NVEO
Natural Volatiles &
Essential Oils

Electricity Generation In Microbial Fuel Cell By Using Beetroot Dye As Mediator

Levin Anbu Gomez[1] , David Paulraj R.S.[2] , Jesse Joel, T.[3*] , Selvarathi, M.[4] , and Ritu Shepherd[5]

[1]Assistant Professor, Department of Biotechnology, Karunya Institute of Technology and Sciences, Coimbatore - 641 114, Tamil Nadu, India

[2]Associate Professor, Department of Biotechnology, Karunya Institute of Technology and Sciences, Coimbatore - 641 114, Tamil Nadu, India

[3*]Assistant Professor, Department of Biotechnology, Karunya Institute of Technology and Sciences, Coimbatore - 641 114, Tamil Nadu, India

[4]Assistant Professor, Department of Mathematics, Karunya Institute of Technology and Sciences, Coimbatore – 641 114, Tamil Nadu, India.

[5]PG and Research Department of English, School of Humanities, Nehru Arts and Science College, Coimbatore - 641 105. Tamil Nadu, India.

ABSTRACT

Microbial fuel cells (MFCs) are shown to be capable of unpolluted energy production through the oxidization of biodegradable organic waste using varied microorganism species as biocatalysts. this study geared toward manufacturing electricity by using beetroot dye as a mediator and treatment of waste water by using MFC.In a Microbial fuel cell (MFC) organic matter is utilized by microorganisms and it results in the production electricity. MFC holds a key in green technology for the production of bioenergy. In this present word a MFC is constructed with two chambers and a salt bridge is used as a separator between the chambers. During this study the beetroot was dried and fine-grained and oxidation-reduction potential was measured using cyclic voltammetry. It absolutely was found that beetroot possesses low redox potential. For MFC setup waste water was used in anode with graphite as electrolyte and salt bridge as membrane and 0.1M potassium permanganate solution as catholyte and copper mesh as electrolyte. The beetroot dye was stuck to the electrode. In another setup Nafion 117 membrane was used rather than salt bridge. maximum voltage obtained was 1278 mV. It can be concluded that beetroot dye acts as a good mediator. In this study we proposed to make an attempt to use readily available and economically viable beetroot dye as a mediator in the microbial fuel cell. And we found that the beetroot dye produces a good result because of its low redox potential.

Keywords (in English): Microbial Fuel Cell, Electricity, Sewage water, Microorganism, MALDI-TOF, redox potential, Cyclic voltammetry.

I. INTRODUCTION

An MFC is a system in which the conversion of energy to electricity is done by microorganisms which acts as a biocatalyst. In MFC energy is produced as a direct utilization of organic biodegradable matter such as sugars, organic acids and biomass [1]. Microbial electric cell consists of an area for the anode and also the cathode, where microorganism grow on the positive conductor (anode), and works on the oxidization of organic matter and inorganic matter at the anode and therefore the cathode once the protein intake and release hydroxyl group non-ionic produces the voltage generated between the poles groups [2]. Based on the various ways in which microorganism transfer electrons in MFC, microorganism will be divided into mediator-less and mediator MFC microorganism. Mediator-less MFC microorganism will directly transfer electrons to anode, in mediator MFC microorganism should transfer electrons to anode via electron mediators, which can be created as mediator MFC [3]. Most of the microorganism in nature are mediator MFC bacteria; the electricity generation of intermediary MFCs according was considerably beyond that of mediator-less MFCs [4].

An ideal mediator present in a very MFC ought to possess the subsequent characteristics [5]: Should form a reversible chemical reaction couple at the electrode

(i) should link to NADH and have a high negative E0− value

(ii) should be stable in each oxidized and reduced type

(iii) soluble in liquid systems

The natural red dye is mainly obtained from beetroot (Beta Vulgaris). Betalain is the red pigment present in the beetroot which gives the red colour and it is classified under the main classification of betalains [6]. The dye betalain is also knows as beetroot red, which is chemically a glycoside which on hydrolysis yields a sugar aldohexose and betanidin [7].

1 MATERIALS AND METHODS

1.1 Isolation of Organism

Bacteria (Pseudomonas aeruginosa and Escheria coli) was isolated from soil sample from Karunya Institute of Technology and Sciences. The samples were serially diluted up to 10-8 dilution. once dilution, the samples were spread plated on the LB Agar medium and incubated for twenty-four hours at 37ºC. Colonies obtained were characterised using MALDI-TOF technique and biochemical Characterization [8].

1.2 Characterization – MALDI-TOF Analysis

BioTyper software (version 3.0) was used for processing the obtained mass spectra, which also includes the BioTyper database version DB-5989 with 5989 reference MALDI-TOF Ms profiles. By using BioTyper a Log(score) and a colour code of green, yellow or red is assigned for the matching of MALDI-TOF MS profiles. From the Log(score) a value exceeding 2.3 which is assigned green colour is highly probable score for species level identification. In general, a Log (score) value in between 2.0 and 2.3 (green colour) suggests a high probability in identification at the genus level and probable identification at the species level. A value

in between 1.7 and 2.0 for the Log(score) is assigned yellow colour and it identifies at the genus level and a Log(score) bellow 1.7 assigned red colour shows there is no similarity is present between the unknown profile and the search database [9].

1.3 Biochemical Characterization

1.3.1 Indole Test:

The culture is fully grown in sulfide-indole-motility (SIM) medium twenty-four hours at 37°C. The result's read after reading Kovac's reagentas seen in Figure 1

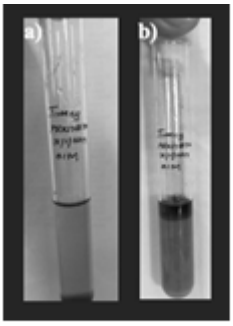

Fig 1: Indole Test

1.3.2 Methyl Red Test:

Figure 2 represents Methyl red–Voges-Proskauer (MR-VP) broth media is prepared to that the culture is inoculated. It is kept for forty-eight hours at 37°C. Incubate all the tubes at 37°C for forty-eight hrs. In to the test tube 3 to 4 drops of methyl red indicator was added.

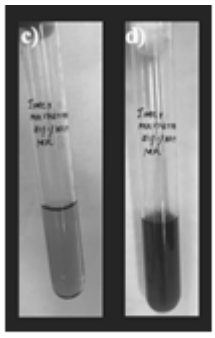

Fig 2: Methyl Red Test

1.3.3 Voges-Proskauer Test:

The organism is inoculated to the methyl red–Voges-Proskauer (MR-VP) broth media. It is incubated for forty-eight hours at 37°C. Once incubated,Barritt's A and Barritt's B reagents are added as cited in Figure 3

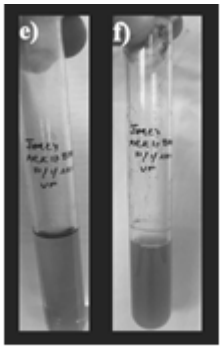

Fig 3. VP Test

1.3.4 Citrate Test:

Figure 4 illustrates Citrate utilization was determined on conventional Simmons citrate agar. The medium was dissolved by steaming and sterilized at 121°C for fifteen min. No reagents were used for this test [10].

Fig 4: Citrate Test

1.4 Extraction of Compounds

The compounds were extracted by using Soxhlet extraction methodology using ethanol as solvent. The dried beetroot powder was loaded into the thimble. Following this, the solvent (250 millilitre of ethanol) was added to a round bottom flask, that is connected to a Soxhlet extractor and condenser. Eight refluxes were taken. The obtained extract was dried under reduced pressure using a rotary evaporator.Water was used to dissolve the dry extract. The extract was used for total phenol assay, total flavonoid test and betanin quantification [11].

1.4.1 Total Phenol Estimation

Folin-Ciocalteu reagent method was used for determining total phenol in the sample. 200 ml of the sample was introduces into test tubes followed by the addition of Folin-Ciocalteu's (1.0 milliliter) followed by 7.5% sodium carbonate solution (0.8 milliliter). The test tubes were incubated at 30℃ for 1.5 h. After incubation the absorbance of the samples were measured at 765 nm.The total phenol results were expressed as milligram of gallic acid equivalent (GAE) per gram of fresh weight (FW) [12]

1.4.2 Total Flavonoid Estimation

The method proposed by Park et at. (2008) was used to determine the total flavonoid content present in the samples. In every experiment, 1.5 mg of methanolic extract or a fraction was dissolved in five millilitres of methanol, from that 300 µL were transferred into 3.4 millilitre of 30 percent aqueous methanol. To the

present mixture, 150 µL each of 0.5M NaNO2 and 0.3 M AlCl3.6H2O were added and mixed completely. The mixture was allowed to stand for 5 minutes and after this 1 M NaOH was also added and mixed well. Immediately after this the absorbance of the sample was measured using a spectrophotometer at 506 nm against a blank.A standard graph was plotted by using rutin as the standard utilizing the same method as mentioned above. The obtained results for the samples were expressed in terms of µg of Rutin per mg of dried mass as calculated by using the following equation. Rutin Equivalent (RE) = Absorbance (506 nm) / 0.0002428 - 0.008141, R2 0.9987 [13].

1.4.3 Photometric Quantification of Betanin

Nilsson's Spectrophotometric method was used for the determination of Betalains,Betacyanins and betaxanthins content present in the extracts. From the liquid samples, the amount of pigment concentrations was determined as absorbance units of a tenth relative to the dry matter of the extract at by measuring the absorbance at 538 and 480 nm for betacyanins and betaxanthins, severally. The amount of betalains content (BLC) in the sample was calculated using the formula as follows: BLC [mg L−1] = (A × DF × MW× 1000) / (ε × 1), where A is the absorption value, DF the dilution factor and one the path length (1 cm) of the cuvette. For the quantification of betacyanins (Bc) and betaxanthins (Bx), the molecular weights (MW) and molar extinction coefficients (ε) was severally, 550 g mol−1 and 60,000 L mol−1cm−1 in H2O: λ = 538 nm for betanin, 339 g mol−1 and forty eight,000 L mol−1cm−1 in H2O: λ = 480 nm for vulgaxanthin I. [14].

1.5 Cyclic voltammetry

Beetroot dye was extracted by grinding and boiling it with hot water. A 100 µM solution of either betanin was placed in a very cyclic voltammeter cell. A 3.3millimetre diameter glassy carbon electrode was used as the working electrode, platinum wire was used as the counter/auxiliary electrode and Ag/AgCl electrode was used as the reference electrode.Sample (1mL) of the pigment (1.4mM) aqueous solution was purged with argon for five min to get rid of gas prior to measurements. The potential was applied linearly to the working electrode at a constant rate (50 mV/s) toward the positive potential (evaluation of reducing equivalents). An electrochemical working station, CV-50W (Bioanalytical Systems, West Lafayette, IN), was used. During operation of the CV, a potential current curve was recorded (cyclic voltammogram) [15].

1.6 Design of MFC

The MFC was made by using 2 plastic containers of working volume (500 ml) which was connected by salt bridge of 15 cm length and 1.5 cm wide. 1M Nacl in 5% Agar was prepared. Electrodes used in anode was graphite of 10cm length and 0.5cm wide and for cathode copper mesh was used. Wastewater was kept in anodic chamber and 0.1M Potassium Permanganate was used in cathodic chamber. In another setup Nafion 117 membrane was used instead of salt bridge. The MFC was operated for 10 days at room temperature of 30ºC. The voltage generation was noted [16].

1.7 Scanning Electron Microscopy

For SEM analysis samples were prepared by the following procedure. A plastic coverslip coated with 1% poly-L-Lysine was taken and a drop of this suspension was above the coated coverslip. Glutaraldehyde was used for fixing the cell to the coverslip for 1h followed by osmium tetroxide for 1h. The coated cells were dehydrated by passing it through different grades of alcohol followed by drying using critical point technique. After drying the samples were trimmed, mounted and coated with gold-platinum and the

samples were used for SEM analysis [17].

II. RESULTS AND DISCUSSION

1.8 Characterization of Micro-Organisms

The micro-organisms isolated werecharacterized by using MALDI-TOF technology and were confirmed to be Pseudomonas aeruginosa. The biochemical characterization of the next colony proved to be Escheria coli by IMVIC test.

The MALDI-TOF characterization of the bacteria reveals it to be Pseudomonas aeruginosa. The biochemical characterization of the bacterial colony proves it to be Escheria coli. It yielded positive result for indole and Methyl red test and negative result for Voges-proskauer and citrate test.

1.9 Total Phenol Estimation

The total polyphenol content in the extract was measured by FolinCiocalteu reagent in terms of gallic acid equivalent (Standard curve equation: (y=0.0052x, R^2 = 0.9994) as given in Figure 5

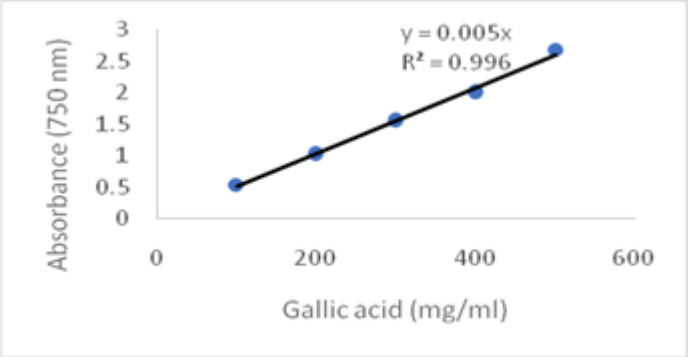

Fig 5: Standard gallic acid curve for estimation of phenol

Concentration of total phenolic compounds in sample was found to be 8.2 + 0.026 mg GAE/ g.

1.10 Total Flavonoid Estimation

Figure 6 representstotal flavonoid content was determined in terms of µg of Rutin Equivalent per mg of the dried material (RE). Graph below shows the flavonoid content of the extract (Standard curve equation: y=0.002x – 0.1848, R^2 =0.9977).

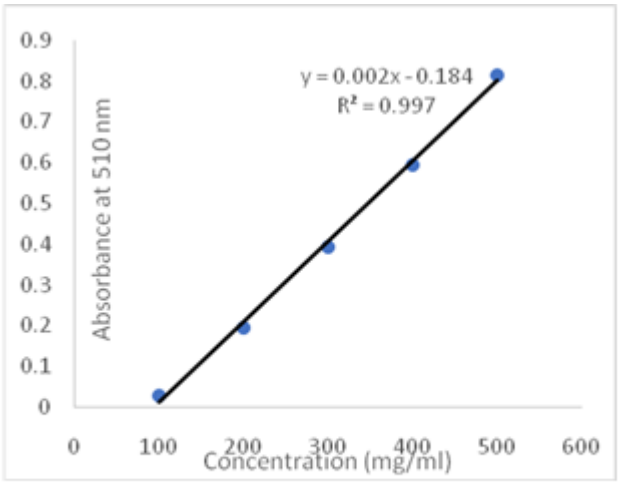

Fig 6. Standard gallic acid curve for estimation of phenol

Concentration of total phenolic compounds in sample was found to be 5.35 + 0.017 mg rutin/ g.

1.11 Betanin Quantification

The betanin was calculated by using the formula (A × DF × MW× 1000) / (ε × 1). Absorbance at 476 nm was found to be 104.40 mg/L.

1.11.1 Redox Potential

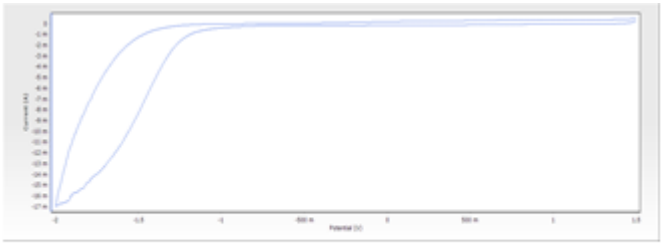

Fig 7. Cyclic voltammetry of Beetroot Dye

The cyclic voltammogram of dye indicates, that it has got less redox potential because in the graph very much less peaks are seen as depicted in Figure 7

1.11.2 Power Generation

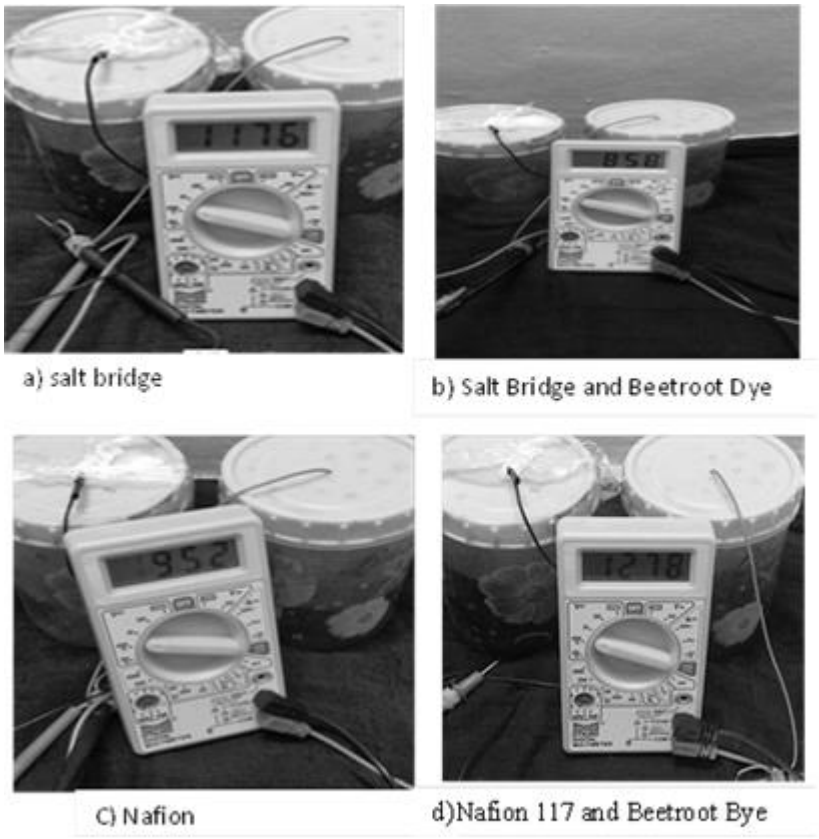

Fig 8. Maximum Voltage Obtained

Current is calculated by using ohms law V = IR, I = V/R and power was calculated by using formula P=VI

TABLE 1: Voltage, Current and Power Generation

TIME (DAY)	VOLTAGE (MV)	CURRENT (MA)	POWER (μW/M^2)
1	109	0.06	6.19
2	221	0.25	55.00
3	320	0.35	112.90
4	473	0.50	235.26
5	520	0.41	211.09
6	668	0.48	319.64
7	770	0.55	424.71
8	858	0.87	743.60
9	704	0.74	523.35
10	516	0.56	287.53

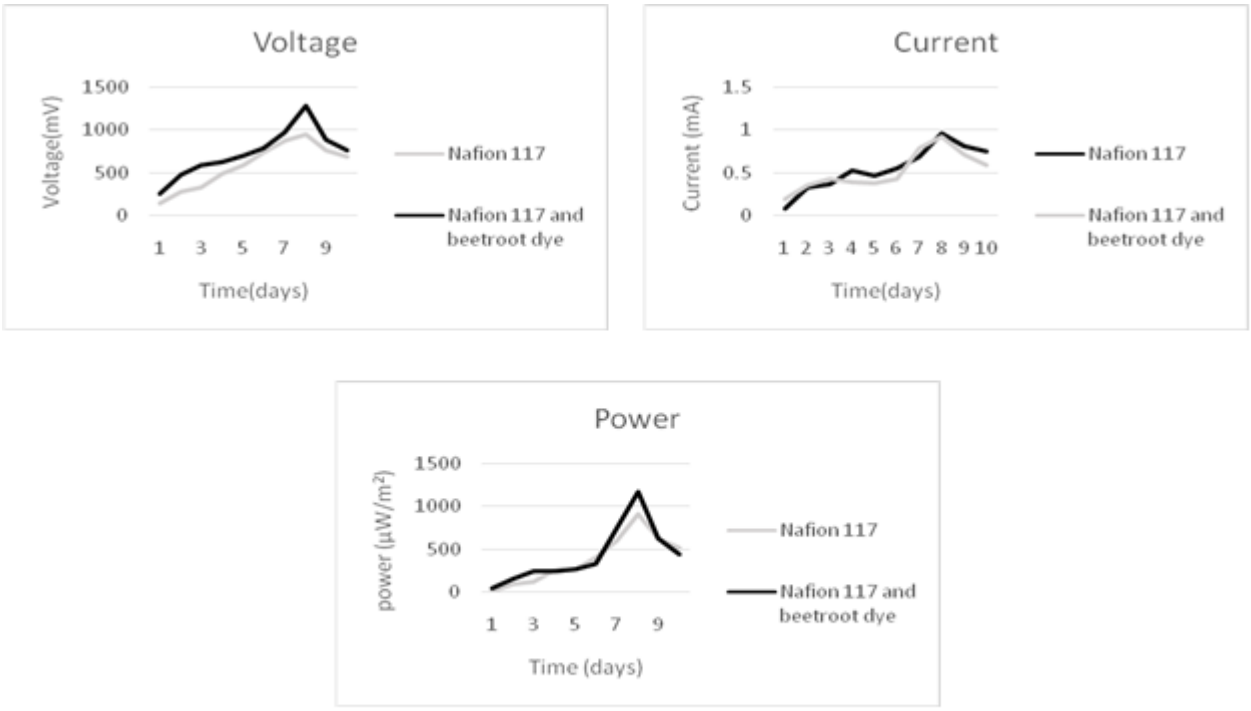

Fig 10: Voltage, Current and Power of Waste Water with Membrane and Beetroot Dye

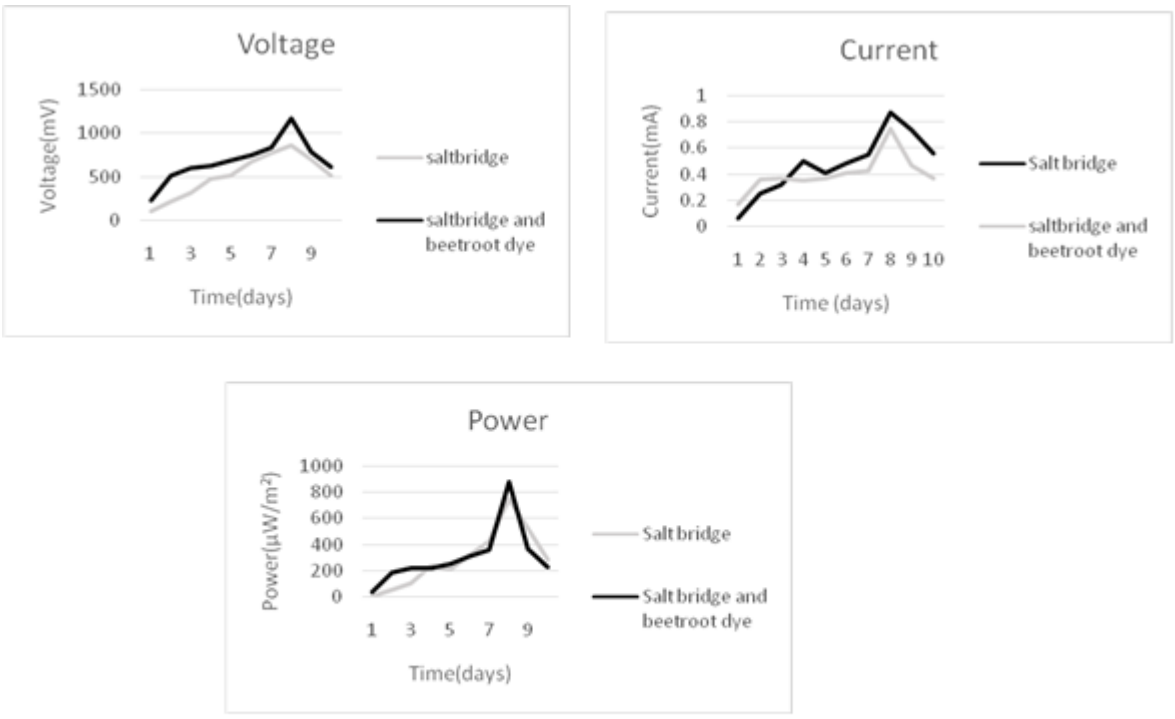

Fig 9. Voltage, Current and Power of W aste Water with Salt Water and Beetroot Dye

The voltage obtained when using saltbridge alone was 858 millivolt, the current produed was 0.87

milliamphere and the power generated was 746.46 µW/m^2 . The power generated is shown in Figure 8 and the consolidated is illustrated in Table 1.When using salt bridge and beetroot dye the maximum voltage obtained was 1170 millivolt, the current produced was 0.75 milliamphere and the power generated was 877.5 µW/m^2 as illustrated in diagrammatic series of Figure 9

The maximum voltage obtained when using membrane alone was 952 millivolts, the current produced was 0.96 milliamphere and the power generated was 913.92 µW/m^2 as given in Figure 8 referringa,b,c and d.

When using membrane and beetroot dye the maximum voltage obtained was 1278 millivolt, the current produced was 0.92 milliamphere and the power generated was 1175.76 µW/m^2 as illustrated in diagrammatic series of Figure 10

Fig 11: Glowing led by Voltage Generation

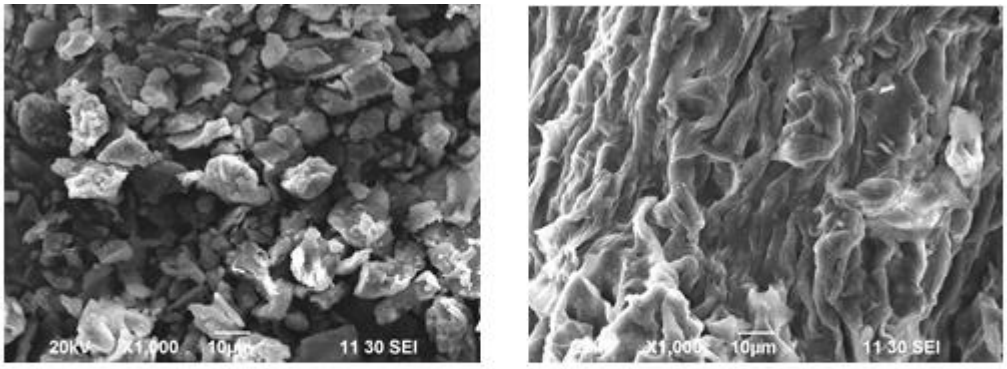

Fig 12. Scanning Electron Microscope (SEM) Result of Beetroot at x1000 before and inoculating it in MFC

III.CONCLUSION

In this study the beetroot was dried and fine-grained and redox potential was measured using cyclic voltammetry. It had been found that beetroot possess low redox potential. For MFC setup waste water was used in anode with graphite as electrolyte and salt bridge as membrane and 0.1M potassium permanganate solution as catholyte and copper mesh as electrolyte. The beetroot dye was stuck to the electrode. In another setup Nafion 117 membrane was used rather than salt bridge. Most voltage obtained was 1278

millivolt. It can be concluded that beetroot dye acts as a good mediator which is finally indicated by the light glowing seen in Figure 11. Figure 12 represents the morphology of change on and after treating the cell with beetroot dye which illustrates the refined and more surface area of the MFC resulting in higher electrical impulse generation.

IV. ACKNOWLEDGEMENT

We express our gratitude and acknowledge KarunyaInstitute of Technology and Sciences and the Department of Biotechnology for providing us a seed grant of Rs.9000/- on the 15th of October 2018 to carry out the work.

REFERENCES

[1] Deval A, Dikshit AK. Construction, working and standardization of microbial fuel cell. APCBEE Procedia. 2013, 1(5):59-63.

[2] Humudat YR, Al-Naseri SK, Kadhim SA. Using Bacteria in Microbial Fuel Cells. Int J Pure App Biosci. 2015, 3(5):21-25.

[3] Lovley DR. Microbial fuel cells: novel microbial physiologies and engineering approaches. CurrOpin Biotech. 2006, 17(3):327-332.

[4] He Z, Minteer SD, Angenent LT. Electricity generation from artificial wastewater using an upflow microbial fuel cell. Environ Sci Technol. 2005, 39(14):5262-5267.

[5]P ark DH, Zeikus JG. Electricity generation in microbial fuel cells using neutral red as an electronophore.Appl EnvironMicrobiol. 2000, 66(4):1292-1297.

[6] Kale RG, Sawate AR, Kshirsagar RB, et al. Studies on evaluation of physical and chemical composition of beetroot (Beta vulgaris L.). Int J Chem Stud. 2018, 6(2):2977-2979.

[7] Von Elbe JH, Attoe EL. Oxygen involvement in betanine degradation—Measurement of active oxygen species and oxidation reduction potentials. Food chem. 1985, 16(1):49-67.

[8] Islam MM, Islam MN, Sharifuzzaman FM, et al. Isolation and identification of Escherichia coli and Salmonella from poultry litter and feed. Int J Nat Soc Sci. 2014, 1(1):1-7.

[9] Nacef M, Chevalier M, Chollet S, et al. MALDI-TOF mass spectrometry for the identification of lactic acid bacteria isolated from a French cheese: The Maroilles. Int J Food Microbiol. 2017, 247:2-8.

[10] Powers EM, Latt TG. Simplified 48-hour IMVic test: an agar plate method. Appl EnvironMicrobiol. 1977, 34(3):274-279.

[11] Ganesan B, Perumal P, Manickam V, et al. Optimization of extraction conditions for embelin in Embeliaribes by UV spectrophotometry. ArchApplSciRes. 2010, 2(2):49-53.

[12] Sun L, Zhang J, Lu X, et al. Evaluation to the antioxidant activity of total flavonoids extract from persimmon (Diospyros kaki L.) leaves. Food ChemToxicol. 2011, 49(10):2689-2696.

[13] Ahmed D, Zara S, Baig H. In vitro analysis of antioxidant activities of Oxalis corniculata Linn. fractions in various solvents. AfrJTraditComplementAlternMed. 2013, 10(1):158-165.

[14] Ben Haj Koubaier H, Snoussi A, Essaidi I, et al. Betalain and phenolic compositions, antioxidant

activity of Tunisian red beet (Beta vulgaris L. conditiva) roots and stems extracts. Int J Food Prop. 2014, 17(9):1934-1945.

[15] Butera D, Tesoriere L, Di Gaudio F, et al. Antioxidant activities of Sicilian prickly pear (Opuntiaficusindica) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. J Agar Food Chem. 2002, 50(23):6895-6901.

[16] Yoganathan K, Ganesh P. Electrogenicity assessment of Bacillus subtilis and Bacillus megaterium using Microbial Fuel Cell technology. Int J Appl Res. 2015, 1(13):435-438.

[17] Sreekanth, D,Arunasree MK, Roy KR, et al. Betanin a betacyanin pigment purified from fruits of Opuntiaficus-indica induces apoptosis in human chronic myeloid leukemia Cell line-K562. Phytomedicine. 2007, 14(11):739-746.