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Bioelectricity generation from coconut husk retting wastewater in fed batch operating microbial fuel cell by phenol degrading microorganism

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ABSTRACT

Dual chamber microbial fuel cell (MFC) operated at fed batch mode for the treatment of retting wastewater has potently achieved both current generation and phenol removal. Hydraulic retention time (HRT) of the reactor was varied from 40 days to 10 days. COD (chemical oxygen demand) removal was 91% at 40 days HRT, with an initial COD concentration of 530 ± 50 g m^{-3}. Retting wastewater with an initial phenol concentration of 320 ± 60 g m^{-3} procured a highest phenol removal of 93% at 40 days HRT of the microbial fuel cell. Maximum power density of 362 mW m^{-2} was achieved using retting wastewater at HRT of 20 days with an internal resistance of 150 Ω in a dual chambered MFC. The bacterial strains in anode region, reported to be responsible for potential phenol removal, were identified as *Ochrobactrum* sp. RA1 (KJ408266), *Ochrobactrum* sp. RA2 (KJ408267) and *Pesudomonas aeruginosa* RA3 (KJ408268) using phylogenetic analysis. The study reveals that, dual chambered MFC effectively removed the phenol from retting wastewater along with power generation.

1. Introduction

Microbial fuel cells (MFCs) are bio electrochemical system offering an integrated approach for energy recovery and wastewater treatment [1,2]. Numerous studies have been carried out in the past 10 years, in the aspect of energy generation from wastewater employing MFC [3–7].

Open retting involves immersion of coconut husks in pond for a period ranging from 6 to 12 months, later the ret water is released from the pond and the retted coconut husk material is used for production of coir fibre. Coconut husk retting effluent containing recalcitrant components like phenol when discharged in pond or river, results in deterioration of water quality and affects the biodiversity of flora and fauna. Carbon-dioxide, salinity and pH of the pond water showed an increasing trend with the mixing of retting effluent. Therefore the phenol removal from retting effluent is more important before their release into receiving water bodies. In the coastal regions of Southern India, the retting wastewater is released

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without treatment into the environment [8]. In comparison with physicochemical methods, the biodegradation methods of phenol reduction are preferred, because of complete mineralization [9]. Nowadays MFC is gaining tremendous importance in the implication of wastewater treatment due to its ability to treat recalcitrant compounds.

The aim of the present study is to (i) evaluate phenol removal efficiency, electricity generation and COD (chemical oxygen demand) consumption rate at different HRT (hydraulic retention time) (days) of the dual chamber MFC in the treatment of retting wastewater and (ii) identification of micro organisms responsible for phenol removal through 16s rRNA sequencing.

2. Materials and methods

2.1. Initial characterisation of retting wastewater

The retting wastewater was collected from colachal (8.15°N 77.14°E), Kanyakumari district, Tamilnadu, India and stored at 4 °C. The physicochemical characterization of wastewater such as total solids, total suspended solids (TSS), total dissolved solids (TDS), phenol, chloride, TCOD (total chemical oxygen demand) and SCOD (soluble chemical oxygen demand) were analysed based on Standard methods detailed in APHA [10] (Table 1).

2.2. Microbial fuel cell setup, operation and analysis

Dual chamber MFC comprised of anode and cathode compartments with working volume of 500 mL. Anode chamber ($L = 15$ cm, $d = 8$ cm) had three ports: inlet port, outlet port for samples and port for electrode. Anode chamber was sparged with nitrogen gas to ensure strict anaerobic conditions. Municipal sewage sludge taken from secondary clarifier was used as inoculums. Retting wastewater was taken in the anode chamber as feed. The wastewater was fed through the injection port of the anode compartment using a peristaltic pump (Watson–Marlow, Falmouth, Cornwall, UK). A 2 mol m^{-3} bromo ethane sulfonate (BES) was added in anode chamber to prevent the growth of methanogenic bacteria.

Table 1 – Initial characteristics of retting wastewater (values represent triplicate measurements of the wastewater sample).

Serial no.	Parameter	Values
1	pH	6.4 ± 0.2
2	Total Chemical Oxygen Demand (TCOD)	530 ± 50 (g m^{-3})
3	Soluble Chemical Oxygen Demand (SCOD)	370 ± 20 (g m^{-3})
4	Chloride	734 ± 50 (g m^{-3})
5	Phenol	320 ± 60 (g m^{-3})
6	Total Solids	3150 ± 200 (g m^{-3})
7	Total dissolved solids	2800 ± 124 (g m^{-3})
8	Total suspended solids	330 ± 50 (g m^{-3})
9	Total Nitrogen	17 ± 5 (g m^{-3})
10	Total Phosphorus	7.5 ± 2.5 (g m^{-3})

Cathode chamber ($L = 15$ cm, $d = 8$ cm) was provided with two ports, one for aeration at the top of the chamber and other for electrode. The cathode chamber filled with distilled water was continuously aerated to provide dissolved oxygen (DO) (6 g m^{-3}) at the cathode. Proton exchange membrane (Nafion – 5 × 5 cm) was placed between anode and cathode chamber. Uncoated graphite sheet with dimension of 12.5 × 7 cm was used as both anode and cathode electrode. The two graphite electrodes were spaced at a distance of 5 cm and were connected via copper wire to an external circuit containing a single resistor. All experiments were performed in triplicates at 26 ± 2 °C and compared with control.

HRT of the retting wastewater in anode chamber was varied from 40 days, 30 days, 25 days, 20 days, 15 days and 10 days. The OLR as the COD was varied from 14 mg d^{-1}, 18 mg d^{-1}, 22 mg d^{-1}, 28 mg d^{-1}, 37 mg d^{-1} and 56 mg d^{-1}(flow rate – 12.5 cm^3–50 cm^3). SS (suspended solids), pH, phenol and COD removal efficiency was determined for every HRT maintained in the MFC for the treatment of retting wastewater.

2.3. Current production and voltage measurement

Polarization curve for the dual chambered MFC was calculated by varying external resistance (50–5000 Ω). Power density (m Wm^{-2}) and current density (m Am^{-2}) were calculated based on total anode surface area. The coulombic efficiency (CE) of the fed batch MFC treating retting wastewater was calculated as stated by Logan [11].

2.4. Isolation of phenol degrading organism from anode biofilm

The whole biofilm attached to anode was scrapped into mineral salt medium (MSM). The mineral salt medium contained KH_2PO_4 – 1.7 g, K_2HPO_4 – 4.35 g, $MgSO_4$ – 0.2 g, NH_4Cl – 2.1 g, $MnSO_4$ – 0.05 g, $FeSO_4.H_2O$ – 0.01 g, $CaCl_2.H_2O$ – 0.03 g, Agar – 20 g dissolved in 1 L distilled water and autoclaved. The bacterial strains were grown in MSM with 400 g m^{-3} of phenol as sole carbon source. DNA from bacterial strains in the medium was extracted.

2.5. DNA isolation and polymerase chain reaction (PCR)

DNA was extracted using Qiagen DNA isolation kit and PCR was performed. The primers used were 27F and 1492R. The PCR conditions were: initial denaturation at 95 °C for 5 min, followed by 32 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s and extension at 72 °C for 1 min and final extension at 72 °C for 10 min. After successful amplification, the PCR products were taken for a second PCR utilizing the 968 F primer with a GC clamp and the 1492 R primers.

2.6. Denaturing gradient gel electrophoresis (DGGE)

DGGE was conducted using the CBS DGGE Scientific system. An 8% polyacrylamide gel with 30–60% gradient of denaturant (7M urea and 40% formamide) and 10–15 μL of PCR products were loaded into the wells [12]. Electrophoresis was carried out at 60 V in 1× TAE buffer for 16 h and bands were visualized