict of interest, according to the authors.

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