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

Karthikeyan Rengasamy, Sheela Berchmans *

Electrodics and Electro Catalysis Division, Central Electrochemical Research Institute (CSIR-CECRI), Karaikudi 630 006, India

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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^3), ethanol (1.97 W/m^3), acetate (1.39 W/m^3) and bad wine (3.82 W/m^3). 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.

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*, *Rhodoferax*, 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_2O_3 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*, *Rhodoferax*, 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

* Corresponding author. Tel./fax: +91 4565 227550.
E-mail address: sheelaberchmans@yahoo.com (S. Berchmans).

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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_2, 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 $CaCO_3$ (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 μA 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 ± 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 × 5 × 0.5 cm). Anolyte is phosphate buffer. Graphite (5×5 × 0.5 cm) was used a cathode. 0.1 M $K_4[Fe(CN)_6]$ in phosphate buffer was used as a catholyte. Glucose (Hi media), Ethanol (Otto Inc.), Sodium acetate (sd finechem) 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 ± 0.2 g/L of COD), ethanol (2.1 ± 0.2 g/L of COD), acetate (2 ± 0.2 g/L of COD) and also bad red wine (7.8 ± 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_2 gas and the pH of the MFC was maintained at 6.4–7.0 at 29 ± 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^3), was calculated using the formula, $i = I/v$, where v is the volume of the anolyte (125×10^{-6} m^3). Power density, P (W/m^3), 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^2 for168 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