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Bioelectricity Production and Comparative Evaluation of Electrode Materials in Microbial Fuel Cells Using Indigenous Anode-Reducing Bacterial Community from Wastewater of Rice-Based Industries

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ABSTRACT. Microbial fuel cells (MFCs) are the electrochemical systems that harness the electricity production capacity of certain microbes from the reduction of biodegradable compounds. The present study aimed to develop mediator-less MFC without using expensive proton exchange membrane. In the present study, a triplicate of dual-chamber, mediator-less MFCs was operated with two local rice based industrial wastewater to explore the potential of this wastewater as a fuel option in these electrochemical systems. 30 combinations of 6 electrodes viz. Carbon (14 cm × 1.5 cm), Zn (14.9 cm × 4.9 cm), Cu (14.9 cm × 4.9 cm), Sn (14.1cm × 4.5cm), Fe (14cm × 4cm) and Al (14cm × 4.5 cm) were evaluated for each of the wastewater samples. Zn-C as anode-cathode combination produced a maximum voltage that was 1.084±0.016V and 1.086±0.028 and current of 1.777±0.115mA and 1.503±0.120 for KRM and SSR, respectively. In the present study, thick biofilm has been observed growing in MFC anode. Total 14 bacterial isolates growing in anode were obtained from two of the wastewater. The dual chambered, membrane-less and mediator-less MFCs were employed successfully to improve the economic feasibility of these electrochemical systems to generate bioelectricity and wastewater treatment simultaneously.

Keywords: Membrane-less, Microbial Fuel Cells, Biofilm, Wastewater, Electrogenic.

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1. Introduction

In recent years, impressive progress has been made in the development of clean energy technologies. Surging demand for fossil fuels and other non-renewable resources has shadowed the success of these clean and green efforts that have been made in past decades. Implementations of energy efficient technologies are greatly hindered by the ever over demanding industrialization, which largely depends on non-renewable resources (Brown, 2001). The scenario of depletion of fossil fuel based resources and its ever increasing demand has made the path for searching for renewable recourses and utilization of waste materials we accumulated in the past few centuries. Energy in the form of electricity is the backbone for the development of any nation. Electricity markets are assumed to undergo massive transformations which are majorly concern towards the low-carbon power generation. The coal and other fossil fuels, larger hydrothermal plants, nuclear power plants have been associated with the adverse environmental consequences (Wei et al., 2010). The urgent need is to think for alternative resources and shifts the industries towards clean energy based on utilization of waste and attract investors in this particular field.

As the industrialization climbed up in an ever seen manner, the amount of waste generated also increased proportionately. This situation leads to crucial environmental as well as economical challenges remarkably accumulation of bulk of the waste, high handling and management coast, sophisticated treatment and disposal operations. According to World Water Development Report (2003), approximately 2 million tons of wastes per day are disposed of within receiving waters from human excreta; agricultural wastes in the form of fertilizers, pesticide residues and industrial wastes and chemicals, etc.

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In the year 1995, the global industrial sectors were estimated to use about 725 km^3 of water, annually. By 2025, a rise expected to about 1,170 km^3 water annually and with this raise, water usage by industrial sectors will represent 24% of all water abstractions. It is estimated that in developing countries 70% of industrial discharges is dumped untreated in water resources. In India, the wastewater generated from medium and large industries is 55,000 million m^3 per day, of which 68.5 million m^3 is dumped directly into local rivers and streams without any treatments (Pangare et al., 2006).

The microbial fuel cell technology offers novel alternative and cost effective approach of energy generation directly from the oxidation of waste organic matter and renewable biomass in the form of electricity with less sludge production as compared to aerobic processes (Ahn and Logan, 2010). A variety of wastewater has been used in MFC as fuel such as domestic sewage (Ahn and Logan, 2010; Liu et al., 2004), paper and pulp (Huang & Logan, 2008), rice mill (Behera et al., 2010), brewery (Feng et al., 2008), swine wastewater (Kim et al., 2008) and phenolic wastewater (Luo et al., 2009) , Oil refinery wastewater (Majumder et al., 2014), Effluent rice mill wastewater (Daniel et al., 2009), chocolate industry wastewater (Patil et al., 2009), and real dye wastewater (Kalathil et al., 2012) etc.

MFCs offer many operational and functional advantages over conventional fuel cells, including the use of waste organic matter as fuels and indigenous microbes as catalysts (Rabaey & Verstraete, 2005). Furthermore, MFCs do not require highly regulated distribution systems and can harvest up to 90% of the electrons from the bacterial electron transport system (Amade et al., 2015).The design and the electrode materials being used in an MFC are the important aspect of the innovation and development. The crucial part of MFCs development is the choice and the coast of the electrode materials which directly impact on the performance of the MFCs, and ultimately leads to the overall reduction of the costs of microbial fuel cells (Feng et al., 2008, Amade et al., 2015). Most frequently used electrode materials are based on carbon and its different forms like, graphite based, carbon cloth, carbon paper, carbon felt (Logan and Regan, 2006), graphite based electrodes like graphite fiber brush, graphite felt (Logan et al., 2008), graphite granules, carbon mesh (Wang et al., 2009) and expensive metal-based materials like Pt-based electrode, platinum, platinum black, activated carbon (Singh and Songera, 2012). Electrodes can be arranged in single or tubular or multi-electrode configurations (Kim et al., 1999). These electrodes should have the properties of biocompatibility, stability, high electrical conductivity and larger surface area (Logan et al., 2008, Singh and Songera, 2012).

The optimization of this technology and their application in real wastewater is a great point towards renewable resources of energy. Looking at this side of the idea, the study was carried out in Chhattisgarh that is an emerging agro-industrial state of India. The state produces tons of seasonal crops. In the year 2012 and 2013, production of 8,127.5 and 1,885.67 thousand metric tons of Kharif and Rabi crop was reported (Chhattisgarh Economic Census, 2013-14). This agro-industrial profile of the state supports many large and medium as well as small size industries. Thus, the waste generated from this region is rich in biodegradable materials. Also, easy availability of the industrial wastes offers the opportunity to work in this concerned field. The present study aimed to check the feasibility of bioelectricity generation using wastewater of rice based industries as a fuel in microbial fuel cells using indigenous anode-reducing bacterial community as well as comparative evaluation of different electrode materials.

2. Materials and Methods

2.1 *Sample Collection*

Wastewater from two local rice based industries has been used throughout the experiment that is Khandelwal Rice Mill, Tatibandh, Raipur, Chhattisgarh (India) and Shree Sita Refiners Pvt. Ltd, Arasnara, Durg, Chhattisgarh (India) designated as KRM and SSR, respectively. Samples were collected in 5 L sterile airtight plastic containers and were stored at 4 ± 1^0C for short term. The wastewater without modification in organic load or pH adjustments was used as inoculum as well as fuel for all MFC operations. The wastewater was analyzed before and after the operation for different physiochemical parameters like-colour, odour, pH, BOD, COD, and TDS using standard methods of APHA.

2.2 *Construction of Microbial Fuel Cells*

H-shaped double-chambered microbial fuel cells were architect using non-reactive and autoclavable plastic containers (15cm long, 8.5cm diameter with a working volume of 750mL) under anaerobic microenvironment. MFC comprised of an anode and an open air-Cathode chambers. A UPVC pipe of dimension 6.1cm × 1.3 cm containing agar salt bridge was used to separate the chambers physically (Figure 1). In the present study, agar salt bridge (sodium chloride, 10% and agar, 5%) was used as proton exchange material offering a cost effective alternative to the proton exchange membranes like –Nafion etc. (Momoh and Naeyor, 2010, Kumar et al., 2012). The aid of adhesive material (M-Seal) was used to fix both the chamber intact.

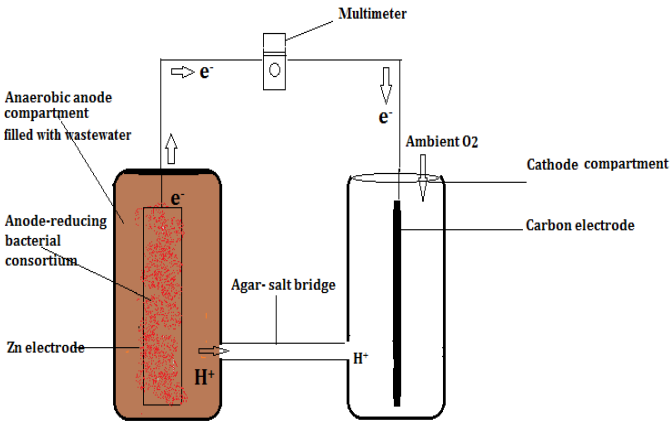

Figure 1. Scheme of MFC construction used in present study

30 combinations from 6 electrodes that are Carbon (14cm × 1.5cm), Zn (14.9cm × 4.9cm), Cu (14.9cm × 4.9cm), Sn (14.1cm × 4.5cm), Fe (14cm × 4cm) and Al (14cm × 4.5cm) were evaluated for each of the wastewater samples assuming that different surface area of electrode may affect biofilm formation and electron transfer of electron between the anode and cathode chambers (Prabowo et al.,2016). These metals were selected because of their easy availability and cost effectiveness that offered an alternative to expensive and sophisticated electrode materials like expensive Pt based electrodes (Logan, 2009).

External copper wires were used to connect the electrodes to the digital multimeter (KUSAM-MECO 603) by alligator clips (Logan, 2005). The anode compartments were subjected to maintain the anaerobic microenvironment by a leak proof sealing of joints and the exposed metal surfaces sealed with a nonconductive epoxy to avoid corrosion (Kumar et al., 2012). The open air-cathode chamber was filled with 50mM phosphate buffer and pH adjusted to 7. In this set up oxygen was employed as the final electron acceptor (Feng et al., 2008). The MFCs were sterilized with saturated ethanol followed by heat sterilization at 85^0C for 2hr and irradiated with UV for 30 min.

2.3 *MFC Operation*

The MFC setup was run in fed-batch mode. The performance of all the MFCs was evaluated by measuring open circuit voltage (OCV) and current along with COD removal efficiency. Constant voltage output and COD removal efficiency were considered as indicators of stable performance of MFC.

The anodic chamber was filled with 750 ml of collected wastewater samples and cathode chamber was filled with phosphate buffer. Anode and cathode were connected to the external wiring to complete the circuit and voltage and current were measured via multimeter. The electrolytic solution is exposed to air for the reduction reaction to occur (Feng et al., 2008, Vignesh and Rani, 2012). In the mediator-less anode compartment, indigenous bacterial community oxidizes fuel, resulting in production of electrons and protons (Gil et al., 2003, Gregory et al., 2004). The electrons travel through the external circuit and the protons are transferred to the cathode compartment through the salt bridge. Throughout the operation anaerobic micro-environment was maintained in the anode compartment (Lovely et al., 1993). MFCs were operated in batch mode at ambient room temperature (32 ± 2^0C).

2.4 *Statistical Analysis*

The voltage and current were recorded in the open circuit using auto-range digital multimeter (KUSAM-MECO 603) after 1hour time intervals for three and five consecutive days for electrode optimization and isolation studies, respectively. The COD and BOD were determined by standard methods described by APHA. EC and TDS were analyzed using EC-TDS analyzer (ELICO CM-183), DO was measured using a DO analyzer (ELICO PE-135) and the pH was measured using a pH meter (ELICO LI-120). All experiments were performed in triplicate. Descriptive analysis of data and calculations were done by SPSS16 and typical values are presented.

3. Results and Discussion

3.1 *Optimization of electrode materials*

One of the major challenges associated with MFCs is the cost of the electrode materials. The present study elucidates the feasibility of comparatively cheap and easily available materials as an alternative to expensive and sophisticated electrode materials (Kim et al., 1999, Logan, 2008, Singh and Songera, 2012). Thirty different pair of electrodes that are Zn-Cu, Cu-Zn, Zn-C, C- Zn, Cu-C, C-Cu, Cu-Sn, Sn-Cu, Sn-C, C-Sn, Zn-Fe, Fe-Zn, Cu-Fe, Fe-Cu, Fe-C, C-Fe, Zn-Sn, Sn-Zn, Al-Cu, Cu-Al, Al-C, C-Al, Al-Fe, Fe-Al, Al-Sn, Sn-Al, Zn-Al, Al-Zn, Fe-Sn and Fe-Sn were used as Anode –Cathode combinations for the optimization of electrode combination for two of the wastewater samples (Table no. 01 and 02). Among the thirty pairs, the Zn-C electrode combination gives best results for both the sample. The maximum voltage generated was 1.084±0.016V and 1.086 ± 0.028 and current of 1.777±0.115mA and 1.503±0.120 for KRM and SSR, respectively (Table 1 and 2). However, Sn-C electrode combination has statistically similar impact on electricity production for the wastewater from KRM. The maximum voltage and current recorded was 1.064±0.016V and 1.777±0.115mA, respectively (Table.1). When comparison made between the

bioelectricity production capacities of these combinations, tremendous trend has been seen. The low voltage and current were recorded for the Al-Fe (0.021±0.001V and 0.030±0.004mA) and Fe-Al combination (0.037±0.008V and 0.052±0.008mA) for WW from KRM. In case of WW from SSR, the lowest voltage and current were recorded for Fe-Sn (0.132±0.025V and 0.415±0.018mA), Fe-Sn combination (0.156±0.032V and 0.250±0.066mA) and C-Cu (0.151±0.017V and 0.386±0.042mA). A moderate bioelectricity generation ranging from 0.549±0.114V–0.957±0.039V and current 0.734±0.115mA–1.484±0.073mA has been seen for the electrode combination Zn-Cu, C- Zn, Fe-C, Al-Cu, Al-C, Sn-Cu, C-Sn, Fe-Cu, C-Al, C-Cu, Cu-Zn and Zn-Fe for wastewater from KRM (Table. 1 and Figure 2).

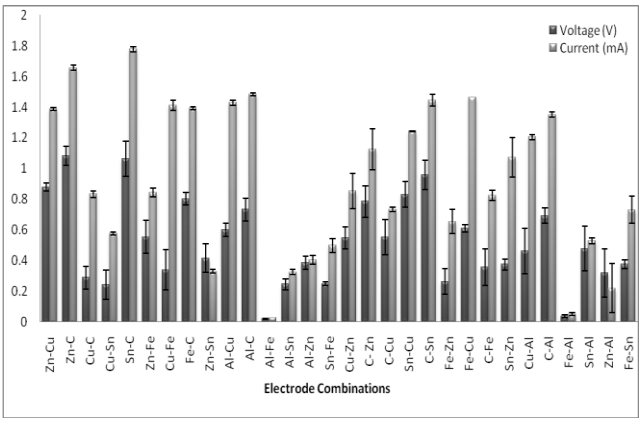

Figure 2. The voltage and current recorded for wastewater from KRM for optimization of electrode combinations

We believe that electricity production was dependent on anode composition that supported attached growth of specific indigenous anode bacteria, high organic load in wastewater and capacity of indigenous anode residing bacteria to harness electricity from the substrate (Park and Zeikus 2002). This combination of conditions affected the overall MFC performance operated with different electrode and wastewater. Wastewater from rice bran oil refinery may have some complex components including poly-unsaturated Fats, mono-saturated fats, saturated fats, rich amount of essential fatty acids, Linoleic fatty acid, Linolenic acids and trace amount of other nutrients that are recommended for edible oils (Ghosh, 2007); whereas wastewater from rice mill is having more amount of starch and lesser of other complex nutrients such as Ash Percentage, Crude Fat, Crude Protein, Crude Fiber and Vitamin B-6 because it is the result of the basic steps of parboiling namely soaking and steaming procedure of paddy in order to obtain commercialized rice. Therefore, it is simple composition of wastewater (Akhter et al., 2014).

Table 1.
Effect of electrode combinations on bioelectricity generation using wastewater from Khandelwal Rice Mill, Tatibandh, Raipur, Chhattisgarh, India

Electrode Combination (Anode-cathode)	Voltage (VΩ)	Current (mA)
Zn-Cu	0.879±0.010^{lm}	1.387±0.020^{hij}
Zn-C	1.084±0.016^{n}	1.657±0.062^{ij}
Cu-C	0.289±0.022^{bc}	0.833±0.275^{defg}
Cu-Sn	0.242±0.010^{b}	0.576±0.176^{bcd}
Sn-C	1.064±0.016^{n}	1.777±0.115^{j}
Zn-Fe	0.555±0.029^{ghi}	0.842±0.307^{defg}
Cu-Fe	0.340±0.035^{bcde}	1.411±0.130^{hij}
Fe-C	0.802±0.010^{kl}	1.394±0.040 ^{hij}
Zn-Sn	0.416±0.013^{cdefg}	0.330±0.092^{abc}
Al-Cu	0.601±0.0169^{hij}	1.430±0.042^{hij}
Al-C	0.732±0.010^{jkl}	1.484±0.073^{hij}
Al-Fe	0.021 ±0.001^{a}	0.035±0.004^{a}
Al-Sn	0.245±0.017^{b}	0.328±0.038^{abc}
Zn-Al	0.388±0.029^{bcdef}	0.405±0.043^{abcd}
Fe-Sn	0.249±0.044^{b}	0.498±0.012^{bcd}
Cu-Zn	0.549±0.114^{fghi}	0.854±0.172^{defg}
C- Zn	0.786±0.134^{kl}	1.125±0.203^{fgh}
C-Cu	0.554±0.012^{ghi}	0.734±0.115^{cdef}
Sn-Cu	0.831±0.004^{klm}	1.243±0.085^{ghi}
C-Sn	0.957±0.039^{mn}	1.445±0.096^{hij}
Fe-Zn	0.263 ±0.084^{bc}	0.655±0.083^{bcde}
Fe-Cu	0.610±0.001^{hij}	1.465±0.024^{hij}
C-Fe	0.357 ±0.034^{bc}	0.823±0.307^{defg}
Sn-Zn	0.374±0.130^{bcde}	1.072±0.037^{efgh}
Cu-Al	0.461±0.018^{defgh}	1.205±0.248^{ghi}
C-Al	0.693±0.019^{ijk}	1.352±0.051^{hij}
Fe-Al	0.037±0.008^{a}	0.052±0.008^{a}
Sn-Al	0.477±0.018^{defgh}	0.529±0.145^{bcd}
Al-Zn	0.318±0.081^{bcd}	0.221±0.158^{ab}
Fe-Sn	0.376±0.089^{bcde}	0.731±0.027^{cdef}

*ANOVA- (for voltage) - df = 29, F= 33.278, Mean Squared = 0.2398, Sig. = 0.00. * ANOVA- (for current) - df = 29, F= 13.026, Mean Squared =0 .7356, Sig. = 0.00. * Means followed by similar superscript letters (a, b, c,…….n) do not differ significantly at 0.05 level by Duncan's Multiple Range Test.

The feasibility of a redox reactions were tested based on the theoretical electrochemical reactions. In electrochemical series Zn is more electro-negativity as compared to Sn. With the help of electromotive series we assumed that the EMF ^{o} comes out to be positive and reaction will take place. In any microbial electrochemical system the electricity generation is dependent on variety of factors that includes type of electrode material, electrical conductivity, the electrode resistance, the substrate composition, high specific surface area (area per volume), the rate and type of the product formed can affect with performance and productivity of the MFC (Logan et al., 2008, Singh and Songera, 2012, Prabowo et al., 2016).

A similar trend has been observed in wastewater from SSR in which electrode combinations Zn-Cu, Cu-Zn, Cu-Sn, Sn-C, Fe-C, Zn-Sn, Al-Cu, Al-C, C- Zn, C-Sn, C-Fe, Cu-Al and C-Al also showed bioelectricity production ranging from 0.573 ± 0.019 V - 0.945 ± 0.043 V and current

0.625 ± 0.032 mA - 1.394 ± 0.069 mA (Table. 2 and Fig. 3). Lower bioelectricity generation seen for Cu-Al, Zn-Sn, Fe-Zn, Cu-C, Cu-Sn, Cu-Fe, C-Fe, Sn-Zn, Al-Sn, Sn-Al, Zn-Al, Al-Zn, Fe-Sn and Fe-Sn electrode combination that ranged from 0.242 ± 0.010 V– 0.477 ± 0.018 V and a current of 0.221 ± 0.158 mA– 1.411 ± 0.130 mA, when tested for wastewater from KRM (Table 1). In case of wastewater from SSR, Cu-C, Sn-Cu, Zn-Fe, Fe-Zn, Cu-Fe, Fe-Cu, Sn-Zn, Al-Fe, Fe-Al, Al-Sn, Sn-Al, Zn-Al, Al-Zn, Fe-Sn and Fe-Sn showed a lowest bioelectricity generation from 0.196 ± 0.070 V - 0.469 ± 0.138 V and 0.169 ± 0.022 mA - 1.126 ± 0.317 mA. For all the combinations, very low electrode fouling was observed and the anode could be used in further experiments without remarkable activity loss.

Table 2.
Showing the effect of electrode combinations on bioelectricity generation using wastewater from Shree Sita Oil Refinery, Arasnara, Durg, Chhattisgarh, India

Electrode Combination (Anode-cathode)	Voltage (VΩ)	Current (mA)
Zn-Cu	0.753±0.137^{ij}	1.003±0.137^{efgh}
Zn-C	1.086±0.028^{m}	1.503±0.120^{i}
Cu-C	0.237±0.033^{abcd}	0.447±0.061^{abc}
Cu-Sn	0.880±0.014^{jkl}	1.394±0.069^{hi}
Sn-C	0.573±0.019^{gh}	1.282 ±0.152^{ghi}
Zn-Fe	0.469±0.138^{fg}	0.555±0.138^{abcd}
Cu-Fe	0.421±0.029^{efg}	0.383±0.048 ^{abc}
Fe-C	0.650±0.010^{hi}	0.635±0.107^{bcde}
Zn-Sn	0.811±0.040^{ijkl}	1.252±0.191^{ghi}
Al-Cu	0.667±0.027^{hi}	0.872±0.210^{defg}
Al-C	0.751±0.010^{ij}	1.276±0.138^{ghi}
Al-Fe	0.256 ±0.041^{abcde}	0.380±0.043^{abc}
Al-Sn	0.196 ± 0.070^{abc}	0.311±0.147^{ab}
Zn-Al	0.461 ± 0.127^{fg}	0.478±0.040^{abcd}
Fe-Sn	0.132 ± 0.025^{a}	0.415±0.018^{abc}
Cu-Zn	0.762 ±0.010^{ij}	0.974±0.107^{efgh}
C- Zn	0.694 ±0.071^{hi}	1.190±0.138^{ghi}
C-Cu	0.151 ±0.017^{ab}	0.386±0.042^{abc}
Sn-Cu	0.422 ±0.011^{efg}	0.655±0.055^{bcde}
C-Sn	0.945 ±0.043^{klm}	1.285±0.146^{ghi}
Fe-Zn	0.331 ±0.003^{cdef}	0.387±0.059^{abc}
Fe-Cu	0.451 ±0.006^{fg}	1.126±0.317^{fghi}
C-Fe	0.718 ±0.049^{hij}	0.871±0.128^{defg}
Sn-Zn	0.272±0.005^{abcde}	0.302±0.030^{ab}
Cu-Al	0.574 ±0.010^{gh}	0.625±0.032^{bcde}
C-Al	0.712 ±0.062^{hi}	1.329±0.172^{hi}
Fe-Al	0.315±0.056^{bcdef}	0.451±0.106^{abc}
Sn-Al	0.203 ±0.063^{abc}	0.169± 0.022^{a}
Al-Zn	0.340 ±0.018^{cdef}	0.756± 0.178^{cdef}
Fe-Sn	0.156 ±0.032^{ab}	0.250± 0.066^{ab}

*ANOVA- (for voltage) df = 29, F= 26.17, Mean Squared = 0.2074, Sig. = 0.00.
*ANOVA- (for current) df = 29, F= 10.3718, Mean Squared = 0.5013, Sig. = 0.00.
*Means followed by similar superscript letters (a, b, c,….m) do not differ significantly at 0.05 level by Duncan's Multiple Range Test.

3.2 Bioelectricity production by indigenous anode-reducing bacterial isolates

Membrane-less MFCs with Zn-C electrode combinations were operated with raw wastewater for the production of electricity in the absence of exogenous electron acceptors. During MFC operation, Zn electrodes (Anode) were removed from assembly and electrodes were scrapped with sterile scalpel and collected in flasks having sterile water. Seven bacterial isolates from each of the MFC operated with two of the wastewater (KRM and SSR) were isolated on nutrient agar plate by spread plate method. The isolates were named as WRK1 to WRK7 and WRS1 to WRS7 in which W stands for wastewater, R for rice industries and K and S for the name of the industry from where wastewater was collected.

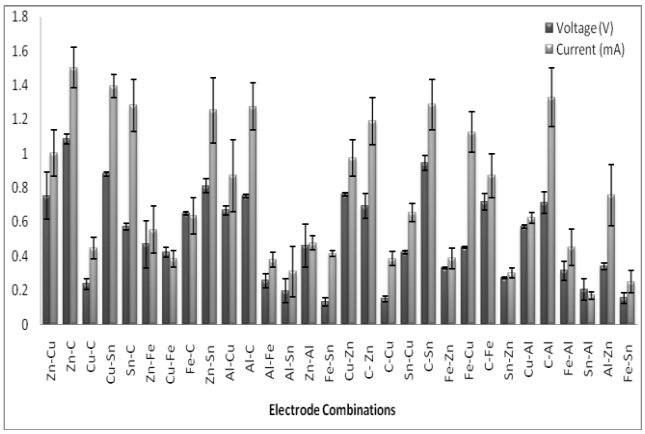

Figure 3. The voltage and current recorded for wastewater from SSR for optimization of electrode combinations.

All the isolates were found to be gram negative and non-acid fast. The isolates were endospore forming except WRK1 and WRK7 (Table 3). Previous reports suggest that gram negative bacteria are good electrogenic candidate for these microbe associated electrochemical systems (Cournet et al., 2010); say for *Shewanella oneidensis* (Logan, 2009), *Shewanella putrefaciens* (Kim et al., 1999), *Geobacter sulfurreducens* (Ishii et al., 2008), *Klebsiell pneumoniae* (Zhang et al. 2008). All the isolates were screened for their electrogenic properties in MFC operated with respective sterile wastewater as well as synthetic wastewater. The electricity generated is directly from microbes and the transfer of electrons from anode to cathode is mediated by microbes itself as no external mediators were used (Lovely et al., 1993, Yi et al., 2009, Bond and Lovley 2003). Literature showed that these MFC systems are dominated by metal- and anode-reducing bacterial communities indigenous in the anode bio-film (Lovely et al., 1993, Yi et al., 2009, Bond and Lovley, 2003).

In overall chemistry, the bacteria utilize the organic components of the wastewater and generate redox-active molecules outside and inside the cells during their metabolic activities that lead to shuttling of electrons between reduced and oxidized compounds. Firstly, when ambient air is employed as oxygen source at the cathode chamber, electrochemical reduced of oxygen takes place due to H^+ ion passage, from anode to cathode through the proton exchange material, forming water on the cathode side. Meanwhile, the oxidation of

fuel leads to generation of electrons that is further transferred to the Zn anode and the oxidation of Zn anode takes place, where Zn is converted to Zn^{2+} when electrons are discharged and transferred through the external wires. The electrons generated on the anode side pass across the external load and goes to the cathode, where the reduction of oxygen takes place. We assume that the direct electron transfer via physical contact of the bacterial cell membrane or a membrane organelle with the fuel cell anode is taking place in the present study because no external redox mediators have been introduced to the electrochemical system that could accomplish the electron transfer between the cells and anode. Relays of electrochemically active redox enzymes present in Cytochromes specifically localized to the outer membrane of intact bacterial cells are believed to allow the electron transfer to an external, solid electron acceptor that is the anode of MFC (Prasad et al., 2007, Bond and Lovley, 2003).

Table 3.
Indigenous Anode bacterial isolates obtained from MFC operated with wastewater of KRM and SSR

Bacterial isolates	Gram staining	Endospore staining	Acid-fast staining
WRK 1	Gram Negative Bacillus	-ve	-ve
WRK 2	Gram Negative Bacillus	+ve	-ve
WRK 3	Gram Negative Streptobacillus	+ve	-ve
WRK 4	Gram Negative Bacillus	+ve	-ve
WRK 5	Gram Negative Coccobacillus	+ve	-ve
WRK 6	Gram Negative Bacillus	+ve	-ve
WRK 7	Gram Negative Staphylococcus	-ve	-ve
WRS 1	Gram Negative Staphylococcus	+ve	-ve
WRS 2	Gram Negative Bacillus	+ve	-ve
WRS 3	Gram Negative Bacillus	+ve	-ve
WRS 4	Gram Negative Bacillus	+ve	-ve
WRS 5	Gram Negative Coccobacillus	+ve	-ve
WRS 6	Gram Negative Streptobacillus	+ve	-ve
WRS 7	Gram Negative Staphylococcus	-ve	-ve

All the fourteen bacterial isolates were found to be electrogenic**.** When mediator-less MFC operated with sterile wastewater (KRM), the isolate WRK 2 produced highest voltage (1.242 ± 0.041 V) and current (2.047± 0.244 mA) as compared to six other isolates. However, all the isolate produced more than 1.00V and current of about 1.6 mA in mediator-less MFCs (Tbale 4, Figure 4 and 5). The results compared with the performance of bacterial isolates in MFC operated with synthetic wastewater less electricity production were observed (Figure 6 and 7).

This pattern might be due to high organic load in raw sterile wastewater as compared to synthetic wastewater that offered range of organic material for growth of bacterial isolates. When mediator-less MFCs operated with sterile wastewater (SSR), no significant difference were noticed between electricity production by the isolate WRS 1, WRS 2, WRS 6 and WRS 7, that was voltage between 1.141 ± 0.032V -1.117 ± 0.001 V and current about 1.254 ± 0.066mA -1.804 ± 0.176 mA (Table 5, Figure 8-11).

Table 4.
Bioelectricity production by Indigenous Anode bacterial isolates operated with synthetic wastewater and sterile wastewater from Khandelwal Rice Mill, Tatibadh, Raipur, Chhattisgarh, India

Bacterial Isolates	Sterile Wastewater		Synthetic Wastewater	
	Voltage (V)	Current (mA)	Voltage (V)	Current (mA)
WRK1	1.08±0.040^{c}	1.509±0.155^{bc}	0.799±0.006^{c}	1.071±0.019^{c}
WRK2	1.242±0.041^{a}	2.047±0.244^{a}	0.678±0.031^{b}	1.043±0.015^{bc}
WRK3	1.156±0.007^{bc}	1.766±0.117^{ab}	0.648±0.006^{d}	0.933±0.029^{d}
WRK4	1.187±0.011^{ab}	1.595±0.108^{bc}	1.051± .014^{b}	1.914±0.420^{b}
WRK5	1.152±0.012^{bc}	1.596±0.054^{bc}	0.396±0.032^{e}	0.301±0.077^{e}
WRK6	1.089±0.007^{c}	1.599±0.066^{bc}	1.148±0.006^{a}	1.428±.071^{a}
WRK7	1.115±0.13^{bc}	1.299±0.340^{c}	1.009±0.036^{b}	1.173±0.030^{bc}

*ANOVA- (for voltage) df = 6, F= 6.17, Mean Squared = 0.4074, Sig. = 0.00. *ANOVA- (for current) df = 6, F= 17.38, Mean Squared = 0.303, Sig. = 0.00. * Means followed by similar superscript letters (a, b, c,….m) do not differ significantly at 0.05 level by Duncan's Multiple Range Test.

Table 5.
Bioelectricity production by Indigenous Anode bacterial isolates operated with synthetic wastewater and sterile wastewater from Shree Sita Oil Refinery, Arasnara, Durg, Chhattisgarh, India

Bacterial Isolates	Sterile Wastewater		Synthetic Wastewater	
	Voltage (V)	Current (mA)	Voltage (V)	Current (mA)
WRS 1	1.117±0.001^{a}	1.796±0.191^{bc}	1.078±0.001^{bc}	1.244±0.032^{ab}
WRS 2	1.117±0.032^{a}	1.303±0.121^{bc}	1.048±0.031^{c}	1.190± 0.025^{c}
WRS 3	1.037±0.003^{ab}	1.461±0.007^{b}	1.112±0.006^{ab}	1.209±0.017^{ab}
WRS 4	0.779± 0.050^{c}	1.009±0.163^{c}	1.102±0.008^{ab}	1.244±0.064^{ab}
WRS 5	0.951±0.038^{b}	0.980±0.061^{c}	1.097±0.012^{bc}	1.224±0.009^{ab}
WRS 6	1.141 ±0.032^{a}	1.804±0.176^{a}	1.161±0 .034^{a}	1.316± 0.027^{a}
WRS 7	1.128± 0.011^{a}	1.254±0.066^{bc}	1.102±0.012^{ab}	1.264±0.024^{ab}

ANOVA- (for voltage) df = 6, F= 20.117, Mean Squared = 0.1074, Sig. = 0.00. *ANOVA- (for current) df = 6, F= 16.7128, Mean Squared 0.7041, Sig. = 0.00. * Means followed by similar superscript letters (a, b, c,....m) do not differ significantly at 0.05 level by Duncan's Multiple Range Test.

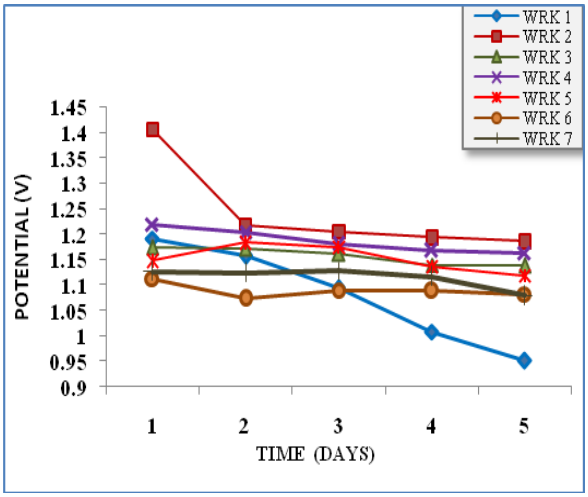

Figure 4. Voltage-time profile of bacterial isolates in MFC Operated with sterile wastewater KRM

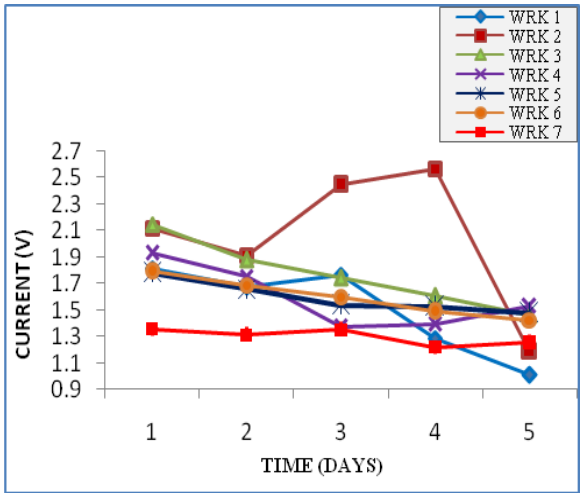

Figure 5. Current-time profile of bacterial isolates in MFC Operated with sterile wastewater KRM

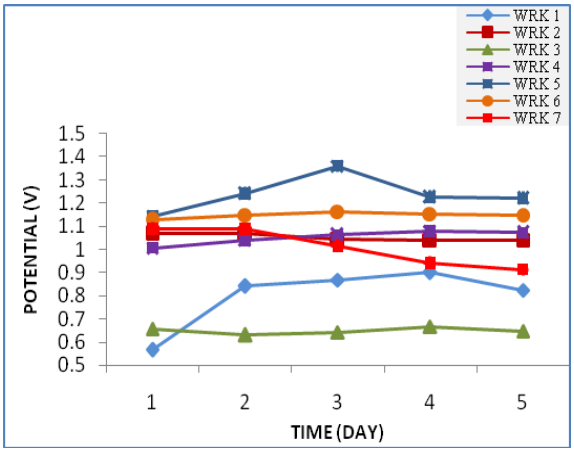

Figure 6. Voltage-time profile of bacterial isolates in MFC Operated with synthetic wastewater

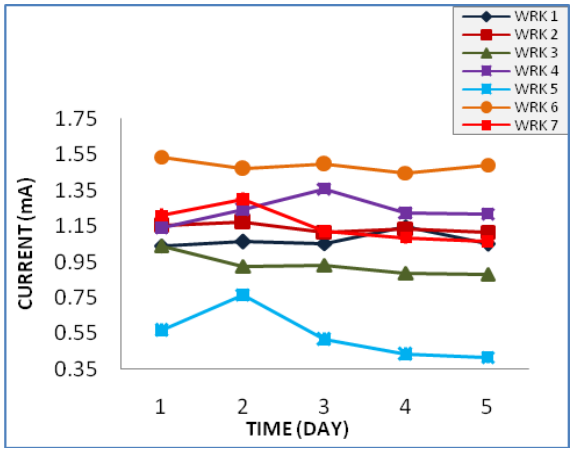

Figure 7. Current-time profile of bacterial isolates in MFC Operated with synthetic wastewater

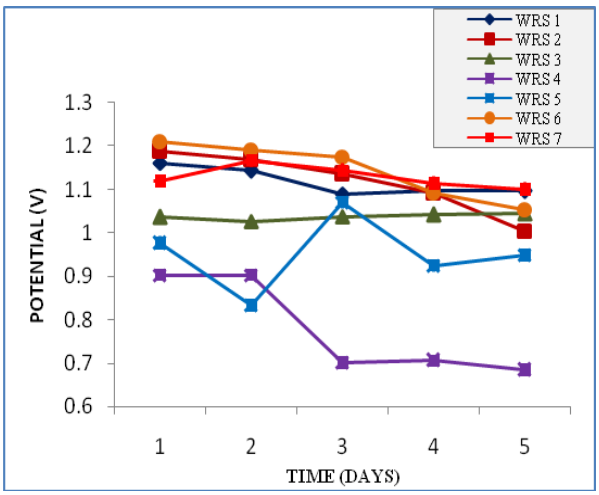

Figure 8. Voltage-time profile of bacterial isolates in MFC Operated with sterile wastewater SSR

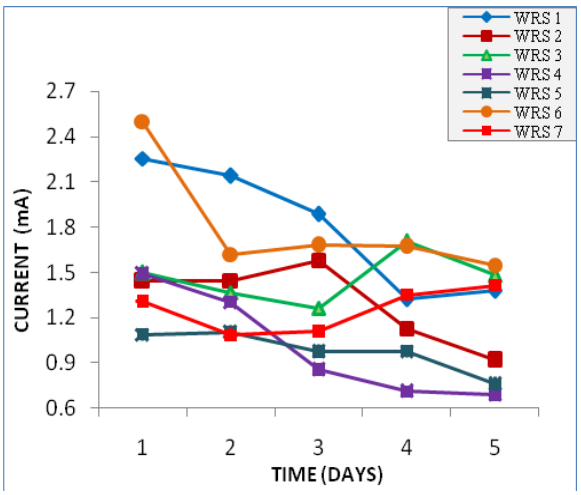

Figure 9. Current-time profile of bacterial isolates in MFC Operated with sterile wastewater SSR

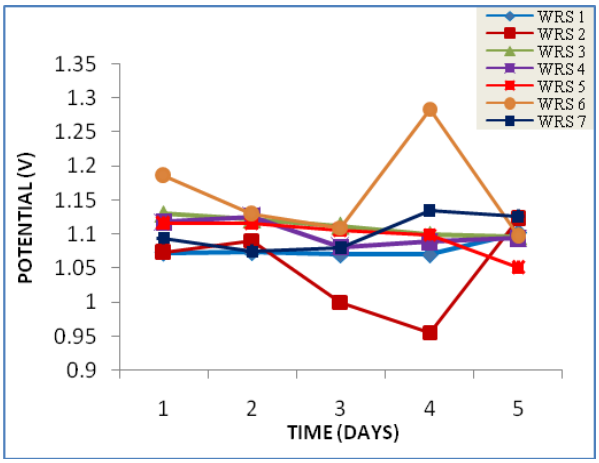

Figure 10. Voltage-time profile of bacterial isolates in MFC Operated with synthetic wastewater

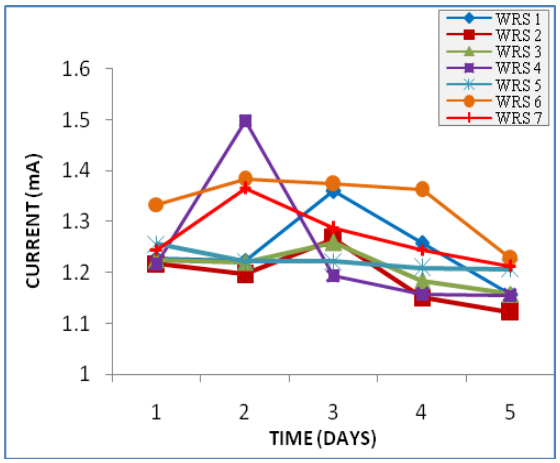

Figure 11. Current-time profile of bacterial isolates in MFC Operated with synthetic wastewater

Our finding support previous study on the wastewater from different industrial sectors that this wastewater can be potential candidate for the fuel in MFCs. A variety of wastewater have been used in MFC as fuel such as domestic sewage with voltages ranging from 0.43–0.44 V (Ahn and Logan, 2010) and 0.32±0.01 V (Liu et al., 2004); Huang and Logan, 2008 were able to produce the power output of 400 mV - 420 mV using paper and pulp wastewater; maximum voltages generated in an MFC using Rice mill wastewater were 0.304 V and 0.172 V (Behera et al., 2010); brewery wastewater was able to produce 0.071 - 0.330 V (Feng et al., 2008); a current of 2.12–2.48 mA were recorded using distillery wastewater (Mohanakrishna et al., 2010); in odor-producing compounds treatment of swine wastewater a maximum of 0.4 V were recorded (Kim et al., 2008); Oil refinery wastewater produced the maximum voltage output of 0.355 V (Majumder et al.,2014). A maximum open-circuit potential of 2.2 V was obtained using rice mill wastewater with the anode in batch-fed mode of operation(Daniel et al., 2009); 0.4908 V achieved using starch processing wastewater (Lu et al., 2009); 0.421V was produced using starch processing wastewater (Park et al., 2001), 0.207±0.03 V to 0.350±0.025 V were recorded using municipal Wastewater (Mohanakrishna et al., 2010); high open circuit voltage (OCV) of 0.810 V were reported using dairy industry wastewater (Lu et al., 2009), 0.689 V was generated using coconut husk retting wastewater (Park et al., 2001), near 1 V open circuit potential (OCV) were

recorded for Palm oil mill effluent (Baranitharan et al., 2013). The present study reports nearly 1 V of electricity from both of the wastewater sample that is higher than the previous reports, suggesting rice based industrial wastewater as a potent candidate for fuel in MFC operations. However, this is not yet known that how much as high as electricity can be produced using these electrogens and an optimization study is needed indeed.

3.3 *Monitoring of wastewater treatment progress*

The typical characteristic of water quality of wastewater from KRM and SSR is represented in Table 6. Progress of wastewater treatment was determined experimentally in terms of various parameters of wastewater characterization *viz.,* pH, DO, BOD, total dissolved solids, and electrical conductivity, before and after operation using standard methods. The colour, temperature and odour were found to be minimized after the MFC operation (Table 6). The temperature became normalized to the ambient room temperature. Electrical Conductivity is the ability of an industrial wastewaters or polluted water to convey an electric current is due to the presence of ionic solutes. We assume that the wastewater from KRM is result of parboiling of rice hence the wastewater is simpler in its composition, having rich organic content as compared to wastewater from SSR which is from rice bran oil refinery. Therefore, the possible reason for the increased values of EC of wastewater from SSR could be the presence of inorganic ions after the bacterial activity. During the MFC operation, organic substances present in the anode chamber have been broken down and dissolved in the water, for both of the cases. Substances dissolved in the water may often include simpler carbohydrates, proteins, esters, mineral salts etc. and its successive metabolized products causing increase in TDS (Uwidia and Ukulu, 2013). Literature shows that these electrochemical systems are dominated by facultative anaerobes. The possible reason for the decrease in DO could be utilization of oxygen molecules by facultative anaerobes as the strict anaerobic conditions were maintained in anode side (Schroder, 2007). However, further investigation is often needed to confirm the microbial behaviour and dynamics inside the anode chamber.

Table 6.
Showing characteristics value of wastewater before and after operation in MFC

Parameter	Quality of Wastewater Before MFC Operation		Quality of Wastewater After MFC Operation	
	SSR	KRM	SSR	KRM
Odour	Pungent	Pungent	Less-odorous	Less-odorous
Colour	Buff	Pale yellow	Translucent	Translucent
Temperature (^{0}C)	34.6 ±2.0	36.8± 2.0	32.4±2.0	31.4±2
pH	2.51± 1.0	4.13±1.36	1.34±0.45	4.34±1
Total dissolve solutes (TDS) (ppt)	35.58±3.21	31.73±2.43	36.11±3.76	32.52±3.22
Electrical Conductivity (mS/cm)	78.68±5.42	64± 3.58	79.56±7.3	53±4.43
Dissolve Oxygen (mg/L)	2.9±1.5	4.13±1.33	1.03±0.07	4.03±1.13
Biological oxygen demand (BOD) (mg/L)	10.60±1.3	26.60±2.3	6.75±0.54	18.75±1.13
COD (mg/L)	2400±302	1120±313	1245±250	544± 31

About 48.125% and 51.43% of COD and about 36.32% and 29.5% of BOD removal has been noticed for the wastewater KRM and SSR, respectively (Table 6). These data showed that MFCs are capable of wastewater treatment efficiently. Our findings support previous report that MFCs are efficient in lowering the chemical load of wastewater; 30% COD removal of oil refinery wastewater (Majumder et al., 2014), 27±2 % to 76±4% TCOD removal of paper and pulp wastewater in an open-circuit control (Huang and Logan, 2008), 98.0% COD removal of starch processing wastewater (Lu et al., 2009), approximately 80% removal of COD from municipal Wastewater (Buitrón and Cervantes-Astorga, 2013), COD and BOD removal efficiency of 45.21% and 45% from unamended palm oil mill wastewater, respectively (Baranitharan et al., 2013), COD removal efficiency around 90% of dairy industry wastewater (Elakkiya and Matheswaran, 2013), 91% removal of COD at 40 days HRT while using coconut husk retting wastewater as substrate (Jayashree et al., 2014). These MFCs can further optimize for enhancing the power output as well as treatment efficiency using a combination of series and parallel connection, addition of mediators and temperature control, co-culture etc. (Behera et al., 2010, Qu et al., 2012).

4. Conclusion

Real field wastewater such as rice-based industrial wastewater has great point in using it in MFCs as fuel. Zn-C electrode combination found to be biocompatible and efficient to support growth of biofilm. Efficiency of the bacteria-associated electrochemical system in

wastewater treatment is established. The MFCs without conventional proton exchange membrane offered much cost-effective and handy MFC operations. The anodic bio-film is the subject matter of further study to elucidate microbial behavior and ecology inside the anode compartment. We further suggest that the high strength rice-based industrial wastewater is potential candidate as fuel in MFC operations and optimization and improvisation of the technology may support the path of future practical applications to meet the demand of electricity, especially in rural areas.

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