Original Research Article

Journal of Biological Research

& Biotechnology

Bio-Research Vol. 22 No.1; pp. 2264-2273 (2024). ISSN (print):1596-7409; eISSN (online):2705-3822

Assessing microbial exoelectrogenicity for enhanced industrial waste water management and power generation

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Abstract

The quest for eco-friendly waste management and renewable sources of energy is rapidly increasing. Microbial fuel cell is promising form of renewable energy, which treat and convert organic matter in wastewater to electricity through the aid of microorganisms present in the wastewater. This study assessed for electrogenic microorganisms that generate power during wastewater treatment at a referenced pH of 8.5 and temperature of 37 0C. The exoelectrogenicity and identity of the microbial isolates were confirmed using microbial fuel cell (MFC) and molecular characterization, respectively. Two bacterial isolates: N4- *Providencia* species, N6- *Proteus* species, and three fungal isolates: S9- *Clavispora lusitaniae*, S10- *Candida parapsilosis* ,S14- *Clavispora lusitaniae* with accession numbers; KX548357.1, KX548358.1 and KX548359.1, KX548360.1, KX548361.1, respectively showed exoelectrogenic properties. *Proteus species* and *Candida parapsilosis* generated relative high-power densities of 1.59 and 1.55 W/m2, respectively. Significant difference ($p < 0.05$) in wastewater treatment was also observed. When compared with the control wastewater, S10 recorded about 38% of contaminant removal with the following parameters; biochemical oxygen demand (536.38mg/l), chemical oxygen demand (1974mg/l), total dissolved solid (640mg/l) and conductivity (512µS/cm). The findings showed that, not only bacteria, but fungi are good exoelectrogenic microorganisms for industrial wastewater treatment and power generation in MFC setup.

Keywords: Contaminants, Electrogenic isolates, Microbial fuel cell, Pathogenicity, Power density

Received September 11, 2023; **Revised** February 17, 2024; **Accepted** February 24, 2024, **Published** March 9, 2024

Journal Homepage: http://www.bioresearch.com.ng.
Publisher*: Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.*

INTRODUCTION

Renewable energy is the key component of sustainable, eco-friendly, and economical means of generation of electricity. The International Energy Agency (IEA) has reported that the global renewable energy demand has increased from year 2019 due to the decrease in the use of fossil fuel to generate electricity (Ang *et al*., 2022). Many industries in under-developed countries that generate wastewater do not have wastewater treatment plant owing to the cost of construction, lack of technical knowhow and power supply. These have constituted a great menace to the environment due to constant environmental pollution resulting from direct discharge of wastewater from industrial, agricultural and domestic sources into water bodies. The adverse effect of this act is deleterious. Issues with climate change and global warming require innovative ways to produce energy from carbon-neutral and renewable sources. Therefore there is need to understand the relationship between economic development and energy consumption (Kabeyi and Olanrewaju, 2022). Currently, many wastewater treatment plants use activated sludge. This is an antiquated, energy intensive, expensive and aerobic process that requires pumping air into a reactor.

Microbial fuel cell technology is a fast-developing technology that promises the benefit of harvesting electricity while biologically treating wastewater. It is an affordable, accessible and ecofriendly technology that uses microorganisms entrenched in wastewater from industrial, agricultural and domestic activities to treat the wastewater and simultaneously produce electricity through anaerobic cellular respiratory mechanism of the microorganisms. Synthetic and natural wastewaters have been applied as substrate in MFC which showed significant removal of organic substances (Xu *et al*., 2015). The energy produced by MFCs is lower when compared to hydrogen fuel cells. The integration of both electricity generations with wastewater decontamination would lessen the cost of treating wastewater. According to Lai *et al.* (2018), Domestic wastewater is capable of generating up to 28 mW/m^2 of power in a small batch system. It is expected that wastewater treatment plants designed for a population of about 100,000 could generate about 0.8 megawatts. This is sufficient to serve up to 500 homes (Logan *et al*., 2015). This technology as a source of producing bio-energy has increased global research with its technical facets being extensively reviewed.

Electrical energy generation with the aid of diverse electroactive microorganisms as applied in microbial fuel cells is one of such carbon neutral and renewable energy source, sustainable and efficient technology that could be employed for powering microelectronics (Logan *et al*., 2019; Liu *et al*., 2022). In a microbial fuel cell system, the inherent microbe oxidises the organic substrates such as glucose and acetate to generate electrons that are transferred to an external circuit, generating electricity. These microorganisms have the capability of producing extracellular electrons onto the electrode surface without mediator chemicals. They are called exoelectrogenic microorganisms. They have high power output and can produce biofilms on the anode surface which accepts electron and transmit the electrons to the cathode thereby producing more energy. Bacteria such as *Stenotrophomonas maltophilia,* , *Achromobacteria xylosoxidans Geobacter sulferreducens, Geobacter metallireducens, Shewanella putrefaciens, Aeromonas hydrophila* and *Acinetobacter bereziniae* can transmit internal cell electrons to extracellular acceptors through biofilms (De La Cruz-Noriega *et al*., 2023). While some microorganisms can transfer electrons directly to anode which acts as terminal electron acceptors, or make use of mediators, others accept electron from cathode (Roy *et al*., 2023).

Although there are extensive reports on the exoelectrogenic nature of some microorganisms in the enhancement of electricity generation using MFC, majority of the reported works focused only on the exploitation of bacteria. This study also evaluated the effectiveness of using bacteria, fungi, and optimized MFC system parameters to improve wastewater treatment and boost power density, which is the primary issue for microbial fuel cells, at a specified pH of 8.5 and temperature of 37°C. This pH and temperature have been established as the optimal environmental conditions for the effective performance of these electrogenic isolates as reported in previous studies by Nwagu *et al*. (2019) in the study of effect of pH and temperature on the performance of MFC.

MATERIALS AND METHODS

Sample collection

Samples of wastewater were collected from abattoir at Gariki market, leachate from landfill at Ocho-udo City and kitchen at Agbaja Unuhu residence using airtight plastic containers at geographic coordinates: latitude longitude (6.2597ºN 8.1086ºE, 6.3291ºN 8.1161ºE, 6.2971ºN 8.1126ºE) of Abakaliki Local Government Area in Ebonyi State, Nigeria, respectively.

Screening and isolation of exoelectrogenic bacteria and fungi

Five (5) double chamber microbial fuel cells were designed according to Padma and Dirk (2012) and used to assess for microbial species in the wastewater that are capable of releasing extracellular electrons using wastewater as substrate. The double chamber was made up of anode and cathode chamber containing graphite rods as electrodes as shown in **figure 1** below. The anode chamber of the MFC contained 250 ml each of wastewaters collected from the 3 different sources while the cathode chamber has 0.1 M of Potassium manganate (vii) solution ($KMnO_4$- Sigma Aldrich, US). After 3 days of observation of release of electrons in form of voltages recorded with digital multimeter (DT9205A), the biofilm produced at the anode electrodes were washed into 5 conical flasks using sterile water. Pure bacteria and fungi cultures were isolated from the biofilm according to the method of Madigan (Madigan, 2012) using Nutrient agar (Oxoid-thermo scientific, UK) and Potato Dextrose Agar (Oxoid-thermo scientific, UK), respectively, according to the manufacturer's specifications. Twenty (20) pure isolates obtained were further screened in 20 MFC setup by inoculating each of the isolates into the anode chamber containing autoclaved kitchen wastewater. An MFC setup that contained no inoculum of the pure isolates served as the control. The five microbial pure cultures that are contained in the MFC setup that generated higher voltages were selected for further analysis at pH of 8.5 and temperature of 37℃

Figure 1. Designed Double Chamber Microbial Fuel Cell (MFC)

Experimental design

Latin square design where subjects are allocated treatments over a given time period where time is thought to have a major effect on the experimental response was utilised in the experiment (Yu *et al*., 2016). The data was analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0. An analysis of variance with one way (ANOVA) was performed on the variables.

Measurement of power density generated by the isolates

The ability of each isolate to generate power and treat wastewater is evidenced by its power density (W/m2) produced at various pH and temperature levels. The product of the voltage (V) and current (I) per area (A) of the graphite rod electrode was used to calculate the power density: Power density (W/m2) = VI / A (Wolfson, 2012).

Measurement of wastewater treatment by the isolates

The wastewater characteristics, before and after treatment were analysed. The following characteristics were assessed: colour, pH, biochemical oxygen demand, chemical oxygen demand electrical conductivity, total dissolved solids (TDS) and total suspended solids (TSS). The rate of contaminant removal was determined by comparing the autoclaved wastewater characteristics of the MFC containing the microbial isolate (that is, treated wastewater) with the “control” (autoclaved wastewater without microbial isolate). The colour was determined using colour chart. The pH and electrical conductivity was measured using a pH and conductivity meter (Rakiro Biotech System Pvt. Ltd), respectively. TDS and TSS was determined using the Indian standard - IS: 3025 (part 16) Reaffirmed (2013) and IS: 3025 (part 17) Reaffirmed (2013), respectively. The COD was determined using the Indian standard -IS: 3025 (part 58) Reaffirmed (2006) while Indian standard - IS: 3025 (part 44) Reaffirmed (2003) was used to determine the BOD.

Identification of bacterial and fungal isolates

Molecular techniques were used to identify the exoelectrogenic isolates (White *et al*., 1999). The ZR fungus/Bacterial DNA MiniPrepTM Kit (Zymo Research, USA) was used to extract DNA from bacterial and fungus isolates. According to Weisburg *et al*. (1991), the 16S rRNA gene of the bacterial genomic DNA was amplified using universal PCR primers: forward: F1 (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse: R5 (5'-ACGGCTACCTTGTTACGACTT-3'), while the 18S rRNA gene of the fungal genomic DNA was amplified using primers ITS1 (5'-CTTGTTCATTTAGAGGA. Electrophoresis of the PCR amplicons were carried out using 1% (w/v) agarose gel according to the method of Nwagu *et al*. (2019).

Purified PCR products were sequenced using the Sanger’s method (PRISM™ Ready Reaction Dye Terminator Cycle Sequencing Kit) and electrophoresed with a model ABI PRISM® 3500XL DNA Sequencer (Applied Biosystems, USA) using the manufacturer’s procedures. BioEdit Sequence Alignment Editor (Hall, 1999) was used to edit the chromatogram from the sequence. The identity of the consensus sequences of the organisms obtained were confirmed from the National Centre for Biotechnology Information database using the Basic Local Alignment Search Tool (BLASTn) algorithm (Altschul *et* al., 1990).

RESULTS

A total of 20 pure cultures where obtained during the screening for exoelectrogenic isolates. Further assessment showed that only five isolates; 2 bacteria (coded as N4 and N6) and 3 fungi (coded as S9, S10, S14) were able to produce significant power densities in the order, N6 (1.59 W/m^2) > S10 (1.55 W/m^2) > S9 (1.51 W/m^2) >S14 (1.49 W/m^2) > N4 (1.44 W/m^2) within 24 h intervals as shown in **Table 1**.

Wastewater characterisation showed significant contaminant removal in the treated wastewater. The fungal isolate, S10 recorded the highest contaminant removal in terms of the pH, conductivity, TSS, TDS, COD and BOD. As shown in **Table 2**, the contaminant removal by the fungal isolates were relatively higher than the bacterial isolate but all the isolates recorded high contaminant removal when compared with the "control" (untreated wastewater).

Polymerase chain reaction (PCR amplification) of the bacterial 16S rRNA and fungal 18S rRNA showed successful amplification of the conserved region as confirmed in the DNA band profile after electrophoresis in **Figure 2**. Their respective sequence analysis showed the true identity of the isolates as N4 (*Proteus* spp), N6 (*Providencia* spp), S9 (*Clavispora lusitaniae*), S10 (*Candida parapsilosis*) and S14 (*Clavispora lusitaniae*) with their assigned accession number as deposited in the NCBI genbank

DISCUSSION

A cocktail of bacterial and fungal isolates were obtained after the preliminary screening. After subculturing, a total of 20 pure cultures of isolates were obtained. Only 5 of the isolates showed the potential of producing extracellular electrons because they produced relatively high voltages which indicates their capacity for wastewater treatment and electricity generation (Nwagu *et* al., 2019). The five exoelectrogenic isolates include 2 bacteria (N4 and N6) and 3 fungi (S9, S10 and S14). The power densities produced by the five isolates at time intervals are shown in **Table 1**.

The isolate, N6 (*Proteus* species) produced the highest power density of 1.59 W/m^2 at 24 h, followed by isolates S10 (*Candida parapsilosis*) and S9 (*Clavispora lusitaniae*) that produced power densities of 1.55 W/m^2 and 1.51 W/m^2 within 24 h, respectively. Several scientific reports on production of extracellular electrons by microbes in MFC concentrated on the exploitation of bacteria such as *Shwanella oneidensis and Rhodopseudomonas palustris* (Park *et* al., 2014; Watson and Logan, 2010). This study showed that some species of fungi are good exoelectrogenic candidates for MFC. Comparatively, the fungal isolates S9 and S10 were more electroactive than the bacterial isolates. Unlike bacteria, the mechanism of electron transfer in fungi has not received intensive study (Sekrecka-Belniak and Toczyłowska-Mamińska, 2018).

The choice of pH of 8.5 and temperature of 37 ^{0}C was adopted following reports from other researchers. In the study of the effect of variations in pH and temperature on power generation by exoelectrogenic isolates, Nwagu *et al*. (2019) reported that higher voltages were produced at pH of 8.5 and temperature range of 35-40 ^{0}C. Also, in a similar study by Sebastia *et al.* (2010) on electricity generation by MFC, power density increased by 80% from 0.36 to 0.66W/m^3 when pH was adjusted from 6 to 9.5, respectively. The power density decreased to 0.5 W/m^3 at the pH of 10. According to Behera *et al*. (2011), at 40℃, maximum coulombic (7.39) and energy (13.14%) efficiencies were obtained, but reduced at 20℃ in MFC. These mean that exoelectrogenicity of microorganisms is highly dependent on the pH, temperature, and availability of nutrients. The decrease in power density at higher time intervals (48 h and above is probably due to decrease in the available substrate (nutrients) in the wastewater required for the activity of the isolates. It is believed that in a typical industrial setting, with constant discharge of rich effluents, inherent exoelectrogenic isolates will remain electroactive for longer time. To maintain or improve power density, biostimulation and bioaugmentation and genetic engineering of isolates would be necessary for any industry that applies MFC technology. According to (Mouhib *et al*., 2023), an engineered *E. coli* showed a significant performance in wastewater collected from a brewery. The modified *E. coli* thrived while other exotic electrogenic microbes faltered, indicating its potential for large-scale waste treatment and energy production.

Table 1. Power density (W/m^2) of isolates at pH of 8.5 and temperature of 37 ^{0}C within time intervals

		Time (h)				
	Isolates	**24**	**48**	**72**	**96**	**120**
Bacteria	**N4**	1.44	0.34	0.24	0.57	0.71
	N6	1.59	0.40	0.80	0.62	0.60
Fungi	**S9**	1.51	1.04	0.64	0.53	0.49
	S10	1.55	0.94	0.66	0.66	0.63
	S14	1.49	0.89	0.51	0.49	0.33

Table 2. Wastewater characteristics after treatment with the isolates

Treatment	Colour	pH at 23^{0}C	Conductivity (µS/cm) at 27^{0}C	TSS (mg/l)	TDS (mg/l)	COD (mg/l)	BOD (mg/l)
N4	Pale lemon	7.70	1104.00	5373.00	1380.00	2150.00	583.90
N6	Pale lemon	6.50	736.00	1475.00	920.00	2500.00	678.40
S9	Pale lemon	6.50	672.00	6062.50	840.00	2840.00	770.20
S10	Pale lemon	7.60	512.00	3925.00	640.00	1974.00	536.38
S14	Pale lemon	6.50	669.00	6030.50	831.00	2800.00	761.65
Control (Untreated)	Pale yellow	5.00	1344.00	6812.50	1680.00	3100.00	840.40

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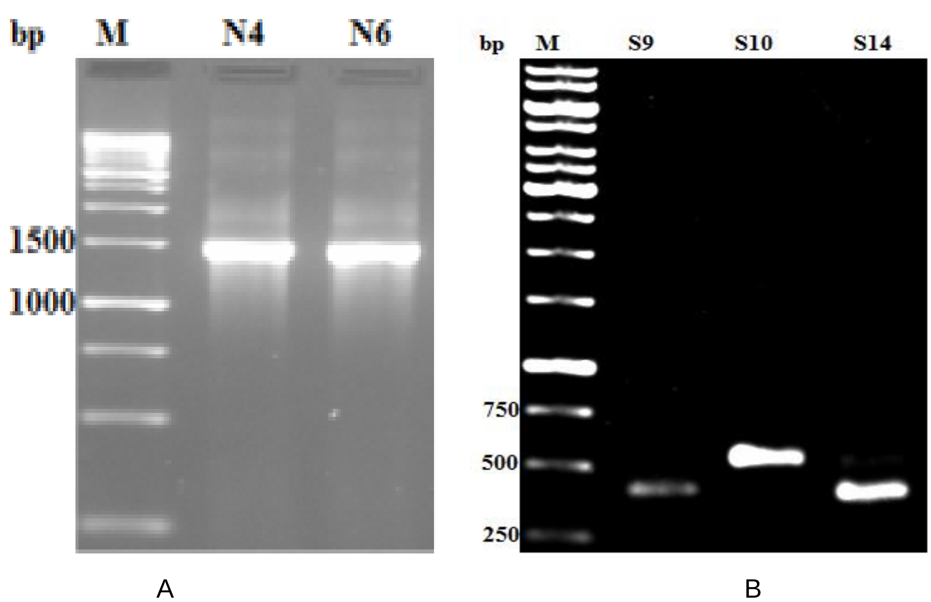

Figure 2. DNA band profile of the bacterial (A) and fungal (B) amplicon. "M" denotes ladder of 2 kb of base pairs.

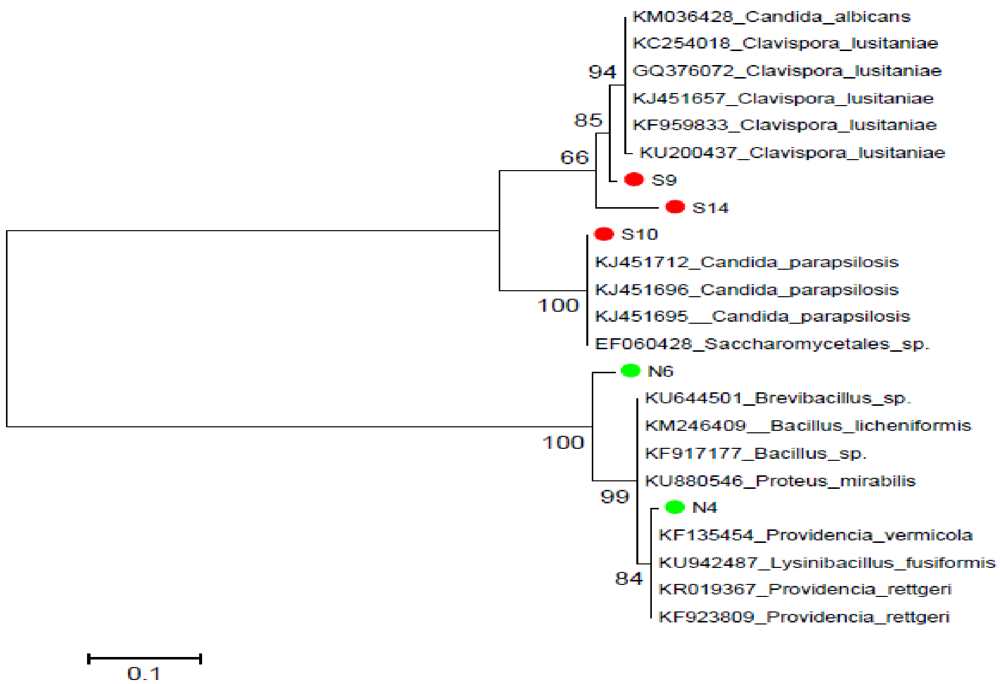

Figure 3. Phylogenetic tree of the isolates. The summary of the identity of the isolates is shown in **Table 3** with the accession number of each isolate as deposited in NCBI database.

Table 3. Sequence identity of the bacterial and fungal isolates.

	Code of Isolate	Number of Base Pairs	Identity in the Database	Assigned Accession Number in NCBI
Bacteria	**N4**	761	*Providencia* species	KX548357.1
	N6	962	*Proteus* species	KX548358.1
Fungi	**S9**	422	*Clavispora lusitaniae*	KX548359.1
	S10	541	*Candida parapsilosis*	KX548360.1
	S14	697	*Clavispora lusitaniae*	KX548361.1

Wastewater analysis before and after use in assessing electrogenicity of isolates showed about 38% contaminant removal. This percentage was obtained by comparing the untreated wastewater (control) with the treated wastewater. Generally, as shown in **Table 2**, the fungal isolates had relatively higher ($p < 0.05$) contaminant removal compared to the bacterial isolates. The fungus S10 (*Candida parapsilosis*) performed better than other isolates. The rate of contaminant removal recorded is related to the power density produced by the isolates. The concentration, composition and type of organic substrate in the wastewater also influence microorganisms and generation of power (Cheng and Logan, 2011). Consequently, the higher the power generated, the higher the contaminant removal and vice versa. Park *et al.* (2014) reported that most isolates used in MFC generate higher power densities in axenic culture than in mixed culture and enhance power generation. Although wastewater generated in industries may contain cocktail of microbial species, reports have shown that single species may perform better than consortium of microorganisms in the release of electron, but contaminant removal from the wastewater would be higher. Das (2015) reported that a mixed culture of *Spirulina* spp and *Chlorella* spp gave a lower voltage in MFC compared to the higher voltage obtained in pure culture. Most MFC research had only focused on power generation without considering the level of wastewater treatment during the process.

The colour of the wastewater changed from pale lemon to yellow while the acidic pH of 5.0 increased in the range of 6.5-7.7 which is close to neutral pH. The pH and conductivity is also dependent on temperature. The higher the temperature, the lower the pH. Other analysed wastewater characteristics (conductivity, TSS, TDS, COD and BOD) showed significant difference between the treated and untreated wastewater (control). Conductivity at 27 ^{0}C in the "control" was reduced from 1344 µS/cm to 512 µS/cm in the wasterwater containing the fungal isolate, S10 (*Candida parapsilosis*). In the same vein, TSS (6812.50 mg/l), TDS (1680.00 mg/l), COD (3100.00 mg/l) and BOD (840.40 mg/l) of the control was reduced to 3925.00 mg/l, 640.00 mg/l, 1974.00 mg/l, and 536.38 mg/l, respectively. The records showed that biological wastewater treatment stage could be achieved using microbial fuel cell. Industries that generate wastewater could adopt this measure for the biological wastewater treatment before secondary and tertiary stage that would ensure desirable contaminant removal. The molecular characterisation, amplification of 16S rRNA and 18S rRNA of the extracted genomic DNA of the isolates showed the DNA banding pattern of the individual isolate's amplicon on an agarose gel after electrophoresis using gel documentation system as shown in Figure 2. The database search for homology of the query sequence with the database sequence through BLAST algorithm showed related database sequence with the name of the isolate. The exoelectrogenic isolates were deposited in NCBI Gene bank with accession number in database as shown in Table 3.

The five identified microorganisms are capable of releasing extracellular electrons; hence, they are exoelectrogenic. From scientific records, these five isolates are pathogenic in nature. Their pathogenicity do not have any effect on the efficiency of MFC and has little or no cause for alarm because there is very low risk of exposure to humans since they are used in a closed chamber. However, safety precautions in handling pathogenic microbes should always be adopted. Zuo *et al.* (2008) reported that a pathogenic bacterium, *Ochobactrum anthropic* and *Pseudomonas aeruginosa* showed electrogenic property in different Microbial fuel cell setups. This implies that electrogenesis could be one of the selective characteristics for opportunistic pathogens; a hypothesis that is subject to further assessment (Osman *et* al., 2010).

CONCLUSION

The research findings have shown that many unknown exoelectrogenic microorganisms exist that could be explored and exploited to enhance power supply and reduce the level of contaminants in wastewaters generated in industries. Some fungal species have been established as great potential exoelectrogenic microorganism. Future studies with fungi-based MFCs should be focused on enhancing the power production in such systems. Increasing the scale and design of the MFC system architecture could improve power density which is the major challenge of MFC. There is need for further research to unravel the gene responsible for the electroactive nature of electrogenic microorganism. Once the responsible gene is known, genetic engineering of such gene in some inert isolates could help solve the problem of power supply in industries. It is also necessary to probe further into the relationship between electrogenicity and pathogenicity of microbial biota.

Conflict of Interest

The authors declare no actual or potential conflict of interest.

Acknowledgments

We appreciate B.R Aremu of North West University, South Africa for her role in the molecular identification of the isolates.

Author Contribution

NKE and AP designed the study, carried out the research work, wrote the first draft of the manuscript and interpreted relevant literature. IAG participated in writing the draft of the manuscript and critically revised the manuscript for important intellectual content and organization. EE and UD carried out the statistical analysis and reviewed the manuscript before submission. All authors read and approved the final draft of the manuscript.

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