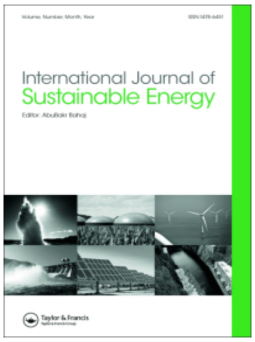

International Journal of Sustainable Energy

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/gsol20

The use of microbial fuel cell for efficient treatment of cauliflower waste and generation of electricity

Rocky Maharjan, Pradip Dhungana, Lakshmaiah Sreerama, Puja Bhatt, Bikram Prajapati, Prabesh Poudel, Pratap Kandel, Mamata Khadka, Apshara Parajuli & Jarina Joshi

To cite this article: Rocky Maharjan, Pradip Dhungana, Lakshmaiah Sreerama, Puja Bhatt, Bikram Prajapati, Prabesh Poudel, Pratap Kandel, Mamata Khadka, Apshara Parajuli & Jarina Joshi (2023) The use of microbial fuel cell for efficient treatment of cauliflower waste and generation of electricity, International Journal of Sustainable Energy, ahead-of-print:ahead-of-print, 304-317, DOI: 10.1080/14786451.2023.2185869

To link to this article: https://doi.org/10.1080/14786451.2023.2185869

© 2023 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group

Published online: 22 Mar 2023.

Submit your article to this journal

Article views: 1592

View related articles

View Crossmark data

Citing articles: 3 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=gsol20

INTERNATIONAL JOURNAL OF SUSTAINABLE ENERGY
2023, VOL. 42, NO. 1, 304–317
https://doi.org/10.1080/14786451.2023.2185869

Taylor & Francis
Taylor & Francis Group

OPEN ACCESS Check for updates

The use of microbial fuel cell for efficient treatment of cauliflower waste and generation of electricity

Rocky Maharjan[a], Pradip Dhungana [a], Lakshmaiah Sreerama[b], Puja Bhatt[a], Bikram Prajapati[a], Prabesh Poudel[a], Pratap Kandel[a], Mamata Khadka[a], Apshara Parajuli[a] and Jarina Joshi[a]

[a]Central Department of Biotechnology, Tribhuvan University, Kirtipur, Nepal; [b]College of Arts and Sciences, Qatar University, Doha, Qatar

ABSTRACT

Microbial fuel cell (MFC) is an alternative way for household organic waste treatment as it produces sustainable clean energy. Chemical parameters of the cauliflower leaf waste before treatment were 143.5 mg/g COD, 0.90 mg/g ammoniacal nitrogen, 67.8 mg/g phosphorous, 214.8 mg/g total reducing sugar and 0.53 mg/g soluble reducing sugar. After eight days of treatment in MFC at the optimised condition of multiwalled carbon nanotubules (MWCNT)′ coated graphite as anode and potassium ferricyanide added phosphate buffer as catholyte with an external resistance of 1000 Ω showed a reduction in COD, ammoniacal nitrogen, phosphorous and total reducing sugar by 24.7, 76.9, 22.5 and 53.4%, respectively, along with a maximum power density of 10.1 W/m^3. Scanning electron microscopy (SEM) showed bacterial adherence in the graphite electrode and its molecular characterisation using 16S rRNA sequencing confirmed as *Bacillus sps*.

ARTICLE HISTORY
Received 2 December 2022
Accepted 17 February 2023

KEYWORDS
Microbial fuel cell; household waste; cauliflower leaf; electricity; chemical oxygen demand

1. Introduction

Kitchen waste is the major household organic waste that causes serious environmental deterioration. This waste can be used as a valuable feedstock in biological and thermochemical conversion processes to produce bioenergy (Soltanian et al. 2022). For the proper management of household organic waste, it needs to be characterised properly so that it can be easily degraded and hence can be applied as raw material to produce various types of value-added products (Dhungana et al. 2022). Waste composition influences the overall yield and kinetics of the biological reaction during digestion (Afifi 2011). The conventional disposal of the waste includes landfill, incineration, composting, anaerobic digestion, etc. (Savini 2021). These disposal processes produce leachate that contaminate the groundwater leaving solid residue with toxic gaseous products in the environment (Nabavi-Pelesaraei et al. 2017) (Figure 1 and Table 1).

Recovery and recycling of waste is a great deal in today's scenario. The discarded items are processed to extract or recover materials and resources or convert them to energy usable heat, electricity or fuel (Santagata et al. 2021). Recycling is the third component of reduce, reuse and recycle waste hierarchy (de Sadeleer et al. 2020). In a recent scenario, the food and energy markets are facing an imbalance of supply and demand (Esfandabadi, Ranjbari, and Scagnelli 2022) resulting in the need to search for alternative carbon-neutral energy sources. Energy waste is one of the

CONTACT Jarina Joshi jarinarjoshi@gmail.com; Pradip Dhungana pradip.dhungana1993@gmail.com

© 2023 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The terms on which this article has been published allow the posting of the Accepted Manuscript in a repository by the author(s) or with their consent.

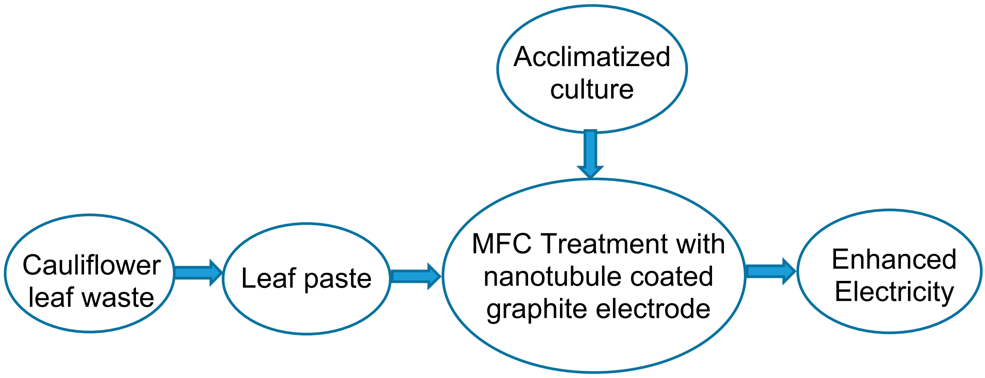

Figure 1. Logical diagram of the research.

good recycling processes which involves converting non-recyclable waste items into usable heat, electricity or fuel through a variety of processes (Ng et al. 2019). This type of energy source is renewable because non-recyclable waste can be used to create energy. It can also help to reduce carbon emissions by offsetting the need for energy from fossil sources (Du and Li 2017).

The microbial fuel cell is one of the techniques to be used for the degradation of waste and the generation of electricity (Joshi et al. 2019). In MFC, electrons are typically transferred by electrogens

Table 1. List of abbreviations and symbols used in this manuscript.

Abbreviations and symbols	Meaning
MFC	Microbial fuel cell
COD	Chemical oxygen demand
SEM	Scanning electron microscopy
MWCNT	Multiwalled carbon nanotubules
PANI/MWCNT	Polyaniline multiwalled carbon nanotubules
LB	Lysogeny broth
CMC	Carboxymethyl cellulose
OCV	Open circuit voltage
PCR	Polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
DNA	Deoxyribonucleic acid
BLAST	Basic Local Alignment Search Tool
NCBI	National Center for Biotechnology Information
g	Gram
mL	Millilitre
Mm	Millimetre
cm	Centimetre
mg/g	Milligram per gram
w/w	Weight by weight
w/v	Weight by volume
mW/m^3	Milliwatt per cubic metre
mg/L	Milligram per litre
v/v	Volume by volume
M	Molar
mV	Millivolt
H_2SO_4	Sulphuric acid
HNO_3	Nitric acid
HCl	Hydrochloric acid
h	Hour
d	Day
°C	Degree celsius
H_2O_2	Hydrogen peroxide
Ω	Ohm
μL	Microlitre
$μm^2$	Square micrometre

directly or indirectly with an exogenous, redox-active mediator. This mediator serves as an electron transporter and delivers the microbes a platform to yield reduced products that are electrochemically active (Adebule, Aderiye, and Adebayo 2018). The use of a microbial fuel cell to treat organic waste and obtain additional electrical capability is possible due to microorganisms and enzymes (Wang et al. 2013). However, the high cost of components for large-scale implementation, limited power generation and lower efficiency are the main challenges to their commercialisation (Ahanchi et al. 2022). Thus, recent advances in MFC technology for simultaneous bioelectricity generation and bioremediation emphasise the types of electrode materials, substrates and different MFC designs (Kumar et al. 2017).

Nanoparticles based on carbon and conductive polymers can promote biofilm formation and enhance electron transfer between the electrodes and the biofilm (Mashkour et al. 2021). In this study, polyaniline multiwalled carbon nanotubule (PANI/MWCNT)-coated graphite electrode is used to study its effect on the performance of the MFC system to generate electricity from cauliflower waste. As cauliflower leaf is the most abundantly found household organic waste in Nepal, it is used in the study. This work is intended to perform as cauliflower leaf waste is generated in a huge amount and this technique has shown efficient degradation of cauliflower leaf waste with less effort and alternately generates electricity.

2. Materials and methodology

All the reagents were of analytical grade. The reagents and media were purchased from Himedia, India Pvt. Ltd. unless stated. Multiwalled carbon nanotubules (MWCNT), aniline, silver sulphate, potassium hexacyanoferrate (II) and mercuric sulphate were purchased from Sigma Co. Cauliflower leaf waste used as the substrate for the study was collected from the central vegetable market, Balkhu, Kathmandu, Nepal. The cauliflower leaf waste (biomass) was air-dried for 24 h and was grounded with a grinding machine to make a fine paste and was used for analysis.

2.1. Physiochemical analysis of biomass

Physical parameters such as pH and moisture content and chemical parameters such as chemical oxygen demand, ammoniacal nitrogen, reducing sugar and phosphorus of the biomass were analysed before and after the treatment in MFC. Moisture content (% w/w) was determined by overnight drying the biomass in a hot air oven at 105°C and ash content (% w/w) was determined by burning the leaf waste in a muffle furnace at 550°C for 2 h. Ammoniacal nitrogen content was determined using the Nessler reagent method as described by Demutskaya and Kalinichenko (2010). Chemical oxygen demand was determined by the spectrophotometric method (Ying et al., 2006) and reducing sugar was determined using the dinitro salicylic acid method (Joshi, Bhattarai, and Sreerama 2018). Similarly, the amount of phosphorus was analysed using the acidified ammonium molybdate method (Ganesh et al. 2012).

2.2. Microbial inoculum preparation

For the growth of the mixed culture of microorganisms, about 10 g of sample (biomass paste) was taken and cultured in 100 mL lysogeny broth (LB). The culture was incubated for acclimatisation at 37°C for 14 d. It was primarily used in MFC for waste degradation.

2.3. MFC construction and operation

A two-chambered MFC was fabricated with a wide-necked plastic bottle each of capacity 600 mL and was separated by Nafion membrane. A platinum wire coil of 0.2 mm diameter and 0.5 m length was used as the anode and 1 cm × 3 cm × 11 cm graphite fibre (Nippon, Japan) was used as the cathode. The anolyte was 10% w/v of finely pasted waste sample in distilled water with 1% microbial inoculum and the catholyte was 0.1 M phosphate buffer, pH 7.6. The wires arising from the anode and the cathode compartment were connected to the multimeter and open circuit voltage (OCV), respectively. However, the closed circuit voltage was obtained by connecting the external resistance of 1000 and 100 Ω.

The MFC was operated for about 7 d. The open circuit voltage reading was taken for the optimisation of MFC performance. Power, voltage and current were obtained from the closed circuit possessing 1000 and 100 Ω resistance. The anodic sample was taken for the determination of reducing sugar, COD, phosphorous and nitrogen. Further improvements in MFC performance were done using the graphite felt coated with PANI/MWCNT as an anode and 1.0% potassium ferricyanide in phosphate buffer at the cathodic compartment. Pieces of normal graphite electrode, modified MWCNT-treated graphite electrode and modified MWCNT-treated graphite electrode after MFC operation were sent to Jawaharlal Nehru University, New Delhi, India to study the surface morphology and adherence of microbial load during MFC operation.

2.4. Electrode modification

In-situ oxidative polymerisation method was adopted for the synthesis of PANI/MWCNT nanocomposites (Abdulla, Mathew, and Pullithadathil 2015). MWCNT (1 mg) was mixed in an aniline-HCl solution (1:1) at 0°C which leads to the adsorption of monomer on the MWCNT surface. The optimised reaction time was 12 h. A solution of ammonium persulphate (0.1 M) in 1 M HCl was added dropwise into the mixture. The polymerisation process was carried out at 0°C for 6 h. The PANI/MWCNT nanocomposite was obtained by washing the material several times by repeated centrifugation with deionised water and methanol to obtain a greenish-black powder. The material was further vacuum-dried at 60°C for 24 h.

Then, the graphite felt was coated with PANI/MWCNT nanocomposite for electrode modification. For this, PANI/MWCNT nanocomposite was mixed with N-methyl-2 pyrrolidone. A clean graphite electrode was dipped in the mixture and sonicated for about 15 min. The graphite electrode then was oven-dried for about 12 h at 60°C.

2.5. Electrode and membrane treatment

The graphite electrode and proton exchange membrane (Nafion) were treated before use. For the electrode treatment, the graphite felt was ultrasonicated with the H_2SO_4 and HNO_3 (3:1, v/v) solution for 6 h. After ultrasonication, it was washed with distilled water until the pH of the washing solution reached 7 and then air-dried (Vaghela and Nath 2020). Similarly, for the treatment of the Nafion membrane successive sonication with 3% H_2O_2 solution, deionised water, 0.5 M H_2SO_4 and finally with deionised water for 1 h each was done. The membrane was then stored in deionised water for short-term preservation to maintain the membrane for good conductivity.

2.6. Statistical analysis

Statistical analysis was done using MS excel and graph pad prism . Cauliflower leaf waste was collected from a local vegetable market where cauliflower arrives from different places in Nepal and India. So data were presented mean of three replicates with standard deviations.

2.7. Isolation and screening of bacteria present in MFC

The acclimatised mixed culture (inoculum) in MFC was serial diluted and cultured on LB agar media plates and incubated at 37°C for 24 h. Different isolated colonies of bacteria on the plate were tested for cellulase production. The inoculum of each isolated colony was made in LB media and about 500 μL of inoculum was inoculated onto a bore made at the centre of the carboxymethyl cellulose (CMC) agar plate. The plate was then incubated at 37°C for 24 h. After incubation, the plate was flooded with 0.1% congo red followed by 1 M NaCl. The observation was done for the clear zone of hydrolysis (Dhungana et al. 2023).

2.8. Molecular characterisation of the isolates

Extraction of DNA from liquid culture was done as described by Nishiguchi et al. (2002). The obtained genomic DNA was verified by running in 1% gel electrophoresis. The gDNA was then amplified using the 16S rRNA universal primer (New England Biolabs). PCR product after amplification was sent to Xceleris Laboratory, Ahmedabad, India for sequencing. The sequences obtained were identified through NCBI BLAST search and sequences were aligned with the help of Bioedit software. The phylogenetic tree was constructed by the neighbour-joining method using MEGA software.

3. Results and discussion

Several physiochemical parameters of the cauliflower leaf waste were determined to see the waste characteristics. The waste was used in MFC for analysing the degradation pattern.

3.1. Physiochemical parameters of biomass

Different types of waste contained variable amounts of chemical parameters. The amount of ammoniacal nitrogen, chemical oxygen demand, reducing sugar and phosphorus present in cauliflower leaf waste was analysed for a feasibility study. The physiochemical parameters of cauliflower leaf waste are given in Table 2. The cauliflower leaf sample had a COD of 143.5 ± 5.60 mg/g, phosphorous of 67.8 mg/g, ammoniacal nitrogen of 0.90 ± 0.4 mg/g and total reducing sugar of 214.8 mg/g. According to Bux et al. (2015), the phosphorous content in cauliflower was 0.61 mg/g; however, the sample was the edible portion of cauliflower. The cauliflower waste contained less amount of total reducing sugar 92 mg/gm of dry sample (Dorge 2018).

3.2. Operation of MFC

The 10% dilution (w/v) of waste biomass as substrate was taken for all MFC operations as a higher concentration of substrate adversely affects MFC operation (Gurung et al. 2012), and also in lower substrate concentration, limitation of the substrate occurs resulting in less OCV. The OCV obtained in MFC at different operating conditions is given in Figure 2. It showed that the maximum OCV of 681 mV was observed when MFC was operated with the PANI/MWCNT-coated anode with potassium ferricyanide added to the catholyte.

Table 2. Physiochemical parameters of cauliflower waste sample.

Analytical parameters of cauliflower waste	Concentration
pH	7.0 ± 0.2
Ash content	9.51 ± 0.61%
Moisture	60.9%
Total reducing sugar (mg/g)	214.8
Soluble reducing sugar (mg/g)	0.53
Chemical oxygen demand (mg/g)	143.5 ± 5.60
Phosphorous (mg/g)	67.8
Ammoniacal nitrogen (mg/g)	0.90 ± 0.4

From the experiment, it is found that the use of PANI/MWCNT-coated graphite fibre showed an improvement in OCV reading, i.e. 426 mV compared with the normal graphite fibre, i.e. 200 mV. This increase in OCV might be due to changes in the physical and chemical properties of graphite after modification of the electrode that might have enhanced the surface area for the microbial attachments and electron transfer (Scott et al. 2007). Thus, modification of the anode materials to increase the affinity of the bio-films proved to be an efficient way to enhance the performance of the MFC as stated by (Yang et al. 2016). Carbon nanotubules are promising alternative materials for MFC electrode performance because of their electrical conductivity, chemical stability, biocompatibility and high specific area (Mustakeem 2015).

Furthermore, the addition of potassium ferricyanide in the catholyte enhanced the exchange of electrons in the cathodic compartment with 681 mV OCV (Table 3) enhancing MFC performance. In one instance, using ferricyanide in the cathode generated 1.5-1.8 fold greater power than using a standard buffer in the cathode because ferricyanide has an excellent electron-accepting capacity and increases power density (Bose et al. 2018). The performance of the mediator (ferricyanide) was probably because ferrocyanide [$Fe(CN)_6^{3-}$] is highly diffusible and can be easily reduced to its ferrous counterpart by the well-defined reversible reaction to increasing the redox potential of the solution (Parkash et al. 2015).

3.3. Power generation in MFC using different resistors

The power generation in MFC during the operation is given in Figure 3. It was operated with 1000 and 100 Ω external resistances when the PANI/MWCNT-coated graphite electrode was used as the anode and potassium ferricyanide was added in the catholyte. Power density with external resistance of 100 and 1000 Ω applied to the system was 0.55 and 10.1 W/m^3, respectively (Table 3). This experiment was done to observe the effect of external resistance on the overall performance of microbial fuel cells using cauliflower leaf as the substrate. Adebule, Aderiye, and Adebayo (2018), also observed the enhanced bioelectricity generation by MFC using kitchen waste as the substrate.

External resistance acts as an integral part of an electrical grid that controls the output of fuel cells (Rismani-Yazdi et al. 2011). The data of two external resistance, viz. 100 and 1000 Ω, were

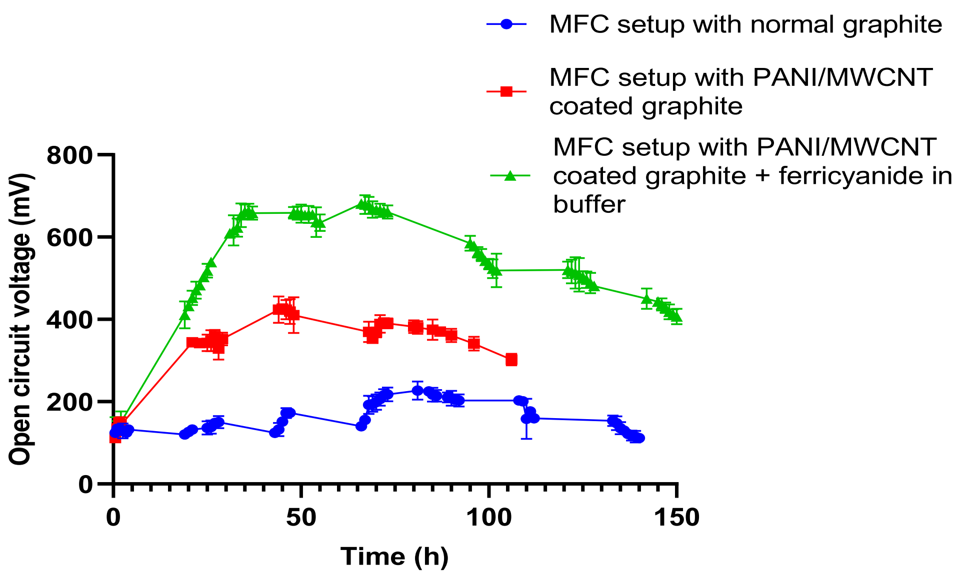

Figure 2. Open circuit voltage (OCV) observed during microbial fuel cell (MFC) operation at different time intervals. MFC was operated with normal graphite and polyaniline multiwalled carbon nanotubule (PANI/MWCNT)-coated graphite for analysis.

Table 3. OCV developed and power density during the MFC operation.

Substrate (anode/cathode)	Electrode (anode/ cathode)	Open circuit voltage (mV)	Resistor (Ω)	Power density (W/m^3)	References
Cauliflower leaf waste/50 mM phosphate buffer	Graphite felt/ Graphite felt	200	–		This study
Cauliflower leaf waste/50 mM phosphate buffer	MWCNT-PANI-coated graphite/graphite	426	-		This study
Cauliflower leaf waste/50 mM phosphate buffer with $K_3[Fe(CN)_6]$	MWCNT-PANI-coated graphite/graphite	681	-		This study
Cauliflower leaf waste/50 mM phosphate buffer with $K_3[Fe(CN)_6]$	MWCNT-PANI-coated graphite/graphite	-	100	0.55	This study
Cauliflower leaf waste/50 mM phosphate buffer with $K_3[Fe(CN)_6]$	MWCNT-PANI- coated graphite/graphite	-	1000	10.1	This study
S. putrefacien- inoculated medium/ 0.05 M $K_3[Fe(CN)_6]$	MWCNT-PANI-coated graphite/graphite	342	1900	-	(Cui et al. 2015)

Note: $K_3[Fe(CN)_6]$: Potassium ferrocyanide.

compared to study optimal conditions for power production. A 1000 Ω external resistance performed better for power generation. This might be because an increase in external resistance might have lowered internal resistance, thus enhancing the overall rate of reaction. The optimal external resistance is usually correlated with the internal resistance of MFC. Internal resistance is not a system constant and depends on the external load applied to the MFC (Manohar et al. 2008). A slight increase in internal resistance can dramatically decrease MFC performance (He et al. 2005). External resistance could also relate to anode potential. Du and Li (2017) studied variations of the anode potential with external resistances and reported that the change in external resistance showed the highest anode potential. When the anode potential is higher, more free energy can be obtained, which could enhance the start-up of electricity generation (Goud and Mohan 2011).

A steady-state power of 7.2 W/m^3 was observed when MFC was operated after 48 h with 1000 Ω external resistance and the PANI/ MWCNT-coated graphite fiber anode, whereas 10.1 W/ m^3 was observed with further addition of potassium ferricyanide in the catholyte. The power density was calculated based on the anodic volume of liquid to better reflect three-dimensional properties of both electrodes and MFC reactors (W/m^3) (Rabaey et al. 2005). Generally, the power density increases with an increase in external resistors from 15 Ω to 2k Ω whereas if the resistance load was too high that limited the flow of electrons through the circuit causing a lower response in

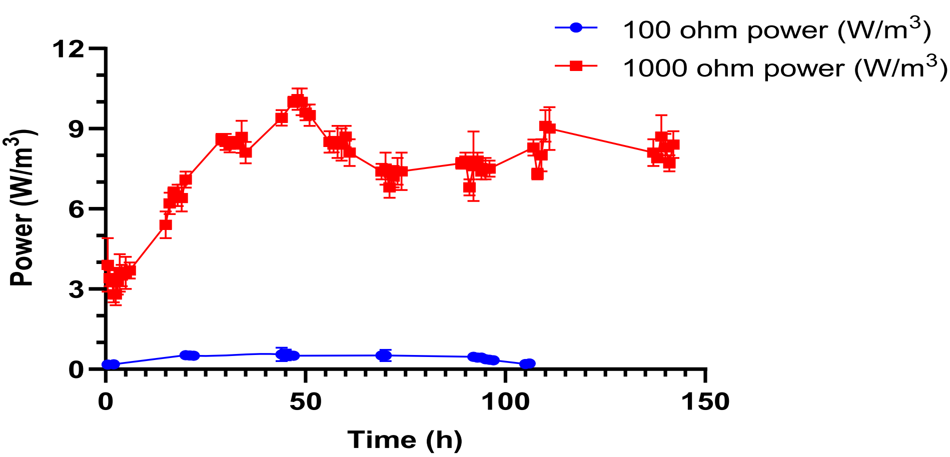

Figure 3. Power generation in a microbial fuel cell using polyaniline multiwalled carbon nanotubule (PANI/MWCNT)-coated graphite fibre with the addition of potassium ferricyanide in catholyte and external resistance of 1000 and 100 Ω.

the current output. For the scenario with water as an electrolyte and graphite electrode, the maximum power density was 13.09 mW/m^3 using 2k Ω, whereas the power density was less than 1 mW/m^3 using 15 Ω (Lopez Zavala and Gutiérrez 2022).

3.4. Change in chemical parameters of the substrate by MFC performance

The degradation of waste biomass and reduction in COD, ammoniacal nitrogen, phosphorous and reducing sugar values after MFC operation at optimised conditions after eight days of operation are given in Table 4. The maximum COD reduction was 24.7% because the raw fresh sample was used in the study. MFCs could achieve up to 69 ± 18% COD removal (Asefi et al. 2019), for which the samples need to be pretreated. There was a huge reduction in ammoniacal nitrogen by 76.9% using coated graphite electrodes than using normal graphite electrodes. The activated carbon sample produced a larger removal potential than any other anode material during the process (Alabiad et al. 2017). Similarly, phosphorus was removed by 22.5% and total reducing sugar by 53.4%.

The graphite felt was a better electrode material to generate a high-voltage output (Cai et al. 2014) and the carbon polymer-based nanoparticle improves biofilm formation. Thus the PANI/MWCNT-coated graphite felt was used for the study, by monitoring the COD removal efficiency as COD is highly proportional to voltage and power density (Abbasi et al. 2016). However, various renewability and cleanness bioenergy technologies still require to analyse the exergo-environmental indices to derive the detailed information regarding the environmental consequences of bioenergy production plants (Aghbashlo et al. 2021). The analysis of energy and exergy within the bioenergy system for optimisation could aid in the system's sustainability (Aghbashlo et al. 2022).

3.5. Scanning electron microscopy of the graphite fibre anode

Scanning electron microscopy (SEM) was used to observe the electrode surface, morphological information and behaviour of the microbial community that adheres to the graphite electrode surface. The pictures were observed, as shown in Figure 4. There was the adherence of a huge amount of microbes in the electrode which could enhance MFC efficiency. SEM images of graphite fibre showed a uniform surface (Figure 4(A)), whereas, after PANI/MWCNT coating (Figure 4(B)) and MFC operation (Figure 4(C)), thick layer bacterial adhesion of approximately 261 μm^2 was observed. SEM images of the PANI/MWCNT-coated graphite electrode after MFC operation showed efficient bacterial adhesion so they showed good MFC operation with lower resistance. The nanofibres of polyaniline are well interconnected with each other forming a highly porous matrix that provides a large surface area for biofilm formation. Also, the long and thin fibre-like structure of MWCNT attachment further increases the surface area for enhanced biofilm formation (Kashyap et al. 2015). The formation of biofilm in the electrode enhances electron transport (Mohamoud 2014).

Table 4. Change in different chemical parameters of the substrate after MFC operation.

Electrode	Buffer	COD reduction (%)	Ammoniacal nitrogen reduction (%)	Phosphorus reduction (%)	Total reducing sugar reduction (%)
Normal graphite electrode	Phosphate buffer	10.75	50.5	10.4	36.5
PANI/MWCNT-coated graphite electrode with a resistor (1000 Ω)	Phosphate buffer with potassium ferricyanide	24.7	76.9	22.5	53.4

Note: COD: chemical oxygen demand; PANI/MWCNT: polyaniline multiwalled carbon nanotubules; MFC: microbial fuel cell.

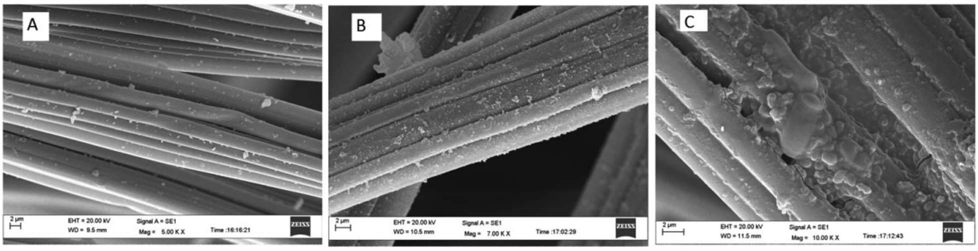

Figure 4. Scanning electron microscopy (SEM) images of the graphite electrode. A: Normal graphite electrode; B: polyaniline multiwalled carbon nanotubule (PANI/ MWCNT)-coated graphite electrode and; C: PANI/MWCNT-coated graphite electrode after microbial fuel cell operation.

3.6. Isolation and characterisation of microbes

The isolated bacterial colonies were observed for their morphological characteristics such as their shape, size, colour, consistency, margin and elevation. All the observed bacteria were circular, small, creamy white, sticky and uniformly margined with convex elevation. Isolated bacteria were Gram-positive.

3.6.1. Screening for cellulase-degrading bacteria

Seven different colonies were isolated from the leaf waste and screened for cellulase production. Among them the colonies labelled R2, R3 and R4 showed clear holozone on the CMC agar plate. The figure of holozone formation for screening of cellulase production for three different isolates R2, R3 and R4 is given in Figure 5.

The pPresence of holozone indicates that potent isolates can produce cellulase enzymes with cellulolytic activities. Bacterial isolates R2, R3 and R4 from the cauliflower waste broth in MFC have great efficiency to convert organic waste into simple molecules due to the presence of cellulase enzyme. (Lu et al. 2006) reported that the mesophilic cellulose-degrading bacteria obtained from vegetable waste showed hydrolytic capacity. Cellulose-degrading organisms help in bioelectricity production by using cellulose as the substrate. Cauliflower contains 16.6% cellulose (Khedkar et al. 2017).

3.6.2. Molecular characterisation of microbes in MFC

The three putative bacterial strains having cellulolytic activity were molecularly characterised. For this, the genomic DNA was extracted and visualised under a UV transilluminator. The concentration of genomic DNA was approximately 100 ng by the nanometre reader and was used as the template for PCR. The strains of bacteria were characterised by the 16S rRNA gene sequence analysis. PCR products showed a visible distinct band of size 1500 bp in the UV transilluminator (Figure 6) and

Figure 5. Holozone test for cellulolytic bacteria in carboxymethyl cellulose (CMC) agar plate by isolates R2, R3 and R4 (left to right) showing cellulolytic activity.

then products were subjected to sequencing. After sequencing, chromatogram files were obtained through Chromas software.

The identity of the bacterial species was determined by comparing the sequences obtained with the gene sequences available in the Genbank database using Basic Local Alignment Search Tool (BLAST) software at the NCBI site. BLAST analysis of R2 and R3 bacterial DNA sequences showed similarity with the *Bacillus* genus of a different strain. However, isolate R4 showed similarity with *Bacillus subtilis* with the gene bank accession number MT040749. This showed there might be the presence of different *Bacillus* species in the waste for the degradation. The phylogenetic tree of the R4 was constructed using the neighbour-joining method (Figure 7) to elucidate the evolutionary relationship of bacteria. *Bacillus subtilis* is highly enriched in various environments having degradation capabilities of different waste- producing enzymes such as protease, lipase, amylase, catalase, etc. (Priest 1977).

For better MFC performance, the strain should be an electro-active microorganism and able to convert chemical energy into electrical energy and transfer electrons by the intermediate of direct, indirect or mediated electron transfer pathways. In this MFC set-up, the community of bacteria was sampled from a real environment of cauliflower waste. Logan et al. (2019) proposed that *Bacillus spp.* are classified as weak exoelectrogens and typically produce quite low current densities in pure cultures. The production of low current densities is associated with unique roles in biofilm microbial ecology (Doyle and Marsili 2018).

Jothinathan and Wilson (2017) studied *Bacillus thuringiensis* DRR-1 from cow rumen, which produced a potential and current when cultivated in an MFC. *Bacillus cereus* was also cultivated on an MFC anode and produced a high current output (Islam et al. 2017). *Bacillus subtilis* in MFC with glucose as a carbon and energy source and 2, 4-dichlorophenol as a pollutant produced

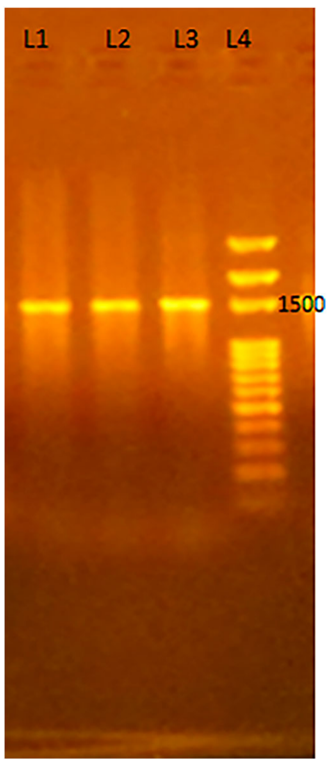

Figure 6. Gel electrophoresis of molecular amplification of 16S rRNA gene of bacterial DNA using a universal primer (1500 bp) in 1% agarose gel. L1: R2 bacteria, L2: R3 bacteria, L3: R4 bacteria and L4: ladder 100 bp.

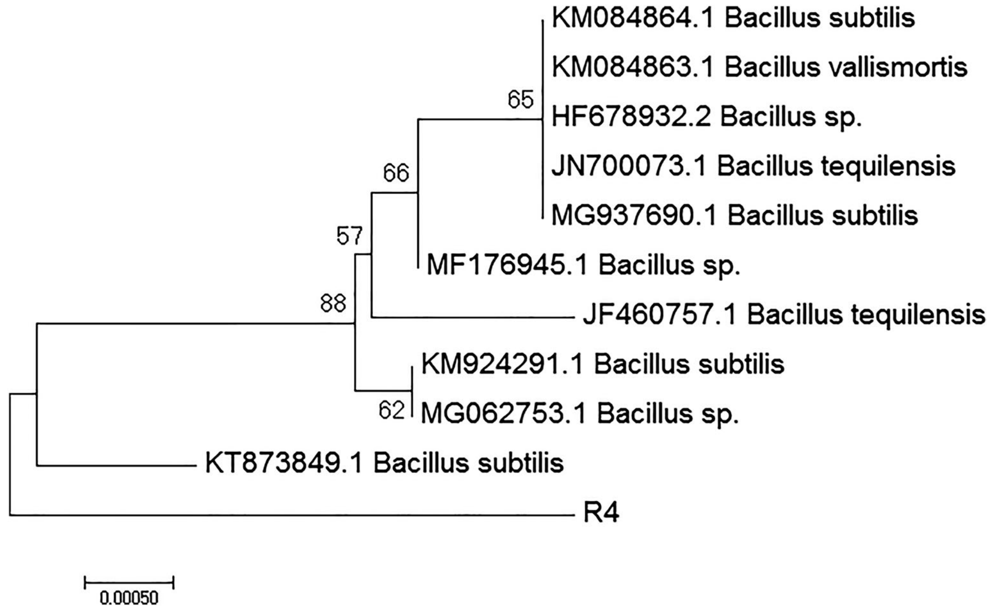

Figure 7. Phylogenetic tree of R4 obtained by the neighbour-joining method using MEGA software. The efficiency of tree resolution was considered successful only when the clades have at least >50% bootstrap value. The allocated gene bank accession number of the R4 isolate is MT040749.

a significant current output (Hassan et al. 2016). Such studies demonstrate that *Bacillus spp.* can perform extracellular electron transfer in an MFC. Also, *Bacillus spp.* can produce cell-free bioactive compounds that make them capable of complete invitro cellulose hydrolysis (Li et al. 2009).

4. Conclusion and prospects

The microbial fuel cell can be applied effectively for electricity generation through organic waste degradation. In this study, cauliflower leaf waste was used as organic waste, and MFC was operated with varied electrode conditions such as normal graphite and nanotubule-coated graphite electrodes. Parameters such as COD, ammoniacal nitrogen, phosphorus and reducing sugar monitored for the analysis showed reduction due to the treatment in MFC-generating electricity which is monitored by measuring open circuit voltage. Open circuit voltage data showed that coating graphite fibre by PANI/MWCNT as the anode and the addition of ferricyanide in phosphate buffer solution at the cathodic chamber enhanced electricity generation. Scanning electron microscopy showed microbial load in the graphite electrode and molecular characterisation showed *Bacillus* sps. involved in the degradation process. Hence, the chemical energy in cauliflower leaf waste can be converted into electrical energy using MFC as an alternative technology. Kitchen wastes can be degraded easily by the technique to convert into alternative energy, ie, an efficient waste valorisation technique. However, further research should be carried out for the mixed substrate as the vegetable wastes and kitchen wastes contain mixed varieties with different degradation efficiency. Also, intensive research should be carried out regarding enhancement in electricity generation. Even though this technology aids in managing waste to worth to some extent it will be fruitful for countries not having their own fossil reserves.

Acknowledgements

University Grants Commission, Nepal faculty research grant (FRG-75/76-5 & T-1), 2019 is highly acknowledged.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was fully supported by University Grants Commission, Nepal faculty research grant (FRG-75/76-5 & T-1), 2019.

ORCID

Pradip Dhungana http://orcid.org/0000-0001-5585-5899

References

Abbasi, U., W. Jin, A. Pervez, Z. A. Bhatti, M. Tariq, S. Shaheen, A. Iqbal, and Q. Mahmood. 2016. "Anaerobic Microbial Fuel Cell Treating Combined Industrial Wastewater: Correlation of Electricity Generation with Pollutants." *Bioresource Technology* 200: 1–7. doi:10.1016/j.biortech.2015.09.088.

Abdulla, S., T. L. Mathew, and B. Pullithadathil. 2015. "Highly Sensitive, Room Temperature gas Sensor Based on Polyaniline-Multiwalled Carbon Nanotubes (PANI/MWCNTs) Nanocomposite for Trace-Level Ammonia Detection." *Sensors and Actuators, B: Chemical* 221: 1523–1534. doi:10.1016/j.snb.2015.08.002.

Adebule, A., B. Aderiye, and A. Adebayo. 2018. "Improving Bioelectricity Generation of Microbial Fuel Cell (MFC) With Mediators Using Kitchen Waste as Substrate." *Annals of Applied Microbiology & Biotechnology Journal* 2 (1): 1–5. doi:10.36876/aamb.1008.

Afifi, M. M. 2011. "Enhancement of Lactic cid Production by Utilizing Liquid Potato Wastes." *International Journal of Biological Chemistry* 5 (2): 91–102. doi:10.3923/ijbc.2011.91.102.

Aghbashlo, M., H. Hosseinzadeh-Bandbafha, H. Shahbeik, and M. Tabatabaei. 2022. "The Role of Sustainability Assessment Tools in Realizing Bioenergy and Bioproduct Systems." *Biofuel Research Journal* 9 (3): 1697–1706. doi:10.18331/BRJ2022.9.3.5.

Aghbashlo, M., Z. Khounani, H. Hosseinzadeh-Bandbafha, V. K. Gupta, H. Amiri, S. S. Lam, T. Morosuk, and M. Tabatabaei. 2021. "Exergoenvironmental Analysis of Bioenergy Systems: A Comprehensive Review." *Renewable and Sustainable Energy Reviews* 149 (July): 111399. doi:10.1016/j.rser.2021.111399.

Ahanchi, M., T. Jafary, A. M. Yeneneh, P. F. Rupani, A. Shafizadeh, H. Shahbeik, J. Pan, M. Tabatabaei, and M. Aghbashlo. 2022. "Review on Waste Biomass Valorization and Power Management Systems for Microbial Fuel Cell Application." *Journal of Cleaner Production* 380: 134994. doi:10.1016/j.jclepro.2022.134994.

Alabiad, I., U. F. M. Ali, I. A. Zakarya, N. Ibrahim, R. W. Radzi, N. Z. Zulkurnai, and N. H. Azmi. 2017, June. "Ammonia Removal via Microbial Fuel Cell (MFC) Dynamic Reactor." *IOP Conference Series: Materials Science and Engineering* 206: 012079. doi:10.1088/1757-899x/206/1/012079.

Asefi, B., S. L. Li, H. A. Moreno, V. Sanchez-Torres, A. Hu, J. Li, and C. P. Yu. 2019, May. "Characterization of Electricity Production and Microbial Community of Food Waste-Fed Microbial Fuel Cells." *Process Safety and Environmental Protection* 125: 83–91. doi:10.1016/j.psep.2019.03.016.

Bose, D., M. Gopinath, and P. Vijay. 2018, May 15. "Sustainable Power Generation from Wastewater Sources Using Microbial Fuel Cell." *Biofuels, Bioproducts and Biorefining* 12 (4): 559–576. doi:10.1002/bbb.1892.

Bux Baloch, A., X. Xia, and S. Ahmed Sheikh. 2015. "Proximate and Mineral Compositions of Dried Cauliflower (Brassica Oleracea L.) Grown In Sindh, Pakistan." *Journal of Food and Nutrition Research* 3 (3): 213–219. doi:10.12691/jfnr-3-3-14.

Cai, J., P. Zheng, M. Qaisar, and Y. Xing. 2014. "Effect of Operating Modes on Simultaneous Anaerobic Sulfide and Nitrate Removal in Microbial Fuel Cell." *Journal of Industrial Microbiology and Biotechnology* 41 (5): 795–802. doi:10.1007/s10295-014-1425-4.

Cui, H. F., L. Du, P. B. Guo, B. Zhu, and J. H. T. Luong. 2015. "Controlled Modification of Carbon Nanotubes and Polyaniline on Macroporous Graphite Felt for High-Performance Microbial Fuel Cell Anode." *Journal of Power Sources* 283: 46–53. doi:10.1016/j.jpowsour.2015.02.088.

Demutskaya, L. N., and I. E. Kalinichenko. 2010. "Photometric Determination of Ammonium Nitrogen with the Nessler Reagent in Drinking Water After its Chlorination." *Journal of Water Chemistry and Technology* 32 (2): 90–94. doi:10.3103/S1063455X10020049.

De Sadeleer, I., H. Brattebø, and P. Callewaert. 2020. "Waste Prevention, Energy Recovery or Recycling - Directions for Household Food Waste Management in Light of Circular Economy Policy." *Resources, Conservation and Recycling* 160: 104908. doi:10.1016/j.resconrec.2020.104908.

Dhungana, P., B. Prajapati, P. Bhatt, D. Regmi, S. Maharjan, U. Lamsal, S. Kathariya, P. Chaudhary, and J. Joshi. 2023. "Production of Bioethanol from Saccharum Spontaneum by Simultaneous Saccharification and Electro-Fermentation Using Mixed Culture of Microbes Saccharification and Electro-Fermentation Using Mixed Culture of Microbes." *Biofuels* 14 (2): 191–199. doi:10.1080/17597269.2022.2126083.

Dhungana, P., B. Prajapati, S. Maharjan, and J. Joshi. 2022. "Current Trends in Lignocellulosic Bioethanol Production." *International Journal of Applied Sciences and Biotechnology* 10 (1): 1–11. doi:10.3126/ijasbt.v10i1.41139.

Dongre, M. 2018. "Nutritive Alteration of Cauliflower Head Infected with *Alternaria Brassicicola* from Maharashtra State, India." September 2017.

Doyle, L. E., and E. Marsili. 2018. "Weak Electricigens: A new Avenue for Bioelectrochemical Research." *Bioresource Technology* 258 (February): 354–364. doi:10.1016/j.biortech.2018.02.073.

Du, H., and F. Li. 2017. "Enhancement of Solid Potato Waste Treatment by Microbial Fuel Cell with Mixed Feeding of Waste Activated Sludge." *Journal of Cleaner Production* 143: 336–344. doi:10.1016/j.jclepro.2016.12.104.

Esfandabadi, Z. S., M. Ranjbari, and S. D. Scagnelli. 2022. "The Imbalance of Food and Biofuel Markets Amid Ukraine-Russia Crisis: A Systems Thinking Perspective." *Biofuel Research Journal* 9 (2): 1640–1647. doi:10.18331/BRJ2022.9.2.5.

Ganesh, S., F. Khan, M. K. Ahmed, P. Velavendan, N. K. Pandey, and U. Kamachi Mudali. 2012. "Spectrophotometric Determination of Trace Amounts of Phosphate in Water and Soil." *Water Science and Technology* 66 (12): 2653–2658. doi:10.2166/wst.2012.468.

Goud, R. K., and S. V. Mohan. 2011. "Pre-fermentation of Waste as a Strategy to Enhance the Performance of Single Chambered Microbial Fuel Cell (MFC)." *International Journal of Hydrogen Energy* 36 (21): 13753–13762. doi:10.1016/j.ijhydene.2011.07.128.

Gurung, A., J. Kim, S. Jung, B. H. Jeon, J. E. Yang, and S. E. Oh. 2012. "Effects of Substrate Concentrations on Performance of Serially Connected Microbial Fuel Cells (MFCs) Operated in a Continuous Mode." *Biotechnology Letters* 34 (10): 1833–1839. doi:10.1007/s10529-012-0979-3.

Hassan, H., L. Schulte-Illingheim, B. Jin, and S. Dai. 2016. "Degradation of 2,4-Dichlorophenol by Bacillus Subtilis with Concurrent Electricity Generation in Microbial Fuel Cell." *Procedia Engineering* 148: 370–377. doi:10.1016/j.proeng.2016.06.473.

He, Z., S. D. Minteer, and L. T. Angenent. 2005. "Electricity Generation from Artificial Wastewater Using an Upflow Microbial Fuel Cell." *Environmental Science and Technology* 39 (14): 5262–5267. doi:10.1021/es0502876.

Islam, M. A., B. Ethiraj, C. K. Cheng, A. Yousuf, and M. M. R. Khan. 2017. "Electrogenic and Antimethanogenic Properties of Bacillus Cereus for Enhanced Power Generation in Anaerobic Sludge-Driven Microbial Fuel Cells." *Energy and Fuels* 31 (6): 6132–6139. doi:10.1021/acs.energyfuels.7b00434.

Joshi, J., T. Bhattarai, and L. Sreerama. 2018. "Efficient Methods of Pretreatment for the Release of Reducing Sugars from Lignocellulosic Biomass Native to Nepal and Characterization of Pretreated Lignocellulosic Biomass Efficient Methods of Pretreatment for the Release of Reducing Sugars from Lignocel." *International Journal of Advanced Biotechnology and Research* 9 (3): 9–23.

Joshi, J., P. Dhungana, B. Prajapati, R. Maharjan, P. Poudyal, M. Yadav, M. Mainali, A. P. Yadav, T. Bhattarai, and L. Sreerama. 2019. "Enhancement of Ethanol Production in Electrochemical Cell by Saccharomyces Cerevisiae (CDBT2) and Wickerhamomyces Anomalus (CDBT7)." *Frontiers in Energy Research* 7 (7): 1–11. doi:10.3389/fenrg.2019.00070.

Jothinathan, D., and R. T. Wilson. 2017. "Comparative Analysis of Power Production of Pure, Coculture, and Mixed Culture in a Microbial Fuel Cell." *Energy Sources, Part A: Recovery, Utilization and Environmental Effects* 39 (5): 520–527. doi:10.1080/15567036.2016.1233306.

Kashyap, D., C. Kim, S. Y. Kim, Y. H. Kim, G. M. Kim, P. K. Dwivedi, A. Sharma, and S. Goel. 2015. "Multi Walled Carbon Nanotube and Polyaniline Coated Pencil Graphite Based bio-Cathode for Enzymatic Biofuel Cell." *International Journal of Hydrogen Energy* 40 (30): 9515–9522. doi:10.1016/j.ijhydene.2015.05.120.

Khedkar, M. A., P. R. Nimbalkar, P. V. Chavan, Y. J. Chendake, and S. B. Bankar. 2017. "Cauliflower Waste Utilization for Sustainable Biobutanol Production: Revelation of Drying Kinetics and Bioprocess Development." *Bioprocess and Biosystems Engineering* 40 (10): 1493–1506. doi:10.1007/s00449-017-1806-y.

Kumar, R., L. Singh, A. W. Zularisam, and F. I. Hai. 2017, June 9. "Microbial Fuel Cell is Emerging as a Versatile Technology: A Review on its Possible Applications, Challenges and Strategies to Improve the Performances." *International Journal of Energy Research* 42 (2): 369–394. doi:10.1002/er.3780.

Li, X. H., H. J. Yang, B. Roy, D. Wang, W. F. Yue, L. J. Jiang, E. Y. Park, and Y. G. Miao. 2009. "The Most Stirring Technology in Future: Cellulase Enzyme and Biomass Utilization." *African Journal of Biotechnology* 8 (11): 2418–2422.

Logan, B. E., R. Rossi, A. Ragab, and P. E. Saikaly. 2019. "Electroactive Microorganisms in Bioelectrochemical Systems." *Nature Reviews Microbiology* 17 (5): 307–319. doi:10.1038/s41579-019-0173-x.

Lopez Zavala, M. A., I. C. C. Gutiérrez. 2022. "Effect of External Resistance, Electrodes Material and Catholyte Type on the Energy Generation and the Performance of Dual-Chamber Microbial Fuel Cells." *SSRN Electronic Journal.* doi:10.2139/ssrn.4267992.

Lu, W. J., H. T. Wang, S. J. Yang, Z. C. Wang, and Y. F. Nie. 2006. "Isolation and Characterization of Mesophilic Cellulose-Degrading Bacteria from Flower Stalks-Vegetable Waste co-Composting System." *Journal of General and Applied Microbiology* 51 (6): 353–360. doi:10.2323/jgam.51.353.

Manohar, A. K., O. Bretschger, K. H. Nealson, and F. Mansfeld. 2008. "The use of Electrochemical Impedance Spectroscopy (EIS) in the Evaluation of the Electrochemical Properties of a Microbial Fuel Cell." *Bioelectrochemistry* 72 (2): 149–154. doi:10.1016/j.bioelechem.2008.01.004.

Mashkour, M., M. Rahimnejad, F. Raouf, and N. Navidjouy. 2021. "A Review on the Application of Nanomaterials in Improving Microbial Fuel Cells." *Biofuel Research Journal* 8 (2): 1400–1416. doi:10.18331/BRJ2021.8.2.5.

Mohamoud, M. A. 2014. "Unexpected Redox Enhancement and Electrochemical Pseudo-Capacitance Performance of Polyaniline/Poly(Vinyl Alcohol) (PAn/PVA) Composite Films." *Electrochimica Acta* 139: 201–208. doi:10.1016/j.electacta.2014.06.174.

Mustakeem, M. 2015. "Electrode Materials for Microbial Fuel Cells: Nanomaterial Approach." *Materials for Renewable and Sustainable Energy* 4 (4): 1–11. doi:10.1007/s40243-015-0063-8.

Nabavi-Pelesaraei, A., R. Bayat, H. Hosseinzadeh-Bandbafha, H. Afrasyabi, and K. wing Chau. 2017. "Modeling of Energy Consumption and Environmental Life Cycle Assessment for Incineration and Landfill Systems of Municipal Solid Waste Management – A Case Study in Tehran Metropolis of Iran." *Journal of Cleaner Production* 148: 427–440. doi:10.1016/j.jclepro.2017.01.172.

Ng, K. S., A. Yang, and N. Yakovleva. 2019. "Sustainable Waste Management Through Synergistic Utilisation of Commercial and Domestic Organic Waste for Efficient Resource Recovery and Valorisation in the UK." *Journal of Cleaner Production* 227: 248–262. doi:10.1016/j.jclepro.2019.04.136.

Nishiguchi, M. K., P. Doukakis, M. Egan, D. Kizirian, A. Phillips, L. Prendini, and H. C. Rosenbaum, et al. 2002. "DNA Isolation Procedures." In *Techniques in Molecular Systematics and Evolution*, edited by Rob DeSalle, Gonzalo Giribet, and Ward Wheeler, 250–287. Basel: Birkhauser.

Parkash, A., S. Aziz, and A. Soomro 2015. "Utilization of Sewage Sludge for Production of Electricity Using Mediated Salt Bridge Based Dual Chamber Microbial Fuel Cell." *Journal of Bioprocessing & Biotechniques* 5 (8): 251. doi:10.4172/2155-9821.1000251.

Priest, F. G. 1977. "Extracellular Enzyme Synthesis in the Genus Bacillus." *Bacteriological Reviews* 41 (3): 711–753. doi:10.1128/br.41.3.711-753.1977.

Rabaey, K., P. Clauwaert, P. Aelterman, and W. Verstraete. 2005. "Tubular Microbial Fuel Cells for Efficient Electricity Generation." *Environmental Science and Technology* 39 (20): 8077–8082. doi:10.1021/es050986i.

Rismani-Yazdi, H., A. D. Christy, S. M. Carver, Z. Yu, B. A. Dehority, and O. H. Tuovinen. 2011. "Effect of External Resistance on Bacterial Diversity and Metabolism in Cellulose-fed Microbial Fuel Cells." *Bioresource Technology* 102 (1): 278–283. doi:10.1016/j.biortech.2010.05.012.

Santagata, R., M. Ripa, A. Genovese, and S. Ulgiati. 2021. "Food Waste Recovery Pathways: Challenges and Opportunities for an Emerging bio-Based Circular Economy. A Systematic Review and an Assessment." *Journal of Cleaner Production* 286: 125490. doi:10.1016/j.jclepro.2020.125490.

Savini, F. 2021. "The Circular Economy of Waste: Recovery, Incineration and Urban Reuse." *Journal of Environmental Planning and Management* 64 (12): 2114–2132. doi:10.1080/09640568.2020.1857226.

Scott, K., G. A. Rimbu, K. P. Katuri, K. K. Prasad, and I. M. Head. 2007. "Application of Modified Carbon Anodes in Microbial Fuel Cells." *Process Safety and Environmental Protection* 85 (5 B): 481–488. doi:10.1205/psep07018.

Soltanian, S., S. A. Kalogirou, M. Ranjbari, H. Amiri, O. Mahian, B. Khoshnevisan, T. Jafary, et al. 2022. "Exergetic Sustainability Analysis of Municipal Solid Waste Treatment Systems: A Systematic Critical Review." *Renewable and Sustainable Energy Reviews* 156: 111975. doi:10.1016/j.rser.2021.111975.

Vaghela, N. R., and K. Nath. 2020. "Reduced Graphene Oxide Coated Graphite Electrodes for Treating Reactive Turquoise Blue 21 Rinse Water Using an Indirect Electro-Oxidation Process." *SN Applied Sciences* 2 (11): 1839. doi:10.1007/s42452-020-03719-6.

Wang, C. T., F. Y. Liao, and K. S. Liu. 2013, August. "Electrical Analysis of Compost Solid Phase Microbial Fuel Cell." *International Journal of Hydrogen Energy* 38 (25): 11124–11130. doi:10.1016/j.ijhydene.2013.02.120.

Yang, Y., D. Ye, Q. Liao, P. Zhang, X. Zhu, J. Li, and Q. Fu. 2016. "Enhanced Biofilm Distribution and Cell Performance of Microfluidic Microbial Fuel Cells with Multiple Anolyte Inlets." *Biosensors and Bioelectronics* 79: 406–410. doi:10.1016/j.bios.2015.12.067.

Ying, L., Hongwei, J., Huizhen, X., & Li, L. (2006). A New Spectrophotometric Method for Measuring COD of Seawater. Journal of Ocean University of China, 5(2), 137–140. doi:10.1007/BF02919212.