



# Simultaneous degradation of bad wine and electricity generation with the aid of the coexisting biocatalysts *Acetobacter aceti* and *Gluconobacter roseus*

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## ABSTRACT

This study describes the cooperative effect of the two biocatalysts *Acetobacter aceti* and *Gluconobacter roseus* for biodegradation as well as current generation. The electro activity of the biofilms of these two microorganisms was investigated by the bioelectrocatalytic oxidation of ethanol and glucose using cyclic voltammetry. Two chamber microbial fuel cells (MFCs) were constructed using single culture of *A. aceti* (A-MFC), and *G. roseus* (G-MFC) and also using mixed culture (AG-MFC). Each MFC was fed with four different substrates viz., glucose, ethanol, acetate and bad wine. AG-MFC produced higher power density with glucose (1.05 W/m<sup>3</sup>), ethanol (1.97 W/m<sup>3</sup>), acetate (1.39 W/m<sup>3</sup>) and bad wine (3.82 W/m<sup>3</sup>). COD removal (94%) was maximum for acetate fed MFCs. Higher coulombic efficiency was obtained with bad wine (45%) as the fuel. This work provides the scope of using these biofuel cells in wineries for performing the dual duty of bad wine degradation along with current generation.

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## 1. Introduction

During the last decade, interest in microbial fuel cell has increased significantly because it produces electricity from the degradable organic matter by using microorganisms (Pant et al., 2009). In the microbial fuel cell the electrons obtained from the substrate oxidation can be transferred onto anode via nanowires of the bacteria attached on anode surface (Reguera et al., 2005; Gorby et al., 2006), by endogenous electron transfer mediators (Rabaey et al., 2005) or through membrane-associated complexes (Kim et al., 2004). In the case of mixed cultures, acting as biocatalysts all these mechanisms may be found to be operative. Mixed-culture MFCs usually provide better electric performance compared to the pure-culture counterparts (Jung and Regan, 2007). This situation may be regarded as a tight alliance amongst the mutually dependent and bacterial species contributing to the consortium aimed at the complete fuel degradation. First group of fermentation bacteria break complex molecules into energy-rich reduced metabolites suitable for the anaerobic respiration of a second bacterial group. Finally, some bacteria in the latter group are able to carry out an extra-cellular respiration when provided with a proper anode material, while the remaining ones take advantage of co-existing bacterial strains to enhance the metabolic breakdown of the complex molecules. The commonly used microorganisms in the MFC research for the construction of mediatorless

microbial fuel cells include members of *Shewanella*, *Rhodospirillum rubrum*, and *Geobacter*. *Geobacter* belongs to dissimilatory metal reducing microorganisms, which produce biologically useful energy in the form of ATP during the dissimilatory reduction of metal oxides under anaerobic conditions in soils and sediments. The electrons are transferred to the final electron acceptor such as Fe<sub>2</sub>O<sub>3</sub> mainly by a direct contact of mineral oxides and the metal reducing microorganisms (Lovley et al., 2004; Vargas et al., 1998). The anodic reaction in mediator-less MFCs constructed with metal reducing bacteria belonging mainly to the families of *Shewanella*, *Rhodospirillum rubrum*, and *Geobacter* is similar to this process because the anode acts as the final electron acceptor just like the solid mineral oxides. Though most of the mediator-less MFCs are operated with dissimilatory metal reducing microorganisms, few exceptions were reported with *Clostridium butyricum* (Oh and Logan, 2006; Park et al., 2001), *Hansenula anomala* (Prasad et al., 2007), *Clostridium* sp., (Prasad et al., 2006), *Gluconobacter roseus* and *Acetobacter aceti* (Karthikeyan et al., 2009) and *Candida melibiosica* 2491 (Hubenova and Mitov, 2010). Another point of interest in the development of biofuel cells is the selection of substrates which influences the power production to a greater extent (Liu et al., 2009). A wide variety of substrates have been utilized to get power from MFC namely, acetate, glucose, lignocellulosic biomass, synthetic wastewater, brewery wastewater, starch processing wastewater, dye wastewater, landfill leachates, cellulose and chitin, inorganic and other substrates (Pant et al., 2009). Recently it has been demonstrated by us that the mixed culture of *A. aceti* and *G. roseus* can be used as biocatalysts in batch type mediatorless MFC (Karthikeyan et al., 2009). *G. roseus* and *A. aceti* are responsible for spoilage

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of wine. These two genera are called as acetic acid bacteria i.e., they oxidize sugars, sugar alcohols, and ethanol with the production of acetic acid as the major end product. It is reported that *Gluconobacter* sp., spoil the grape and *Acetobacter* sp., spoil the wine. Acetic acid bacteria are present at all stages of wine making, from the mature grape through vinification to conservation (Joyeux et al., 1984). Low levels of *A. aceti* are present in the wine and they exhibited rapid proliferation on short exposure of the wine to air and caused significant increase in the concentration of acetic acid. Higher temperature of wine storage and higher wine pH favored the development and metabolism of these species (Joyeux et al., 1984). Free SO<sub>2</sub>, used as a preservative of red wines, does not sufficiently protect against the metabolism of acetic acid bacteria. Such a wine loses its freshness and the usage of such wine creates wine allergy problem (Bartowsky and Henschke, 2008). The bad wine thus produced can be converted to useful electrical energy in MFCs. In this work we have shown the effect of power production and coulombic efficiency by the coexisting bacterial strains over a range of substrates like glucose, ethanol, acetate and bad wine. The direct electron transfer of the mixed culture biofilm in presence of the fuels glucose and ethanol is demonstrated by cyclic voltammetry.

## 2. Methods

### 2.1. Biofilm formation

In order to understand the electron transfer property of mixed culture, (*A. aceti* and *G. roseus*) biofilms were formed by applying a constant anodic current density galvanostatically (Busalmen et al., 2008). *A. aceti* (NCIM No. 2116) and *G. roseus* (NCIM No. 2049) were procured from NCL, Pune, India. Sub-culturing was carried out using the following media composition: Tryptone (1 g), yeast extract (1 g), glucose (1 g) and CaCO<sub>3</sub> (1 g) in 100 mL of distilled water. The biocatalysts (1:1 composition of mixed culture containing wet weight of 0.15 g *A. aceti* and 0.15 g *G. roseus*) were suspended in the 100 mM phosphate buffer, pH 7.0 (25 mL) containing 25 mM of glucose (6 g/L) in the three electrode electrochemical cell. The glassy carbon (GC) working electrode (WE, 3 mm dia) is used for biofilm formation. Before the experiments the WE was polished using a polishing cloth and alumina powder. A platinum electrode was used as the counter electrode, and a normal calomel electrode (NCE) was used as the reference electrode. Buffers were purged with nitrogen gas for at least 30 min before the experiments, and a nitrogen environment was then kept above the solution in the cell to protect the solution from oxygen. A constant current of 50  $\mu$ A was anodically applied for a period of 168 h. After that the GC was gently removed from the culture medium and washed with phosphate buffer (pH 7) to remove loosely held microorganisms on the electrode. Cyclic voltammetry was used to study the direct electron transfer of the biofilm. The cyclic voltammograms were recorded (PARSTAT) with a potential range from  $-1$  V to  $1$  V with respect to NCE (Normal calomel electrode) at a scan rate of 50 mV/s. The electrocatalytic oxidation of the biofilm was analyzed by the addition of various concentration glucose and ethanol. All experiments were performed at room temperature ( $28 \pm 2$  °C).

### 2.2. MFC construction

A dual chamber microbial fuel cell was constructed, separated by nafion 117 membrane (Aldrich). Each chamber is made up of perspex sheet and each chamber has the volume of 125 mL. The anode is a piece of carbon felt ( $5 \times 5 \times 0.5$  cm). Anolyte is phosphate buffer. Graphite ( $5 \times 5 \times 0.5$  cm) was used a cathode.

0.1 M K<sub>4</sub>[Fe(CN)<sub>6</sub>] in phosphate buffer was used as a catholyte. Glucose (Hi media), Ethanol (Otto Inc.), Sodium acetate (sd fine-chem) were used as received. Red wine (Golconda Ruby wine, United spirits Ltd., India. 16% v/v ethanol) was exposed to air for one hour to allow spoilage of wine and was used for experiments and hereafter referred to as bad wine. Twelve fuel cells were operated by different substrates (glucose, ethanol, acetate and bad wine) with these biocatalysts namely pure cultures of *A. aceti* (A-MFC), *G. roseus* (G-MFC) and a mixed culture of both the species (AG-MFC).

Each MFC was fed with four different substrates, 25 mM of glucose ( $5.8 \pm 0.2$  g/L of COD), ethanol ( $2.1 \pm 0.2$  g/L of COD), acetate ( $2 \pm 0.2$  g/L of COD) and also bad red wine ( $7.8 \pm 0.2$  g/L of COD). All the MFCs were operated for a period of 72 h to compare the substrate oxidation under the operating external resistance. In order to evaluate the long time performance and the reproducibility of bad wine fed MFCs period of operation was extended to 144 h for one cycle. Three cycles of operation were carried out to check the reproducibility of results for the bad wine fed MFC (each cycle 144 h, for three cycles 432 h). During the operation the anode chamber was completely deaerated by N<sub>2</sub> gas and the pH of the MFC was maintained at  $6.4\text{--}7.0$  at  $29 \pm 2$  °C.

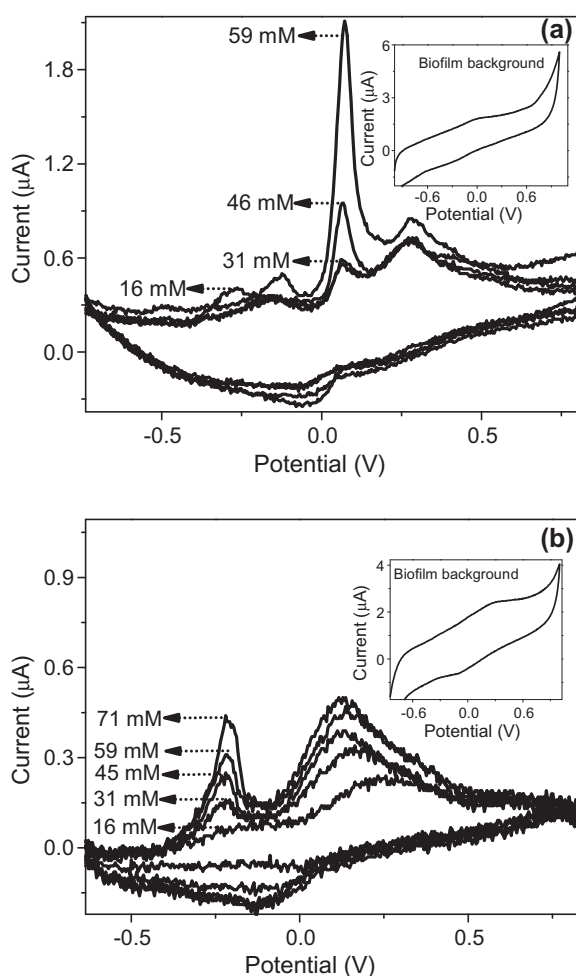
### 2.3. Analysis and calculation

The voltage difference between the anode and the cathode was measured across the fixed external resistance for every 5 or 10 min interval by using the data logger (Agilent acquisition 34970A data acquisition/switch unit). The data were collected automatically by a data acquisition program and a personal computer. Polarization tests were carried out by applying the variable resistance in the circuit and recording the resulting steady state voltage (Yazdi et al., 2011). Current ( $I$ ) was calculated on the basis of Ohm's law ( $I = V/R$ ), where  $V$  is voltage and  $R$  the applied resistance and current density,  $i$  (A/m<sup>2</sup>), was calculated using the formula,  $i = I/v$ , where  $v$  is the volume of the anolyte ( $125 \times 10^{-6}$  m<sup>3</sup>). Power density,  $P$  (W/m<sup>2</sup>), was calculated by multiplying the current by voltage and dividing with anolyte volume,  $P = IV/v$ . Ohmic resistance was calculated from the slope of polarization curve at the linear (ohmic) region (Fan et al., 2008). It is understood that the Ohmic resistance (internal resistance) of the MFC collectively refers to resistance of electrodes, electrolytes and interconnections to electron and proton transport process. Coulombic efficiency (CE) was calculated based on  $CE = (C_e/C_t) \times 100\%$ , where  $C_e$  is the total coulombs calculated by integrating the current generated over the total time of operation, and  $C_t$  is the theoretical amount of coulombs available based on the measured COD removal in the MFC (Logan et al., 2006).

## 3. Results and discussion

### 3.1. Direct bioelectrocatalysis

The electrochemical activity of the AG-biofilm on GC formed by imposing a constant current density of 0.71 mA/cm<sup>2</sup> for 168 h, was investigated by cyclic voltammetry. The biofilm exhibits redox peaks at 0.0716 V and  $-0.1098$  V (vs. NCE). The presence of redox peak could indicate the presence of electroactive redox enzymes present in the biofilm itself. In order to visualize the bioelectrochemical oxidation of glucose directly through electroactive biofilm the voltammograms were recorded at pH 7 (Fig. 1a). The biofilm exhibits increasing catalytic oxidation current with the addition of glucose. The oxidation of glucose occurs at two peaks, indicating that different electron transfer mechanisms are active at different potentials and also it favors electronic coupling of at

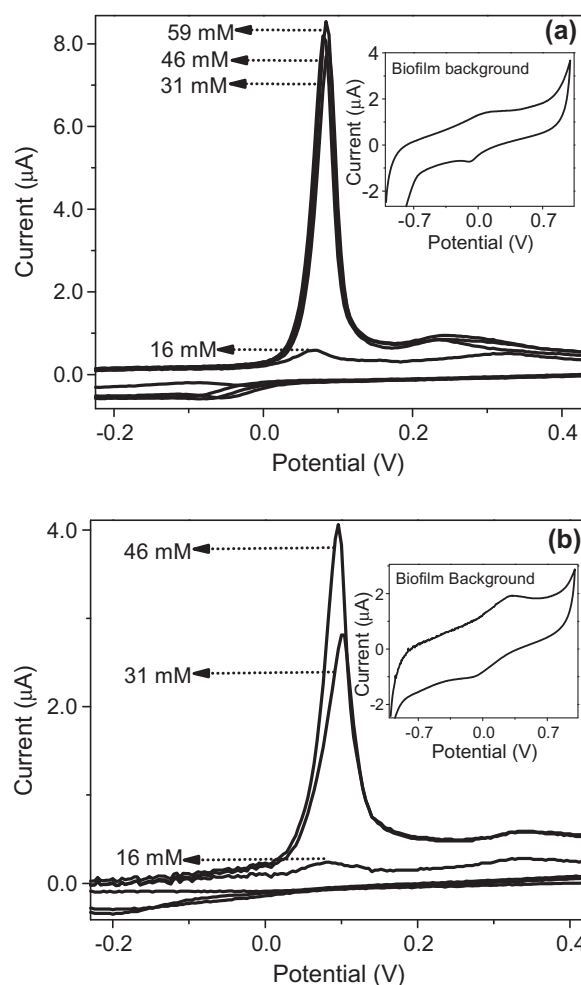


**Fig. 1.** Background current subtracted voltammogram (50 mV/s) of the biofilm for different additions of glucose (in mM) at pH 7 (a) and at pH 4 (b), inset: cyclic voltammogram recorded for the biofilm only.

least two sub units containing few heme c sites (Tkac et al., 2009). While increasing the concentration of glucose above 59 mM, no increase of oxidation current is observed (results not shown) which indicates saturation effect of the electroactive biofilm with glucose. The oxidation of glucose at pH 4.0 (Fig. 1b) buffer medium shows a lower catalytic oxidation current compared to the currents observed at pH 7.0. An oxidation current of only 0.5 μA was observed for 71 mM of glucose at pH 4.0 where a current of 1.7 μA was obtained for 59 mM of glucose at pH 7.0.

Fig. 2(a) and (b) show the background current subtracted voltammogram of biofilm for different additions of ethanol at pH 7 and pH 4. In this case, the oxidation current increased nearly 4 times compared to that of glucose oxidation (1.7–8 μA i.e., 25–113 μA/cm<sup>2</sup>) at pH 7. The same observation could be observed at pH 4 (0.5–4.07 μA i.e., 7.1–58 μA/cm<sup>2</sup>, ≈8 times). It is inferred that the mixed culture of *A. aceti* and *G. roseus* exhibits better catalytic/degradation effect on ethanol compared to glucose which is expected naturally. Under alkaline conditions the biofilm did not show any oxidation current with glucose and ethanol (figure not shown).

*A. aceti* can oxidize ethanol favorably due to the presence of membrane bound alcohol dehydrogenase (ADH) in the cells which acts as a mediator between the enzyme and anode of the fuel cell (Ikeda et al., 1997). *G. roseus* will oxidize the ethanol and glucose due to the presence of quinoxinoprotein ADH and quinoprotein glucose dehydrogenase (GDH) in the bacterial cell membrane



**Fig. 2.** Background current subtracted voltammogram (50 mV/s) of the biofilm for different additions of ethanol (in mM) at pH 7 (a) and at pH 4 (b), inset: cyclic voltammogram recorded for the biofilm only.

(Ikeda et al., 1992) which act as mediator between enzyme and anode of the fuel cell. Electrochemically active biofilm on the electrode can be formed by either under constant applied potential or current. In both cases biofilm formation was induced by the electron sink nature of the electrode surface which attracts the negative surface charges of the bacteria (Wang et al., 2009). It was recently found that in the case of *Geobacter sulfurreducens*, biofilm formation induced under open circuit voltage (OCV) condition did not oxidize the acetate, though it exhibits redox behavior. Biofilm formed under applied potential (0.2 V or 0.4 V) showed the presence of surface confined redox species (Katuri et al., 2010) and oxidized the acetate. These constraints are avoided by forming the biofilm under galvanostatic conditions.

### 3.2. Electricity generation from glucose fed MFC

Maximum operating cell voltage (0.324 V) was found at 900 Ω for AG-MFC<sub>glu</sub> with a resulting power density of 1.05 W/m<sup>3</sup> (3.24 A/m<sup>3</sup>) which is higher than the power generated by A-MFC<sub>glu</sub> and G-MFC<sub>glu</sub> (Fig. S1 of Supporting information). It supports our previous experiments with the mixed culture of *A. Aceti* and *G. roseus* MFC (AG-MFC) (karthikeyan et al., 2009). The literature contains information about different types of MFCs with glucose using specific bacteria such as *Escherichia coli* (Qiao et al., 2008), *Pseudomonas aeruginosa* (Rabaey et al., 2004), *Rhodospirillum rubrum*

**Table 1**

COD removal and MFC power output for a period of 72 h.

MFCs	COD removal ( $\pm 1\%$ )	Coulombic efficiency ( $\eta\%$ )	Optimal external resistance ( $\Omega$ )	Ohmic resistance ( $\Omega$ )	Steady state current density ( $A/m^3$ )
A-MFC <sub>glu</sub>	89	1.2427	900	742 $\pm$ 108	2.26
G-MFC <sub>glu</sub>	93	0.9271	1800	2186 $\pm$ 24	1.44
AG-MFC <sub>glu</sub>	86	3.8513	900	637 $\pm$ 104	3.24
A-MFC <sub>et</sub>	73	4.9853	900	1170 $\pm$ 81	2.584
G-MFC <sub>et</sub>	68	2.31	1700	1453 $\pm$ 7	2.12
AG-MFC <sub>et</sub>	62	8.75	500	568 $\pm$ 4	5.12
A-MFC <sub>act</sub>	92	6.39	900	807 $\pm$ 40	3.15
G-MFC <sub>act</sub>	94	5.94	900	946 $\pm$ 29	2.8178
AG-MFC <sub>act</sub>	90	6.20	900	790 $\pm$ 18	3.336
A-MFC <sub>wn</sub>	59	16.27	900	767 $\pm$ 51	4.06
G-MFC <sub>wn</sub>	50	12.41	1400	946 $\pm$ 195	2.2457
AG-MFC <sub>wn</sub>	41	45.18	600	438 $\pm$ 47	7.133

Note: glu-glucose, et-ethanol, act-acetate, wn-bad wine.

*ferrireducens* (Chaudhuri and Lovley, 2003), Yeast *Saccharomyces cerevisiae* (Walker and Walker, 2006) and *Actinobacillus succinogenes* (Park and Zeikus, 1999, 2000; Park et al., 1999). The direct comparison of these MFC outputs may not provide any conclusion (Pant et al., 2009) because of the variable parameters such as electrode designs, electrode materials, operating conditions, surface area, electrolyte conductivity, biocatalyst, units employed for power output etc., that were used in these MFCs.

### 3.3. Electricity generation from ethanol fed MFC

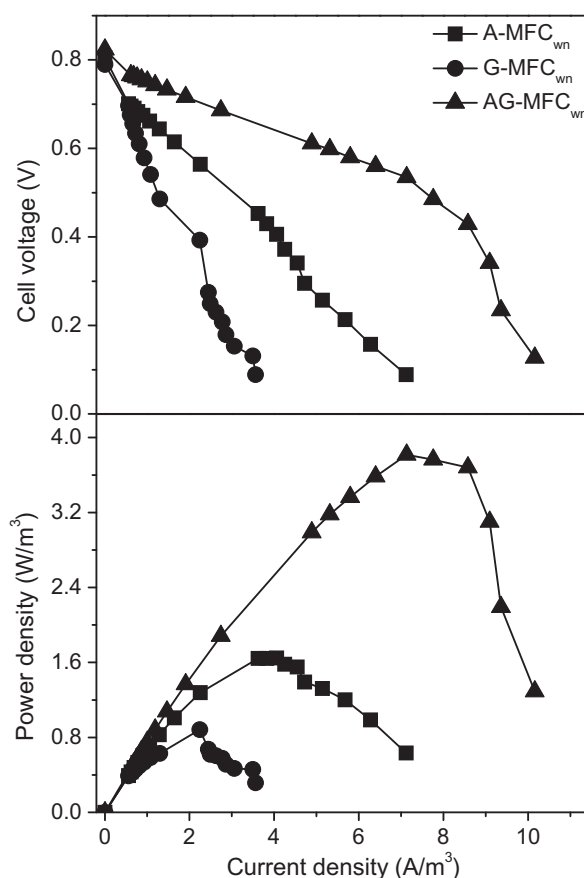
The maximum operating cell voltage (0.384 V) was found at 500  $\Omega$  for AG-MFC<sub>et</sub> (Fig. S2 of Supporting information) with a power output of 1.97 W/m<sup>3</sup> (5.12 A/m<sup>3</sup>) which is higher than the power generated by A-MFC<sub>et</sub> and G-MFC<sub>et</sub>. It should be noted that the optimum operating resistance of ethanol fed MFCs has considerably decreased to 500  $\Omega$  when compared to glucose fed MFCs which implies the rate of electron transfer in this fuel cell configuration is more when compared to glucose fed MFCs (MFCs<sub>glu</sub>). Further it can be added that ethanol is one of the metabolic pathway product during the glucose degradation and being a small molecule can be effectively degraded compared to glucose. The number of electrons released during the complete oxidation of glucose, ethanol and acetate are 24 e<sup>-</sup>/mol, 12 e<sup>-</sup>/mol and 8 e<sup>-</sup>/mol, respectively.

### 3.4. Electricity generation from acetate fed MFC

Acetate is a simple molecule which has been extensively studied in electricity generation using electroactive bacteria (Bond et al., 2002). Due to its inertness towards alternative microbial conversions (fermentations and methanogenesis) at room temperature, acetate can be used as a benchmark fuel in MFCs (Aelterman, 2009). More over acetate is the end product of several metabolic pathways (e.g., Entner–Doudoroff pathways for glucose metabolism) for higher order carbon sources (Biffinger et al., 2008). The OCV was found to be 0.687–0.728 V and operating maximum steady cell voltage of 0.417 V was observed at 900  $\Omega$  for AG-MFC<sub>act</sub> generating a power density of 1.34 W/m<sup>3</sup> (at 3.34 A/m<sup>3</sup>) which was comparable to the power generated by A-MFC<sub>act</sub> and G-MFC<sub>act</sub> (Fig. S3 of Supporting information). The optimum operating resistance (900  $\Omega$ ) is relatively higher compared to the case of ethanol (Table 1). Though acetate involves only two carbon atoms, the electron transfer kinetics is not favorable when compared to ethanol.

### 3.5. Electricity generation from bad wine fed MFC

It is clear that these biocatalysts (*A. aceti* and *G. roseus*) can recover the electrons from the substrates such as glucose, ethanol and acetate. Since the biodegradation conditions are favorable for ethanol, the ability of the mixed culture for the degradation of

**Fig. 3.** Polarization and power generation of bad wine based MFCs.

bad wine has been evaluated. Fig. 3 shows the polarization curve of wine fed MFCs with three types of biocatalysts. The OCV was found to lie between 0.790 V and 0.823 V. The maximum operating cell voltage of 0.535 V was determined at 600  $\Omega$  ( $R_{ohm}$  438  $\pm$  47  $\Omega$ ) for AG-MFC<sub>wn</sub>. As a result AG-MFC<sub>wn</sub> can generate a power density of 3.82 W/m<sup>3</sup> (7.13 A/m<sup>3</sup>) which is higher than the power generated by A-MFC<sub>wn</sub> and G-MFC<sub>wn</sub>. The optimum operating resistance of the wine fed MFCs was higher when compared to ethanol fed MFCs and lower when compared to glucose fed MFCs.

Table 1 shows the fuel depletion and efficiency of MFC with optimal external resistance during 72 h. It indicates that higher COD removal (90–94%) was achieved with acetate fed MFCs and lower COD removal (59–41%) was accomplished with bad wine. The electron transfer yield is reflected in the values of coulombic efficiency. Glucose fed MFC shows lower efficiency (0.9–3.8%).



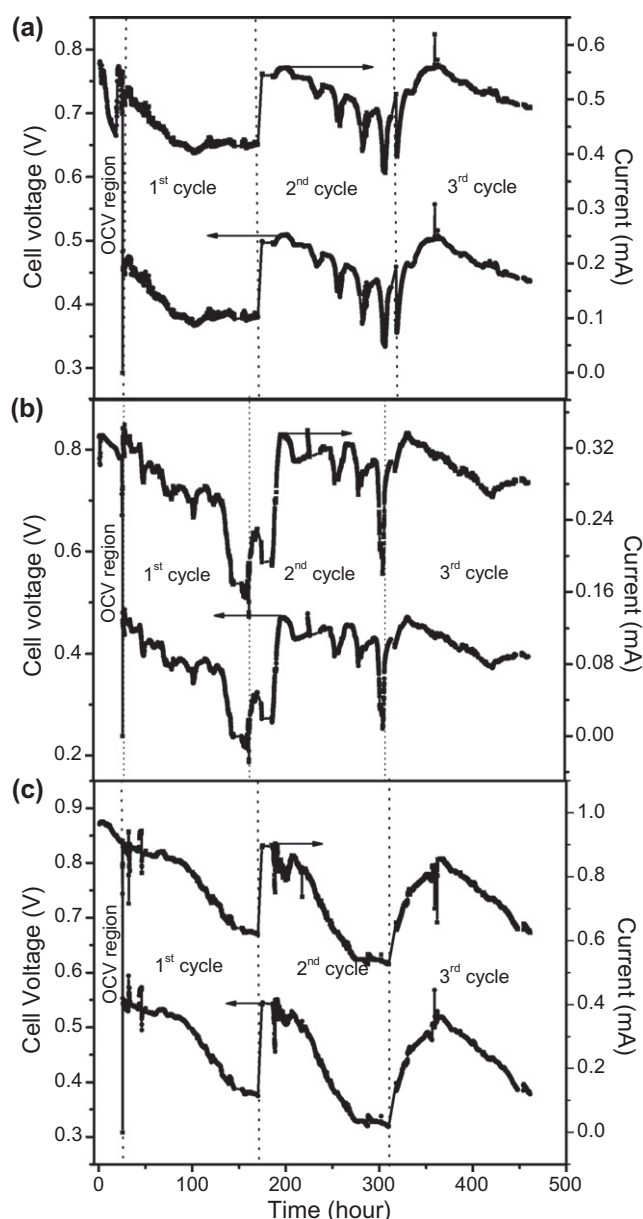


Fig. 4. Current and voltage profile of A-MFC<sub>wn</sub> (a), G-MFC<sub>wn</sub> (b) and AG-MFC<sub>wn</sub> (c) for a period of 432 h (1–3rd cycle) under optimal external resistance.

Bad wine fed MFC exhibits a coulombic efficiency of 12–45% with optimum current production of 4–7 A/m<sup>3</sup>. The optimum operating external resistance was found to be the same in all acetate fed MFCs and similar power output (0.9–1.3 W/m<sup>3</sup>) was obtained in all the acetate fed MFCs. It indicates that acetate can be oxidized equally well in all fuel cell configurations (A, G, AG-MFC<sub>act</sub>). Lower coulombic efficiency and power density were obtained for glucose fed MFCs. It can be explained due to the fact that glucose is a fermentable substrate implying its consumption by diverse competing metabolisms such as fermentation and methanogenesis (Pant et al., 2009; Chae et al., 2009).

Fig. 4(a)–(c) shows the current and voltage profile vs. time for A-MFC<sub>wn</sub>, G-MFC<sub>wn</sub> and AG-MFC<sub>wn</sub>. The current derived from wine fed MFC follows the order of AG-MFC<sub>wn</sub> (0.89 mA) > A-MFC<sub>wn</sub> (0.55 mA) > G-MFC<sub>wn</sub> (0.32 mA) and the power density vs. time at an optimum external resistance indicates AG-MFC<sub>wn</sub> produces the maximum power density (3.5–3.9 W/m<sup>3</sup>) in all cycles (Fig. S4 of Supporting information). The COD removal and the

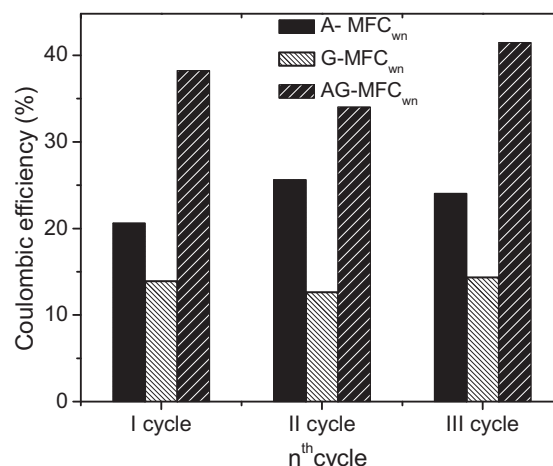


Fig. 5. Coulombic efficiency of wine fed MFCs for 3 cycles (each cycle 144 h).

corresponding coulombic efficiencies for wine degradation are presented in Fig. S5 (Supporting information) and Fig. 5, respectively for all the three cycles. It is inferred that while increasing the operating period of bad wine fed MFC from 72 h to 144 h the COD removal increased from 41% to 87.5% while the coulombic efficiency decreased from 45.2% to 41%. More recently researchers developed a power management system (PMS) that enables a sediment microbial fuel cell (SMFC) to operate a remote sensor consuming 2.5 W of power (Donovan et al., 2001). They designed a custom PMS to microbial energy in capacitors and use the stored energy in short bursts using two DC/DC converters and a digital logic circuit to convert low-level power from a SMFC (maximum  $0.38 \text{ V} \times 64 \text{ mA} = 24 \text{ mW}$  and a average continuous power of 3.4 mW) to 2.5 W power for 5 s. Similar electronic circuitry can be developed to deliver power output of the order of watts from our MFCs.

In this study, AG-MFC<sub>wn</sub> shows a sustainable operating voltage of 0.535 V with a current output of 0.89 mA. Hence the power output will be  $470 \pm 5 \mu\text{W}$ . This power will be 500 times higher than the power required to run a wall clock which requires only 8–10  $\mu\text{W}$  at minimum voltage of  $1.3 \pm 0.2 \text{ V}$  (Supporting information). When two MFCs are connected in series, the power output obtained is sufficient to run devices like wall clock normally run with 1.5 V dry cell.

#### 4. Conclusions

In conclusion mixed cultures of *A. aceti* and *G. roseus* can be used as biocatalysts to perform the dual duty of biodegradation and current generation. Based on the experimental results bad wine fed mixed culture fuel cell (AG-MFC<sub>wn</sub>) generates a power density of  $3.8 \pm 0.2 \text{ W/m}^3$  with 45% coulombic efficiency. While increasing the period of operation (72–144 h) bad wine fed AG-MFC<sub>wn</sub> exhibited an increase in the COD removal from 41% to 87.5%. The results indicate the effectiveness of the cooperative effect of the two microorganisms in facilitating current generation and biodegradation and the utility of these MFCs for current generation in wineries.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.10.092.

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