Original Research Paper

Volume - 11 | Issue - 01 | January - 2021 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

GREEN APPROACH FOR THE GENERATION OF BIOELECTRICITY PERFORMANCE, CHARACTERIZATION AND CAPACITY IMPROVEMENT

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ABSTRACT Microbial fuel cells (MFCs) or Biological fuel cells are the elevation in the field of science that generates bio-electricity from the organic matter using bacteria as biocatalyst. In this study, activated sludge is used as substrate, the percentage of organic matter in a sample estimates the electrical capacity of MFCs fueled. From the experiment conducted, we have observed that higher the percentage of organic matter in the sample, results in higher the electricity production of MFCs powered. Physicochemical analysis and the concentration of metal ions present in the sludge were also analyzed. Single chamber MFC was constructed with different combination of electrodes. Microbes associated with fuel production were isolated from the anode surface. The isolate was found to be Myroides xuanwuensis through morphological, biochemical and molecular characterization. Double chamber MFC was designed, constructed with the isolated strain using salt bridge as a membrane with the varying concentrations of agarose to produce maximum voltage. Four MFCs connected in series showed maximum voltage of 2.26V under optimized conditions found to be sufficient to glow a LED bulb.

KEYWORDS : Microbial fuel cell, Bioelectricity generation, Myroides xuanwuensis, Salt bridge, Activated sludge.

INTRODUCTION

Production of electrons by microbial metabolism results in the production of electricity which is obtained and the electrons produced can be captured to maintain a stable or continuous form of energy production (Akshay et al. 2016). MFCs (Microbial Fuel Cells) have the ability to convert chemical energy into electrical energy by means of oxidation process where the micro-organisms present in the organic wastes by oxidation give to photosynthesis which is coupled with electricity production (Rosenbaum et al. 2010). This technology plays as an alternative tool for the current treatment on waste water system which helps in transforming waste materials into energy-saving technique (Ma et al. 2018; Rozendal et al. 2008; Liu et al., 2005; Fornero et al. 2008; Dong et al. 2012). MFCs working principle is same as that of other battery or fuel cells which consists of an anode and an electrode. Micro-organisms plays a vital role from the part of anode by oxidizing the organic materials and thus liberates electrons and protons and the entire circulating process is commonly called as electricigen (Ma et al. 2018).

Energy requirement, especially electrical consumption is increasing in today's world day-by-day and fossil fuels have led their usage in one or other way (Venkatesh Chaturvedi, and Pradeep Verma 2016). Eco-friendly and cheaper availability for the production has become the prime necessity and thus by using micro-organisms have paved the way for the same (Venkatesh Chaturvedi, and Pradeep Verma 2016; Logan 2004). There are different types of MFCs present namely single, double chambered MFCs and the substrates also varies widely in terms of their usage (Chae et al. 2009). Low molecular weight substrates like sucrose, fructose, glucose and maltose are also used in the reforming of this technology along with organic acids like propionate, acetate and lactate (Chaudhuri and Lovely 2003; Kim et al. 2000a; Kim et al. 2000b). Major drawback in the MFC technology is found to be that the low power density where, there is a need to isolate a particular micro-organism which have the efficiency to transfer the electrons to anode or in another case by engineering of the organisms through DNA recombinant technology to show the improved electron transfer to the anode (Aelterman et al. 2006). Thus, in the present study, an individual micro-organism have been isolated from the activated sludge and by means of MFCs using salt bridge, a cost effective model has been carried for the production of electricity under optimized conditions to obtain maximum voltage for an LED bulb to glow.

1. Materials and methods

1.1. Physicochemical parameters

The physicochemical parameters of the activated sludge such as color, odor, and temperature were analyzed immediately after collection. The analyses of moisture, total solids (TS), total suspended solid (TSS), total dissolved solid (TDS), sludge volume index (SVI), chemical oxygen demand (COD) and detection of metal of metal ions were determined using standard method of the United Stated Environmental Protection Agency (USEPA 1994) and the standard methods of American Public Health Association (APHA 2005) (Xavier Alberico Freitas et al. 2019).

1.2. Detection of heavy metals

The total heavy metal concentration was determined by initial drying for 48 h at room temperature until air-dried and then to a constant mass at 105ºC and finally the sludge obtained was milled in a mortar grinder (Malwina Tytła 2019). 0.2 g of sample was digested with nitric acid (HNO_3) and hydrochloric acid (HCl) in a Teflon flask, using a microwave digestion system. Obtained solution was then filtered through fine filters (0.45 µm) and diluted with 5% HNO_3 to a volume of 50 ml. Sample was stored at 4°C prior to analysis. The total heavy metal concentrations were determined using Inductively Coupled Plasma Optical Spectrometry.

1.3. Construction of single chambered MFC

Single chambered MFC was constructed using a 500ml plastic container. The activated sludge sample was added till the rim of the container. Copper wire was used as an anode and Aluminium mesh as cathode. The chamber was closed to maintain anaerobic conditions. Electricity production was measured using digital multimeter (Wang et al. 2008).

1.4. Construction of double chambered MFC

A two chambered Microbial fuel cell was constructed using two 1000ml plastic containers and marked anode and cathode (Remya Nair et al. 2013). A side opening of 1.25cm radius was made at a height of 6cm from the bottom of the container and connected with a PVC pipe. 12% Agarose along with 4% Potassium chloride (KCL) salt was prepared by heating it in a water bath and the molten agarose was allowed to cool down and poured into the PVC pipe and sealed at one end using cello tape. The agarose was left undisturbed to solidify. The PVC pipe containing the salt-agarose mixture was fixed between the two containers using epoxy material and behaved like the salt bridge assisting in the proton transfer mechanism. Activated sludge sample was added to anode chamber and anaerobic conditions were maintained. The cathode chamber contained 0.5M Phosphate buffer. Copper wire and Aluminum mesh were used as the electrodes. Electricity production was measured using digital multimeter.

1.5. Combination of electrodes

Different electrode materials were collected and pretreated by washing with sterile distilled water and followed by ethanol wash for 10 minutes to remove the possible metal and inorganic contaminations and to neutralize the electrodes (Parkash et al. 2015). The electrodes were dried in hot air oven for 5 minutes and these electrodes were relatively inexpensive and easily available. Combinations of electrodes such as, Copper wire + Aluminum mesh, Copper wire + Stainless steel mesh, Copper wire + Pencil lead, Aluminum mesh + Stainless steel mesh, Aluminum mesh + Pencil lead and Stainless steel mesh + Pencil lead were used for the study.

1.6. Isolation and identification of bacterial strain

Pure culture was grown on slants by stab and streak method for subsequent identification and biochemical characterization of the bacterial isolates. For long term storage, isolates were taken in Nutrient broth and covered with 10% glycerol and stored at -20°C and the isolates were numbered as KNC 01 - KNC 04. The isolates were identified at generic level using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology (Cappuccino and Sherman 2002).

1.7. Genomic DNA isolation

CTAB method was used to extract large quantities of heavy molecular weight DNA from bacteria (Parkash et al. 2015). Growth from 3 days old bacterial cultures was inoculated aseptically into a 5ml of nutrient broth and incubated in a rotary shaker 120 rpm at 30°C for 24hrs. The culture was then centrifuged at 10,000rpm for 5 minutes and the supernatant was discarded. The cells were resuspended with 567µl of TE buffer and 5µl RNAse A in a clean centrifuge tube, mixed well and incubated for 5 min at room temperature. 15µl of 10% SDS, 4µl Proteinase K was added, mixed thoroughly and incubated at 37°C for 1hr. After incubation, 100µl of 5M NaCl, 80µl of CTAB/NaCl (10% w/v; 0.7M) solution was added, mixed thoroughly and incubated at 65°C for 10min. approximately equal volume of chloroform/isoamyl alcohol was added and centrifuged for 10min at room temperature. The supernatant was transferred to a fresh eppendorff and equal volume of phenol/chloroform/isoamyl alcohol was added and centrifuged at room temperature for 5 min. The pellet obtained was washed with 70% ethanol, dried and dissolved in 50µl TE buffer with pH 7 or water.

1.8. SEM analysis of anode

The anode surface morphologies were studied using Scanning Electron Microscope (SEM) (Hitachi, SS70; Japan) (Samuel Raj 2013). The anodic samples were collected and fixed overnight with 2.5% paraformaldehyde and 1.5% glutaraldehyde in a buffer solution (0.1 M cacodylate, pH 7.5 at 4°C, and then washed twice followed by stepwise dehydration in a gradient series of water/ethanol solutions (25, 50, 70, 85, 95, 100%), and then the critical-point dried (carbon dioxide).

1.9. Statistical analysis

The statistical analysis of data was carried using SPSS (v.16) and represent as mean ± SD.

2. RESULTS AND DISCUSSION

2.1. Preliminary parameters of chamber construction and isolation bacterial strains

Table (1) represents the physicochemical parameters of the activated sludge such as color, odor, pH (pH meter Model Ec10), temperature (Thermometer) were analyzed immediately after collection. The analyses of Moisture, Total Solids (TS), Total Suspended Solid (TSS), Total Dissolved Solid (TDS), Sludge Volume Index (SVI), Chemical Oxygen Demand (COD) exceeds the CPCB permissible levels. This may be due to the contamination of toxic substances effluxed out from the tannery, pharmaceuticals and textile industries. However, activated sludge is a rich source of micronutrients and macronutrients thereby contributes in the production of cost effective biofuels and biofertilizers by an alternative process known as anaerobic biodigestion (Roni et al. 2015). The construction of single chamber was represented in the figure (1,2). Generally, the cathode in the single chamber is direct contact with air which eradicates the necessity of cathode part; however, the double chamber cathode is exposed to water. Over the construction of single chamber MFCs double chamber showed various advantages like regulation of pH, oxygen generation, addition of electrons mediators, thus these contributes towards the enhanced production of MFCs. Ashoka and his group of researchers have demonstrated the construction of various combinations of electrode and their significance in the microbial fuel cells for the production of electricity. From their results, among all types of electrodes used, Cu/Zn, Al/SS, C/C and SS/SS combination of electrodes produced higher voltage than others (Ashoka 2012). Our results was consistent with the previous studies that copper wire and aluminum mesh combination serves best from their ability to collect electrons produced by the bacteria for a period of 5-7 days and utilized for further electricity production (Table 2: Figure 3). To demonstrate an evolutionary relationship between species, the phylogenetic tree was constructed using the BLAST tree tool (figure 4). A group of genes related through a process of divergent evolution from a common ancestor or the result of convergent evolution was demonstrated. The gene bank accession number is **MT311689 (***Myroides xuanwuensis)* (Table 3; Figure 5,6). Using the isolated strain KNC 01 the maximum output of 0.40 V was obtained at 216 hours after which the voltage decreased gradually.

3.2. Optimization for MFC generation

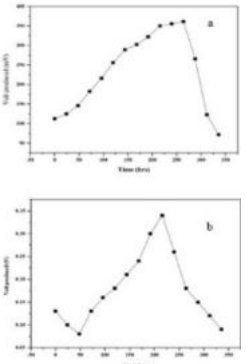
Double chamber MFC performance in UV light treated sludge was evaluated under varying pH concentration, Nitrogen source, Agarose Concentration, activated sludge concentration. The voltage across the external resistor in an MFC was measured using a multi meter till voltage drop was observed.

The power generation varied with increasing pH, 7.0 found to yield maximum power. Gradual decrease in power was observed after 5 days of incubation time. The biological and electrochemical reactions modulate the production of microbial fuel cell as represented in Figure (7). The continuous metabolism of bacteria may produce weak acid to maintain their physiological pH. From the previous literature if the pH is at 7 is auspicious for methanol production which therefore contributes towards the removal of COD (Martin et al. 2010). However, pH more than 7 decreases the coulombic efficiency directly involved in the inhibition of bacteria due to alkalinity nature (Kumar and Mungray. 2017). Based on the reports compared to acidic and neutral pH of MFC alkaline pH are more often produces higher voltage (Naina Mohamed et al. 2018). The power generation varied with nitrogen source, yeast extract found to be the best nitrogen source along with the organic pollutants present in the activated sludge sample to yield maximum power. Gradual decrease in power was observed after 5 days of incubation time. The power generation varied with the varying concentration of agarose in the salt bridge, 10% agarose concentration found to be the best to yield maximum power generation (Figure 8). The power generation was reduced as the activated sludge sample was diluted 2 times, 4 times and 8 times. Though the power generation was reduced the isolate was capable to produce electricity at minimum level. Four double chamber MFC were connected in series under optimized conditions. The anode of the first MFC was connected to the cathode of second MFC, anode of second MFC to the cathode of third MFC and anode of third MFC to the cathode of fourth MFC. At the end the Cathode of the first MFC and anode of the fourth MFC were connected to the multi meter and the voltage generation was measured using multi meter (Figure 9).

Figure 1

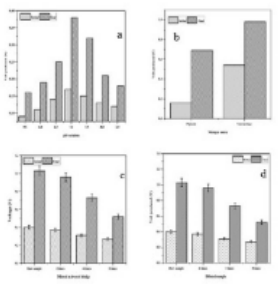
a. Single chambered MFC
b. Double chambered MFC
c. Activated sludge treated with UV for 2h
d. Four double chambered MFC's

Figure 2



a. Single chamber MFC
b. Double chamber MFC

Figure 3



a. Optimization of pH concentration
b. Optimization of nitrogen source
c. Optimization of activated sludge
d. Optimization of agarose concentration

Figure 4

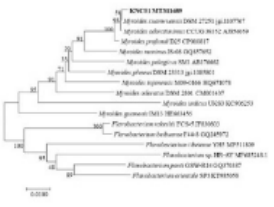


Figure 5

Figure 3

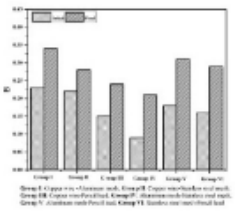


Figure 6

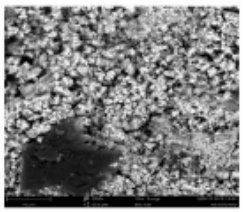


Figure 7

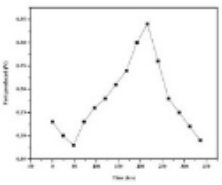


Figure 9

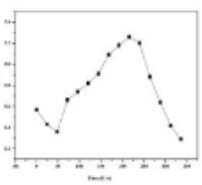


Table 1:

Sl.NO	Parameters	Activated sludge	CPCB I
1	Color	Blackish	NA
2	Smell	Offensive	NA
3	pH	6.4±0.1	5.5-9.0
4	Temperature	28±1.0°C	28-30°C
5	Total Solids(mgL^{-1})	104000±120.2	1000
6	Total Suspended Solids (mgL^{-1})	800±5.0	100
7	Total Dissolved Solids (mgL^{-1})	103200±115.2	500-20(
8	Sludge Volume Index (mgL^{-1})	468.75±6.2	NA
9	Moisture (%)	99.92	NA

Table 2:

Sl. No.	Metal ($mgKg^{-1}$)	Activated Sludge ($mgKg^{-1}$)	WHO standard ($mgKg^{-1}$)
1	Cr	**1.45±0.43**	**0.05**
2	Cu	1.97±0.25	1.0
3	Zn	**1.98±2.11**	**5.0**
4	Ni	0.31±0.02	-
5	Cd	0.17±0.22	0.05
6	Pb	0.27±0.04	0.1

Table 3:

CHARACTERS	KNC 01	KNC 02	KNC 03	KNC 04
Gram stain	-	-	+	+
Shape	Rods	Rods	Rods	Rods
Motility	Non-Motile	Motile	Motile	Motile
Spore	-	-	+	+
Indole	-	+	-	-
MR	-	+	-	+
VP	+	-	+	-
Citrate	+	-	+	-
Catalase	+	+	+	-
Oxidase	+	-	-	-
H_2S	-	-	-	+
Urease	+	-	-	-
Identification	Myroides sp.	E. coli	Bacillus sp.	Clostridium sp.

CONCLUSIONS

The study performed concludes that the strain Myroides *xuanwuensis* - MT311689 isolated form activated sludge has the potential to generate sufficient amount of electricity which can be used for small domestic requirements. Though MFC produces minimal electricity, parameters such as substrate, catholyte and electrode design can be modified to improve the voltage output. The usage of salt bridge instead of proton exchange membrane is more cost effective and easily available. Microbial fuel cells in near future might bring a beneficial result and hence are of utmost concern to clean up the environment and add wastewater to the list of new renewable energy of bio-energy.

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