Sustainable Energy Generation in Microbial Fuel Cell Catalyzed with Bacillus Subtilis Species

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Abstract
Microbial fuel cell (MFC) attracts growing efforts as a kind of environmentally friendly biotechnology. In this study, the aerobic bacterium Bacillus subtilis has been utilized in a dual-chambered upflow microbial fuel cell fueled with actual domestic wastewater. The MFC system was continuously operated for 75 days. The performance of the UMFC was mainly evaluated in accordance with the COD removal and maximum power generation as well as BOD reduction. The results revealed that the microorganism Bacillus subtilis is electrochemically active culture. The maximum observed efficiency of COD removal and power generation were 90% and 270 mW/m², respectively.

Keywords: MFC, Anaerobic treatment, Power generation, Wastewater, and Bacillus subtilis

1. Introduction
Microbial fuel cell (MFC) can be considered as a novel bioreactor utilize microorganisms as catalysts to convert chemical energy stored in organic substrates to electricity by the electrochemical technology [1]. Many researchers have reported the generation of electricity using activated sludge as the source of microorganisms. However, when using a mixed community, the electrochemical activity of a few bacterial species enhances the power output of the whole system. However, fewer studies using single type of microorganisms have been previously reported. Nimje et al. [2] studied the energy generation by a strain of Bacillus subtilis in a MFC fed with synthetic glucose-based wastewater. The maximum observed power density was 1050 mW/m². Rahimnejad et al. [3] used a dual chambered air–cathode MFC fed with glucose solution as a substrate having an initial concentration of 30 g/L. The system was inoculated with anaerobic pure culture of Saccharomyces cerevisiae. Maximum produced power density was 283 mW/m². Fatemi et al. [4] evaluated electricity generation in a dual chambered MFC inoculated with Saccharomyces cerevisiae. Glucose with initial concentration of 4 g/l was used as an electron donor. The power density and maximum COD removal efficiency were 10.1 mW/m² and 54%, respectively. In this study the performance of a two-chambered MFC fueled with actual domestic wastewater and biocatalyzed with an anaerobically grown bacterium Bacillus subtilis has been evaluated.
2. MATERIALS AND METHODS

An upflow MFC consisted of a dual rectangular chambers made of transparent acrylic parallelepiped having dimensions of 52 x 9.4 x 9.4 cm was used in this study. The anode and cathode chambers contained graphite plain electrodes; each had a surface area of 60 cm². The two chambers were separated by a cation exchange membrane (CEM) type CMI-7000, supplied by membrane international INC., NJ. The CEM sheet of dimensions 10X10 cm was placed between two perforated glass sheets. The MFC was inoculated with *Bacillus subtilis* and continuously fed with *actual domestic wastewater* at a rate 0.1 mL/min and average initial COD concentration of 380 mg/L. The *Bacillus subtilis* was isolated from a mixed culture of activated sludge freshly collected from a local sewage treatment plant. The schematic diagram of the microbial fuel cell as well as the ports details are given in Figs. 1 and 2, respectively. The UMFC was operated at temperature 28 ± 2°C. The freshly collected wastewater was primarily clarified and settled before entering the upflow MFC. The composition of the primarily clarified actual wastewater performed in this study with its constituents average concentration expressed in (mg /L) was as follows: BOD (200), COD (400), total phosphorus (12.8), nitrate nitrogen (9), ammonium nitrogen (24), chloride (30), sulfate (200), and pH (7.0). To start up the MFC operation, the anodic chamber was flushed with nitrogen, and then bacterium *Bacillus subtilis* was placed in the anodic compartment of the UMFC without aeration for 7 days in order to favor the enrichment of the cultures. The *Bacillus subtilis* was previously isolated from an activated sludge collected from the deep bottom of an aeration tank in a local sewage treatment plant. The wastewater was not pumped into the MFC during this conditioning period, thus the only substrate available for the microorganisms was obtained from the endogenous metabolism. After 7 days, the UMFC was fed with actual wastewater, at the same time the air was injected through the cathode chamber. Dissolved oxygen concentrations were measured in the anodic and cathodic compartments. The measured values of dissolved oxygen in the cathodic chamber indicated saturated concentrations. On the contrary, in the anodic chamber the oxygen concentration was observed to be very low indicating anaerobic conditions in this compartment. An external resistance of 300 Ω was placed between the anode and cathode.

![Schematic diagram of the MFC](image1)

**Fig. 1** Schematic diagram of the MFC

![Microbial fuel cell with membrane](image2)

**Fig. 2** Microbial fuel cell with membrane, Port 1: wastewater inlet, port 2: nitrogen flushing, port 3: outlet of wastewater, port 4: replacement of catholyte, port 5: aeration, port 6: catholyte feeding, 7 and 8 are the perforated glass sheets.
3. RESULTS AND DISCUSSION

COD Removal
MFC was continuously operated for 15 days before achieving the steady state condition. COD reduction was up to 90% upon achieving steady conditions as shown in the Fig. 3. Average initial COD concentration was 400 mg/l and volumetric COD loading rate to this MFC was 0.847 kg COD/m³ d. After achieving steady state, the average effluent concentration of COD was 40 mg/l. The observed COD removal efficiency was more than the reported efficiency of 54% [4], using (Saccharomyces cerevisiae) as a pure microorganism for anodic inoculation, and 83% COD removal reported by Chaudhuri and Lovley, [5] using Rhodobacter ferrireducens.

Current Generation
In this study, the MFC system generated maximum stable power outputs of 270 mW/ m² and maximum obtained Coulombic efficiency was 49.6%. The results proved that the pure microorganism Bacillus subtilis is electrochemically active and the performance of MFC inoculated with this pure culture is comparable, even higher to those inoculated with mixed cultures. For electricity generation, the current increased rapidly for the first 5 days, then increased slowly for the next 7 days to a maximum constant value of 3.60 mA as shown in Fig. 4. The current was maintained stable for more than 60 days under the given conditions. The open circuit potential was 0.81volt and the maximum closed circuit voltage drop a cross continuous external resistance 125 Ω was 0.45 volt Fig. 5. The electrochemical activity of the applied species Bacillus subtilis type of species is one of the most commonly used hosts in fermentation production, because it is simple to cultivate. Also, its products, the protein and metabolites are often secreted in the growth medium, M9 [2]. Additionally, the composition of M9 medium supplemented with glucose was employed to culture bacteria in the anode with biofilm might cause maximum productivity.
Polarization Curve

The UMFC inoculated with pure culture *Bacillus subtilis* produced a power density due to the existing of electrogenic bacteria which transfer electrons to the electrode without interfering mediator in the anode chamber (Fig. 6). The electrogenic bacteria existing in pure cultures have higher electrochemical activity than mixed culture leading to more power generation in MFC inoculated with pure culture.

![Polarization curve](image1)

**Fig. 6 Polarization curve**

**BOD removal**

Although the performance of microbial fuel cells were mainly evaluated in term of chemical oxygen demand (COD), measurements of biological oxygen demand (BOD) was also performed. Results revealed that the reduction in BOD concentration was up to 82% after 20 days operation as given in Fig. 7.

**Granulation of biomass in UMFC**

Upon reaching steady state conditions in the MFC, the electrode in the anodic chamber was almost fully covered with a thick layer of biofilm and the biomass granules formation was visible in the anode chamber. After 75 days of continuous operation, the MFC was emptied and small portions of the biofilm were scratched from the electrode surfaces and collected for microscopic examination from anode chamber. The anodic biofilm was partially granulated having 1-2 mm size of granules. The Scanning Electron Microscopy (SEM) images of the biofilm (Figs. 8 and 9) revealed the porous and spongy structure with some cracks on the surface. Similar observations were previously reported by Fang et al. [6] and Ghangrekar & Shinde [7]. They suggested that the porous structure of granules with multiple cracks on the surface is likely to facilitate the passage of nutrients and substrates as well as the release of hydrogen, which had a very limited solubility of 1.58 mg/l in water, thus these granules did not exhibit a layered structure because of the simplicity of the acidification process.

**4. CONCLUSION**

This study demonstrated and evaluated the performance of an upflow dual-chambered mediator-less microbial fuel cell (UMFC) catalyzed with *Bacillus subtilis* consortium for simultaneous wastewater treatment and power generation. The biofilms which grow under anaerobic environment and attached to the surfaces of the anode were the main contributors to the electricity generation. The results demonstrated that the aerobic Gram-positive species *Bacillus subtilis* was able to grow anaerobically and produce a biofilm in
microbial fuel fell, which generated a long-term power output.

Fig. 8 SEM image for anode surface before granulation of biomass

Fig. 9 SEM image for anode surface after granulation of biomass

5. REFERENCES


