Chapter 4

Bioelectricity generation by using cellulosic waste and spent engine oil in a concentric photobioreactor-microbial fuel cell

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4.1 Introduction

Spent engine oil (SEO) is a considerable environmental pollutant. It is loaded with toxic polycyclic aromatic hydrocarbons and heavy metals. Heavy metals and polynuclear aromatic hydrocarbons have elevated the levels of toxicity and impart a significant cancer risk in human beings. Every year, SEO contributes to pollution significantly [405]. SEO pollutes more than petroleum as its main sources are industrial units and automobiles. In addition to hydrocarbons and heavy metals, SEO also includes lubricant additives [406]. Spent engine oil contamination in groundwater arises from improper disposal, leaks, and urban runoff. Spent engine oil contamination in groundwater is common in areas lacking regulations. This pollution poses environmental risks, necessitating strict waste management practices, monitoring, and remediation efforts to safeguard groundwater quality. The pollution caused by crude oil and SEO are two separate aspects. Researchers have paid massive attention to the pollution due to crude oil and its detrimental effects on the environment. On the other hand, the removal of SEO has been scarcely studied. It is extremely challenging to remove water emulsified SEO and it is a serious threat to the aquatic environment [406].

MFC is a pollutant remediation system and renewable source of bioelectricity. The MFC has a cation exchange membrane or salt bridge separating the anode and cathode chambers, like any chemical fuel cell [76]. The microbes in anode chamber bioremediate wastewater and transform chemical energy into bioelectricity. In most of the cases, pollutants may be removed either at the anode or cathode, via the microbial oxidation or reduction [407]. MFC operates on following mechanisms for the elimination of pollutant (i) oxidation of pollutants (agricultural waste, food waste) at anode (iii) their adsorption on biofilms (iv) effect of

electrical field on chemical formulation of pollutant (v) transfer of proton towards cathode (reduction) which changes the pH of catholyte [19]. Cellulose is an essential cell wall polymer that is continually produced in nature during photosynthesis. Nevertheless, the majority of cellulose is considered as waste in pre and post agriculture harvest including food processing sector [408]. The waste management techniques encourage the recovery of useful by-products like bioenergy before it is disposed [409]. The implementation of MFC could be a strategic approach for the valorisation of cellulosic waste biomass in the production of bioelectricity. The microbes must be anaerobic, electroactive and able to hydrolyse cellulose for the direct conversion of cellulosic waste in to bioelectricity [13], [325]. Cellulosic waste biomass is a by-product from various industries like forestry (leaf, grass), pre and post agriculture harvest (corn stover, rice husk, sugarcane bagasse, straw, rotten fruits, fruit and vegetable waste) and others (wood and paper industry waste) [410]. There are several reports on the use of cellulosic biomass (such as lemon peel, sweet lemon peel, banana peel, blueberry waste, orange peel, paper and pulp industry wastewater in MFC [337], [350]. Dry newspaper powder is rich in cellulose, a complex organic polymer. Untreated newspapers worldwide would lead to excessive landfill accumulation, emitting methane and contributing to climate change. Decomposition releases pollutants, risking soil and water contamination. The loss of resource recovery opportunities and the environmental impact underscore the importance of proper waste management for sustainability. Cellulose is a common carbon source that can be degraded by cellulolytic microorganisms [337], [350]. The degradation of cellulose can release electrons as byproducts of microbial respiration [337], [350]. Algal biomass is a source of organic matter, including lipids, proteins, and carbohydrates. Microorganisms can break down these complex organic compounds, releasing electrons in the process. In the MFC, microorganisms will degrade the dry algal biomass, utilizing the various organic components for energy. As a result, electrons will be generated during the microbial degradation of algal

biomass, supporting the electrogenic activity of the microbial community[371]. The use of bacteria in bioremediation is an effective approach. However, the biomass produced post remediation is not valuable in anyway. In the present work, a novel concentric dual chamber photobioreactor MFC was designed. This concentric photobioreactor MFC accomplishes multiple goals by targeting following objectives: (i) organic waste valorization at anode chamber (ii) bioremediation of simulated SEO wastewater, (iii) microalgae biomass production and (iv) bioelectricity generation. At anode, newspaper powder (NP), mixed fruit peel powder (FP) and dried microalgae powder (MB) have been used as cellulosic substrate with an isolated cellulolytic bacterium. At anode, *Enterobacter cloacae* IIT BHU M2V2 degraded cellulosic waste and generated bioelectricity. An acclimatized microalgae culture was used at cathode for the SEO bioremediation along with enhancing the oxygen reduction rate (ORR).

4.2 Material and methods

4.2.1 Sample Collection

Microalgae samples were collected from a pond (lotus lake, university campus) and fruit peels (FP: banana, papaya, sweet lemon, orange and pomegranate peels) were collected from a juice shop present in the university campus, during month of September 2022. Pond water has yellowish-green in color containing viable microalgae biomass and few phytoplankton. Pond water was stored in refrigerator at 4 ºC till further use. Old newspapers (NP) were collected from a local shop situated near university campus. The microalgae biomass (MB) (*Chlorella pyrenoidosa*) was grown in laboratory and then dried (115 ± 20 mg/L) in a hot air oven (Imperial Biotech make, India) at 80 ºC for 5 hours.

4.2.2 Pretreatment of Cellulosic Waste

NP was soaked in tap water for 3 days at room temperature. Soaking of NP remove maximum portion of ink during preprocessing. And hence it could not interfere with microbial growth.

The wet pieces of NP were milled using mixer grinder which resulted in a fine slurry. Obtained slurry was drained using a mesh and dried in a hot air oven (Imperial Biotech make, India) at 105 ºC for overnight. Dried newspaper powder was sieved through 44 mesh sized sieves (Retsch AS200 make, Germany). After sieving, fine NP powder was stored in an air tight container at room temperature (30 ºC). NP was used as a cellulosic substrate at anode. FP and MB were dried using a hot air oven at 105 ºC for overnight. FP and MB biomass were milled separately till a fine powder was obtained and sieved (mesh size 44). Thereafter, fine FP and MB powder were stored in an air tight container at room temperature. Alkali pretreatment has been done for each FP, MB and NB by using 3% (w/v) NaOH solution followed by heat treatment in autoclave [411].

4.2.2.1 Characterization of Cellulosic Waste

Elemental analysis of the NP, FP and MB was done by CHNS (O) Analyser (Euro EA 3000, Elemental analyser make, Italy). Proximate analysis was used in accordance with the procedures outlined by the American Society for Testing and Materials (ASTM) [379] to investigate ash [376], moisture [377] and volatile content [378]. Crude fibre content was analysed by AOAC 978.10 method [412].

4.2.2.2 Anolyte Preparation and Characterization

20g of each NP, MB and FP were mixed with 30-gram NaOH in 1000 ml distilled water (for alkali cellulosic pretreatment, 3% (w/v) NaOH solution). 10 grams of yeast extract was added into each NP, MB and FP and autoclaved at 121°C and 15 psig. The Anthron method was used to measure the total sugar content of NP, MB, and FP. The reducing sugar was estimated by DNSA (3,5-Dinitrosalicylic acid) technique. A digital pH metre (Eutech pH tutor make, USA) was used to measure the substrate's pH. Chemical oxygen demand (COD) of NP, MB and FP

were estimated by the closed-reflux method provided by American Public Health Association [380].

4.2.3 Microalgae Cultivation

Sample collected from pond was inoculated in to a 200 ml Bold basal medium (BBM) in 500 ml erlenmeyer flask. It was placed into a shaker-cum-incubator at 180 rpm under 2000 lux fluorescent light. After a week, microalgae culture was washed thrice with phosphate buffer saline to eliminate microorganisms present in the microalgae. Washed algae cells were serially diluted up to 10^{-3} times. The diluted sample was spread on the agar plates. Single colony was picked up for streaking on agar plates. Pure culture was inoculated in 200 ml BBM media under 2000 lux fluorescent light. The purity of the isolate was ensured by repeated plating and observation under a fluorescent microscope (Olympus fluorescence microscope BX53 make, Japan). After 7 days, isolated microalgae cells were transferred into 200 ml BBM media containing 2 ml of SEO. They were incubated at 2000 lux fluorescent light and aerated using an air pump (flow rate: 3L/min) for acclimatization. This was repeated twice by increasing SEO concentration up to 10 ml/L.

4.2.3.1 DNA Extraction, Amplification and Sequencing of Microalgae

Chromosomal DNA of resultant microalgae culture was extracted by using spin column kit (HiMedia make, India). Algal 18SrRNA gene (1500 bp) was amplified using polymerase chain reaction (PCR) [413]. Purified amplicons were sequenced by Sanger method in ABI 3500xl genetic analyzer (Life Technologies make, USA). Sequences were further analysed by Basic Local Alignment Search Tool (BLAST) with closest culture sequence retrieved from the National Centre for Biotechnology Information (NCBI, USA) database [414]. The BLAST algorithm was used to find phylogenetic relationships between sequences and to identify

members of gene families. Further multiple sequence alignment and phylogenetic analysis were used for the prediction of accurate species and evolutionary relationship [415], [416]

4.2.4 Preparation of Catholyte

BBM media was prepared for the cultivation of microalgae. 10 ml culture media was prepared with following composition: (1) 25 g/L $NaNO_3$, 17.5 g/L KH_2PO_4, 7.5 g/L K_2HPO_4, 2.5 g/L $CaCl_2{\cdot}2H_2O$, 2.5 g/L NaCl, 7.5 g/L $MgSO_4{\cdot}7H_2O$ and (2) 1 ml of media was prepared with following composition: 4.98 g/L $FeSO_4{\cdot}7H_2O$, 50 g/L anhydrous EDTA, 1g/L H_2SO_4, 31 g/L KOH, 11.4 g/L H_3BO_3, 8.82 g/L $ZnSO_4{\cdot}7H2O$, 0.71 g/L MoO_3, 1.44 g/L $MnCl_2{\cdot}4H_2O$, 1.57 g/L $CuSO_4{\cdot}5H_2O$, 0.49 g/L $Co(NO_3)_2{\cdot}6H_2O$. SEO water emulsion was prepared by adding 15 ml of SEO in 985 ml of BBM media. Thereafter, the catholyte was called as BBM-SEO.

4.2.4.1 Characterization of catholyte

Standard procedures outlined in the [380] were used in order to ascertain the levels of COD, ammonium-nitrogen, nitrate-nitrogen, and phosphate-phosphorus. Oil and grease (OG) estimation was done by partition gravimetry (Method 1664) [417]. At the end of operation, N-hexane was used to wash algae biomass obtained from cathode chamber of each MFC unit. Post washing, the microalgae biomass was collected on filter paper and dried at 105 ° C for overnight. Final weight of the dried filter paper was measured in order to calculate dry weight of algae biomass.

4.2.5 Anode Inoculum and Characterization

Enterobacter cloacae IIT BHU M2V2 (accession number OM214000) isolated in previous study was used as inoculum at anode [325]. *Enterobacter cloacae* IIT BHU M2V2 showed cellulolytic activity [325]. Inoculum has been added at the beginning of the acclimatization

period. Reinoculation was done after each cycle during the operational period. Metagenomic sequencing was done to characterize microbial community developed on anode biofilms.

4.2.6 MFC Construction and Operation

A mini aquarium tank (made up of acrylic fiber) of 18 x 14 x 16 cm was used as cathode chamber. Another mini aquarium tank (acrylic fiber) 14 x 11 x 8 cm worked as an anode chamber. Agar salt bridge (2-5% agar in 1M KCl (w/v)) was prepared by joining a silicon tube (0.5 mm diameter) of length 3 cm into anode chamber. The salt-bridge in MFCs offers a more affordable substitute for the extensive costly proton-exchange membranes. The rate of oxygen passage in an agar salt bridge is too low that it is effectively inaccessible [418]. Anode chamber was fixed in the middle of the cathode compartment. A stainless-steel mesh (mesh size 13) 60 cm^2 and a cylindrical graphite rod (0.5 cm radius and 10 cm length) were used as anode and cathode, respectively. Insulated copper wire was used to connect anode and cathode. A 1000Ω resister was used as an external load. An air pump was used for aeration at cathode chamber. Three co-centric dual chamber MFCs were prepared as mentioned above and named as F-MFC, M-MFC, and N-MFC. Figure 4. 1(a) represents graphical illustration of co-centric combined photobioreactor- MFC and Figure 4. 1(b) shows all MFC setups prior to experiments.

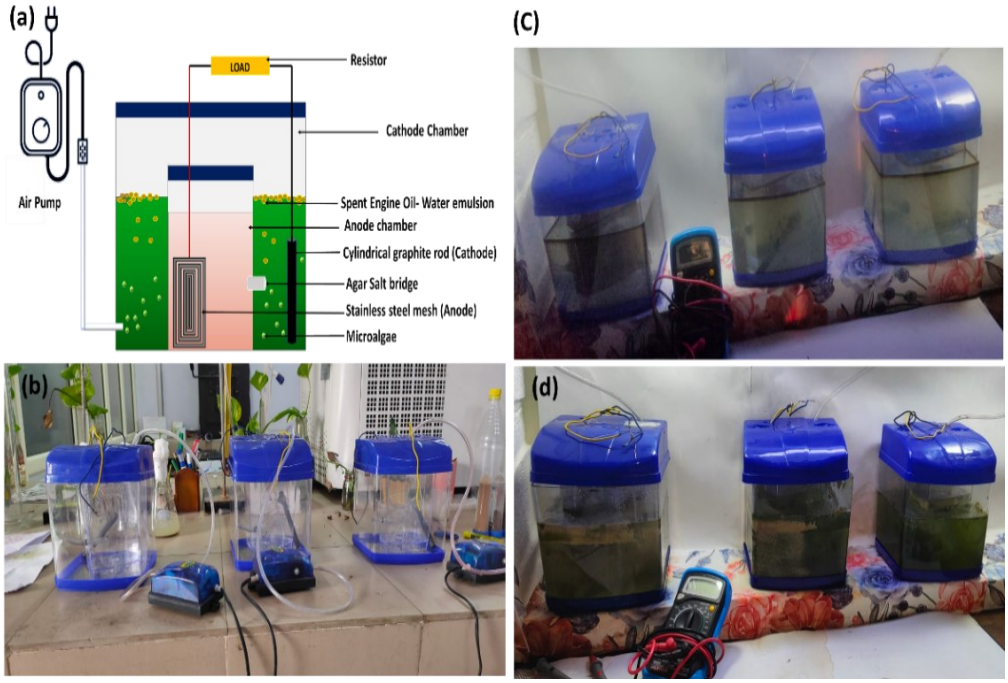

Figure 4. 1. Co-centric combined photobioreactor- MFCs (a) graphical illustration (b) MFC setup prior experiment (c) MFC setups day 1 (cycle 1) (d) MFC setup day 15 (cycle 1)

NP, FP and MB anolytes were filled in anode chamber of N-MFC, F-MFC and M-MFC respectively. All the MFCs were inoculated with 1% *E. cloacae* IIT BHU M2V2. In all MFCs cathode compartment was filled with 1 L of BBM media and inoculated with 10% (v/v) of isolated microalgae culture. Cathode chamber of all MFCs were kept at continuous lightning (24 hours/day) of 2000 lux fluorescent light and aerated for 3 hours/day by an air pump sparger during initial 15 days of acclimation. The air pump has been run continuously (24 hours/day) to create a homogeneous medium inside the cathode chamber. SEO floated on the top layer of the catholyte medium. Hence, it was necessary to aerate them for appropriate mixing. Each MFC setup was operated in the batch mode for three consecutive cycles. Initial 15 days were considered as an acclimation period. The operation period was of 10 days/cycle excluding acclimatization. After every 10 days anode chamber was refilled with substrates and re-

inoculated with 1% *E. cloacae* IIT BHU M2V2 for the next cycle. Cathode chamber was kept same throughout the operation (30 days).

4.2.6.1 MFC characterization

Voltage (V) was measured by a digital multimeter after acclimation period (Mextech Mas 8301 make, India) at 1000 Ω. Polarization studies were performed during the 2nd cycle. In order to observe the polarisation behaviour, voltage was recorded after stable voltage generation. External resistances ranging from 15000 to 10 Ω were used in polarization studies. Ohm's law was applied to calculate current (I) and power (P) [419]. The internal resistances (R_{int}) of each F-MFC, N-MFC and M-MFC were estimated from the slope of the I vs V plots [325].

The ammonium-nitrogen (mg/L), nitrate-nitrogen (mg/L), phosphate-phosphorous (mg/L) and COD (mg/L) levels of the feed sample were estimated at the end of each MFC cycle. The total removal efficiency (TE) of COD was calculated by Eq. 4.1 [387].

$$\mathrm{TE} = 100 \times \frac{\mathrm{COD_{t=0}} - \mathrm{COD_{t=f}}}{\mathrm{COD_{t=f}}} \tag{4.1}$$

where, $\mathrm{COD_{t=f}}$ and $\mathrm{COD_{t=0}}$ are the final and initial COD of the wastewater. Similarly, the removal percentages of nitrate-nitrogen, phosphate-phosphorous and ammonium-nitrogen were also determined. Columbic efficiency (CE) was estimated from Eq. 4.2:

$$\mathrm{CE} = \frac{8 \int_0^t \mathrm{I}\, \mathrm{d(t)}}{\mathrm{F\, V_{anode}\, \Delta COD}} \times 100 \tag{4.2}$$

where, 8 is a constant used for COD, F is the Faraday's constant (96485 C-mole^{-1} - electrons), V_{anode} is the volume of anode chamber and ΔCOD is the variation in the COD over time (t).

4.2.7 Characterization of Microbial Community at Anode

Study on microbial community was executed in order to explore the indigenous microbial community grown on anode biofilm in each M-MFC. Anode biofilm samples were centrifuged

(10,000 rpm for 10 min) and stored at -80 °C. DNA extraction was carried out by commercially available kit. The extracted DNA was nano dropped and gel checked prior to PCR amplification. The nano drop readings between 260-280 at value of 1.8-2.0 were used to control the quality of DNA. The forward and reverse primer 16s F:5′AGAGTTTGATGMTGGCTCAG3′ and 16s R:5′TTACCGCGGCMGCSGGCAC3′ were applied for the PCR amplification of bacterial 16S rRNA genes having V3 to V4 variable regions. During the amplification, 40 ng of the isolated DNA and 10 pM of each primer were used. The purified amplicons were obtained using Ampure beads. Eight cycles of PCR using Illumina barcoded adapters were carried out for achieving library sequences. Ampure beads were utilised to produce purified libraries. The Qubit dsDNA high sensitivity test kit (Thermo Fisher Scientific) was used for the quantification of the libraries. An Illumina Miseq with a 2x300PE v3 sequencing kit was used for DNA sequencing. After the amplification by 16s PCR, the product was purified and checked through GEL Check and Nanodrop for quality control (QC). The QC on the raw data was done with the help of the software Fastqc (Version 0.11.9) and Multiqc (Version 1.10.1). Thereafter, adapters and low-quality readings were removed with the help of TRIMGALO+21RE. Once the reads were trimmed, they underwent further processing. This consisted of combining the paired-end reads, removing the chimaeras, calculating the OTU abundance and correcting the estimate. This was accomplished by using the QIIME, MOTHUR, KRAKEN, and BRACKEN workflows. NCBI's database was utilised for the 16s V3–V4 region of the genome.

4.2.7.1 Preparation for Scanning Electron Microscopy (SEM) Analysis

Anode biofilms were fixed by using 50 mM Hepes buffer solution of 2% glutaraldehyde and left overnight in a refrigerator at 4 °C. Thereafter, sequential concentrations of ethanol (50%, 70%, 80%, 90%, and 100%) were used to dry the anodes, for half hour each. The anode biofilms with the nano porous structure were gold sputtered before SEM.

4.2.8 Statistical Analysis

All experiments were carried out thrice and the average of results have been reported. Microsoft Excel (Version 2021) was used to plot graphs along with calculation of mean values, standard deviation, and standard error.

4.3 Results and Discussion

4.3.1. Proximate and Ultimate Analysis of Dried Substrates

Table 4. 1 shows the % of carbon (C), hydrogen (H), nitrogen (N), sulphur (S), moisture content, ash, volatile content, fixed carbon and crude fibre content present in the NP, FP and MB on dry weight basis.

Table 4. 1. Ultimate and proximate analysis

	Amount (%)		
	NP	FP	MB
C	43.77	32.62	33.05
H	5.26	8.69	5.021
N	-	-	-
S	-	-	-
Moisture content	3.61 ± 0.06	3.52 ± 0.05	2.76 ± 0.7
Ash	0.46 ± 0.15	0.29 ± 0.19	0.80 ± 0.00
Volatile content	95.43 ± 0.37	91.27 ± 0.29	96.34 ± 0.06
Fixed carbon content	0.48 ± 0.29	4.90 ± 0.50	0.620 ± 0.27
Crude fibre content	1.69 ± 0.07	13.95 ± 0.06	9.42 ± 0.36

Table 4. 2 reflects the values of total sugar, reducing sugar and COD of anolyte. It also represents COD, ammonium-nitrogen, nitrate-nitrogen, phosphate-phosphorus and oil-grease concentration in catholyte.

Table 4. 2. Characterization of anolyte and catholyte

	Concentration (mg/L)			
	NP	**FP**	**MB**	**BBM-SEO Catholyte**
Total sugar (mg/L)	13125 ± 62.5	14458 ± 95.47	8854 ± 93.94	-
Reducing sugar (mg/L)	270.83 ± 12.02	3000 ± 62.50	145.83 ± 36.08	
Initial COD (mg/L)	8440 ± 222	6826 ± 283	7600 ± 14	11240 ± 461
Oil and Greece (mg/L)	-	-	-	14066 ± 37.85
Ammonium-nitrogen (mg/L)	-	-	-	56.41 ± 2.24
Nitrate-nitrogen (mg/L)	-	-	-	368.14 ± 0.917
Phosphate-phosphorus (mg/L)	-	-	-	267.06 ± 0.90
pH	7.8	6.5	7.2	9.1

Elemental analysis showed that the NP, FP and MB were ideal cellulosic substrates for *E. cloacae IIT BHU M2V2* as they contained all the essential growth-supporting components (**Table 4. 1** and **Table 4. 2**). FP, NP and MB had pH of 6.5, 7.8 and 7.2 respectively. Dried lignocellulosic biomass of corn bran, banana peel, orange peel and lemon peel have been also explored as substrate in MFC [23], [336], [338]. Microalgae biomass consist of starch (chloroplast) and cellulose/polysaccharides (cell wall). Dried microalgae biomass has been also observed as a suitable substrate for fermentation as it contains ample amount of starch, lipid,

cellulose and protein [420]. Newspaper has been widely accepted as cellulosic substrate for the production of second-generation biofuels [421].

4.3.2 Identification of Microalgae Strain

The phylogenetic tree representing the closest species with the isolated microalgae strain is shown in **Figure 4. 2**.

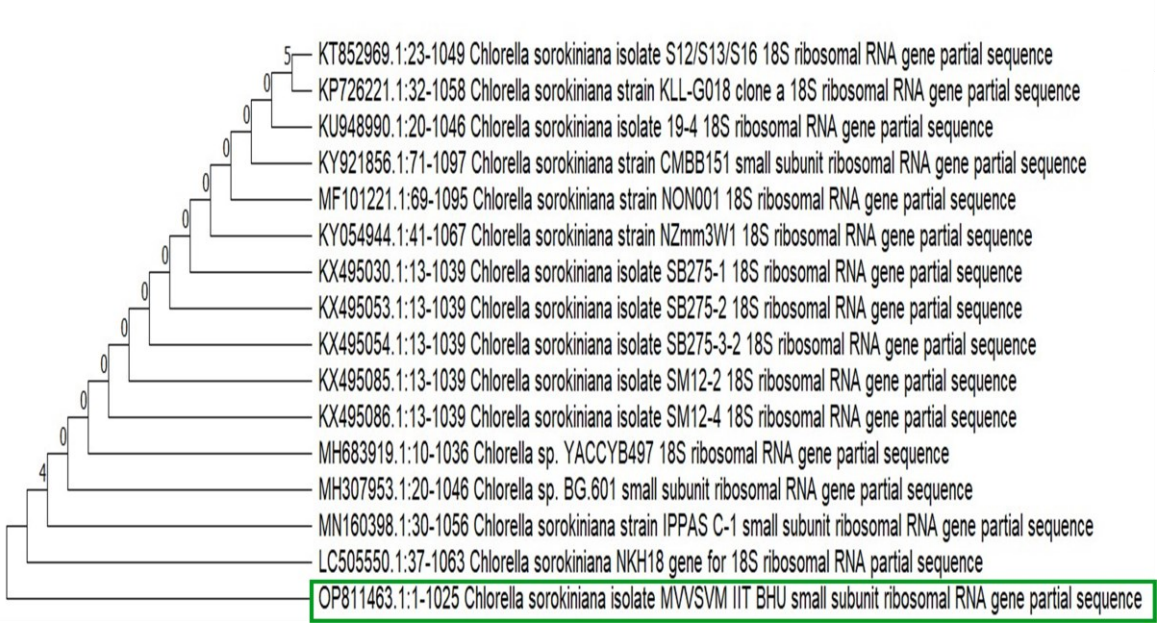

Figure 4. 2. Phylogenetic tree of Chlorella sorkiniana MVVSVM IIT BHU (accession number OP811463).

Isolated microalgae revealed closest homology with *Chlorella sp*. (closer to *sorokiniana*) (99.61%). Thus, microalgae strain was designated as *Chlorella sorkiniana* MVVSVM IIT BHU. This strain was submitted to NCBI under the accession number OP811463. *Chlorella sorokiniana* has been well explored for the treatment of palm oil mill effluents using microalgae biomass [422]. However, it has been rarely observed for the SEO remediation using photo-MFC.

4.3.3 Power generation

NP, FP and MB were evaluated as anodic substrates with *E. cloacae* IIT BHU M2V2 and other indigenous microorganisms. Stable generation of electricity was attained at the end of acclimation period (15 days). The open circuit voltage (OCV) vs. time profile of F-MFC, N-MFC, M-MFC were evaluated in the duration of 30 days (3 cycles) (Figure 4. 3A). The data obtained showed that there was a steady rise in OCV during 1^{st} - 4^{th} day. OCV values got stabled with passage of time and declined after 7^{th} - 8^{th} day. These outcomes revealed that the highest OCV was generated from N-MFC (370 ± 1.5 mV) during 3^{rd} cycle. F-MFC and M-MFC generated maximum OCV of 280 ± 1.5 mV (3^{rd} cycle) and 284 ± 1.0 mV (2^{nd} cycle), respectively. Figure 4. 3(B) and Figure 4. 3(C) represent voltage vs time (at 1000 Ω) and current vs time (at 1000 Ω) profile, respectively.

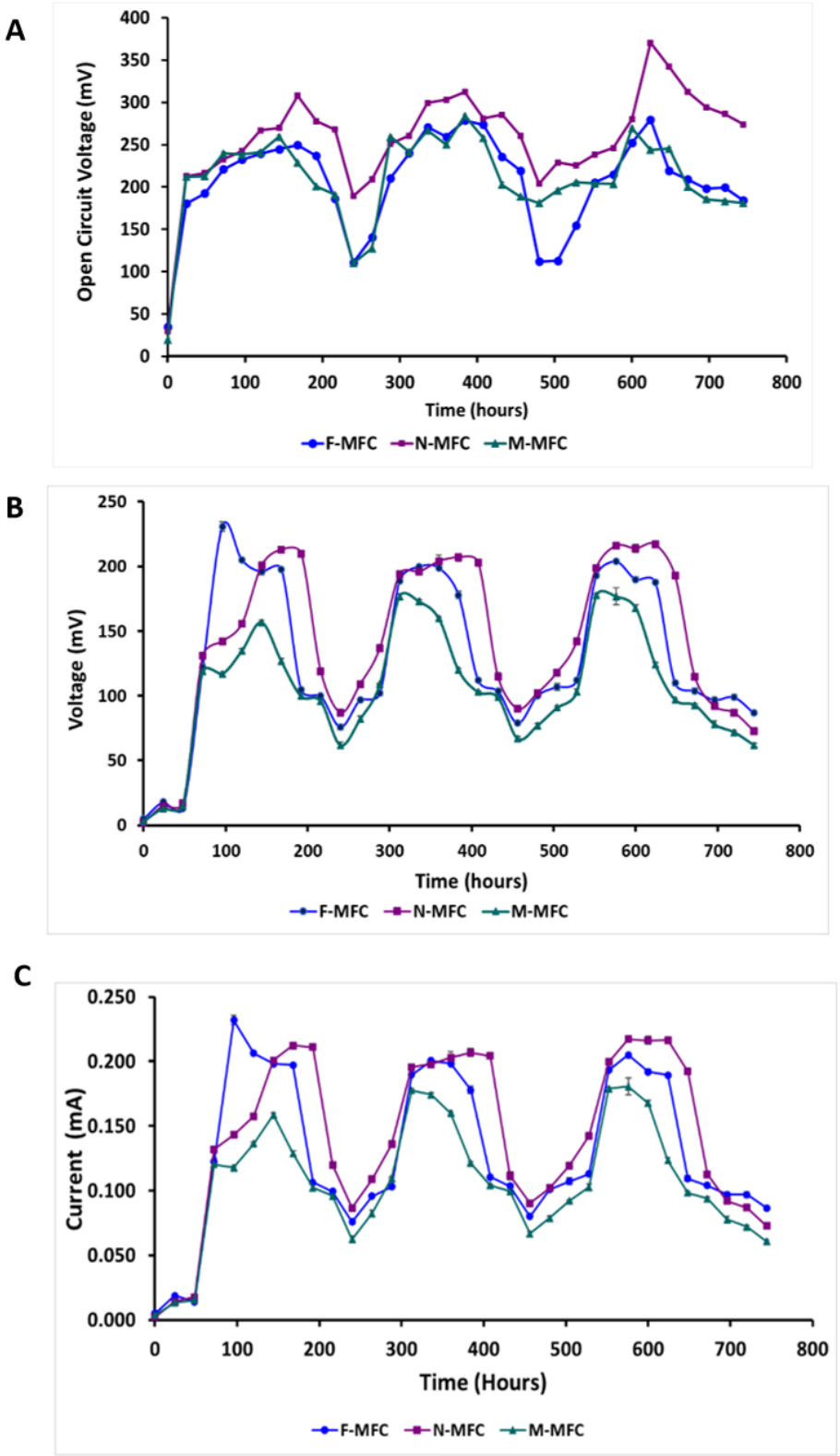

Figure 4. 3. Voltage and current variations; (A) Open circuit voltage vs Time (B) Voltage vs Time and (C) Current vs Time

There was an increase in voltage and current level upon the addition of substrate in MFCs (Figure 4. 3 B and C). Across 1000 Ω, highest voltage 232 ± 3.6 mV was obtained from F-MFC which was followed by N-MFC (217 ± 1.1 mV). M-MFC generated maximum voltage of 181 ± 6.3 mV. The difference in voltage in each cycle might be due to following reasons [423] (i) biological variability, (ii) the initial colonization and development of the anode biofilm can vary from cycle to cycle, (iii) factors like biofilm detachment and sloughing, influencing its electrochemical performance and leading to variation in voltage outputs, (iv) the measurement and recording of voltage values themselves may introduce some degree of variability. Similarly, F-MFC generated highest current 0.232 ± 0.003 mA. Similarly, F-MFC generated highest current 0.232 ± 0.0 mV. Thereafter, the MFC's microbial population either died off or the substrate was exhausted. This resulted in progressive drop in each cycle's voltage and current output [337]. *E. cloacae* IIT BHU M2V2 and other possible indigenous microorganisms consumed biodegradable compounds present in the substrate. Consequently, electricity was produced using substances that were hard to degrade. Makhtar and Tajarudin (2020) reported a stable voltage of 271 mV using banana peel powder and 176 mV from corn bran in membrane-less MFC [424]. The paper industry wastewater was exploited by *Cellulomonas iranensis* in a MFC unit and a steady voltage of 173 mV was obtained [425]. A study compared the performance of fruit pulp and defatted microalgae biomass as substrate in a photo-MFC [426]. A stable voltage of 250 mV was reported from lipid extracted microalgae. On the other hand, fruit pulp generated 230 mV [426]. These results were parallel with the present investigation. M-MFC showed highest internal resistance (370 Ω) among all the MFCs. M-MFC showed highest internal resistance (370 ± 0.10 Ω) among all the MFCs. F-MFC and N-MFC had internal resistance of 285 ± 0.44 Ω and 303 ± 0.39 Ω correspondingly. In order to reduce internal resistance and boost power production in the MFC, an appropriate biofilm attachment must be established over the anode surface. Besides this, the value of the internal

resistance and current production are strongly influenced by the anode biofilm, substrate and electrode-electrolyte proximity. Figure 4. 4 depicts SEM images of anode biofilms from each MFC.

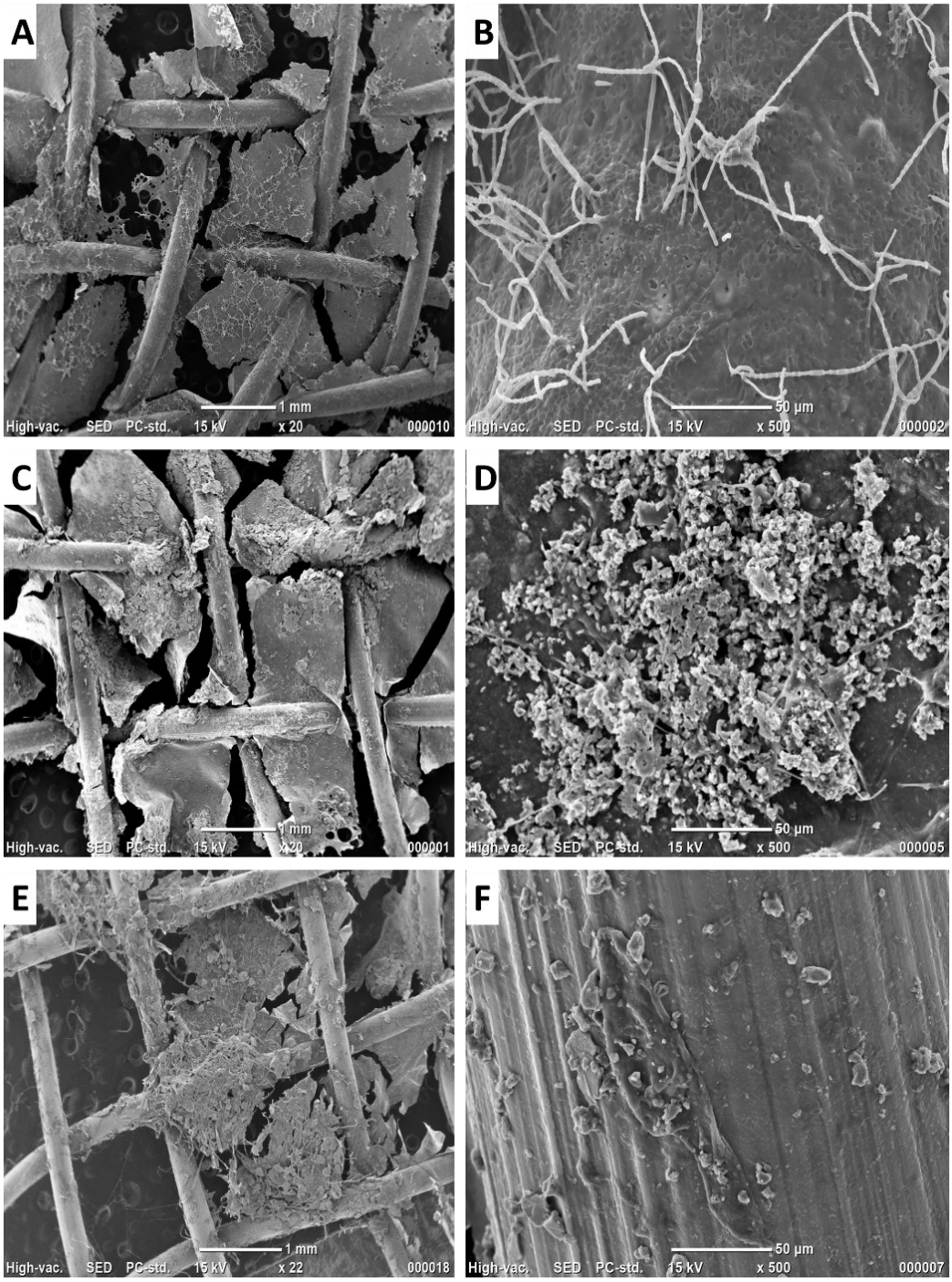

Figure 4. 4. SEM images of anode biofilm: (A, B) F -MFC; (C, D) N-MFC; (E, F) M-MFC

The SEM images demonstrated that microorganisms were grown on the stainless-steel mesh surface as biofilm. Microbial clusters were detected over the anode surface. The SEM images showed densely-packed thick biofilms and irregularly distributed microbial cells along with several cell aggregates. SEM images of F-MFC anode biofilms depicted some filamentous microbial attachments to the anode surface. SEM images of N-MFC described round aggregates of microorganisms on 500X resolution (Figure 4. 4 D). Also, few microbial clusters along with filamentous attachments were noticed on the anode surface of M-MFC (Figure 4. 4 E and F). Polarisation curves for F-MFC, N-MFC and M-MFC have been shown in Figure 4. 5.

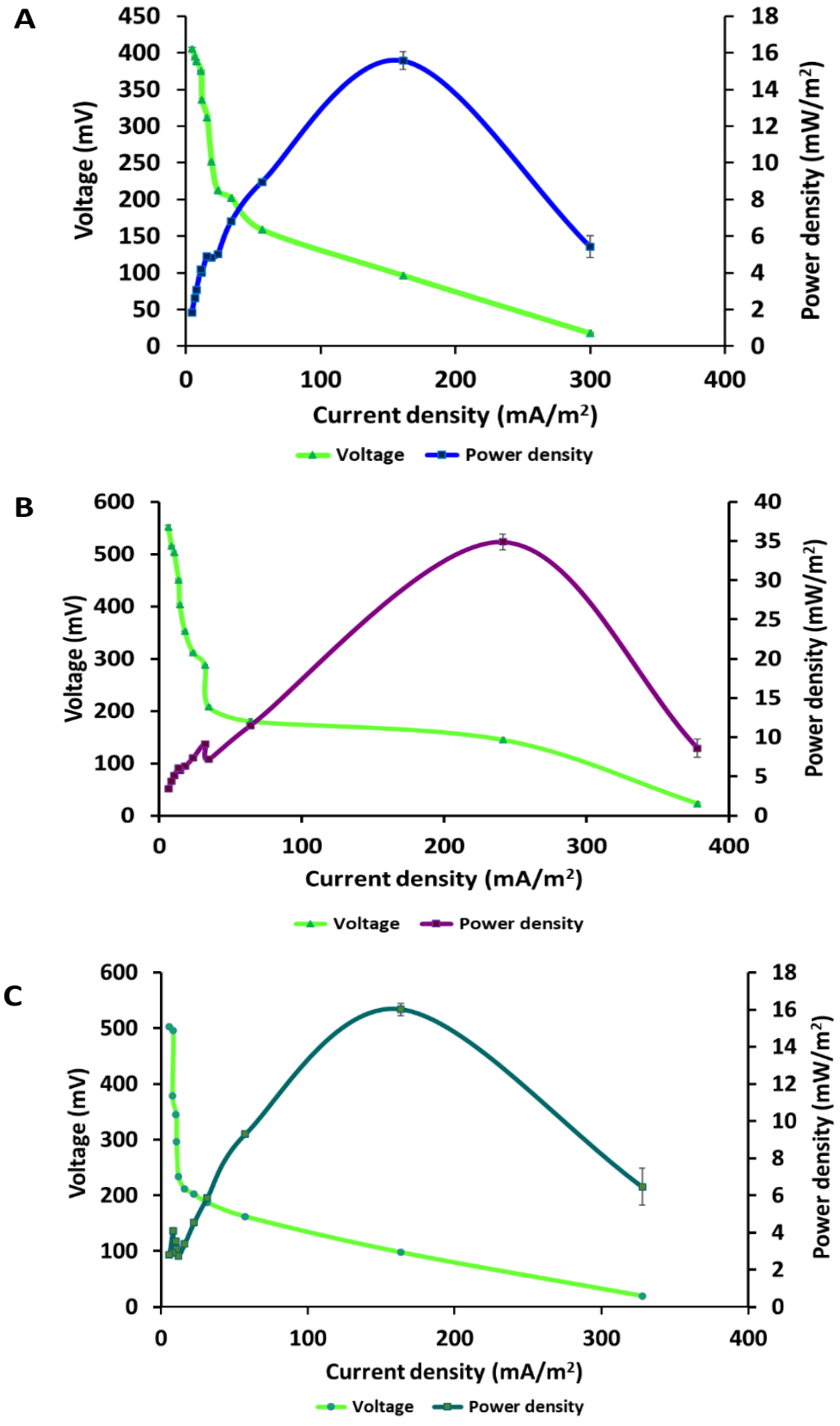

Figure 4. 5. Polarization curve (A) F-MFC; (B) N-MFC; (C) M-MFC

In the present work, production of cumulative voltage, current and power was evaluated under steady-state condition for various R_{ext} values (10-15000 Ω). As the values of the external resistance were increased, the voltage across the MFCs escalated substantially. The cumulative power density increased with a drop in the voltage and external resistance (15,000 to 10 Ω). N-MFC was able to achieve highest power density (34.88 ±1.00 mW/m^2) among all the MFCs coupled with a current density of 241.11 ± 3.46 mA/m^2. A study reported maximum power density of 44.05 mW/m^2 using *Cellulomonas iranensis* LZ-P1 from paper industry effluent [425]. On the other hand, F-MFC and M-MFC achieved almost similar maximum power density of 15.57 ± 0.49 mW/m^2 (at 161.1 ± 2.54 mA/m^2) and 16.00 ± 0.32 mW/m^2 (at 163.3 ± 1.66 mA/m^2), correspondingly. The chosen substrates, NP, FB and MB may have lower energy content. This can result in reduced metabolic activity and lower power output by the microbial community in the anode chamber. Also, mass transfer limitations, high internal resistance of salt bridge might be a reason of lower power densities in this study. Makhtar and Tajarudin, (2020a) recorded power density of 23.75 mW/m^2 and 12.65 mW/m^2 from banana peel powder and corn bran, individually [424]. A study reported maximum power density of 8.67 W/m^3 using microalgae biomass as a substrate at anode chamber [427]. *Chlorella vulgaris* and *Dunaliella tertiolecta* were fed in the anode chamber of MFC and maximum power density of 15.0 mW/m^2 and 5.3 mW/m^2 were achieved separately [428]. In the present work, MFC fed with microalgae powder at anode generated maximum power density of 16.00 ± 0.32 mW/m^2.

4.3.4 Substrate Degradation and Nutrient Removal

F-MFC, N-MFC and M-MFC were employed to generate electrical energy by using FP, NP and MB as cellulosic substrates at anode chamber. BBM-SEO catholyte was treated by using microalgae. High substrate degradation and high-power yield are crucial parameters to evaluate MFC performance on the basis of waste treatment and electricity generation. In order to

evaluate the biodegradability of the FP, NP and MB, the removal efficiency of COD, reducing sugar, total sugar and coulombic efficiency of MFCs were investigated. In third cycle, F-MFC, N-MFC and M-MFC have final total sugar concentration up to 9354 ± 31.82 mg/L, 6187 ± 125.00 mg/L and 5666 ± 72.16 mg/L respectively. Final reducing sugar remaining in the F-MFC, N-MFC, and M-MFC were 2708 ± 130.10 mg/L, 104 ± 63.64 mg/L and 83 ± 36.08 mg/L. At anode, pH values decreased to 5.1, 5.7 and 5.3 for F-MFC, N-MFC and M-MFC respectively. At cathode pH have been increased to 10.7, 11.2 and 11.7 for F-MFC, N-MFC and M-MFC, respectively. Table 4. 3 shows the COD removal and coulombic efficiency of each MFC together with the final amount of nitrate-nitrogen, ammonium-nitrogen, phosphate-phosphorous and OG remaining in BBM-SEO.

Table 4. 3 Substrate degradation profile of anolyte and catholyte

MFC	COD removal or TE (%)	Oil and grease (mg/L) (3rd cycle)	Nitrate-nitrogen (mg/L)	Ammonium-nitrogen (mg/L)	Phosphate-phosphorous (mg/L)	Coulombic efficiency (%)
F-MFC	Anode: 65.13%	-	-	-	-	2.7%
	Cathode: 57.33%	8753 ± 20.81	69.96 ± 2.46	1.97 ± 0.34	15.58 ± 0.68	
N-MFC	Anode: 74.38%	-	-	-	-	2.1%
	Cathode: 54.17%	8103 ± 25.16	81.25 ± 4.96	1.38 ± 0.34	23.47 ± 0.68	
M-MFC	Anode: 86.25%	-	-	-	-	1.5%
	Cathode: 71.56%	9960 ± 30	87.67 ± 0.43	1.18 ± 0.59	25.24 ± 0.90	

At anode chamber of all the MFCs, COD removal efficiencies were between 65 to 86%. Moderate COD removal was observed at cathode chamber in all the MFCs which was between 54-71%. Very low OG removal was observed in all the MFCs in a time period of 30 days. The OG percentage removal were 37.7%, 42.3% and 29.1% for F-MFC, N-MFC and M-MFC respectively. Microalgae have the ability to absorb hydrocarbons from the wastewater through a process called bioaccumulation [429]. The oil compounds are adsorbed onto the cell surfaces or taken up into the cells. The absorbed hydrocarbons are metabolized by the microalgae through various enzymatic pathways [429]. Microalgae have the ability to metabolize and degrade hydrocarbons found in petroleum oil contaminated wastewater. However, complete decomposition of oil solely through microalgae activity is debatable [430]. Microalgae can significantly reduce the concentration of oil contaminants, complete decomposition may require a combination of biological, chemical, and physical processes[429]. Up to 76-80% nitrate-nitrogen, 96-97% ammonium-nitrogen, 90-94% phosphate-phosphorous removal was observed in all the MFCs. Microalgae recover nitrate, phosphate, and ammonia through active transport mechanisms and subsequent metabolic processes. Nitrate is actively up taken through nitrate transporters on cell membranes, reduced to nitrite by nitrate reductase, and converted to ammonium by nitrite reductase [431]. Phosphate is taken up via phosphate transporters and utilized for energy production, nucleic acid synthesis, and phospholipid formation. Ammonia can be directly absorbed by cells through passive diffusion or actively transported via ammonium transporters [431]. Ammonia is used for the synthesis of amino acids, proteins and other nitrogen-containing compounds [431] .

4.3.5 Dried Algae Biomass Yield

At the end of third cycle, 280 mg/L, 320 mg/L and 286 mg/L of dried algae biomass were obtained from F-MFC, N-MFC and M-MFC respectively. Gajda et al. (2015) developed a membrane fuel cell (MFC) with anaerobic biofilm at the anode half-cell that produced current and algae biofilm at the cathode [404]. This arrangement enhanced ORR. Authors reported 250 mg/L of algal biomass from the cathode. Another study reported a yield of 240 mg/L of *Chlorella pyrenoidosa* biomass using diluted cow urine as catholyte [325].

4.3.6 Microbial Community and Diversity

V3-V4 amplicon region of F-MFC, N-MFC, and M-MFC had GC content of 54.5%, 52.5% and 52.5% respectively. The main phyla in all three anodic biofilms were Proteobacteria, Firmicutes and Bacteroidetes. In F-MFC, 53.8% Proteobacteria, 41.9% Firmicutes and 3.8% Bacteroidetes were found. While N-MFC had 41.1% Proteobacteria, 26.1% Firmicutes and 32.5% Bacteroidetes. M-MFC comprises 43.9% Proteobacteria, 29.4% Firmicutes and 26.4% Bacteroidetes. Figure 4. **6** to Figure 4. **9** depicts taxonomic classification of microbial community analysis of anode biofilms.

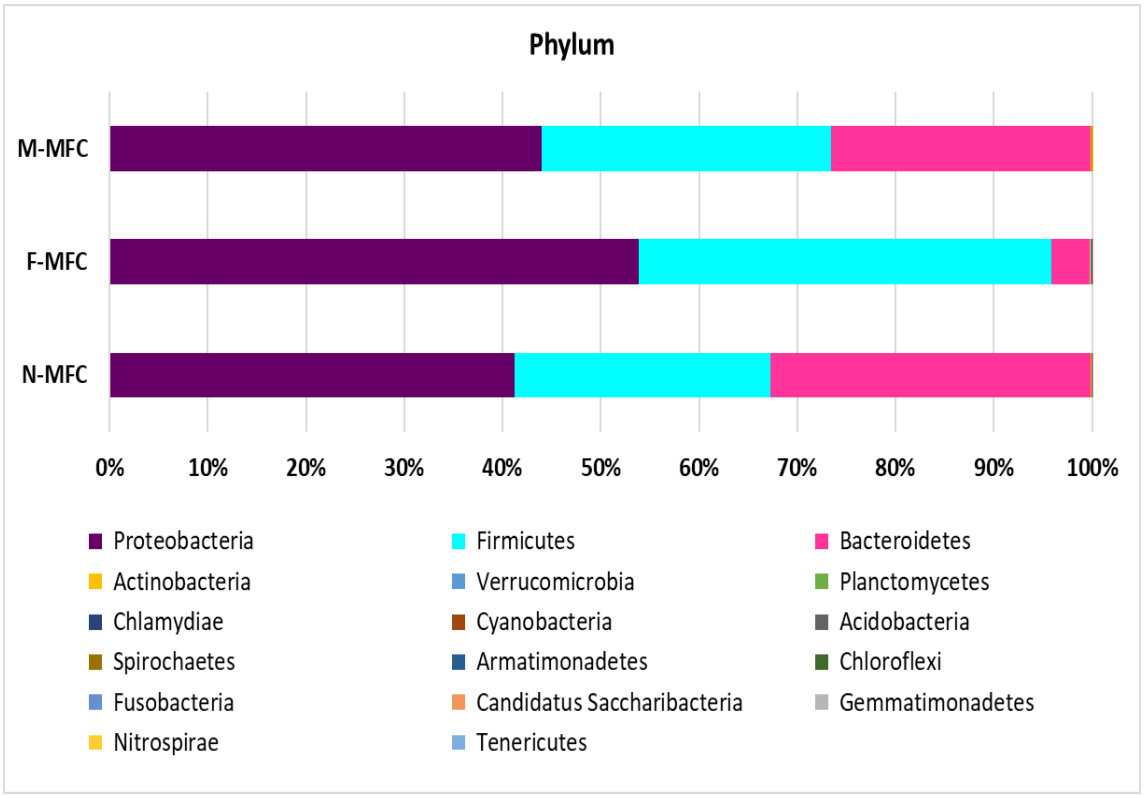

Figure 4. 6. Phyla obtained in microbial community analysis of anode biofilms

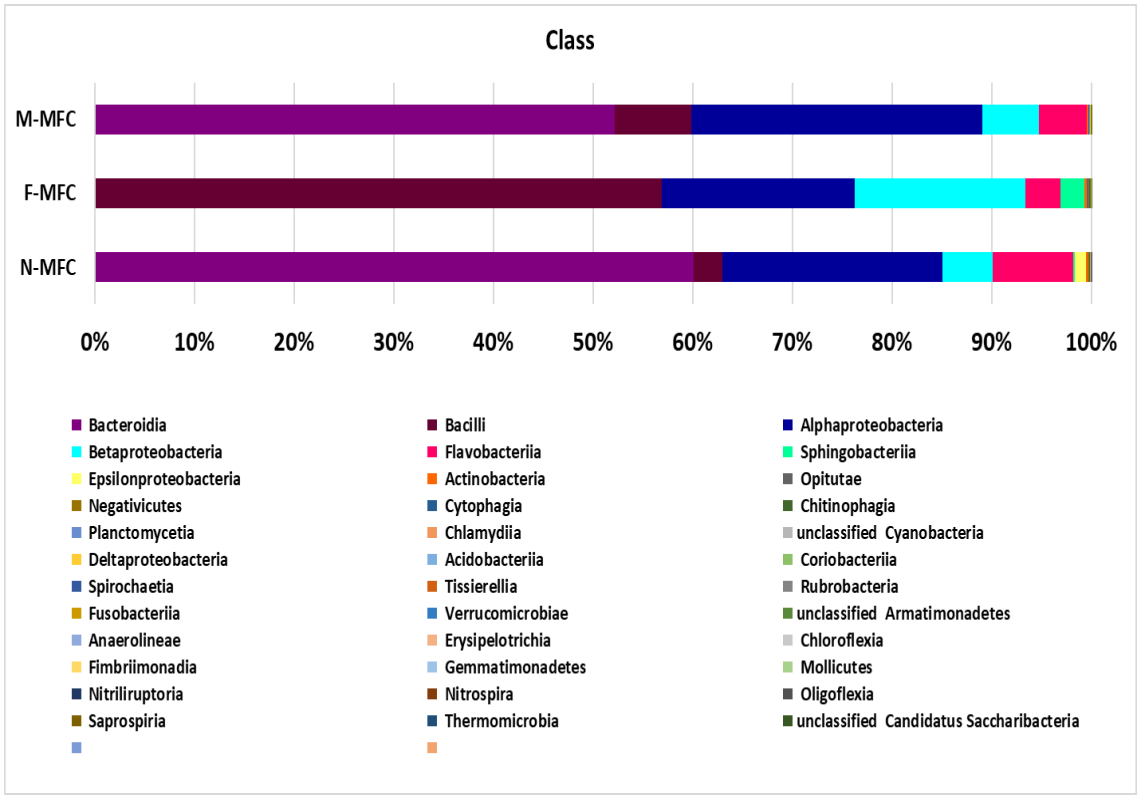

Figure 4. 7 Most abundant classes of microorganism present in anode biofilms

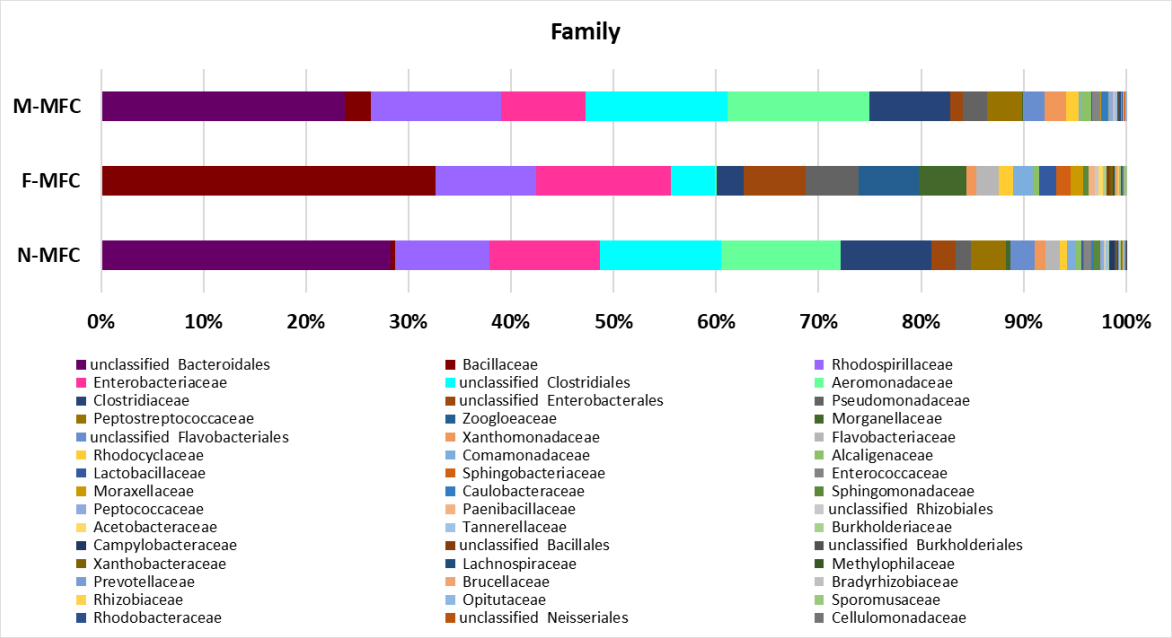

Figure 4. 8 Microbial community analysis of anode biofilms at family level

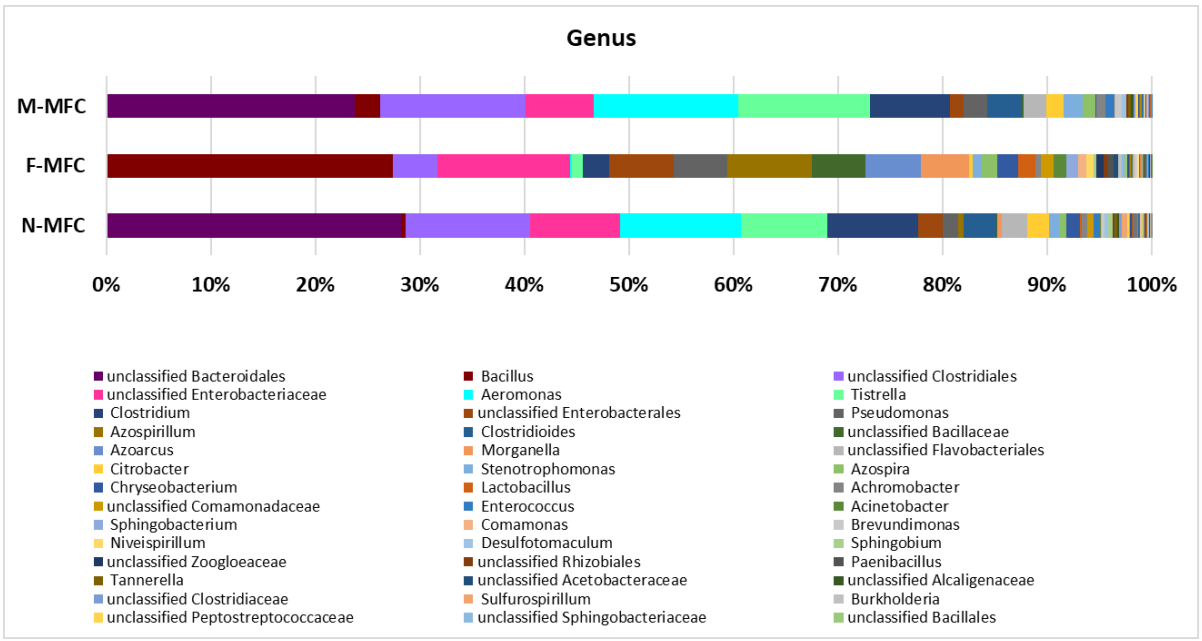

Figure 4. 9 Microbial community analysis of anode biofilms at genus level

The phylum Proteobacteria is the one that is found in anodic biofilm commonly followed by the Firmicutes and Bacteroidetes [432]. At the class level, Gammaproteobacteria (27-31%), Clostridia (6-25%), Bacteroidia (0.08-28%), Bacilli (1-34%), Alphaproteobacteria (10-13%), Betaproteobacteria (2-10%), Flavobacteria (2-3%), Sphingobacteriia (0.04 -1%) and Epsilonproteobacteria (0-0.5%) were the most abundant classes observed in all the MFCs. Microbial analysis of the genera disclosed that the most dominant genera in all the three anodic biofilms were unclassified *Bacteroidales* (0.03-28%), *Bacillus* (0.4-27%), unclassified *Clostridiales* (4-13%), unclassified *Enterobacteriaceae* (6-12%), *Aeromonas* (0.1-13%), *Tistrella* (1-12%), *Clostridium* (2-8%), unclassified *Enterobacterales* (1-6%) and *Pseudomonas* (1-5%). Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and deltaproteobacteria are well known class in the electrogenic microbial community [432]. In order to examine the differences in the microbial community structures at the genus level of the anodic biofilms, a heat map was created using cluster analysis.

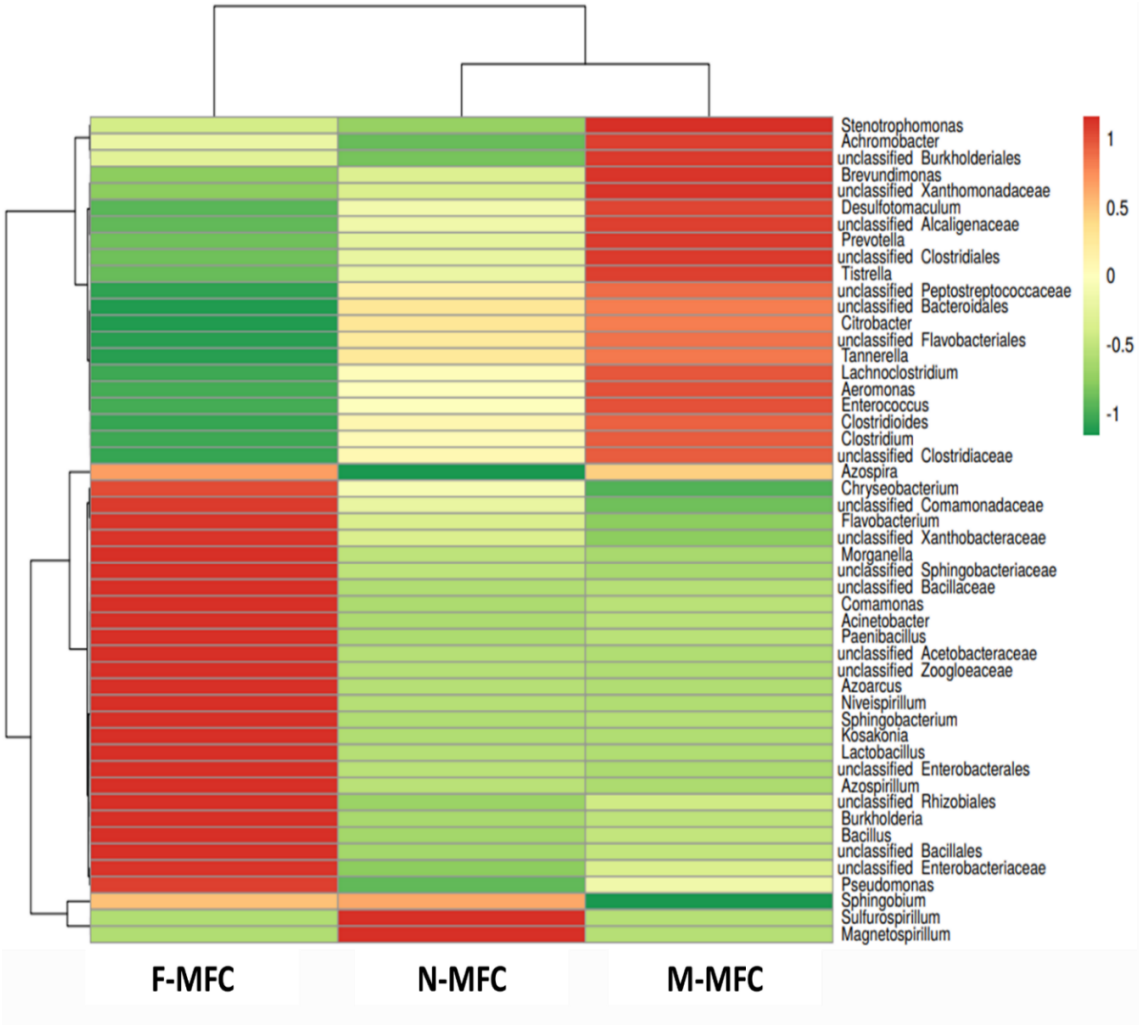

Figure 4. 10. Phylogenetically clustered heat map of representative genera in anodic biofilms. At the top right, the intensity of color in each panel describes the relative abundance (%) of a genus.

It became evident from Figure 4. 10 showed there was a shift in the bacterial genera present within the microbial assemblies of biofilm at the level of the genus. Microbial community analysis of anodic biofilms of all the MFCs revealed potential association of electrogens such as *Clostridium, Pseudomonas, Lactobacillus* and *Enterobacter*. The electroactive biofilm of F-MFC was found to have more species as compared to N-MFC and M-MFC's anodic biofilm. *Pseudomonas and Enterobacter* are a well-recognized electrogenic bacteria, found in the anode biofilms, usually. Well known exoelectrogen, *Geobacter* and *Shewanella* were not observed in

the anode biofilm of any MFC. The amount of variation present within the microbial community is closely correlated with the organic source consumed by the microorganisms. These organic substrates are suitable for the utilisation by fermentative or electrogenic bacteria, which produce metabolites that electrogenic microbes consume.

4.4 Conclusions

In the present work, newly designed concentric-dual-chambered photo-MFC has successfully illustrated its effectiveness for the breakdown of SEO and cellulosic waste (FP, NP and MB). The power density of N-MFC was the highest among all the MFCs (34.88 ± 1.00 mW/m^2). The COD removal efficiency of the anode chambers of the all MFCs varied from 65 to 86%. M-MFC was able to achieve a maximum COD removal at the anode chamber, which was 86.25%. The percentages of oil and grease that were removed by *Chlorella sorkiniana* MVVSVM IIT BHU in F-MFC, N-MFC, and M-MFC were 37.7%, 42.3% and 29.1% respectively. In each MFC, 76-80% of the nitrate–nitrogen, 96-97% of the ammonium–nitrogen, and 90-94% of the phosphate–phosphorous were removed. Microbial community analysis reported Proteobacteria, Firmicutes and Bacteroidetes were the predominant phyla in all the anodic biofilms. However, more improvements in the scale up by stacking arrangement of such microalgae photobioreactor combined with MFCs are needed in future. CO_2 generated by the degradation of dried microalgae powder at anode could be supplied to the fresh microalgae cells at cathode to enhance the microalgae growth rate also.