*Environmental Engineering and Management Journal* December 2014, Vol.13, No. 12, 2945-2954
http://omicron.ch.tuiasi.ro/EEMJ/

*"Gheorghe Asachi" Technical University of Iasi, Romania*

# ENHANCED TANNERY WASTEWATER TREATMENT AND ELECTRICITY GENERATION IN MICROBIAL FUEL CELL BY BACTERIAL STRAINS ISOLATED FROM TANNERY WASTE

**Abhilasha Singh Mathuriya**

*Anand Engineering College, Department of Biotechnology, N H-2, Keetham, Agra-282007, India*
*Email: imabhilasha@gmail.com; Phone: +919897261545; Fax: 05613-272027*

**Abstract**

Toxic chemical nature of tannery wastewater makes it difficult to be treated by routine biological treatment processes. Microbes, already present in tannery wastewater can adapt to these conditions and degrade the organics in tannery wastewater. In the present contribution, three bacterial electrogenic strains, tolerant to tannery environment, were isolated from soil contaminated with tannery waste and named as *BS1, BS2,* and *BS3.* Tannery wastewater was treated with these pure and mixed consortia of three bacterial strains in different microbial fuel cells. Comparative analysis was made by treating the tannery wastewater with foreign microbial consortia (activated sludge inoculum) and with plain wastewater containing only natural habitat microbes, already present in wastewater. Mixed consortia of electrogenic strains gave best results. Up to 10.38mA current and 94.3 per cent of Chemical Oxygen Demand (COD) removal was obtained during 30 days of operation.

*Key words:* bioelectricity, COD, Microbial fuel cells, microorganisms, tannery wastewater

*Received: December, 2011; Revised final: July, 2012; Accepted: July, 2012*

## 1. Introduction

Leather Industry, occupies a distinct place in the world economy, in terms of potential for employment, growth and exports. Leather processing requires various chemicals to treat and soften hides. Every tanning process step, with exception of the crust finishing operations, produces wastewater (Ozgunay, 2007). This wastewater contains fat, protein, preservatives, lime, ammonium salts, sulphides, chromium, polyphenolic compounds, dye and solvent chemicals (Tunay et al., 1995). In fact, after conventional treatment (i.e., chromium precipitation–primary sedimentation–biological oxidation–secondary sedimentation), effluents still do not meet the required limits, at least for some parameters such as BOD, COD, salinity, ammonia and surfactants (Bartlett and James, 1988). On the other hand, tannery wastewater is the source of diverse extremophilic microbial flora, which has amazing adaptation and flexibility for surviving in extreme tannery environment (Aono and Inoue, 1998; Horikoshi, 1998). These microorganisms have ability to protect themselves from heavy metal toxicity by various mechanisms such as adsorption, uptake, methylation, oxidation, and reduction (Megharaj et al., 2003).

Despite the high concentrations of Chromium (Cr) in contaminated soils and sediments, occurrence of a substantial quantity of bacterial populations has been reported (Aono and Inoue, 1998). Microbial fuel cell (MFC) is an emerging technology, which enjoys the benefit of electricity generation during wastewater treatment by utilizing bacterial metabolism (Jang et al., 2004; Liu et al., 2004; Logan, 2004; Min et al., 2005; Mathuriya and Sharma, 2009; Mathuriya 2014). A typical two chamber MFC consists of anode and cathode separated by a cation specific membrane. In the anode compartment electrons and protons are generated from the oxidation of the substrate by microorganisms. Electrons are transferred through an external circuit while the protons diffuse through the solution to the cathode via proton exchange membrane, where electrons combine with protons and oxygen to form water (Delaney et al., 1984; Park and Zeikus., 2000). Oxygen is superior to other electron acceptors for its unlimited availability, easy handling and high redox potential (Zhao et al., 2006).

Although MFCs exhibit less efficiency, when operating on wastewaters than pure compounds; yet the electricity generation during wastewater treatment would reduce the cost of treating primary effluent wastewater. The benefits of using MFCs include: clean, quiet performance, low $CO_2$ emissions, higher efficiency, and direct electricity recovery. However, the availability of effective toxic tannery environment tolerant organisms is an essential prerequisite for the bio-based treatment of extreme tannery wastewater. The use of microorganisms able to grow in highly concentrated tannery environment and transform the waste into an easily recycled bioproduct during current generation, offers a promising perspective. Till now, many studies of MFCs have either focused on the ability of pure cultures of electrochemically active bacteria (Bond et al., 2002; Chaudhuri and Lovley, 2003; Kim et al., 1999; Pham et al., 2003) or the colonization of the anode by bacteria derived from a range of inocula (Bond et al., 2002; Kim et al., 2004; Lee et al., 2002; Phung et al., 2004). While this has been tried to some extent in the past, it is difficult to compare the results of previous studies which used different MFC architectures, inoculums and enrichment media.

In this study, three considerations were evaluated: (i) Isolation of electrochemically active microorganisms from tannery waste, for providing maximum effort to tannery wastewater treatment during electricity generation. (ii) Enrichment of MFCs as individual and mixed consortia of these isolates for electricity generation. (iii) Comparison of electricity generation with plain wastewater and enrichment with foreign microbial communities (activated sludge inoculum). This was perhaps the first attempt on the treatment of tannery wastewater and simultaneous electricity generation employing isolated bacterial strains from tannery waste itself individually and in mixed consortia.

## 2. Materials and methods

### *2.1. Wastewater sample*

Tannery effluent wastewater sample was collected from a commercial tannery at Agra, India. Since the process of tanning is batch, after finishing process (12 h), spot samples were collected and transported to laboratory for physicochemical analysis. These parameters include pH, total dissolved solids, total suspended solids, color, COD and BOD. Wastewater sample was kept in a refrigerator at 4ºC, when not in use. The plain tannery wastewater (without any modifications such as addition of nutrients, mediator, any other microbial inoculum or trace metals), was used as the inoculum for all MFC tests except as indicated. Experiments were conducted using two-fold diluted wastewater (Feng et al., 2008; Min et al., 2005; Wen et al., 2009), at 30ºC, pH 7.0 and stagnant condition.

### *2.2. Bacterial strains*

Bacterial strains resistant to extreme tannery environment, were isolated from a contaminated soil (0–15 cm depth) collected from a long-term tannery waste disposal site at Agra (India). The bacterial strains were isolated using Thioglycollate medium supplemented with (g/l): 30.0, $K_2Cr_2O_7$; 6.0, $Na_2HPO_4$; 3.0, $KH_2PO_4$; 1.0, $NH_4Cl$; 0.5, NaCl; 0.246, $MgSO_4.7H_2O$; 0.01, $CaCl_2$, and 0.5, glucose as a carbon source. The physicochemical characteristics of the soil were: pH, 7.9; electrical conductivity, 0.67 dS/m (1:5, soil to water ratio); total Cr, 61,785 mg/kg soil; and organic carbon, 10.1 per cent. The bacterial strains were isolated by enrichment culture technique. The sterilized medium was inoculated with 0.2 per cent of the tannery contaminated soil samples, and incubated at 30 °C for one week in an Anaerobic jar (Himedia). Sub-culturing was done by transferring 5 per cent of the inoculum to fresh medium under similar conditions.

Bacterial isolates were characterized based on colony morphology, Gram-staining, physiological characteristics and biochemical tests and named Bacterial Strain (BS); BS1, BS2, and BS3. The isolates were purified by streak plate method and the purified bacterial isolates were then transferred to the anaerobic blood agar slants and used for further studies.

### *2.3. MFC construction and operation*

The MFCs were constructed from glass (16x16x10 cm). Total volume of each chamber was 1000 ml, and working volume was 700 ml. Both anode and cathode were separated by a glass, containing hole (6x6 cm) on which, a proton exchange membrane (Nafion ™ 117, DuPont Co., USA) was pasted.

Three electrode arrangements consisting of plain carbon paper (6x6cm) as anode and graphite (6x6 cm) as cathode were immersed in their respective chambers. The electrodes were attached using copper wire with all exposed metal surfaces sealed with a nonconductive epoxy. The anode chamber was filled (600 mL) with tannery wastewater and was continuously flushed with $N_2/CO_2$ (80:20) to sustain anaerobic conditions. Aerobic cathode chamber was filled with 100mM Phosphate Buffer and pH adjusted to 7.0 by 0.5 N NaOH. The cathode chamber was provided with air that was passed through a 0.45µm pore size filter.

After setting the experiment, all two-chambered mediator-less MFCs were operated with tannery wastewater samples at different operating conditions. The MFCs were continuously monitored during experiment and readings were taken after each

24 hr. Inoculation time was considered as time 0. Initially MFCs were inoculated with artificial wastewater containing glucose as carbon source. After two cycles, feed solution containing 50 per cent artificial wastewater and 50 per cent tannery wastewater sample, inoculated into MFCs. Then, after two cycles, feed solution was switched to tannery wastewater sample. Subsequently, all the microbial fuel cells were inoculated with plain wastewater, isolated pure bacterial cultures, activated sludge inocula and as mixed consortia of isolates and operated with tannery wastewater for three cycles (30 days for each cycle).

The experimental setup was run in fed batch mode. The performance of all the microbial fuel cells was evaluated by measuring current, current density, potential and power density along with COD removal efficiency (Figs. 1-3). Stable current output was achieved after two cycles. Constant substrate (COD) removal efficiency and current output were considered as indicators to assess the stable performance of the MFC.

*2.4. Analytical methods*

Current ($I$) and potential ($V$) measurements were recorded using a Digital Multimeter (Kusam electrical industries, India, Model – 108) by connecting with 100Ω external circuit. For polarization, current generation was monitored at various external resistances (200 to 20Ω) connected for a few minutes and readings were noted after stabilization of voltage.

Power (m$W$) was calculated using the equation $P = IV$, where $I$ is in mA and $V$ is in mV. Power density (mW/cm$^2$) and current density (mA/cm$^2$) were calculated by dividing the obtained power and current with the surface area (cm$^2$) of the anode. Power yield (W/kg of COD removed) was obtained by dividing power with the amount of COD removed. All samples were filtered through a 0.22 μm (pore diameter) membrane filter prior to pH, BOD5 and COD measurements. COD was determined with potassium dichromate oxidation method (Hach Heating System, Hach Corporation, USA), and BOD5 was determined with manometric respirometry (OxiTop IS 6, Germany).

The pH values were measured pH meter (Fisher Scientific accumet- model 10) (Greenberg et al., 1992). COD removal per cent was calculated as $E_{COD} = [COD_{in}–COD_{out}/COD_{in}]$ x 100 per cent, where $COD_{in}$ is the influent COD and $COD_{out}$ is the effluent COD. All experiments were conducted using three separate microbial fuel cells and results were presented as average values or a typical result.

## 3. Results and discussion

*3.1. Results*

*3.1.1. Growth optimization of bacterial isolates*

Optimization experiments were performed for growth of isolated bacterial strains on shaker at 120rpm to determine the growth factors, and higher biomass yield.

Among nine strains isolated, three were selected on the basis of their growth in modified medium. Inoculum concentration, temperature, pH and agitation were the parameters optimized. Samples were collected at regular time intervals; the biomass growth was monitored by recording the absorbance at 600 nm in Spectrophotometer (Shimadzu Scientific Instruments). The absorbance values were plotted against time to obtain growth curves (results not shown). The optimized parameters for growth of selected bacterial strains are shown in Table 1.

**Table 1.** Optimized parameters for growth of isolated bacterial strains

| *Bacterial Strain* | *Inoculum conc. (v/v)* | *Temperature (°C)* | *pH* | *Agitation rate (rpm)* |
|---|---|---|---|---|
| *BS1* | 5% | 32 | 8 | 150 |
| *BS2* | 8% | 38 | 7.5 | 150 |
| *BS3* | 6% | 34 | 7.5 | 120 |

*3.1.2. Characterization of tannery wastewater*

The characteristics of the wastewaters are shown in Table 2. The results of this study showed that the wastewater of tannery process is one the major sources of environment pollutants as the concentration of harmful material in the wastewater was extremely high. This is in conformity with many other studies (Tunay et al., 1995; Song et al., 2004).

**Table 2**. Characteristics of tannery wastewater sample

| *S. No.* | *Parameters* | *Units* | *Value* |
|---|---|---|---|
| 1 | pH | - | 7.8 |
| 2 | COD | mg/L | 2839 |
| 3 | BOD | mg/L | 1528 |
| 4 | TSS | mg/L | 3854.6 |
| 5 | TDS | mg/L | 15831.5 |
| 6 | Odor | - | Foul |
| 7 | Color | - | Brownish |

*3.1.3. Electricity generation*

When tannery wastewater was used as anolyte in MFCs, a lag phase was observed which was followed by a gradual rise in the current. This initial increase of current can be assigned to the presence of easily degradable components that were utilized by microorganisms present in the wastewater. When these easily degradable substrates were exhausted, the current outputs began to decrease.

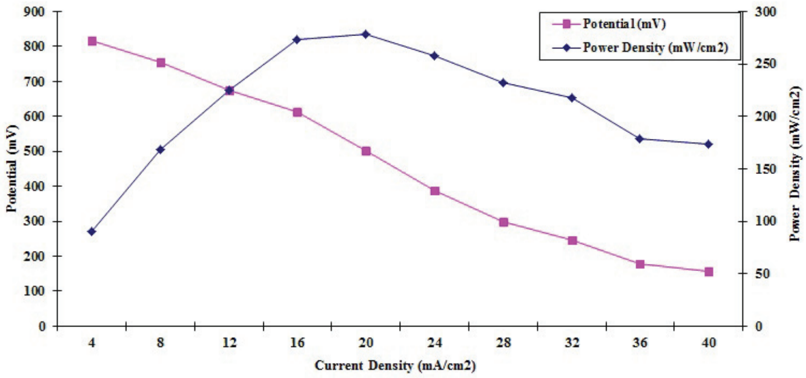
Meanwhile, degradation of complex components was continued, therefore lower efficiency was observed. Fresh feed was supplemented when remarkable current drop was observed. Before changing the feed, inoculum was allowed to settle down (1 h) and exhausted feed (300 mL) was replaced with fresh feed under anaerobic condition. The anode chamber was sparged with oxygen free $N_2$ gas for a period (4 min) to maintain anaerobic microenvironment after every feeding event. A steady increase in current generation was observed with additional feed.

Figs. 1(a-f) show the polarization curve as a function of potential, current and power density measured at variable resistances (20–200 Ω). Current generation in different resistors was observed once the stable voltage was obtained. A decreasing current trend with increase in resistance was observed, which claims a typical fuel cell behavior and is consistent with the reported literature (Min and Logan, 2004; Venkata Mohan et al., 2008).

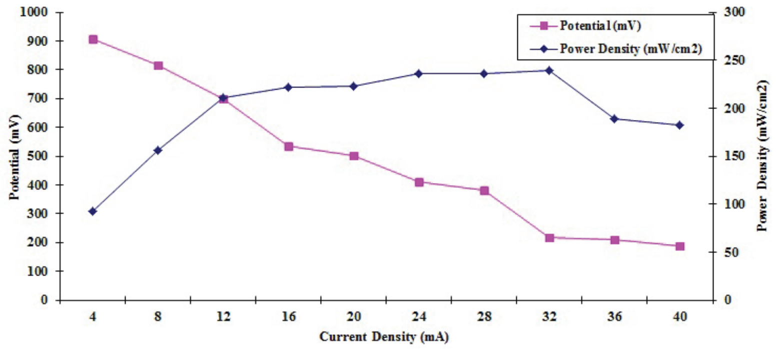
Higher resistance exhibited relatively less power density. Oxidation of substrates by microbes was found to be more at lower resistance than at higher resistance (Mano et al., 2003; Liu et al., 2005; Venkata Mohan et al., 2007). The trend was similar in all MFCs but MFC running on plain wastewater sample showed inferior results (Fig. 1a). It can be seen in Fig. 2, that the current generation response was poorest in plain wastewater sample, this sample showed a larger lag period and attained the value of 3.86mA on 5$^{th}$ day, and after many fluctuations the peak value generated was 8.54mA on 25$^{th}$ day, and thus could not be a feasible candidate for electricity generation. Samples with monotype isolates BS1, BS2, BS3 and activated sludge inoculum showed better response, shorter lag period and achieved peak value of 9.35mA, 9.53mA, 9.11mA and 9.49mA on 26$^{th}$, 25$^{th}$, 20$^{th}$, and 15$^{th}$ day of operation.

Mixed microbial flora MFCs showed relatively stronger response to current generation than MFCs operating with plain wastewater, monotype isolate inoculated MFCs and MFC with activated sludge inoculum. The average peak current generated from mixed microbial consortia was 10.38mA on 16$^{th}$ day which was about 21.5, 10.0, 8.0, 12.0 and 10 per cent higher than those generated from systems operating on plain wastewater, BS1, BS2, BS3 and foreign inoculum.

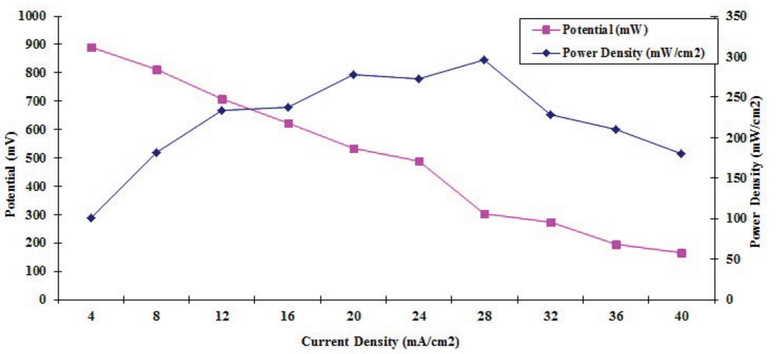
Although it took a long time to develop stable microbial consortia and to generate steady current response (0.97mA up to 2 days), mixed microbial consortia exhibited significantly higher current yield than all monocultures.




a)




b)




c)

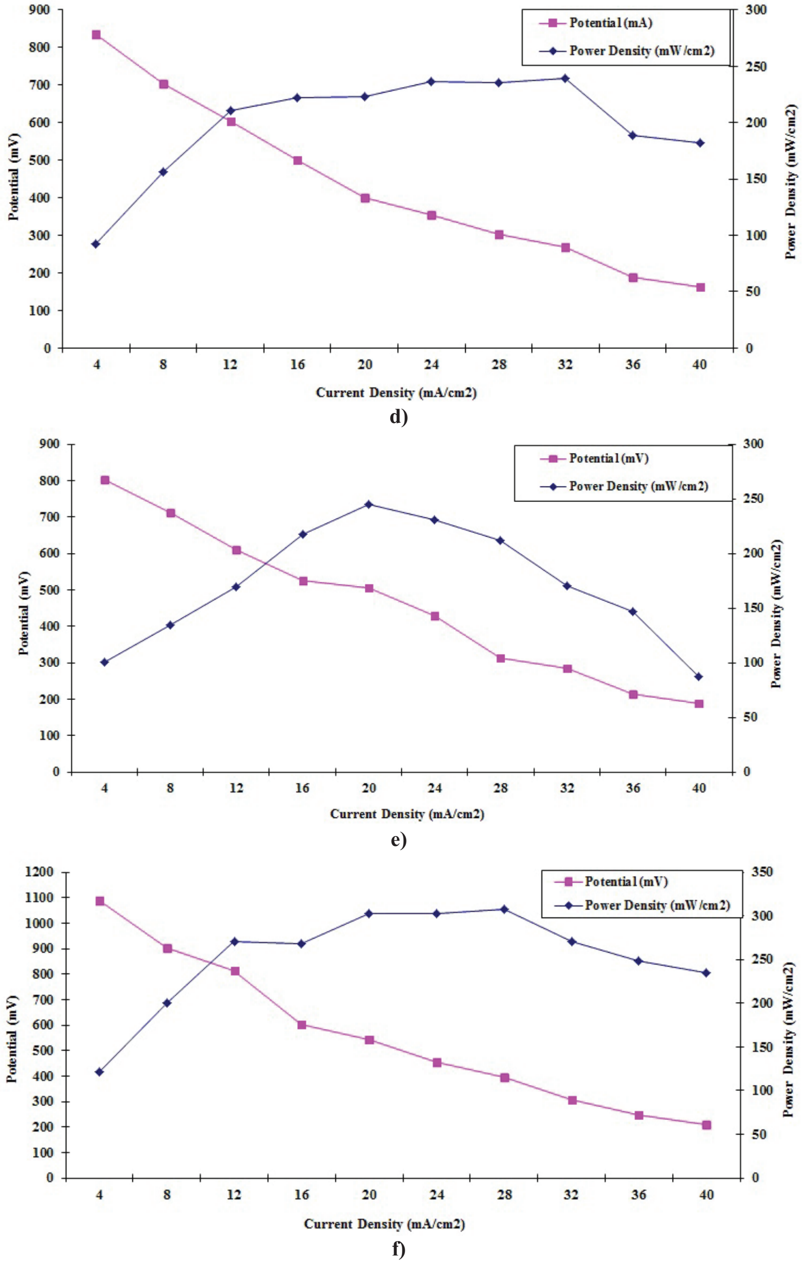



**Fig. 1.** Polarization Curve obtained from MFC operating on (a) plain wastewater (b) Bacterial isolate BS1 (c) bacterial isolate BS2 (d) bacterial isolate BS3 (e) sludge inoculum (f) mixed microflora of isolates

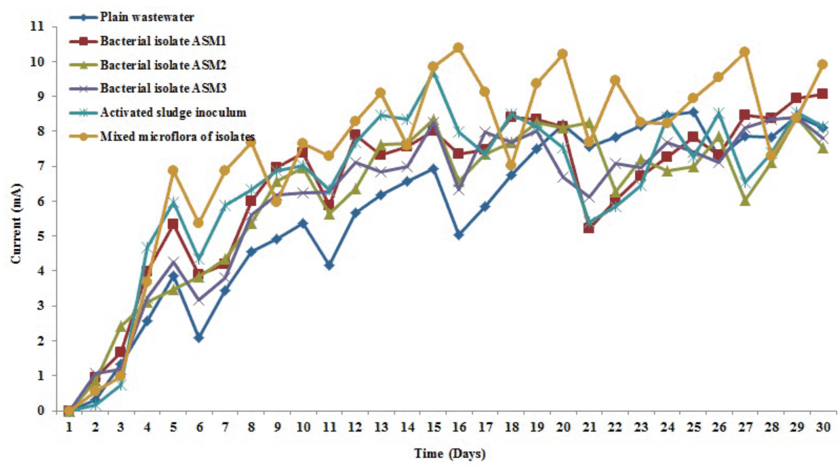



**Fig. 2.** Current generation from MFCs operating on plain wastewater, bacterial isolate BS1, BS2, BS3, sludge inoculum and mixed microflora of isolates

Furthermore, although addition of newly supplied electron providers lowered the current response, mixed bacterial communities showed high stability by matching to earlier current quickly (major current drops on 9th, 14th, 18th, 21st, and 28th day with values of 5.96, 7.60,7.02,7.68 and 7.38mA and regained on 10th, 15th, 18th, 21st and 29th day with values 7.66, 9.83, 9.36, 9.44, and 8.36mA). While the activated sludge inocula not only consumed longer adaptation period initially (0.75mA in 2 days) and after feed replacement every time, but generated inferior results than mixed microflora throughout. In contrast all monocultures (BS1, BS2, and BS3) inoculated MFCs, initially showed relatively stronger response and generated up to 0.94, 0.85, and 1.08mA current respectively in 24 hr. But ultimately the current response was limited to 9.35, 9.53, and 9.11mA with subsequent wastewater replacement in overall operation.

These observations suggested that established mixed culture was able to harness efficient electricity from tannery wastewater with enhanced treatment efficiency. The results are in conformity with earlier studies. Kan et al. (2011) reported the electricity generation in Microbial fuel cells using enriched wastewater by microbial consortia of its wastewater, while, Kim et al. (2008) enriched the MFC operating on swine wastewater by leaving it for 10 days using full-strength wastewater. On other hand, Yokoyama et al. (2006) used cow slurry itself as source of bacteria in MFC treating cow waste slurry.

Therefore, it can be mentioned that monoculture can set a good model for mechanistic studies, but in general it is agreed that mixed microbial consortia hold greater promise for large scale applications (Logan, 2007).

COD removal efficiency of all samples is shown in Fig. 3. By 30th day of MFCs operation, 83.4, 87.8, 86.3, 84.7, and 94.3 per cent COD removal efficiency observed in samples operating on plain wastewater, BS1, BS2, BS3, activated sludge inocula and mixed microflora respectively. These results suggested that the electrochemically active bacteria propagated in the microbial fuel cell and that the wastewater itself contained electrochemically active bacteria at a lower concentration.

Microbes enriched for 30 days in a microbial fuel cell removed organic contaminants in wastewater almost completely, with the concomitant generation of electricity. Further experiments revealed that the current generation was stable for many days (data not shown).

### *3.2. Discussion*

Plain wastewater contains vast variety of microbial population. The major bacterial population in typical anaerobic wastewater sludge is believed to consist of fermentative bacteria, methanogens, and sulfate reducers (Angenent et al., 2002; Dollhopf et al., 2001; Snaidr et al., 1997). Electricigens, like iron-reducing bacteria have been estimated to less than 3 per cent of the total bacteria in activated sludge (Nielsen et al., 2002). Thus, it is possible that non-electrochemically active bacteria can occupy space on the electrode during the initial inoculation step preventing efficient power generation in the MFC.

In addition, acidogenesis (production of acetate and propionate) was also found to be suppressed under electrogenic conditions (Ishii et al., 2008). Fermentative microorganisms typically convert a fermentable fuel, such as glucose, to small chain organic acids, hydrogen, and carbon dioxide. Electricity production results from the interaction of reduced compounds produced under low redox conditions generated during fermentation or possibly by direct electron transfer between the fermentative microorganisms and the anode surface.

Furthermore at high rate of metabolism, which results from the growth of mixed microorganisms, the accumulation of fermentation acids in the system can inhibit the growth of microorganisms. Also most of the electrons initially present in the fuel are recovered in the fermentation acids rather than electricity, and thus columbic efficiency remains low in these systems, and MFC cannot be operated for a long period of time (Ishii et al., 2008).

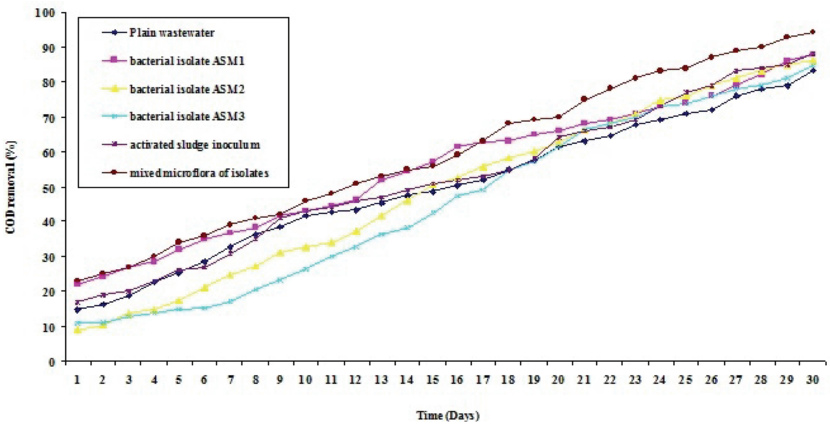



**Fig. 3.** COD removal percentage from MFCs operating on plain wastewater, bacterial isolate BS1, BS2, BS3, sludge inoculum and mixed microflora of isolates

Additionally, in fed-batch systems, where the carbon source is added periodically, a substantial excess of the carbon source exists initially. This promotes growth of non-electricigens, which convert the available carbon to other byproducts, thus leads to lower performance.

Therefore, the presence of at least one electricigen is required for microbial fuel cells to effectively convert organic fuels to electricity. Electricity generation with electricigens has a number of advantages including the high columbic efficiency that results from these microorganisms being able to completely oxidize organic fuels to carbon di oxide with an electrode serving as the sole electron acceptor. The enrichment of electricigenic biofilm-forming organisms resulted in development of a consortium capable of generating electricity at a high power density. In fact, until an anode material that can effectively and sustainably catalyze the abiotic oxidation of fermentation products is developed, it will be impossible to have a microbial fuel cells that can have high columbic efficiency without employing an electricigen. This is because even if nonelectrigens, such as fermentative or methanogenic microorganisms, carry out the initial metabolism of the organic fuel, at least one electricigen that can effectively recover the electrons from the metabolic products of the nonelectricigen will be required in order to achieve high electron recoveries as electricity.

Another benefit to electricigen-powered microbial fuel cells is their sustainability. This results from the fact that electricigens conserve energy for maintenance and growth from electron transfer to anodes. Earlier electricigen-based microbial fuel cells were run for more than 2 years without any remarkable decline in power output (Ishii et al., 2008).

On the other hand, the superior behavior of mixed microbial consortia may be due to their nutrient adaptability to handle a broad substrate range, stream with multiple substrates present at the same time and concentrations present in wastewater and resistance to stresses. This is supported by many previous studies (Holmes et al., 2004; Jung and Regan, 2007; Rabaey et al., 2004; Ki et al., 2008).

Utilizing mixed anaerobic cultures (activated sludge inoculum in this case) for production of electricity is a more useful phenomenon and has practical advantages. But factors limiting current generation with foreign microbial inoculum may include: i) A limited extent of adaptation to highly toxic tannery wastewater, leading to long lag phase during current generation (only 0.75mA up to 3 days). ii) Microbial cells disruption or inactivation due to sudden shift in ionic strength from activated sludge to tannery wastewater ultimately caused significance reduction in system performance as even with acclimatized culture; satisfactory performance required a constant ionic environment.

Therefore, extreme tannery wastewater should be treated at either lower food to microorganism (F/M) ratio, or higher than usual bacteria mixed liquor volatile suspended solids (MLVSS) concentrations or by self-tolerant microbial flora tannery wastewater itself. Foreign inoculum also needs a longer Hydraulic Retention Time (HRT) as every time, after older wastewater replacement with fresher, there was more adaptation time needed. Other workers concluded that this limited efficiency was due to an inability to produce thick biofilms on the anode surface (Kim et al., 2002; Lanthier et al., 2005). Therefore, of the microorganisms known to contribute to electricity production in microbial fuel cells, electricigens isolated from similar environment to be treated offer the possibility of highest efficient, self-sustaining conversion of waste organic matter and renewal biomass to electricity perhaps due to stability and adaptability in wastewater environment.

Adapted mixed microbial culture operated microbial fuel cell show the competitive response with other conventional wastewater treatment technologies viz. activated sludge process, sequential batch reactors, and membrane bioreactors. Durai et al., (2010) conducted aerobic digestion of tannery wastewater (initial COD 6240mg/L) using mixed microbial culture obtained from self-environment (Common Effluent Treatment Plant treating tannery wastewater) and obtained up to 74 per cent COD removal under optimum conditions. Lefebvre et al., (2005) achieved 95 per cent COD removal (initial COD 2200mg/L) in a lab scale Sequencing Batch Reactor using adapted microbial flora in 5 days HRT. Bera et al., (2012) studied tannery wastewater treatment using activated sludge process with working capacity of 25L and achieved up to 65.2 per cent COD removal (initial COD 800mg/L) using *Bacillus cereus* $M^{1}_{16}$ in 12 days of operation. In another study, Mazumdar, (2010) treated tannery wastewater in a shaft type activated Sludge Reactor and achieved up to 88 per cent COD removal efficiency. In an interesting study, Iaconi et al., (2002) used a periodic submerged filter sequential batch biofilm reactor of 16 L working capacity for tannery wastewater (initial COD 3500-4000 mg/L) treatment and obtained up to 97 per cent COD removal in overall 60 days of operation.

While in present study the COD removal efficiency reached up to 94.3 per cent in 30 days of operation which claims efficient candidature of microbial fuel cells as wastewater treatment devices. In addition to waste (COD) removal, microbial fuel cells offer 10.38mA current generation which makes this technology superior than other competitors, as other technologies are energy incentive. Other advantages which make this technology a choice are: a) MFCs do not consume much energy in comparison of activated sludge process (Watanabe, 2008) and do not require highly regulated distribution systems like the ones needed for Hydrogen fuel cells.

MFCs operate efficiently at ambient temperature. b) MFCs exhibit safe and quite performance (Rabaey and Verstreates, 2005). MFCs do not require gas treatment, as the off-gas of MFCs is mainly carbon dioxide ($CO_2$) and normally have no useful energy content (Jang et al., 2004). c) In addition, MFCs would not generate more $CO_2$ than typical biological wastewater treatment processes, thus their substitution for fossil fuel power plants would result in a net reduction of $CO_2$ emissions. d) The amount of power generated by MFCs in the wastewater treatment process can potentially halve the electricity needed in a conventional treatment process that consumes a lot of electric power aerating activated sludge. e) MFCs yield 50–90% less solids to be disposed of (Du et al., 2007) and the generated sludge is more stable than aerobic treatment process (Kim et al., 2007).

The power output and waste treatment efficiency of MFCs have been improved dramatically in past few years. MFC technology holds promise towards sustainable power generation and wastewater treatment along with application in several areas including: a) biosensors for detection of various oxidizable compounds (Karube, 1985), b) rapid estimation of bacterial food contaminants (Patchett et al., 1988) c) onsite power generation in remote areas and power supply for sensors using indigenous biodegradable fuels (Fan et al., 2007); and d) detection of microbial cell population in polluted water streams (Maoyu and Zhang, 1989).

Although waste treatment aura of MFC is increasing day by day, still MFC technology has not yet been applied to practical waste material treatments, primarily because it is a growing technology, which is generally limited up to small laboratory scale and much time is required for its technical maturation. Other major factors, which limit the candidature of wastewater in MFCs, are: a) Process scale up, which results into less efficiency (Clauwaert et al., 2007) and causes inability to fulfill the requirement of treating larger quantity of industrial wastewater. b) MFCs fabricated with PEM receive limited acceptance for wastewater treatment due to fouling from suspended solids and soluble contaminants in a large wastewater treatment process (Duteanu et al., 2010). c) The biodegradation of extreme wastes can only be achieved up to a certain concentration, after which it leads to inhibition in biological treatment process and can kill microbial flora (Abbas et al., 2009; Mayen-Mondragon et al., 2008). d) Addition of mediator, sometimes may lead inactivity of pure culture microbes. e) Low coulombic efficiency. f) MFCs operating on wastewaters have another problem of un-uniform substrate distribution and biofilm generation on anode due to random concentration on wastewater, which creates kinetic and mass transfer limitations. g) Most of the wastewaters require pre- treatment strategies like pre-fermentation before their treatment in MFCs for optimal efficiency (Goud and Venkata Mohan, 2011). h) Long start up and Hydraulic retention time compared to other processes (Duteanu et al., 2010) is another limitation. Overcoming all these limitations may explore MFC technology as promising future waste-to-power technology.

## 4. Conclusions

This study extensively investigated the effect of inoculum type on power generation and waste removal in MFCs. Among four types of inocula used in the study, microbial strains obtained from self-tannery environment as mixed microbial flora exhibited best performance with an efficient value of 10.38mA along with 94.3 per cent COD removal.

The fuel cell operated with individual microbial strain alone showed relatively lower current output and substrate degradation compared to the mixed consortia.

The correlation between the power production and electrochemical characteristics of MFCs was established on the basis of results. It was observed that the inoculum types had significant effects on the MFC performance. While still the influence of and biology on electricity generation in microbial fuel cells is not fully understood, this study shows that appropriate enrichment methodologies can influence the power density and wastewater treatment in microbial fuel cells.

Further improvements can be made to MFC configurations to improve energy recovery or to increase substrate degradation, resulting in new technologies that will make electricity generation using MFCs a practical method of wastewater treatment. On overcoming these issues, MFC technology can prove itself as the sustainable future waste to power technology.

## References

Abbas A.A., Guo J., Liu Z., Pan Y.Y., Al-Rekabi W.S., (2009), Review on landfill leachate treatments, *American Journal of Applied Science*, **6**, 672–84.

Angenent L.T., Zheng D., Sung S., Raskin L., (2002), Microbial community structure and activity in a compartmentalized, anaerobic bioreactor, *Water Environment Research,* **74**, 450–461.

Aono R., Inoue A., (1998), *Organic Solvent Tolerance in Microorganisms*, In: *Extremophiles: Microbial Life in Extreme Environments*, Horikoshi K., Grant W.D. (Eds.), Wiley-Liss Inc, New York, 287–310.

Bartlett R., James B.R., (1988), Mobility and bioavailability of chromium in soils, *Advances in Environmental Science and Technology,* **20,** 267–304.

Bera D., Chattopadhyay P., Ray L., (2012), Continuous removal of chromium from tannery wastewater using activated sludge process-Determination of kinetic parameters, *Indian Journal of Chemical Technology.* **19**, 32-36.

Bond D.R., Holmes D.E., Tender L.M., Lovley D.R., (2002), Electrode reducing microorganisms that harvest energy from marine sediments, *Science*, **295**, 483–485.

Chaudhuri S.K., Lovley D.R., (2003), Electricity from direct oxidation of glucose in mediator-less microbial fuel cells, *Nature Biotechnology*, **21**, 1229-1232.

Clauwaert P., Rabaey K., Aelterman P., De Schamphelaire L., Pham T.H., Boeckx P., Boon N., Verstraete W., (2007), Biological denitrification in microbial fuel cells. *Environmental Science & Technology*, **41**, 3354-60.

Delaney G.M., Bennetto H.P., Mason J.R., Roller S.D., Stirling J.L., Thurston C.F., (1984), Electron-transfer coupling in microbial fuel cells. II. Performance of fuel cells containing selected microorganism mediator combinations, *Journal of Chemical Technology and Biotechnology*, **34B**, 13–27.

Du Z., Li H., Gu T., (2007), A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy, *Biotechnology Advances*, **25**, 464–82.

Durai G., Rajamohan N., Karthikeyan C., Rajasimman., M., (2010), Kinetics studies on biological treatment of tannery wastewater using mixed culture, *International Journal of Chemical and Biological Engineering*, **3**, 105-109.

Duteanu N.M., Ghangrekar M.M., Erable B., Scott K., (2010), Microbial fuel cells- an option for wastewater treatment, *Environmental Engineering and Management Journal*, **9**, 1069-87.

Dollhopf S.L., Hashsham S.A., Dazzo F.B., Criddle C.S., Tiedje J.M., (2001), The impact of fermentative organisms on carbon flow in methanogenic systems under constant low substrate conditions, *Applied Microbiology and Biotechnology,* **56**, 531-538.

Fan Y., Hu H., Liu H., (2007), Enhanced Coulombic efficiency and power density of air-cathode microbial fuel cells with an improved cell configuration, *Journal of Power Sources*, **171**, 348-54.

Feng Y., Wang X., Logan B.E., Lee H., (2008), Brewery wastewater treatment using air-cathode microbial fuel cells, *Applied Microbiology and Biotechnology*, **78**, 873–880.

Goud R.K., Venkata Mohan S., (2011), Pre-fermentation of waste as a strategy to enhance the performance of single chambered microbial fuel cell (MFC), *International Journal of Hydrogen Energy*, **36**, 13753 -13762.

Greenberg A., Clesceri L.S., Eaton A.D., (1992), *Standard Methods for the Examination of Water and Wastewater*, 18$^{th}$ Edition, American Public Health Association, Washington D.C.

Holmes D.E., Bond D.R., O'Neil R.A., Reimers C.E., Tender L.R., Lovley D.R., (2004), Microbial communities associated with electrodes harvesting electricity from a variety of aquatic sediments, *Microbial Ecology*, **48**, 178–190.

Horikoshi K., (1998), *Alkaliphiles*. In: Horikoshi K., Grant W.D. (Eds.), *Extremophiles: Microbial Life in Extreme Environments*, Wiley-Liss Inc, New York, 155–179.

Iaconi C.Di, Lopez A., Ramadori R., Pinto Di A.C., Passino R., (2002), Combined chemical and biological degradation of tannery wastewater by a periodic submerged filter (SBBR), *Water Research*, **36**, 2205–2214.

Ishii S., Hotta Y., Watanabe K., (2008), Methanogenesis versus Electrogenesis: Morphological and Phylogenetic Comparisons of Microbial Communities, *Bioscience Biotechnology and Biochemistry*, **72**, 286–294.

Jang J.K., Pham T.H., Chang I.S., Kang K.H., Moon H., Cho K.S., Kim B.H., (2004), Construction and operation of a novel mediator-and membrane-less microbial fuel cell, *Process Biochemistry*, **39**, 1007–1012.

Jung S., Regan J.M., (2007), Comparison of anode bacterial communities and performance in microbial fuel cells with different electron donors, *Applied Microbiology and Biotechnology,* **77**, 393–402.

Kan J., Hsu L., Cheung A.C., Pirbazari M., Nealson K.H., (2011), Current production by bacterial communities in microbial fuel cells enriched from wastewater sludge with different electron donors, *Environment Science and Technology,* **45**, 1139-46.

Karube I., (1985), *Biosensors in Fermentation and Environmental Control*, Technomic Publ Co., Basel.

Ki D., Park J., Lee J., Yoo K., (2008), Microbial diversity and population dynamics of activated sludge microbial communities participating in electricity generation in microbial fuel cells, *Water Science and Technology*, **5**, 2195–2201.

Kim B.H., Park H.S., Kim H.J., Kim G.T., Chang I.S., Lee J., Phung N.T., (2004), Enrichment of microbial community generating electricity using a fuel-cell type electrochemical cell, *Applied Microbiology and Biotechnology*, **63,** 672–81.

Kim B.H., Chang I.S., Gadd G.M., (2007), Challenges in microbial fuel cell development and operation, *Applied and Microbial Biotechnology,* **76**, 485-94.

Kim H.J., Hyun M.S., Chang I.S., Kim B.H., (1999), A microbial fuel cell type lactate biosensor using a metal-reducing bacterium, *Shewanella putrefaciens*, *Journal of Microbiology and Biotechnology,* **9**, 365–7.

Kim H.J., Park H.S., Hyun M.S., Chang I.S., Kim M., Kim B.H., (2002), A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefacians*, *Enzyme Microbiology Technology,* **30**, 145-152.

Kim J.R., Dec J., Bruns M.A., Logan B.E., (2008), Removal of odors from swine wastewater by using microbial fuel cells, *Applied and Environmental Microbiology*, **74**, 540–2543.

Lanthier M., Juteau P., Lépine F., Beaudet R., Villemur R., (2005), *Desulfitobacterium hafniense* is present in a high proportion within the biofilms of a high-performance pentachlorophenol-degrading, methanogenic fixed-film reactor, *Applied and Environmental Microbiology*, **71**, 1058–1065.

Lee S.A., Choi Y., Jung S., Kim S., (2002), Effect of initial carbon sources on the electrochemical detection of glucose by *Gluconobacter oxydans*, *Bioelectrochemistry,* **57**, 173–8.

Lefebvre O., Vasudevan N., Torrijos M., Thanasekaran K., Moletta R., (2005), Halophilic biological treatment of tannerysoak liquor in a sequencing batch reactor, *Water Research* , 39, 1471–1480.

Liu H., Ramnarayanan R., Logan B.E., (2004), Production of electricity during wastewater treatment using a single chamber microbial fuel cell, *Environment Science and Technology,* **38,** 2281-2285.

Liu H., Cheng S., Logan B.E., (2005), Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell, *Environment Science and Technology*, **39**, 658–662.

Logan B.E., (2007), *Microbial Fuel Cells*, John Wiley & Sons, Inc. New Jersey.

Logan B.E., (2004), Extracting hydrogen and electricity from renewable resources, *Environment Science and Technology*, **38**, 160A-167A.

Mano N., Mao F., Heller A., (2003), Characteristics of a miniature compartment-less glucose-$O_2$ biofuel cell and its operation in a living plant, *Journal of American Chemical Society*, **125**, 6588–6594.

Maoyu Y., Zhang Y., (1989), Electrode system for determination of microbial cell populations in polluted water, *Applied and Environmental Microbiology*, **55**, 2082-85.

Mathuriya A.S., Sharma V.N., (2009), Bioelectricity production from paper industry waste using a microbial fuel cell by *Clostridium species*, *Journal of Biochemical Technology*, **1**, 49-52.

Mathuriya, A.S., (2014), Eco-affectionate face of Microbial Fuel Cells, *Critical Reviews in Environmental Science and Technology*. **44**, 97-153.

Mayen-Mondragon R., Ibanez J.G., Vasquez-Medrano R., (2008), Simultaneous electrochemical oxidation and reduction of representative organic pollutants. *Fresenius Environmental Bulletin*, **17**, 1294–99.

Megharaj M., Avudainayagam S., Naidu R., (2003), Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste, *Current Microbiology*, **47**, 51–54.

Min B., Logan B.E., (2004), Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell, *Environment Science and Technology,* **38,** 5809–5814.

Min B., Kim J., Oh S., Regan J.M., Logan B.E., (2005), Electricity generation from swine wastewater using microbial fuel cells, *Water Research,* **39**, 4961-4968.

Nielsen J.L., Juretschko S., Wagner M., Nielsen P.H., (2002), Abundance and phylogenetic affiliation of iron reducers in activated sludge as assessed by fluorescence in situ hybridization and microautoradiography, *Applied and Environmental Microbiology*, **68**, 4629–4636.

Ozgunay H., Colak S., Mutlu M.M., Akyuz F., (2007), Characterization of leather industry wastes, *Polish Journal of Environmental Study,* **16**, 867-873.

Park D.H., Zeikus J.G., (2000), Electricity generation in microbial fuel cells using neutral red as an electronophore, *Applied and Environmental Microbiology,* **66**, 1292–1297.

Patchett R.A., Kelly A.F., Kroll R.G., (1988), Use of a microbial fuel cell for the rapid enumeration of bacteria, *Applied and Microbial Biotechnology*, **28**, 26-31.

Pham C.A., Jung S.J., Phung N.T., Lee J., Chang I.S., Kim B.H., Yi H., Chu J., (2003), A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Aeromonas hydrophila*, isolated from a microbial fuel cell, *FEMS Microbiology Letters*, **223,** 129–34.

Phung N.T., Lee J., Kang K.H., Chang I.S., Gadd G.M., Kim B.H., (2004), Analysis of microbial diversity in oligotrophic microbial fuel cell using 16S rDNA analyses, *FEMS Microbiology Letters*, **233**, 77–82.

Rabaey K., Boon N., Siciliano S.D., Verhaege M., Verstraete W., (2004), Biofuel cells select for microbial consortia that self-mediate electron transfer, *Applied and Environmental Microbiology*, **70,** 5373–5382.

Rabaey K., Verstraete W., (2005), Microbial fuel cells: novel biotechnology for energy generation, *Trends in Biotechnology*, **23**, 291–98.

Snaidr J., Amann R., Huber I., Ludwig W., Schleifer K.H., (1997), Phylogenetic analysis and in situ identification of bacteria in activated sludge, *Applied and Environmental Microbiology*, **63**, 2884–2896.

Song Z., Williams C.J., Edyvean R.G.J., (2004), Treatment of tannery wastewater by chemical coagulation, *Desalination*, **164**, 249-259.

Tunay O., Kabdasli I., Orhon D., Ates E., (1995), Characterization and pollution profile of leather tanning industry in Turkey, *Water Science and Technology,* **32**, 1–9.

Venkata Mohan S., Veer Raghavulu S., Srikanth S., Sarma P.N., (2007), Bioelectricity production by mediatorless microbial fuel cell under acidophilic condition using wastewater as substrate: Influence of substrate loading rate, *Current Science*, **92,** 1720-1726.

Venkata Mohan S., Saravanan R., Raghavulu S.V., Mohanakrishna G., Sarma P.N., (2008), Bioelectricity production from wastewater treatment in dual chambered microbial fuel cell (MFC) using selectively enriched mixed microflora: Effect of catholyte, *Bioresource Technology*, **99,** 596-603.

Watanabe K., (2008), Recent developments in microbial fuel cell technologies for sustainable bioenergy, *Journal of Bioscience and Bioengineering,* **106**, 528–36.

Wen Q., Wu Y., Cao D., Zhao L., Sun Q., (2009), Electricity generation and modeling of microbial fuel cell from continuous beer brewery wastewater, *Bioresource Technology*, **100**, 4171–4175.

Yokoyama H., Ohmori H., Ishida M., Waki M., Tanaka Y., (2006), Treatment of cow waste slurry by a microbial fuel cell and the properties of the treated slurry as a liquid manure, *Animal Science Journal*, **77**, 63.4–638.

Zhao F., Harnisch F., Schro¨der U., Bogdanoff P., Hermann I., (2006), Challenges and constraints of using oxygen cathodes in microbial fuel cells, *Environmental Science and Technology,* **40**, 5193-5199