

A Study on Bioelectricity Production by the Synergistic Action of *Bacillus tequilensis* DMR-5 and *Pseudomonas aeruginosa* DMR-3 Isolated from Rumen Fluid

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ABSTRACT

The present study focuses on the bioelectricity production from pure culture of *Bacillus tequilensis* DMR-5 and *Pseudomonas aeruginosa* DMR-3. Both the cultures were isolated from the anodic biofilm of pre-run MFC's with rumen fluid as anodic substance. They were checked for the power production for 10 days both as pure isolates and co-culture. *Bacillus tequilensis* and *Pseudomonas aeruginosa* produced 250 mV and 20 mA, 310 mV and 10 mA respectively. Both these bacteria when used as mixed culture (110×10^5 CFU/mL) produced 450 mV and 40 mA. The biofilm of the anode was taken for cyclic voltammetry study and the oxidation and reduction peaks observed in both forward and reverse scan confirmed the electrochemical nature of the bacteria. Based on the power readings measured and cyclic voltammograms obtained, it has been found that the co-culture produced more power than the pure cultures when used individually in the Microbial fuel cell.

Keywords: Electrochemically Active Bacteria, Rumen Fluid, Cyclic Voltammetry, *Pseudomonas Aeruginosa*

1. INTRODUCTION

Increasing population and food demands has urged the world to find an alternative source of sustainable energy. The current energy consumption has almost depleted the fossils fuels and this has created a big threat to the future society. Green energy is one of the options which can hopefully help us in an effective way. Microbial fuel cell is the emerging technology where the microbes generate power by the oxidation of the organic matter in the anode chamber.

Microbial Fuel Cells (MFCs) are devices that can use bacterial metabolism to produce an electrical current from a wide range of organic substrates. Due to the promise of sustainable energy production from organic wastes, research has been increasing in this field in the last few years.

Many groups of bacteria have been experimented in MFC and they were found to be electrochemically active in the anode region. For instance, *Geopsychrobacter electrodiphilus* isolated from marine sediment was found to be electrochemically active and thus the name derived (Holmes *et al.*, 2004). *Shewanella oneidensis* MR-1 grown in MFC converted lactate to acetate and produced more electrons (Lanthier *et al.*, 2008). *Shewanella oneidensis* was found to have Nanowires which helped in the transfer of electrons (Gorby *et al.*, 2006). The most studied bacteria in the MFC field were *Geobacter* and *Shewanella* sp. (Kim *et al.*, 1999) which were known to have the electrochemical activity. *Pelobacter carbinolicus* isolated from sediment microbial fuel cell was found to produce electricity when it was coupled with *Geobacter sulfurreducens* in the presence of ethanol. The ethanol metabolism of *Pelobacter* sp

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initiated when *Geobacter* sp consumed the hydrogen (Richter *et al.*, 2007).

The current study focuses on comparing the power generation by MFC developed with pure culture and co-culture of *Bacillus tequilensis* and *Pseudomonas aeruginosa* in the anodic chamber. This will help us in determining the electrochemical activity of the bacterium when it is cultured as pure and in combination. There are very few reports in this area and the researchers are working in depth to know their contribution in electricity generation.

2. MATERIALS AND METHODS

2.1. Isolation of Bacteria from the Anodic Biofilm

Bacteria were isolated from the anodic biofilm of a double-chambered pre-run MFC with rumen fluid as anodic substance. They were isolated by serial dilution method and colonies were taken from dilution 10^{-6} to 10^{-8} based on the colony morphology. The bacteria were then sub cultured many times to obtain pure culture which was maintained in nutrient agar slants for further use.

2.2. Construction of MFC

A two chambered MFC made up of plastic with the dimension of 15 cm height X 7 cm diameter has been designed for the experiments. Carbon electrodes of diameter 1.5 and 13 cm long served as both anode and cathode. The electrodes were initially treated with 0.1N HCl overnight before using for the experiment and cleaned with 0.1N NaOH after the completion of experiment. They were suspended in the liquid from the top in both the chambers. The anode compartment was filled with 500 mL of nutrient broth and 1ml of *Bacillus tequilensis*, *Pseudomonas aeruginosa* and a mixed culture of both the bacterium in three different MFC's respectively. The cathode compartment was filled with distilled water which acted as air cathode. Both the compartments were connected with a 0.5 cm diameter and 14 cm long tube which was filled up with salt bridge made of sodium chloride and agar in the ratio of 1:2.

2.3. Identification of Bacteria by Biochemical Tests and 16srRNA Sequencing

Two isolates which were taken for the study in Microbial fuel cell was tested in various biochemical tests and 16srRNA sequencing was done to identify the genus and species of the bacteria using the primers: Forward-8F (5'-GAGTTTGATCATGGCTCAG-3') Reverse-1495r(5'-CTACGGCTACCTTGTTACG-3'). The bacterial morphology was studied with the help of microscope.

2.4. Pure Culture MFC

Three different models of Microbial fuel cell namely one MFC with pure culture of *Bacillus tequilensis*, one MFC with pure culture of *Pseudomonas aeruginosa* and other with a co-culture of *Bacillus tequilensis* and *Pseudomonas aeruginosa* were constructed and the potential and current readings were measured using a digital multimeter every 24 h for 10 days.

2.5. Electrochemical Activity of the Bacterial Biofilm

To confirm the presence of electrochemical activity of the anodic bacteria, the electrodes were taken after 10 days and the cyclic voltammograms were recorded in 100 mM phosphate buffer pH-7.0 (61.5 mL of 1M Dipotassium hydrogen phosphate and 38.5 mL of 1M Potassium dihydrogen phosphate were taken and made up to 1 litre with distilled water). The redox potential was measured in the presence of phosphate buffer pH-7.0 in the absence of microorganisms by cyclic voltammetry using a PC4/750 potentiostat (Gamry Instruments) (Park *et al.*, 2001; Cuong *et al.*, 2003). Measurements were made at different scan rates of 2mV/s, 5mV/s and 10mV/s in a three-chambered electrochemical cell consisting of working electrode (Anode of surface area 61.28 cm²), a counter electrode (platinum electrode) and an Ag/AgCl reference electrode.

3. RESULTS

3.1. MFC Model

The MFC model constructed was feasible for the bacterial inoculation. The oxygen (air cathode) acted as an oxidizing agent. No oxidizing agents were used for the MFC performance. The model showing a voltage of 0.47 has been shown in Fig. 1.

3.2. Identification of the Micro-Organisms

The bacteria which were isolated from the anodic biofilm of pre-run rumen fluid MFC were identified as *Bacillus tequilensis* and *Pseudomonas aeruginosa* by 16srRNA sequencing. The photomicrograph of the bacteria has been given in the Fig. 2a and b.

3.3. Operation of MFC with Pure Cultures and Co-Culture

The anodic chamber of MFC was inoculated with 1mL culture (110×10^5 units/mL) of *Bacillus tequilensis*, *Pseudomonas aeruginosa* and co-culture of these bacteria in three separate MFC's on the same day.

The potential and current were measured with the multimeter and the results were represented in the Fig. 3a and b) respectively. When inoculated as pure culture *Pseudomonas aeruginosa* showed a maximum of 310 mV and 20 mA.

3.4. Power Generation of Co-Culture

The co-culture of *Bacillus tequilensis* and *Pseudomonas aeruginosa* has shown a maximum of 450mV and 40mA. The power density was found to be 254mW/cm². When compared to the pure cultures, co-culture produced high power density.

3.5. Cyclic Voltammograms of the Bacteria Isolated From Biofilm

The voltammograms obtained for individual culture and mixed culture reveals that the oxidation and reduction has occurred more when the bacteria are inoculated as co-culture (combined). *Bacillus tequilensis* which produced less voltage showed no prominent peaks in different scan rates. However, *Pseudomonas aeruginosa* produced a little high voltage than the former and showed an oxidation peak at -0.398V and reduction peak at -0.157V. Figure 4a and b shows the cyclic voltammograms of pure cultures of the bacteria in different scan rates.



Fig. 1. MFC model showing a voltage of 0.47

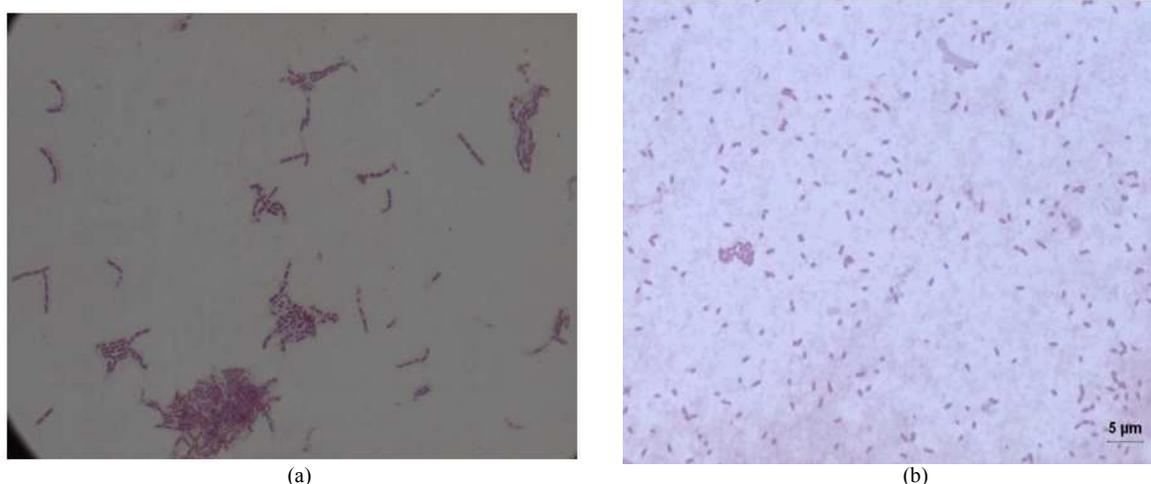


Fig. 2. (a) and (b) Photomicrograph of *Bacillus tequilensis* and *Pseudomonas aeruginosa* (1000X)

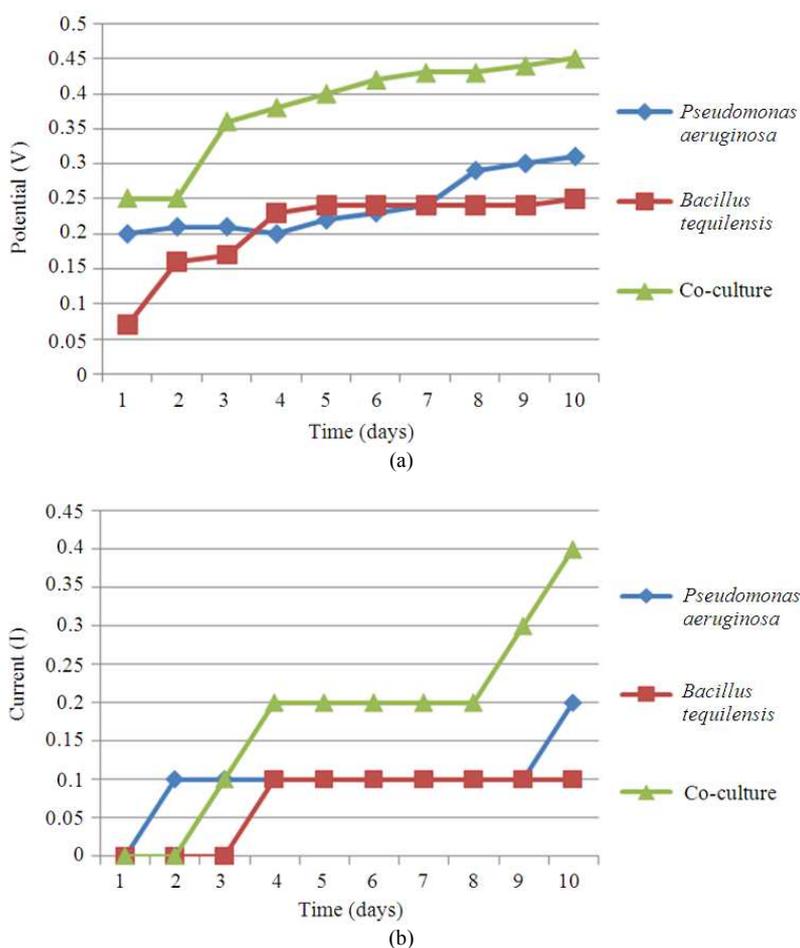
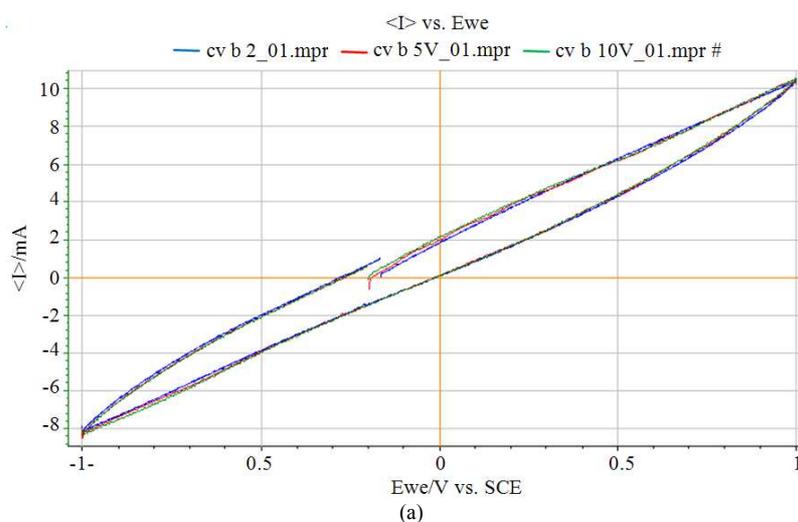


Fig. 3. (a) Cell voltage of *Bacillus tequilensis*, *Pseudomonas aeruginosa* and co-culture of the bacteria. (b) Current output of *Bacillus tequilensis*, *Pseudomonas aeruginosa* and co-culture of the bacteria in 24hr intervals



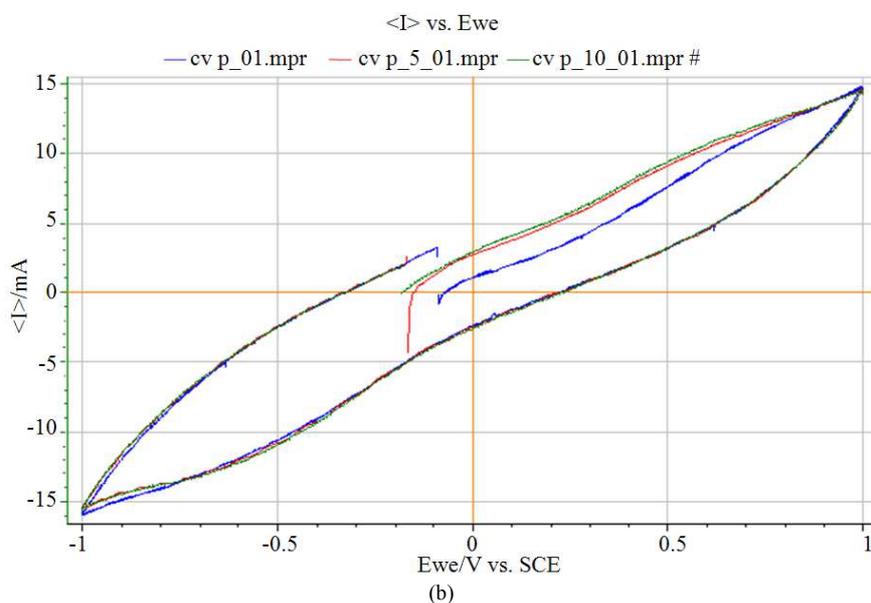


Fig. 4. (a) Cyclic voltammograms of *Bacillus tequilensis* at scan rates of 1mV/s, 5mV/s and 10mV/s (b) Cyclic voltammograms of *Pseudomonas aeruginosa* at scan rates of 1mV/s, 5mV/s and 10mV/s

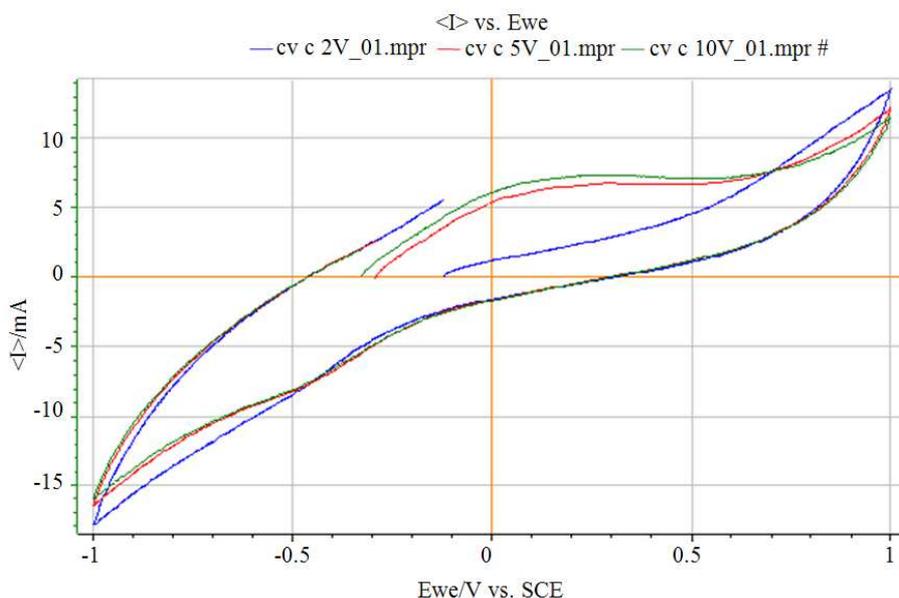


Fig. 5. Cyclic voltammograms of co-culture at scan rates of 1mV/s, 5mV/s and 10mV/s

3.6. Cyclic Voltammograms of Co-Culture of *Bacillus tequilensis* and *Pseudomonas aeruginosa*

The voltammogram obtained for the co-culture in different scan rates revealed that the oxidation has

taken place and the oxidation peaks were observed at - 0.45V and 0.5V, reduction peak at 0.16V. Among the different scan rates, 10mV/s gave a proper graph. **Figure 5** shows the cyclic voltammogram of co-culture in different scan rates.

4. DISCUSSION

Microbial fuel cell performance differs for each and every bacterium. 10.89 mA and 10.45 mA current were generated by *Saccharomyces cerevisiae* and *Clostridium acetobutylicum* after 10 days of operation (Mathuriya and Sharma, 2009). On an other side, *Aeromonas hydrophila* inoculated in LB with ferric citrate was used to check the electrochemical activity. An aircathode MFC with *Enterobacter aerogenes* produced a maximum power density of 2.51W/m³ where no mediators were used (Zhuang *et al.*, 2011). *Geobacter sulfurreducens* and *Geobacter metallireducens* exhibited lower current densities of 110±7 A/m³ (Call *et al.*, 2009). *Shewanella oneidensis* DSP10 grown in medium with lactate exhibited 24 mW/m² for reticulated vitreous carbon and when external mediators were used the current and power increased by 30-100%. Ringeisen *et al.* (2006). *Hansenula anomala* gave 2.34 W/m³ when graphite-felt was used as the anode material and deaerated suspension of nutrient broth in phosphate buffer was filled in anodic chamber (Prasad *et al.*, 2007).

The cyclic voltammogram is a characteristic feature which confirms the electrochemical activity of the biofilm or individual bacteria. Hence, this technique has been widely used for the studies involving microbial fuel cell. This gives the data of the redox potential that has happened in the anode compartment and also information about the direct electron transfer. For instance, the electrochemical activity of two enzymes has been demonstrated in a study where *Hansenula anomala* produced less peak currents when lactate has been added (Prasad *et al.*, 2007).

5. CONCLUSION

The present study emphasizes that the co-culture produce comparatively good voltage than the pure ones. Though the bacteria are of different genus they adapt very well in the anodic chamber and helps in the transfer of electrons. There exist a mutualism among the bacterial population and hence to conclude *Bacillus tequilensis* and *Pseudomonas aeruginosa* produces more power when they are in a co-culture form.

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