Chapter 5

Bioelectricity generation by microbial degradation of banana peel waste biomass in a dual-chamber *S. cerevisiae*-based microbial fuel cell

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5.1 Introduction

Electricity has a crucial role in the human lifestyle. The rise in human population and the industrial sector have enhanced the electricity demand. Electricity production depends largely on the consumption of fossil fuels [433]. Dependency on fossil fuels brings many negative consequences to the sustainable development of the environment. Many efforts are being made continuously to acquire a sustainable and efficient source of renewable energy. Renewable energy technologies which utilize waste materials (agricultural waste, food waste and animal waste) to generate energy are highly expected to tackle environmental issues like pollution and climate change. Fuel cell technology has engrossed more interest due to its optimistic qualities, such as compatibility, cleanliness and compactness. It is also a convenient source of power generation [77]. Microbial fuel cell (MFC) is a division of fuel cell technology that generates sustainable bioelectricity from biomass using microorganisms. MFC is an innovative method for valorising organic substrates/waste to generate bioelectricity. Waste management policies also advocate the concept of energy recovery from waste before its direct disposal [409]. MFC setup is like a battery consisting of an anode and a cathode. Oxygen is the most common electron acceptor at the cathode (a non-toxic waste product) and forms water by combining with protons generated from the anode [77]. Glucose, lactate, acetate, sucrose and wastewater are the most commonly used substrates in MFCs [410]. Lignocellulosic waste like agriculture debris, rotten fruit or vegetables and their peels are the abundantly available bio-conversable substrates. The financial viability and productivity of transforming waste into bioenergy depend on the components and characteristics of the waste material. Researchers have explored lignocellulosic waste in the form of hydrolysates or powder to produce bioelectricity [434].

Fruit peel waste is also a form of lignocellulosic waste. It is made up of too many carbon sources like cellulose, sucrose, glucose, Fructose protein, flavonoid, vitamins, etc. Hence, it is a source of potential energy in the electrogenic microorganisms for bioelectricity production [336]. Grape waste has been evaluated as substrate in single chamber MFCs using zinc, copper, magnesium electrodes, thionine and toluidine (red and blue) as mediators. Thionine based MFC generated comparatively higher voltages (2.5 V) than toluidine [339]. MFCs have also been evaluated by using papaya waste as substrate with carbon felt and magnesium oxide as anode and cathode, respectively. Papaya based MFC generated power density up to 0.75 to 0.81 mW cm^{-2} [340]. Miran et al. (2016a; 2016b) used orange and lemon peel waste as substrates in MFC [23], [338]. As a result, Utilization of fruit and vegetable peel waste as a carbon source in MFC for power generation is an ensuring option for food waste management and energy recovery. Bananas (*Musa paradisiaca*) are abundantly accessible in tropical and subtropical zones across 130 nations. In 2019, 116 million tonnes of bananas were produced worldwide, and the fruits was available all-round the year. The typical fruit weighs around 125 grams, of which 75% is water and 25% is dry substance. China produced 11.6 million tonnes and India 30.4 million tonnes of bananas in 2019. Indonesia was third with 7.2 million tons, followed by Brazil and Ecuador with 6.8 and 6.5 million tonnes, respectively. BW is available throughout the year in India and neighbouring countries. The massive quantity of BW originates from fruit markets and fruit processing industries every day. This shows that managing fruit peel waste is huge project for the environmental safety. BW comprises 30 - 40% weight out of whole weight of banana fruit. It is made up of lignin (5-10 %), hemicellulose (6 - 8 %) and cellulose (60 - 65 %) [435]. Banana peel waste (BPW) is an organic waste rich in carbohydrates, proteins, phenolic compounds and essential macro or micronutrients, which promotes microbial growth [435]. BPW is accessible throughout the year in India and nearby countries. BPW can be a proper value-added substrate instead of their

direct disposal leading to environmental pollution. Moreover, banana peel is abundant in nutrients, which makes it an appropriate substrate for microbial growth and electricity generation in MFCs. *S. cerevisiae* (a eukaryote) is non-pathogenic, easy to handle and tolerant to extreme environmental conditions [16]. The involvement of *S. cerevisiae* is supposed to obtain alcohol after fermentation. The yield of alcohol is also advantageous as fuel if obtained as a by-product at the end of MFC operation [16]. In this chapter comparative analysis of the banana peel waste (BPW) and its potential to generate electrical energy in dried powdered and slurry forms under the influence of *S. cerevisiae* and indigenous microbial consortia have been investigated. A binder-free coating of activated charcoal on stainless steel mess anode has been done to enhance power generation. To the best of our knowledge, no study has yet analysed *Saccharomyces cerevisiae* as an anode biocatalyst with BPW as a substrate in MFC.

5.2 Materials and Methods

5.2.1 Substrate Collection and Preparation

Banana peel (*Musa paradisiaca*) waste was collected from the fruit juice shop inside the university campus in September 2022 (37ºC). Banana peel used in MFC was processed as dried banana peel powder and banana peel slurry.

5.2.1.1 Preparation of Dried Banana Peel Powder

The waste banana peel was dried in a hot air oven at 105°C overnight in order to reduce the moisture content. The dried peels were then ground into a ball mill and the powder obtained was sieved through 44 mesh-sized sieves (Retsch AS200, make Germany). The powder obtained after sieving was packed into airtight polyethene bags till further use.

5.2.1.2 Preparation of Banana Slurry

The fresh banana peel was washed and ground in a mixer grinder. Banana peel paste obtained after grinding was used for the preparation of the substrate.

5.2.1.3 Substrate Preparation

60.0 g of banana peel powder was added into 3L of distilled water to prepare 20 $g.L^{-1}$ dried banana peel wastewater (BDW) which was used as an anodic substrate for MFCs. The banana slurry wastewater (BSW) was prepared by mixing 100 mL of banana peel paste in 1.9 L of distilled water. BDW and BSW were stored in a refrigerator (at 4ºC) for repeated use in all cycles of MFC operation. Another substrate, Yeast Extract-Peptone-Dextrose (YPD) medium (yeast extract 10 $g.L^{-1}$, Peptone 20 $g.L^{-1}$, Dextrose 20 $g.L^{-1}$) was prepared for comparative analysis.

5.2.1.4 Substrate characterization

Elemental analysis of substrate was done by CHNS (O) Analyzer (Euro EA 3000, Elemental analyser, made in Italy). Ash [376], moisture [377], volatile content [378] and total carbon content were analysed by proximate analysis as provided in American Society for Testing and Materials methods (ASTM) [379]. The total sugar content of BDW and BSW were determined by the Anthron method and reducing sugar was determined by the DNSA (3,5-Dinitrosalicylic acid) technique. The pH of the BDW and BSW is determined by a pH meter (Eutech pH tutor, USA). The chemical oxygen demand (COD) of prepared BDW and BSW is determined by the closed reflux method [380]. Standard methods of APHA were used to analyse the concentration of total dissolve solids (TDS), total suspended solids (TSS), total fixed solids (TFS), total volatile solids (TVS) and total solids (TS) present in the BDW and BSW [380].

5.2.2 Anode biocatalyst

Baker's instant yeast *Saccharomyces cerevisiae* (KOTHARI'S-Four Season Instant yeast, make India) was used as inoculum at the anode.

5.2.3 MFC construction

Dual-chambered H-shaped MFCs (Figure 5. 1) was constructed by using two 500 mL containers with similar anode and cathode characteristics in terms of surface area, material and orientation. A traditional "H" shape dual chamber MFC comprised of two bottles of the same volume, connected by a tube holding a separator inside, which is usually a proton exchange membrane or a basic salt bridge [436]. The anode is prepared with rectangular (60 cm^2) 304 stainless steel mesh (mesh size 13) modified with binder-free activated charcoal (Himedia, India). Activated carbon was dispersed in ethanol to make an even dispersion without additional additives. Single-layer activated charcoal-stainless steel (AC-SSM) anodes were set up by soaking followed by drying process [437]. However, in the present work, activated charcoal was used instead of carbon black due to the higher surface area-to-volume ratio [438]. The AC-SSM electrode was prepared by treating stainless-steel mesh with 1 M H_2SO_4 for 24 hours; in order to remove surface oxides and to obtain a coarse surface. Further, it was soaked in 10 g L^{-1} activated charcoal dispersion in ethanol. The electrode was allowed to dry at room temperature (25±2 ^oC). AC-SSM was prepared, after dipping and drying for five consecutive cycles [437]. Cylindrical graphite rods of 10 cm length and 0.5 cm radius were used as cathodes. Copper wires were used as connectors between electrodes. An external resistance of 1000 Ω was applied to each MFC. 0.1 M phosphate buffer ($Na_2HPO_4.7H_2O$ and $NaH_2PO_4.H_2O$) of pH 7.4 was used as catholyte.

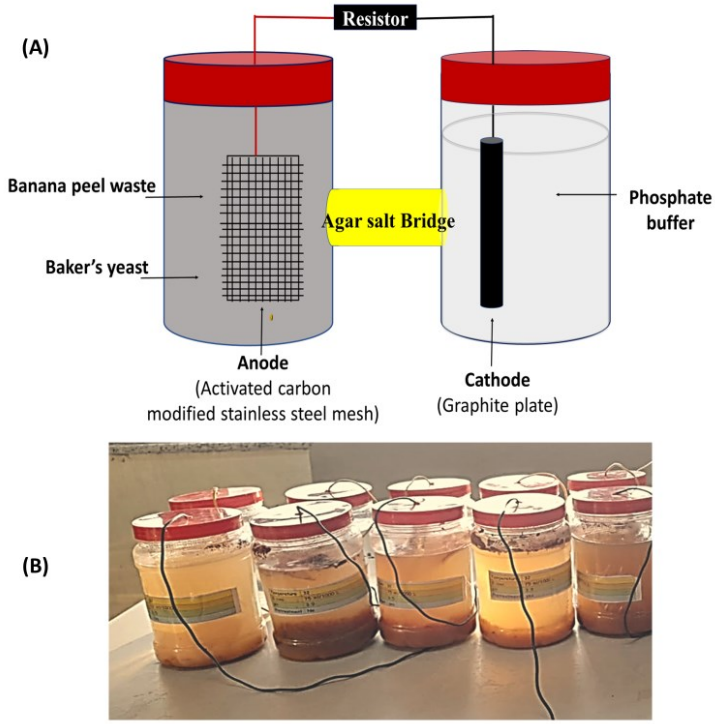

Figure 5. 1. (A) Graphical illustration of H-shaped dual-chamber MFC setup; (B) Actual setup of H-shaped MFCs

5.2.4 MFC operation

Five dual-chamber H-shaped MFCs (working volume 300 mL) was designed, fabricated and operated as shown in Table 5. 1. Unsterilized dried banana peel powder (20 $g.L^{-1}$) and banana peel slurry (50 $ml.L^{-1}$) were used as substrate at the anode inoculated with *S. cerevisiae* (1%) and named as BDY- MFC and BSY- MFC, respectively. Dried banana peel (20 $g.L^{-1}$) (BD-MFC) and banana peel slurry (BS-MFC) (50 mL. L^{-1}) were used as substrate at anode without any inoculum (indigenous microbes might be present). Another MFC was prepared by using a YPD medium as substrate, inoculated with *S. cerevisiae* inoculum. YPD-grown *S. cerevisiae* based MFC was used for the purpose of comparison and performance evaluation of banana peel waste as a substrate. Table 5. 1 shows the substrate and inoculum combination used in this study.

Table 5. 1. Substrate and inoculum used at the anode

MFC	Substrate	Anode biocatalyst
Y-MFC	YPD Medium	*Saccharomyces cerevisiae*
BD- MFC	Dried banana peel powder (20 $g.L^{-1}$)	-
BDY- MFC	Dried banana peel powder (20 $g.L^{-1}$)	*Saccharomyces cerevisiae*
BS- MFC	Banana peel slurry (50 $mL.L^{-1}$)	-
BSY-MFC	Banana peel slurry (50 $mL.L^{-1}$)	*Saccharomyces cerevisiae*

All the MFCs were operated in batch mode at room temperature and acclimatized for 5 days (stable voltage generation) with their respective substrate and inoculums. Observation was recorded after 5 days of acclimation. Once, the acclimation period was over, all MFCs were repeatedly refilled after every 10 days with their respective inoculant (*Saccharomyces cerevisiae*) and substrate (BSW or BDW), thereby forming an operation cycle. All MFCs were operated continuously for 30 days.

5.2.5 MFC characterization

Voltage was recorded by using a multimeter (Mextech Mas 830l, make India). Once the voltage was stabilized for several hours, polarization study was performed. MFCs were connected to resistors ranging from 15000 Ω to 10Ω for studying the polarization behavior of the MFC. A multimeter was used to measure the voltage of MFC at each resistance. Current (I) was calculated by using Ohm's law (Eq. 5.1)

$$I = \frac{V}{R} \tag{5.1}$$

where V denotes the voltage (mV), R denotes resistance in ohm (Ω) and I represents the current (mA). Power (P) is calculated by Eq. 5.2:

$$P = I.V \tag{5.2}$$

In order to obtain power density (PD) in $mW.m^{-2}$, P was divided by the surface area (A) of the anode (0.006 m^2) (Eq. 5.3) [387].

$$PD = \frac{P}{A} \tag{5.3}$$

The current density (J) in $mA.m^{-2}$ was calculated by Eq. 5.4 and 'I' was divided by the surface area (A) of the anode (0.006 m^2) [387].

$$J = \frac{I}{A} \tag{5.4}$$

The internal resistance (R_{int}) was calculated from the slope of the current vs. voltage plots [386] (Eq. 5.5):

$$V = E_{cell} - I\,R_{int} \tag{5.5}$$

where, E_{cell} is the electromotive force and R_{int} is the internal resistance of MFC.

At the end of the operation, the anolyte was withdrawn from the MFC and the COD (mg. L^{-1}) was measured. The COD removal efficiency represents the percentage of COD eliminated throughout the operational time. It was determined by Eq. 5.6 [387].

$$COD\ \% = 100 \times \frac{COD_i - COD_f}{COD_i} \tag{5.6}$$

where, COD_i and COD_f are the initial and final COD of the wastewater. Columbic efficiency (CE) was calculated by integrating the current (I) measured over time (t) and comparing it with the theoretical current on the basis of change in chemical oxygen demand (△COD) removal (Eq. 5.7) [388]:

$$CE = \frac{8 \int_0^t I \, d(t)}{F V_{anode} \Delta COD} \times 100 \quad (5.7)$$

where, 8 is a constant used for COD, based on MO_2= 32 g $mole^{-1}$, 4 electrons exchanged per mole of oxygen, F is the Faraday's constant (96485 $Cmole^{-1}$ - electrons), V_{anode} is the volume of anode chamber and ΔCOD is the change the COD over time (t).

5.2.6 Sampling, PCR amplification, Metagenomic analysis

Microbial community analysis was carried out for banana slurry fed into the BS-MFC. The sample was centrifuged at 10,000 rpm for 10 minutes and preserved at 80 °C. DNA extraction is done by using the commercially available kit. DNA extraction was done as per the manufacturer's instructions. Extracted DNA from the samples was subjected to Nano Drop and GEL Check before being taken for PCR amplification: The Nano Drop readings of 260 to 280 at an average value of 1.8 to 2.0 is used to determine the DNA's quality. Primers 16sF:5'AGAGTTTGATGMTGGCTCAG3' and 16sR:5'TTACCGCGGCMGCSGGCAC3' were used for the PCR amplification of bacterial 16S rRNA genes containing V3 to V4 variable regions. 40 ng of extracted DNA is used for amplification along with 10pM of each primer. 25 cycles of the PCR were carried out. Ampure beads were used to remove unused primers to obtain purified amplicons. In order to prepare the sequencing libraries 8 cycles of PCR were performed by using Illumina barcoded adapters. Ampure beads were used to prepare purified libraries. Libraries were quantified by Qubit dsDNA high sensitivity assay kit (Thermo Fisher Scientific). Illumina Miseq with 2x300PE v3 sequencing kit was used for sequencing. The amplified 16s PCR product was purified and subjected to GEL Check and Nanodrop QC. Raw data quality control (QC) was done by using Fastqc (Version 0.11.9) and Multiqc (Version 1.10.1) tools, followed by trimming of adapters and low-quality reads by TRIMGALO+21RE. The trimmed reads were further processed, which included the merging of paired-end reads, chimera removal, OUT abundance calculation and estimation correction.

Above mentioned processing was achieved by QIIME, MOTHUR, KRAKEN and BRACKEN workflows. These workflows enable highly accurate investigations at the genus level. The database used for the 16s V3-V4 region in NCBI. The 16S workflow is useful in identifying pathogens in a mixed sample and understanding the composition of a microbial community.

5.2.7 Statistical Analysis

All experiments were carried out in triplicate and average values were reported. All results and error bars were plotted using Microsoft Excel (Version 2019). The calculation of mean values, standard deviation and standard error was calculated by Microsoft Excel (Version 2019).

5.3 Results and Discussion

5.3.1 Anode modification by activated charcoal

Figure 5. **2**A and 5.2B show the scanning electron micrograph of unmodified SSM and modified AC-SSM anodes, respectively. AC-SSM composite anodes were made by adsorbing a single thin layer of activated charcoal onto the SSM surface by means of a binder-free dipping and drying method. The thin layer of activated charcoal on SSM improves the microbial adhesion at the surface of the anode and makes extracellular electron transfer easier between the *S. cerevisiae* and the anode. It is helpful in boosting the current generation [437]. SEM images of anode biofilm from each MFC are shown in Figure 5. 2. The AC-SSM anode biofilm was characterized (after 30-days) by SEM to determine the possibility of *S. cerevisiae* anode biofilm attachment. Figure 5. **2**B shows the SEM of bare AC-SSM anode before the experiment, whereas Figure 5. **2**C, 5.2D, 5.2E, 5.2F and 5.2G shows the SEM image of Y-MFC, BDY-MFC, BS-MFC, BSY-MFC and BD-MFC respectively. Figure 5. 2 exposed the presence of plentiful microbial adhesion on the AC-SSM anode biofilm surface as compared

to the bare AC-SSM anode. The difference in the anodophillic morphology was due to the adhesion of particles from BSW, BDW, *S. cerevisiae and* other indigenous bacteria.

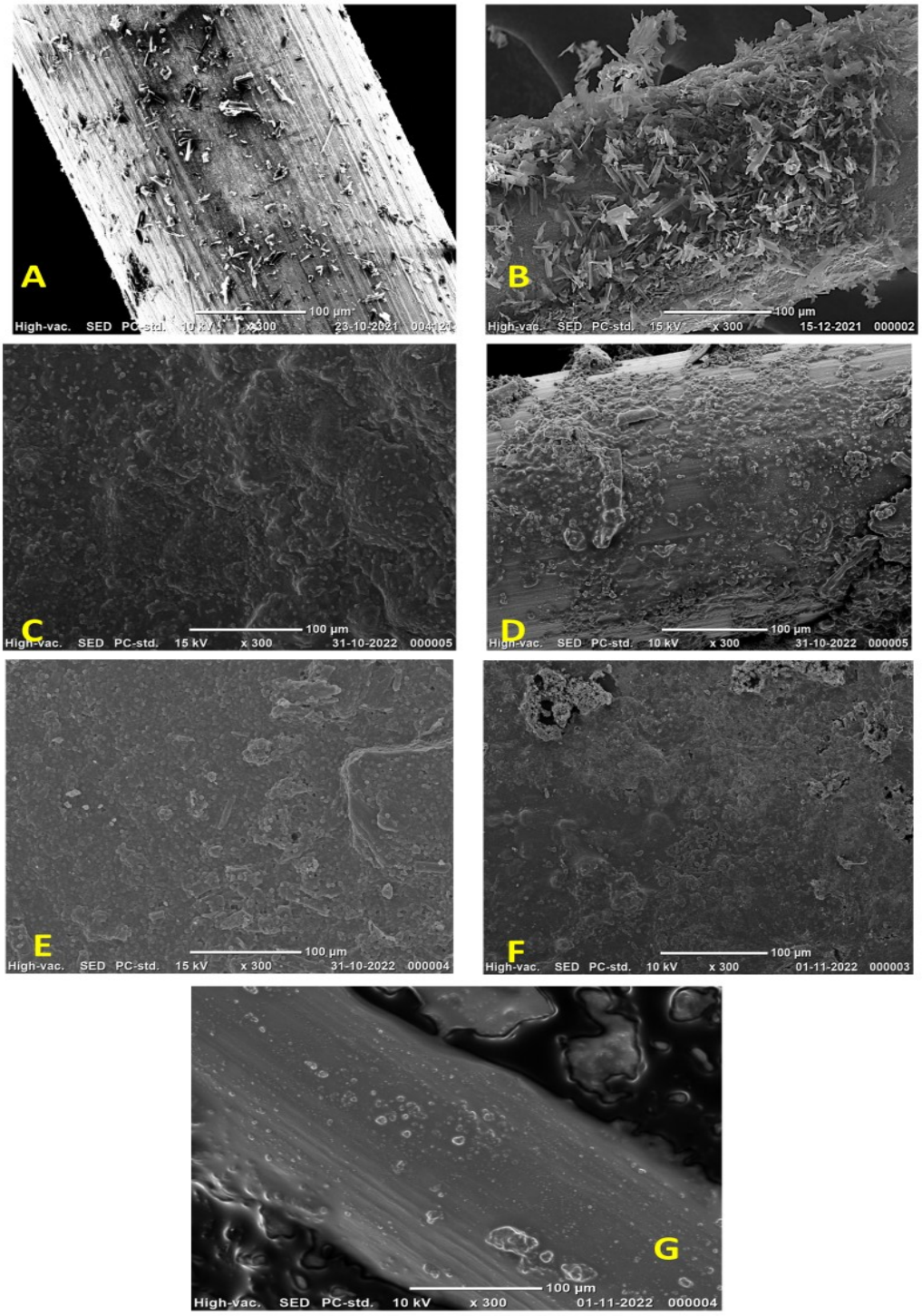

Figure 5. 2. SEM images of AC-SSM anode biofilm. (A) Unmodified SSM (B) Modified AC-SSM (C)Y-MFC; (D) BDY-MFC; (E) BS-MFC; (F) BSY-MFC; (G) BD-MFC

5.3.2 Ultimate and Proximate Analysis of Banana Peel

Table 5. 2 shows the % of C, H, N, S, moisture, ash, volatile and fixed carbon content present in the banana peel powder on the dry weight basis.

Table 5. 2. Ultimate analysis of Banana peel

	Amount (%)
C	41.65
H	6.80
N	2.18
S	0.78
Moisture content	4.5 ± 0.0
Ash	0.5 ± 0.0
Volatile content	94 ± 0.0
Fixed carbon content	0.7 ± 0.0

Elemental composition revealed that the banana peel powder was a suitable substrate for the microbial growth as it contained all the fundamental growth-supporting components. Makhtar and Tajarudin, (2020) also reported similar elemental composition in dried banana peels. Authors analysed the nutritional values of banana peel and observed ample amounts of crude carbohydrates, protein, lipids, fibers, macronutrients and micronutrients [439]. Table 5. 3 shows total sugar content, reducing sugar content and COD of BDW and BSW.

Table 5. 3. Characterization of banana peel-derived synthetic wastewater

	Wastewater using banana peel powder (BDW)	Banana slurry wastewater (BSW)
Total sugar (mg. L^{-1})	21458 ± 433	30416 ± 939
Reducing sugar (mg. L^{-1})	5964 ± 82	13684 ± 248
Initial COD (mg. L^{-1})	1126 ± 41	1366 ± 64
TDS (mg. L^{-1})	466 ± 57	1500 ± 100
TSS (mg. L^{-1})	1600 ± 100	2000 ± 173
TFS (mg. L^{-1})	600 ± 100	833 ± 115
TVS (mg. L^{-1})	1466 ± 208	2666 ± 57
TS (mg. L^{-1})	2066 ± 152	3500 ± 100
pH	7.5	7.9

BDW and BSW had COD of 1126 ± 41 mg. L^{-1} and 1366 ± 64 mg. L^{-1} respectively. BDW and BSW had total sugar content of 21458 ± 433 mg. L^{-1} and 30416 ± 939 mg. L^{-1}. The high sugar content in the BPW makes it a lucrative substrate for *S. cerevisiae*, which leads to amplification in power generation. The initial pH of BDW and BSW was 7.5 and 7.9, respectively. TDS, TSS, TFS, TVS and TS for BDW was 466 ± 57, 1600 ± 100, 600 ± 100, 1466 ± 208 and 2066 ± 152 mg L^{-1} respectively. TDS, TSS, TFS, TVS and TS were 1500 ± 100, 2000 ± 173, 833 ± 115, 2666 ± 57 and 3500 ± 100 mg. L^{-1} respectively, for BSW.

5.3.3 Analysis of Power Generation from MFC

BDW and BSW were evaluated as anodic substrates with or without *S. cerevisiae*. Stable generation of electricity was attained after the completion of acclimation period (5 days). The

Open circuit voltage (OCV) vs. time profile of YMFC, BD-MFC, BS-MF, BSY-MFC and BDY-MFC in the duration of 30 days (3 cycles) are shown in

Figure 5. **3**.

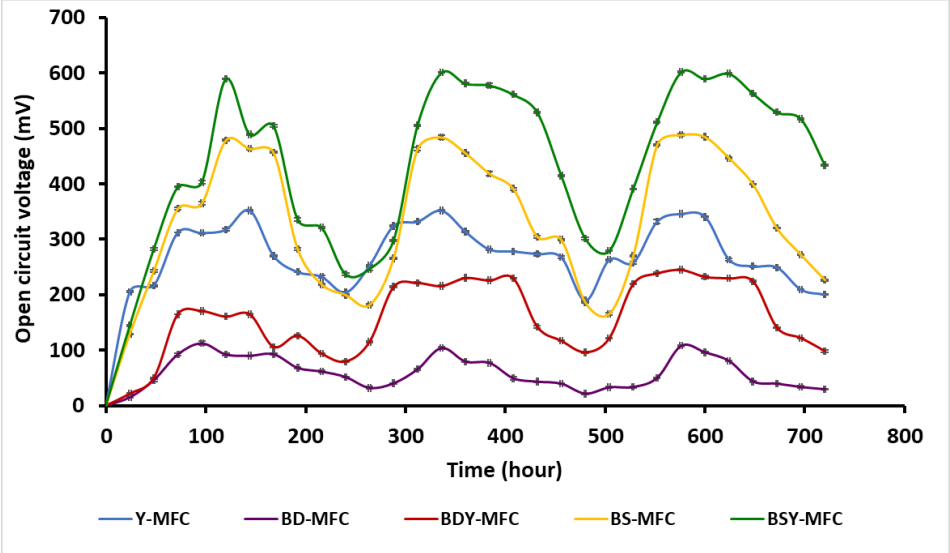

Figure 5. 3. Variation in open circuit voltage vs. time

The data obtained showed that there was a steady rise in OCV with the passage of time. The outcomes revealed that the highest OCV was produced from BSY-MFC (602 ± 1.5 mV) as compared to YMFC (352 ± 1.1 mV), BDY-MFC (245 ± 1.0 mV), BD-MFC (113 ± 3.2 mV) and BS- MFC (488 ± 1.5 mV). In BS-MFC, the increase in voltage occurred after 5th day and increase continuously till 8th day (first cycle) and achieved a maximum voltage of 488 ± 1.5 mV (third cycle), which is greater than the OCV obtained from YMFC in all the three cycles. The rise of high OCV in BS-MFC was probably due to the growth of indigenous microorganisms. Makhtar and Tajarudin, (2020) designed membrane-less MFC with dried banana peel powder as substrate and anaerobic sludge was added as inoculum at the anode. The authors obtained 271 mV of OCV [424]. However, in this study, BDY-MFC with dried

banana peel powder and *S. cerevisiae* obtained OCV of 245 ± 1.0 mV. Figure 5. 4 and Figure 5. 5 represent voltage and current profile over time at external resistance of 1000Ω.

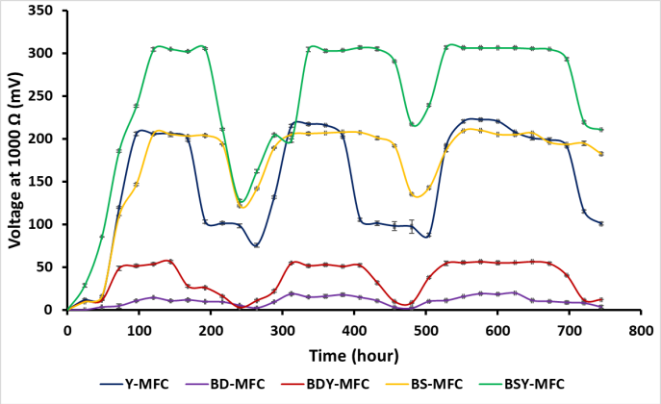

Figure 5. 4. Variation in voltage at 1000Ω (mV) vs. time

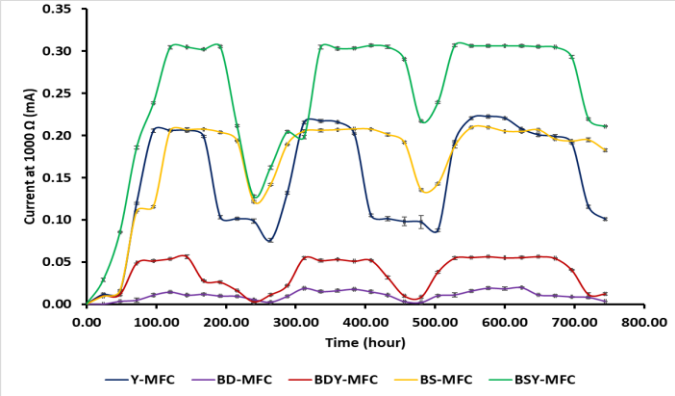

Figure 5. 5. Current at 1000 Ω (mA) vs. time profile

As shown in Figure 5. 4 and Figure 5. 5, voltage and current increased rapidly after the substrate was refilled into MFC (0 hours to 96 hours). Maximum output voltage reached approximately 222 ± 1.5 mV (Y-MFC), 20 ± 0.5 mV (BD-MFC), 56 ± 1.5 mV (BDY-MFC), 210 ± 0.5 mV (BS-MFC) and 307 ± 1.5 mV (BSY-MFC) with an external resistance of 1000 Ω. BSY-MFC achieved a maximum current output of 0.31 mA with an external resistance of 1000 Ω. Y-MFC and BS-MFC gained almost similar stable current outputs of 0.22 and 0.21 mA, respectively. In the case of BD-MFC and BDY-MFC, the current outputs were 0.02 and 0.06 mA, which was very insignificant. In the first cycle, voltage and current remained stable

for approximately 72 hours for Y-MFC and 100 hours for BDY-MFC, BS-MFC and BSY-MFC. In the case of BS-MFC and BSY-MFC, the voltage was stable for more than 120 hours during the second and third cycle. Thereafter, the voltage and current output decreased gradually in each cycle due to substrate depletion or death of the microbial population in MFC [440], [441]. Microorganisms consumed biodegradable compounds present in the substrate. Subsequently, compounds that were difficult to degrade were used to generate electricity. The internal resistance of the BD- MFC increased up to 555.5 Ω, followed by Y-MFC (344.8 Ω). BDY- MFC, BS-MFC and BSY-MFC had internal resistance of 101 Ω, 196.0 Ω and 144.9 Ω, respectively. Miran et al. (2016a) observed 237.3± 21.0 Ω of internal resistance from orange peel. Anode biofilms have a vital role in dropping the internal resistance and in increasing the power generation in the MFC [400], [401]. In addition to this, substrate, anode biofilm and electrode-electrolyte spacing also influence the magnitude of internal resistance of the MFC and current generation [402]. Attainment of optimal R_{ext} is an influential aspect of gaining maximum power density as it directly affects the anode potential, charge transfer and current generation in MFCs [395]. Figure 5. 6 represents the voltages across different external resistance for MFC. Voltage was recorded against 10 to 15000 Ω during the 3rd cycle (24th day).

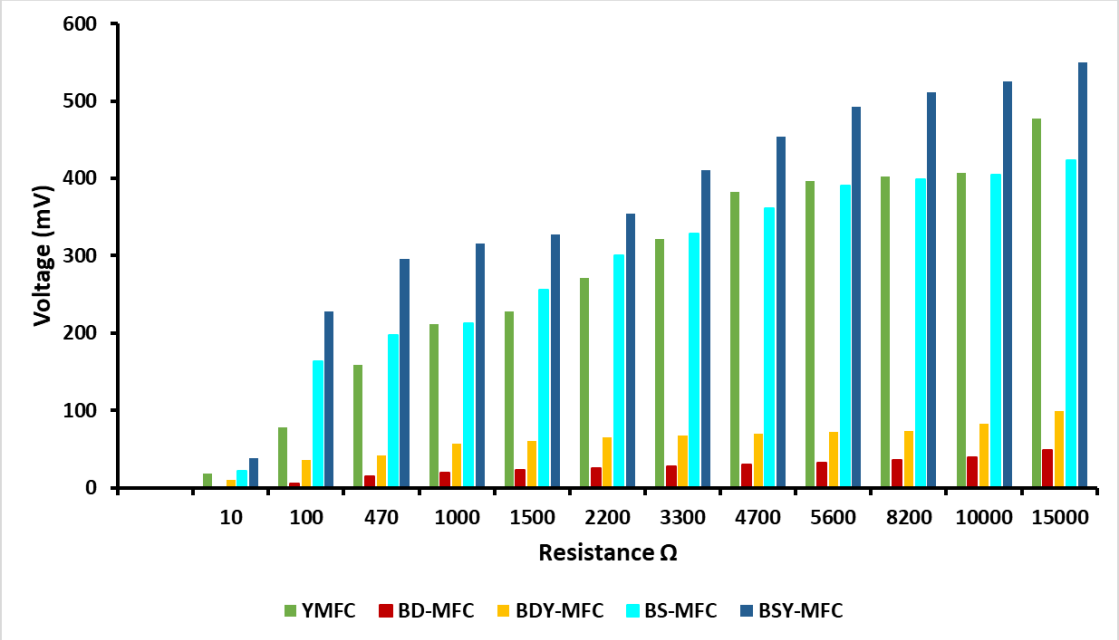

Figure 5. 6. Voltage profile of MFCs at different resistance

It was observed that escalation in resistance values increased the voltage across MFCs and cumulative power improved with a decline in voltage from 15000 Ω to 10 Ω. The external resistance is an essential parameter in evaluating the MFC performance as it affects biocatalyst activity and power generation [442]. The cell voltage decreases with the decrease in the external resistance of MFC [443]. The external resistance influences the ratio of the current generation to the cell voltage. Moreover, high external resistance leads to a higher cell voltage but lowers the generation of current and vice versa [443].

5.3.3.1 Polarization Curve

In the present work, the cumulative power and voltage generation were measured under steady conditions during 3rd cycle (24th day) for various R_{ext} values (10 Ω, 470 Ω, 1000 Ω, 1500 Ω, 2200 Ω, 3300 Ω, 4700 Ω, 5600Ω, 8200Ω, 10000Ωand 15000Ω). Polarization curves for YMFC, BDY-MFC, BD-MFC, BS-MFC and BSY-MFC are shown in Figure 5. 7 to Figure 5. 11.

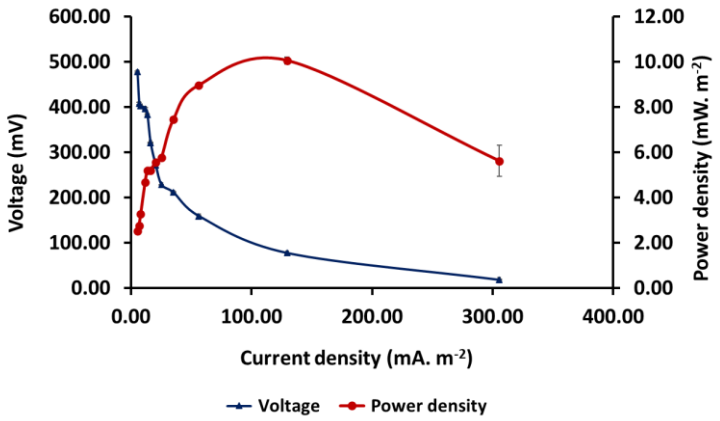

Figure 5. 7 Polarization curve for Y-MFC

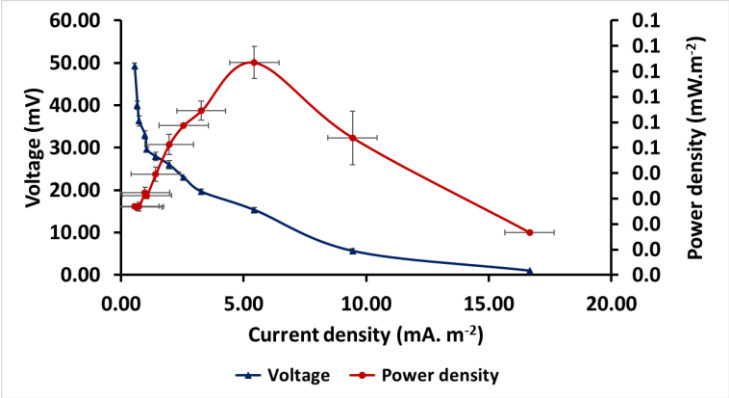

Figure 5. 8 Polarization curve for BD-MFC

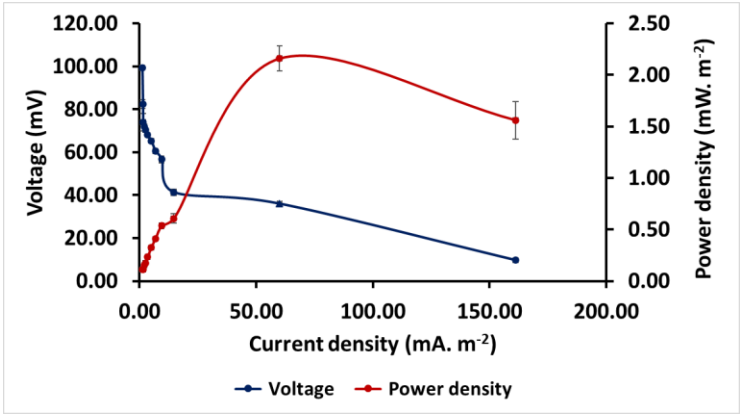

Figure 5. 9 Polarization curve for BDY-MFC

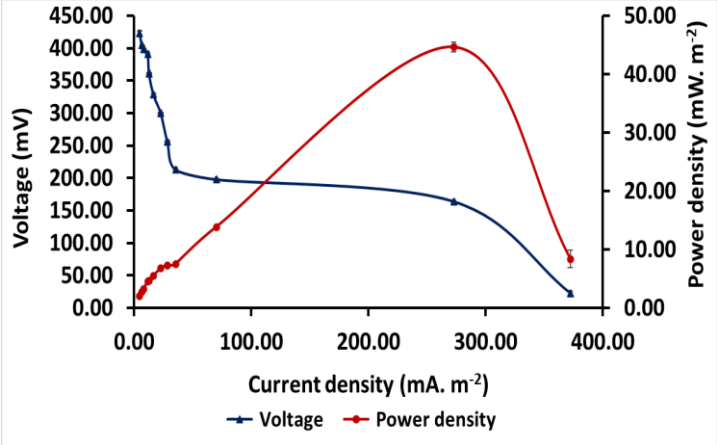

Figure 5. 10. Polarization curve for BS-MFC

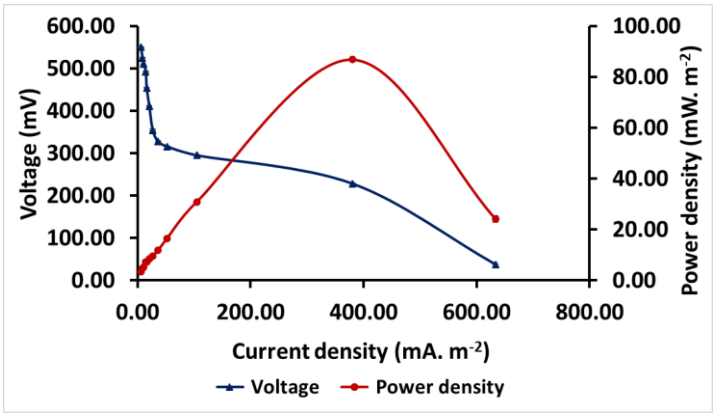

Figure 5. 11 Polarization curve for BSY-MFC

The maximum power density was attained by BSY-MFC (86.9 ± 0.4 mW. m^{-2}) with a current density of 380.6 ± 1.0 mA. m^{-2}. Contrary to this, maximum power densities in Y-MFC and BDY-MFC were recorded as 10.0 ± 0.1 mW. m^{-2} (at 129.4 ± 1.0 mA. m^{-2}) and 2.2 ± 0.1 mW.m^{-2} (at 60.0 ± 1.7 mA. m^{-2}). However, MFCs without inoculum generated a power density of 0.1 ± 0.0 mW. m^{-2} and 44.6 ± 0.8 mW. m^{-2} in BD-MFC and BS-MFC. Indigenous microorganisms present in banana slurry were responsible for the significant higher power density in BS-MFC (44.6±0.8 mW. m^{-2}) as compared to Y-MFC, BDY-MFC and BD-MFC. Mohd Zaini Makhtar and Tajarudin, (2020) obtained a maximum power density of 3.75 mW m^{-2} together with a current density of 192.8 mA m^{-2} by using dried banana peel powder as

substrate and sludge as an inoculum [336]. In this study, BDY-MFC (dried banana peel powder with *S. cerevisiae*) achieved a maximum power density of 2.2 ± 0.1 $mW.m^{-2}$ (at 60.0±1.7 $mA. m^{-2}$). The power and current density recorded in BSY-MFC were quite higher whereas BDY-MFC attained lesser power and current density than Makhtar and Tajarudin, (2020) [336]. Polarization curves exhibited a sharp drop in voltage at higher current densities, indicating the existence of conventional ohmic and mass transfer losses in MFCs. BSY-MFC showed significant improvement as compared to other *S. cerevisiae*-based MFCs. Table 5. 4 shows the comparison of results obtained in the present work with the other fruit and vegetable waste based MFCs.

Table 5. 4. Comparison of power generation with other fruit and vegetable waste MFC

Substrate	MFC	Inoculum	Power density	Reference
Citrus peels	Air cathode single chamber MFC	Microflora from anaerobic digester	71.1 mW m^{-2}	[334]
Blueberry waste	Single-Chamber MFC	*C. boidinii*	3.155 ± 0.24 W cm^{-2}	[24]
Dried waste mango peels	Dual chamber MFC	*S. cerevisiae*	4.48 mW m^{-2}	[335]
Lime waste	Single-Chamber MFC	-	66 mW cm^{-2}	[22]
Orange waste	Single-Chamber MFC	-	62.5 $mWcm^{-2}$	[22]
Tangerine waste	Single-Chamber MFC	-	72 mW cm^{-2}	[22]
Corn bran	Membrane-less MFC	Microbes isolated from dewatered sludge	12.65 mW m^{-2}	[336]

Banana peel	Membrane-less MFC	Microbes isolated from dewatered sludge	23.75 mW m^{-2}	[336]
Orange peel	Dual chamber MFC	Anaerobic consortia	358 ± 15.6 mW m^{-2}	[23]
Lemon peel waste	Dual chamber MFC	Anaerobic consortia	371 ± 30 mW m^{-2}	[338]
Potato pulp waste	Air cathode single chamber MFC	Indigenious microbial consortia	32.1 ± 0.5 W m^{-3}	[444]
Dried banana peel powder	Dual chamber MFC	*S. cerevisiae*	2.2±0.1 mW.m^{-2}	This study
Banana peel slurry	Dual chamber MFC	*S. cerevisiae* with indigenous microorganisms	86.9±0.4 mW. m^{-2}	This study
Banana peel slurry	Dual chamber MFC	Indigenous microorganisms	44.6±0.8 mW. m^{-2}	This study

Flores et al. (2020) evaluated the tangerine, orange and lime waste as a substrate in MFC and obtained maximum power densities of 72, 62.5 and 66 mW. cm^{-2} [22]. Flores et al. (2021) evaluated blueberry waste as a substrate with *Candida boidinii* as an anode biocatalyst in the MFC and obtained a maximum power density of 3.155 ± 0.24 W. cm^{-2} [24]. Miran et al. (2016) examined the performance of dual chamber MFC with orange and lemon peel as a substrate. The authors obtained a maximum power density of 358.8 ± 15.6 and 371 ± 30 mW m^{-2} from

orange and lemon peel [23], [338]. With the use of banana peel slurry with *S. cerevisiae*, this study showed a significant improvement in power density as compared to previous research where *S. cerevisiae*-based MFCs were fed with glucose or lactose [178], [242], [445], [446]. The high-power density released from BSY-MFC was due to the result of the growth of indigenous microorganisms as mixed microbial consortia [447].

5.3.4 COD Removal and Substrate Digestion

MFCs were used to generate electrical energy by using banana peel wastes in the form of substrates. High removal rate and high-power output were required for better MFC performance. In order to assess the biodegradability of the BDW and BSW, the removal rates of COD, total sugar, reducing sugar and coulombic efficiency of MFCs were studied in this work. Figure 5. 12 shows the COD removal efficiency of each MFC along with the final amount of total and reducing sugar remaining in BDW and BSW anolytes.

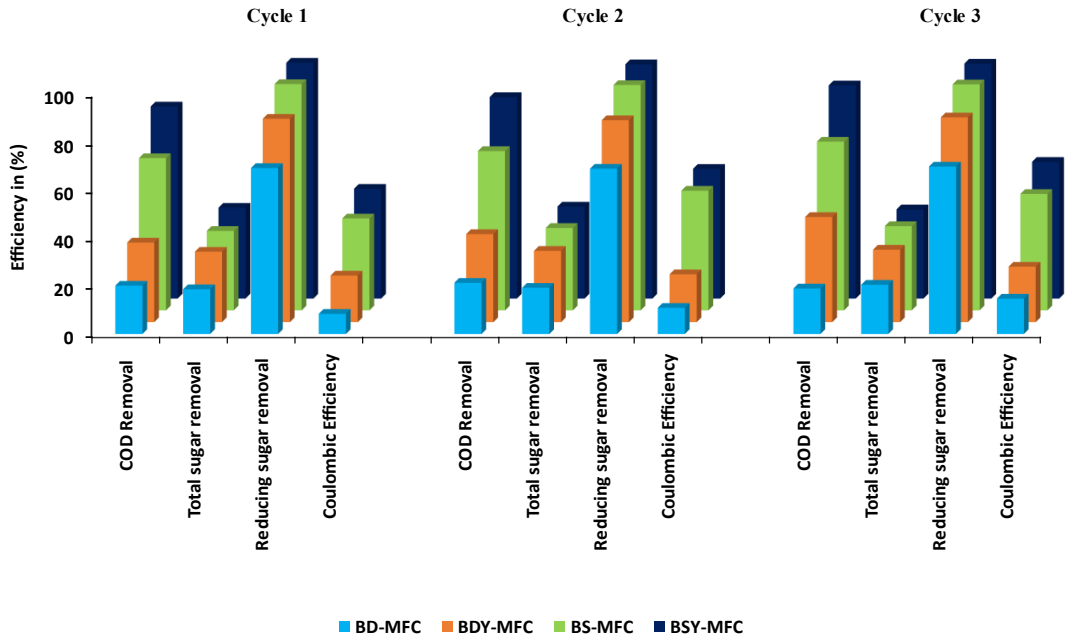

Figure 5. 12. Removal of organics from different MFC

Table **5. 5** reports the removal of organics and substrate consumption at the end of the third cycle.

Table 5. 5. Removal of organics and substrate consumption

MFC	COD removal efficiency (%)	Coulombic efficiency (CE%)	Final-total sugar content (mg. L^{-1})	Final reducing sugar content (mg. L^{-1})
BD-MFC	18.9	14.7	14979 ± 77	1807 ± 80
BDY-MFC	43.8	23.1	17063 ± 88	877 ± 61
BS-MFC	70.2	48.4	19750 ± 29	807 ± 30
BSY-MFC	88.8	56.9	19125 ± 59	298 ± 80

In the present work, the performance characteristics of the dual chamber MFC operated by BDW and BSW were studied in terms of COD removal. Some reducing sugars and organic substances might be present in BSW and BDW that exceed the COD content (Table 5. 3) before the MFC operation. During anaerobic treatment in MFC, macromolecular substances in the BDW and BSW were broken down into small molecular compounds, which are easily degraded by *S. cerevisiae* and indigenous microorganisms. However, several complex organics present in the BDW and BSW are challenging to biodegrade by indigenous microorganisms in MFCs. High COD removal and coulombic efficiency indicate that the indigenous microorganism present in BSY-MFC and BS-MFC have effectively utilized the organic materials available in BSW [332]. In cycle 3, maximum COD removal was obtained from BSY-MFC (88.8%), which was 18% higher than the BDY-MFC (45%). In some of the studies, COD removal rates have reached 70% - 80% or above 80% [448]–[450]. Jia et al. (2013) studied the food waste based MFC with various organic loading rates and obtained a

maximum power density of 556 $mW.m^{-2}$ and maximum COD removal of 86.4% [449]. Rikame et al. (2012) observed the performance of dual-chambered MFC fed with food waste leachate. At optimum conditions, food waste leachate based MFC gained a current density of 66.75 A. m^{-3} and a power density of 15.14 W. m^{-3}, along with 90% COD removal [451].

5.3.5 Analysis of Indigenous Microbial Consortia

The microbial community structure of the banana slurry was analysed on the basis of 16S rRNA gene pyrosequencing. The metagenomic analysis is valuable as it can reveal the dominant operational taxonomic unit (OTUs) and the rare microbial communities.

Table **5. 6** summarize the 16s metagenomic data of bacterial communities present in the banana slurry.

Table 5. 6. 16s metagenomic data of bacterial communities present in the banana slurry

16s metagenomic data and statistical analyses	Banana slurry
Total number of sequences	319492
Sequences after trimming	316880
Average sequence length	301
Average sequence length after trimming	278
The standard deviation of sequence length	11.5
Operational taxonomic unit (OTU)	598
No. of phyla	25
No. of genera	285

The microbial communities present in the banana slurry belong to 25 phyla. A total 598 OTUs were obtained. The most abundant phyla in banana slurry were *Proteobacteria* (50.16%), *Bacteroidetes* (25.91%), *Firmicutes* (10.35%) and *Chloroflexi* (3.07%), *Actinobacteria*

(5.50%). Figure 5. 13 represents the taxonomic classification of the microbial community. Microbial community analysis showed the presence of Firmicutes (38.7%), Bacteroidetes (28.5%) and Proteobacteria (28.1%) as dominant phyla present in food waste based MFCs [452]. Metagenomic analysis of the genera level disclosed that the most dominant genera were *Pseudomonas* (21%), *Chryseobacterium* (20%), *Flavobacterium* (17%), *Stenotrophomonas* (15%), *Brevundimonas* (6%), *Chryseolinea* (5%), *Rhodococcus* (5%), *Rhizobium* (4%) and *Acinetobacter* (4%). *Pseudomonas* is a well-recognized electrogenic bacteria, usually found in the anode biofilms [23], [453]. Miran et al. (2016) also reported the presence of *Pseudomonas* in microbial community of orange peel fed MFC [23]. Exoelectrogen, like *Shewanella* and *Geobacter* were not observed in BS-MFC. As the banana slurry is expected to go through fermentation, fermentative microorganisms might have played a vital role in the overall MFC performance [23]. The high diversity of the microbial community depends on the organic source consumed by the microbes. These organic substrates can be directly utilized by electrogenic or fermentative microbes and produce metabolites, which get consumed by electrogenic microbes [454]. The most prominent fermentative bacteria detected in the banana slurry were *Acinetobacter* and *Flavobacterium*. Tian et al. (2017) observed an abundance of *Flavobacterium* in the microbial community analysis of potato pulp waste-fed MFC [444].

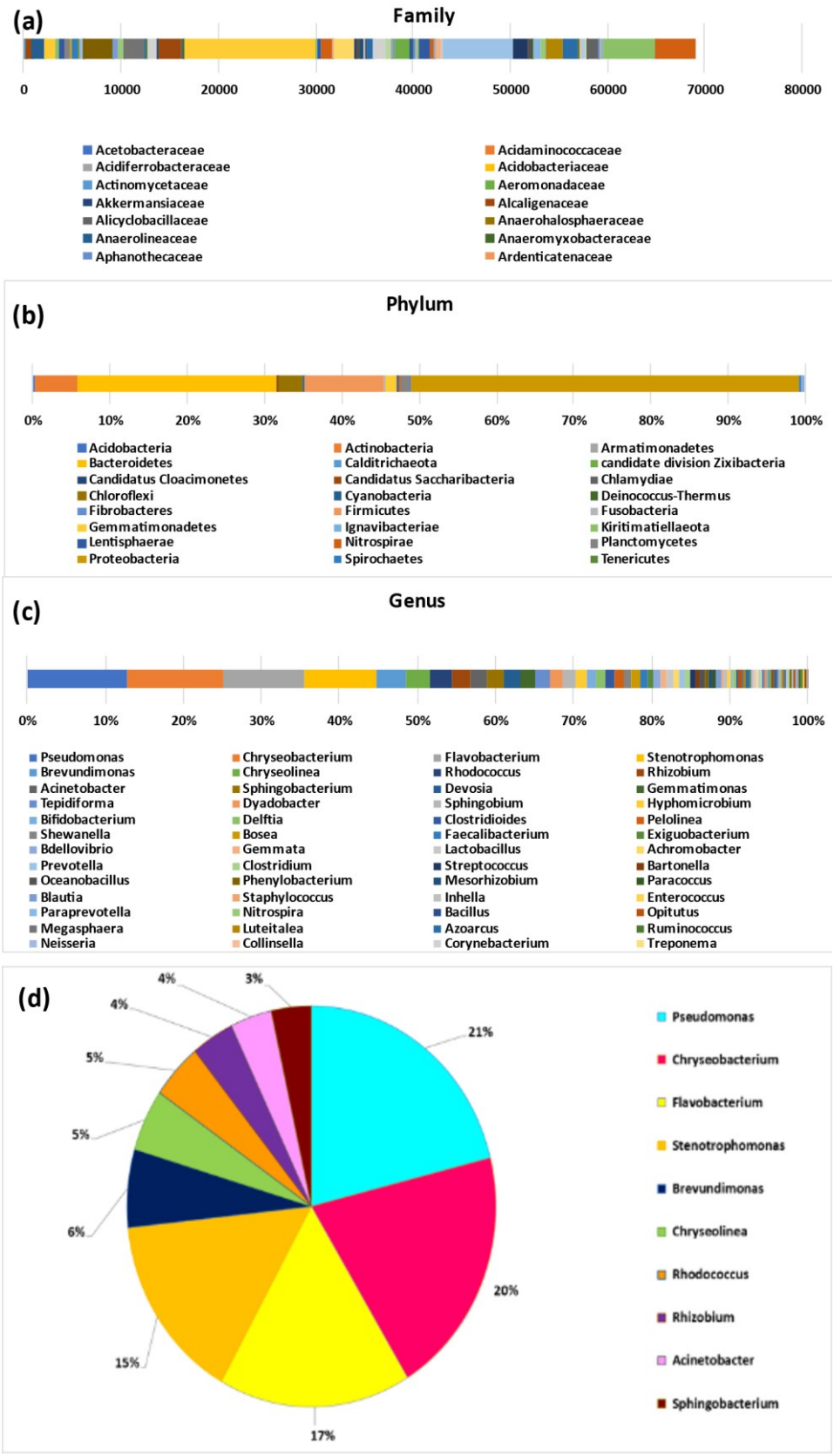

Figure 5. 13. Taxonomic classification of microbial community at a) family; b) phylum; c) genus; d) most dominant genera present in the banana slurry

There is another probability that many unclassified genera might have played an essential role in banana slurry degradation and power generation. Overall, the combination of fermentative, electrogenic and other functional bacteria play a significant role in the effective degradation of substrates for bioelectricity generation.

5.4 Conclusion

In the present work, the possibility of electricity generation was effectively demonstrated from the degradation of banana peel waste in dual-chambered H-shaped MFC by using *S. cerevisiae* as a biocatalyst. Considerable performance was observed from BSY-MFC and BS-MFC setup, which indicates that banana slurry supports the growth of microorganisms than the dried banana peel powder. The maximum power density attained by BSY-MFC was 86.9 ± 0.4 $mW.m^{-2}$, whereas BS-MFC (without any inoculation) generated a power density of 44.6 ± 0.8 $mW. m^{-2}$. Indigenous microorganisms present in the banana slurry were the key players behind the high-power outputs in BS-MFC and BSY-MFC as compared to Y-MFC, BDY-MFC and BD-MFC. Microbial community analysis of banana slurry revealed that the most dominant genera were *Pseudomonas*, *Chryseobacterium*, *Flavobacterium*, *Stenotrophomonas*, *Brevundimonas*, *Chryseolinea*, *Rhodococcus*, *Rhizobium* and *Acinetobacter*. Up to 88.8% of COD elimination was observed from BSY-MFC followed by 70.2% of COD removal in BS-MFC. Outcomes from BSY-MFC and BS-MFC are good enough to obtain high power densities and substrate removal efficiency. The present study can be further tested in a continuous mode of MFC operation, or multiple MFCs can be stacked together to obtain a high-power output. Also, the proposed MFC systems can be optimized for the yield of ethanol by using *S. cerevisiae* and banana slurry.