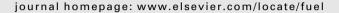


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Utilization of soak liquor in microbial fuel cell

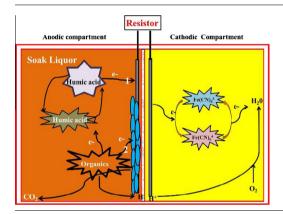


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HIGHLIGHTS

- The usefulness of soak liquor in microbial fuel cell was explored.
- No additional substrate and mediator was used in the study for energy production.
- The energy output was studied with simple graphite plates instead of expensive electrodes.

G R A P H I C A L A B S T R A C T



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ABSTRACT

Soak liquor is a primary effluent from tannery industry. It poses a threat to the environment and it is necessary to treat the effluent. The predominant tannery effluent bacteria were isolated, identified and used for the electricity generation. The present study investigates the use of soak liquor for the first time as a substrate for electricity generation in Microbial Fuel Cell (MFC). The high salinity and rich organic content of soak liquor increase the efficiency of MFC. Various electrochemical characterizations such as polarization curve, cyclic voltammetry, chronoamperometry, electrochemical impedance spectrometry were performed to analyse the efficacy of the soak liquor. MFC produced a maximum power density (P_{max}) of 44.02 mW/m² with a current density 140.34 mA/m². The chemical oxygen demand (COD) reduction rate was found to be 93% ± 4.7% in a cycle period of 168 h. The presence of humic acid was identified in soak liquor, which might be involved in shuttling of electrons from the microorganisms to the electrode.

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1. Introduction

MFC is one of the sustainable technologies to meet the energy requirements, in which organic matter can be converted to electricity using electroactive microorganisms [1-6]. The knowledge of current production by electrogenic microorganism was reported in early 1911 by Potter [7] however, interest and development came into this field in the recent years. MFC using pure compounds such as acetate [8], glucose [9,10], sucrose [11], amino acid (cysteine) [12] and bovine serum albumin [13] were well documented in the literature. It is also shown that a mixture of

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nutritional substrates can result in higher extractable current output when compared with their individual counterparts [14].

Power generation via MFC using wastewater was investigated by many investigators [1,13,15-17]. Zuo et al. [18,19] preferred the application of low-cost electrodes and mixed cultures to develop an environmentally sustainable process. The presence of ammoniacal nitrogen hinders the application of industrial waste in MFC [20]; subsequently, the power yield was also low when compared to pure substrates. There are varieties of factors that affect the overall performance of MFC, such as the type of organic matter, solution conductivity, alkalinity, pH, temperature and MFC architecture [21,22]. The internal resistance of a bioelectrochemical system (BES) is contributed by the ohmic resistance of the solution as well as from the polarization behaviour of anode and cathode [23]. The major contribution of internal resistance is favoured from the solution resistance [24]. Thus increasing the electrolyte conductivity will decrease the solution resistance. which increases the power density of MFC [1,21,25]. Hence, the anolyte plays one of the major role in performances of MFC. The presence of mediating compounds like humic acids is also found to enhance the performance of MFC [26]. Mediators can either be naturally present in the analyte or chemicals secreted by microbes or it can be added externally. A natural mediator reduces the cost of operation and it is non-toxic. Selecting an analyte with natural mediators or the electrogenic bacteria secreting mediators are essential for the successful MFC operation [21].

Soak liquor the effluent from tannery industry contains 2–4% of sodium chloride by weight and biodegradable substance such as flesh, blood, skin and other suspended particles [27]. The presence of high salt concentration and rich organics are the major concern with the effluent, which demands the need for treatment. According to the Central pollution control board of India about 3000-4000 L of soak liquor is generated per ton of skin. The existing treatment involves solar evaporation pans [28] and electrooxidation of soak liquor [27] which is a time-consuming and energy-intensive process. The high salt content and rich organics present in soak liquor were never used optimistically. In the present study, soak liquor was used as an anolyte in MFC for the first time. The organics present in soak liquor were converted to electrical energy, which was monitored and characterized by various electrochemical techniques. The organic content in the soak liquor was monitored initially and after MFC, on the basis of chemical oxygen demand (COD), protein and lipid removal efficiency to ensure the treatment of the effluent.

2. Materials and experimental techniques

2.1. Sample collection

Soak liquor was collected from leather processing division, Central Leather Research Institute – Council of Scientific and Industrial Research (CSIR) Chennai, Tamilnadu. The sample was kept under cold storage condition to avoid the degradation of organic constituents present in wastewater. Soak liquor was filtered using blotting paper to remove debris such as hair and sand, aseptic conditions were maintained throughout the experiment.

2.2. Characteristics of soak liquor

The pH of soak liquor was determined using OAKTON pH 700 series. Further, chemical oxygen demand (COD), the concentration of protein [29] and lipid [30] were measured using American Public Health Association (APHA) standard procedures [31]. The interference of chloride during COD measurement was overcome by adding 10/1 weight ratio of mercuric sulphate to chloride [32]. The

variation in the functional group was determined using FT-IR (Bruker Optik GmbH, model no – Tensor 27).

2.3. Bacterial isolation, 16S rRNA gene sequencing and phylogenetic analysis

The soak liquor effluent was enriched in nutrient broth and subsequently plated on nutrient agar plates and was incubated at 37 °C for 24-48 h. The morphologically dissimilar well-isolated colonies were selected randomly, further streaked on nutrient agar plates and purified. The pure cultures were maintained on nutrient agar slants at 4 °C to keep the microbial strain viable. For further investigations, the cultures were grown overnight in nutrient broth at 37 °C. The cultures were centrifuged at 5000g for 15 min. The bacterial pellets were used for the genomic DNA extraction. Genomic DNA extracted according to the method described by Marmur [33] and the small subunit rRNA gene was amplified using universal primers 16S1 (5'-GAGTTTGATCCTGGCTCA-3') & 16S2 (5'-CGG CTACCTTGTTACGACTT-3'). The PCR products were visualized on 1.5% agarose gel and gel purified using QIA quick gel extraction kit (Qiagen, Germany). The purified PCR product, approximately 1.5 kb in length, was sequenced using five forward and one reverse primer as described in our earlier paper [34]. The deduced sequence was subjected to blast search for the closest match in the Genbank. The 16 s rRNA gene sequence of bacteria were submitted in Genbank. The phylogenetic analysis was carried out using the neighbor-joining (NJ) method. The biofilm formed by the isolated bacterial strains on the anode surface was analysed by Scanning Emission Microscope (SEM). A small portion of the anode from the MFC was cut and immersed in distilled water to remove the physical adsorptions, then fixed immediately using 2.5% of glutaraldehyde in 0.1 M phosphate buffer for 8 h at 4 °C. The anode was rinsed with deionized water and dehydrated by rinsing in increasing concentration of ethanol (20%, 40%, 60%, 80%, and 100%) for about 1 min in each concentration. The dried samples were gold sputtered and analysed using SEM (TESCAN).

2.4. MFC construction and operation

The experimental setup of MFC used in this study is shown in Fig. 1. A dual chamber MFC separated by Nafion 117 membrane (DuPont) was constructed. Each chamber was made by Perspex sheet of 10 mm thickness with a dimension of $6.5 \times 5 \times 10 \text{ cm}^3$ with the total volume capacity of 350 mL. The Nafion 117 membrane of dimension 7.5×5 cm was exposed to the electrolyte in MFC. The working volume of MFC in the experiment was 300 mL. A head space of 2.5 cm was maintained throughout the experiment. The chambers were provided with sample ports, inlet and outlet ports, and wire point inputs at the top. Graphite was used as the anode and cathode of dimension $7 \times 3 \times 0.2$ cm³. The electrodes were soaked in deionized water for 24 h before use [35]. The anolyte was soak liquor and the catholyte was 0.05 M K₄ [Fe (CN)₆] in phosphate buffer. The anode chamber was agitated at 150 rpm using a magnetic stirrer for proper mixing of organics. The MFC was operated until a decline in voltage was observed. Four cycles were carried out to check the reproducibility of the results. After each cycle, the supernatant was replaced with the fresh feed, along with the leftover sludge to sustain the microflora [19]. The anode chamber was maintained under anaerobic condition during MFC operation.

2.5. Analysis and calculations

The voltage difference between the anode and the cathode was measured across the fixed external resistance of 1000 Ω [36] for every 10 min by using Hewlett-Packard bench link 16 channel data

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