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Research article

Treatment of seafood processing wastewater using upflow microbial fuel cell for power generation and identification of bacterial community in anodic biofilm

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ABSTRACT

Tubular upflow microbial fuel cell (MFC) utilizing sea food processing wastewater was evaluated for wastewater treatment efficiency and power generation. At an organic loading rate (OLR) of 0.6 g d^{-1}, the MFC accomplished total and soluble chemical oxygen demand (COD) removal of 83 and 95%, respectively. A maximum power density of 105 mW m^{-2} (2.21 W m^{-3}) was achieved at an OLR of 2.57 g d^{-1}. The predominant bacterial communities of anode biofilm were identified as RB1A (LC035455), RB1B (LC035456), RB1C (LC035457) and RB1E (LC035458). All the four strains belonged to genera *Stenotrophomonas*. The results of the study reaffirms that the seafood processing wastewater can be treated in an upflow MFC for simultaneous power generation and wastewater treatment.

1. Introduction

The contamination of water bodies with organic materials is a major environmental crisis throughout the world. Strengthened environmental regulations have accelerated the development of wastewater treatment technologies which are directed to recover valuable products and energy, while concurrently achieving the objective of pollution control. One of the revived bio-electrochemical concept and promising technology that address all these aspects is microbial fuel cell (MFC). Microbial fuel cells (MFC) are bio – electrochemical reactors in which microorganisms mediate the direct conversion of chemical energy in organic substrates to electrical energy and it has gained tremendous importance due to its simultaneous application in wastewater treatment with reduced sludge generation and energy production (Logan and Regan, 2006; Huang and Logan, 2008; Kim et al., 2008; Choi and Ahn, 2013). Many studies concerning the application of real wastewater such as the swine wastewater (Min et al., 2005), domestic wastewater (Choi and Ahn, 2013; Min and Logan, 2004), chemical wastewater (Mohan et al., 2009), rice mill wastewater (Behera et al., 2010), and refinery wastewater (Zhang et al., 2014) have been reported previously in MFC for electricity generation.

Sea food processing industry is involved in the processing and packaging of varieties of fishes, shrimp, crabs and squids. Organic content of this sea food processing wastewater is relatively high due to contamination with blood, fish heads, intestinal remains and flesh pieces. The effluent quality of the seafood processing industry greatly depends on the type of fish being processed and type of processing undertaken. Pollution generated from the processing of oily fish species is much higher than white fish species (UNEP, 2000). The release of this wastewater without treatment into water resources leads to eutrophication and coastal pollution. The deterioration of the organic compounds in the wastewater leads to oxygen depletion and generates obnoxious odour (Scott and Hui, 2004).

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Previously two studies has been concerned in the treatment of the seafood processing wastewater, one is MFC with modified anoxic/oxic architecture (You et al., 2010) and another is up-flow bio-filter circuit (UBFC) without membrane (Sukkasem and Laehlah, 2013). The later study investigated the treatment of the seafood process wastewater at specific organic loading rate (30 g d^{-1}) with carbon fibre brush as anode. In this study upflow MFC was evaluated in terms of power generation and organics removal from seafood processing wastewater at different organic loading rate for a total period of 205 days and in addition the micro-organisms developed in the anode during MFC operation was also analysed. Activated carbon fibre felt (ACFF) was decided to be used as electrode material in the upflow MFC. In general ACFF is widely applied in wastewater treatment (Yi and Chen, 2007; Yi et al., 2008) due to high specific surface area that favours dense bio film formation, high adsorption capability, better electrical and catalytic properties (Fan et al., 2008).

The aim of this study were to i) investigate the effect of organic loading rate (OLR) on the performance of upflow MFC operated on sea food processing wastewater, ii) determine the influence of phosphate buffer as catholyte in current generation and iii) identify the microbial communities in anode biofilm of the MFC through 16s rRNA sequencing.

2. Materials and methods

2.1. Wastewater collection and characterization

The wastewater was collected from the seafood processing industry located at Tuticorin district (8.81°N 89.14°E), Tamil Nadu, India and stored at 4 °C until further use. The initial characteristics of the wastewater such as pH, total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), alkalinity, chloride, soluble chemical oxygen demand (SCOD) and total chemical oxygen demand (TCOD) were determined as described in Standard Methods (APHA, 2005) (Table 1). The seafood processing effluent was predominantly enriched with blood rather than oils or any other suspended materials. The salinity of the wastewater was 209 g m^{-3}.

2.2. Microbial fuel cell setup and operation

Tubular upflow microbial fuel cell consists of an anode and cathode chamber with dimensions (L = 19 cm, b = 15 cm, d = 6 cm) as described earlier by He et al. (2005) with some modifications. Anode and cathode chambers had a working volume of 450 cm^3 and 500 cm^3, respectively. Activated carbon fibre felt (ACFF) served as both anode and cathode electrode material (Fig. 1). The projected surface area of activated carbon fibre felt (ACFF) was 180 cm^2. The anode and cathode electrode (ACFF) was placed at a distance of 2 cm separated by proton exchange membrane (PEM) (Per fluorinated membrane – Nafion 5 × 5 cm). The cathode chamber filled with distilled water was continuously aerated to provide dissolved oxygen (DO) (6 g m^{-3}) at the cathode.

Wastewater had a TCOD concentration in the range of 4000 ± 200 g $m^{-3}$and therefore it was diluted with distilled water, to achieve a final TCOD concentration in the range of 700 ± 50 g m^{-3} before it is being fed into the anode chamber as feed. The inoculum used in this study was a mixture of sludge from pre-acclimatized MFC that has been operated for 6 months (Jayashree et al., 2014) and waste activated sludge from secondary clarifier of municipal wastewater treatment plant. A 2 mol m^{-3} of BES (2 – bromoethane sulfonate) was added to the anode chamber to selectively inhibit the growth of methanogenic organism.

OLR as the COD of the seafood processing wastewater used in the study was 0.6 g d^{-1}, 0.81 g d^{-1}, 1.2 g d^{-1}, 1.56 g d^{-1}, 1.8 g d^{-1}, 2.57 g d^{-1} and 4.5 g d^{-1}. Correspondingly, the hydraulic retention time (HRT) in the anode chamber of MFC varied from 30 h, 22 h, 15 h, 11.5 h, 10 h, 7 h and 4 h. After upflow MFC achieved stable performance it was operated at every OLR for duration of 30 days and the pH, TSS, TCOD and SCOD removal from the sea food processing wastewater were evaluated periodically. The experiments were conducted in duplicates at room temperature of 27 ± 2 °C and compared with control.

Table 1
Initial characteristics of seafood processing wastewater.

Parameter	Value
Total solids (TS)	12.4[a] ± 0.12 (Kgm^{-3})[b]
Total dissolved solids (TDS)	5512 ± 67 (gm^{-3})
Total suspended solids (TSS)	6962 ± 139 (gm^{-3})
Total chemical oxygen demand (TCOD)	4000 ± 120 (gm^{-3})
Soluble chemical oxygen demand (SCOD)	2700 ± 60 (gm^{-3})
Chloride	432 ± 50 (gm^{-3})
Alkalinity	205 ± 23 (gm^{-3})
pH	9

[a] Values represent average of 3 samples except pH.
[b] Values in parentheses represent standard deviation.

2.3. Electrochemical measurements

Power density (mW m^{-2}) and current density (mA m^{-2}) were calculated according to the projected activated carbon fibre felt (ACFF) surface area. Polarization curve were plotted using set of variable external resistances (11200 – 20 Ω) (Logan, 2008).

2.4. Coulombic efficiency

Coulombic efficiency was calculated using the following equation, CE = I·delta(t)/(F·n·W/M), Where I is the current (A), F is the Faraday number (C/mol), n is the number of electrons per mol of COD (e-/mol), M is the molecular weight of wastewater (g), W is the weight of CODs removed per day (g) and delta(t) is the time interval (e.g. for daily removal it is 86400 s).

2.5. Phylogenetic analysis

2.5.1. DNA isolation and polymerase chain reaction (PCR)

The genomic DNA extraction was performed as described in Qiagen manufacturers protocol. After 205 days of continuous operation, the anode biofilm was scrapped from the anode and suspended in 2 cm^3 of sterile distilled water before DNA extraction. The sample was then centrifuged at 12,000 rpm for 10 min. The DNA pellet was washed with chilled 70% ethanol by centrifuging for 10 min at 10,000 rpm. The mixture was resuspended in 30 μL of sterile Tris-EDTA buffer. The extracted genomic DNA was used as template for nested PCR. Nested PCR involves two PCR reactions. The primers used in first PCR are 27F, 1492R and in second PCR, the forward primer was replaced by 968F GC. The PCR conditions were: initial denaturation at 95 °C for 10 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 1 min followed by final extension at 72 °C for 20 min. The first PCR product was used as template for second PCR and program is same but annealing is at 50 °C for 30 s and extension at 72 °C for 50 s.

2.5.2. Denaturing gradient gel electrophoresis (DGGE)

A 40% acrylamide gel with 30%–60% of denaturant was used. Electrophoresis was carried out at 70 V for 14 h and bands were visualized by silver staining. The bands were eluted and amplified using second PCR protocol for conformation. DNA sequencing was performed by Chromous Biotech (Bangalore, India). The 16s rRNA