



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.

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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 ($8.81^{\circ}\text{N } 89.14^{\circ}\text{E}$), 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 ($L = 19 \text{ cm}$, $b = 15 \text{ cm}$, $d = 6 \text{ cm}$) 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 $5 \times 5 \text{ cm}$). 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 $4000 \pm 200 \text{ g m}^{-3}$ and therefore it was diluted with distilled water, to achieve a final TCOD concentration in the range of $700 \pm 50 \text{ g m}^{-3}$ 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 $27 \pm 2^{\circ}\text{C}$ and compared with control.

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 ($11200 - 20 \Omega$) (Logan, 2008).

2.4. Coulombic efficiency

Coulombic efficiency was calculated using the following equation, $CE = I \cdot \Delta t / (F \cdot n \cdot W / M)$, 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 Δt 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 $30 \mu\text{L}$ 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

Table 1
Initial characteristics of seafood processing wastewater.

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

^a Values represent average of 3 samples except pH.

^b Values in parentheses represent standard deviation.

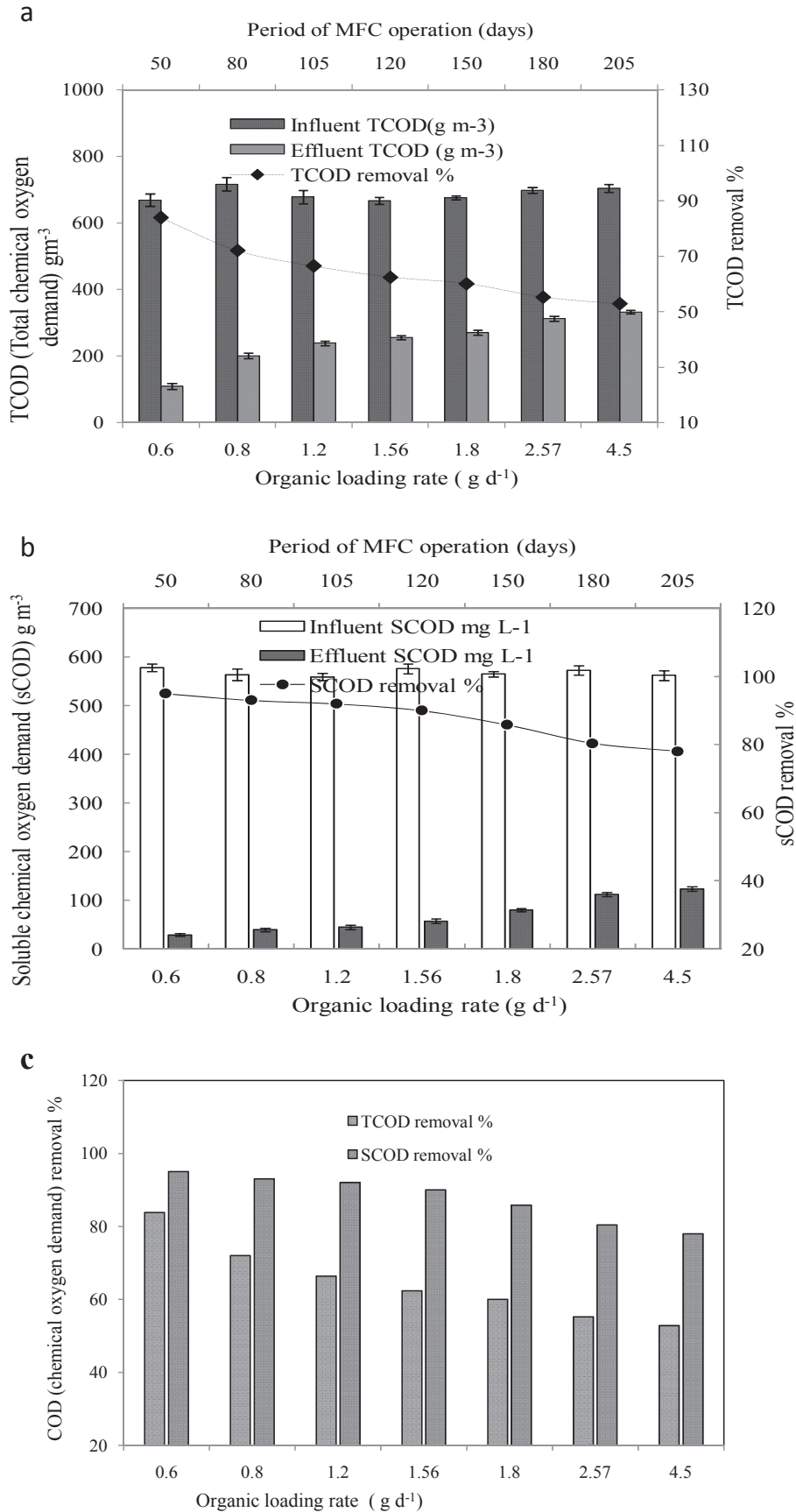


Fig. 2. Organic removal efficiency at different OLR. TCOD removal efficiency of the MFC at different OLR (a), SCOD removal efficiency of the MFC at different OLR (b), and comparison of TCOD and SCOD removal efficiency (c).

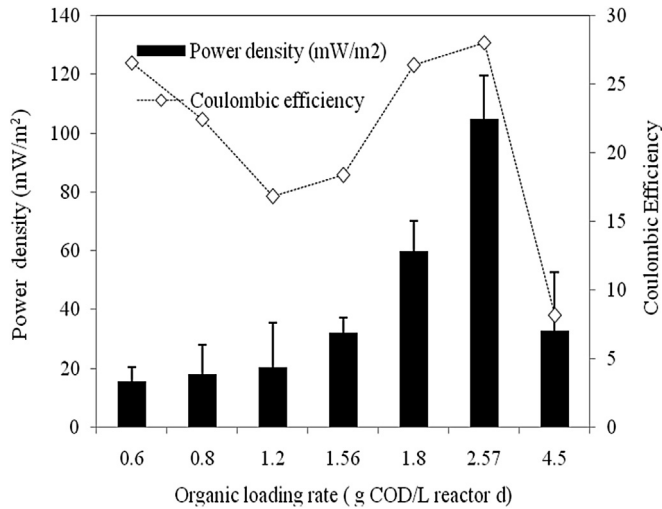


Fig. 3. Power density and coulombic efficiency of the MFC at different OLR.

Polarization curves were plotted for each OLR by connecting series of external resistances for a period of 1 h (Figs. 4 and 5). Loading rate of the wastewater plays a crucial role in power generation of the MFC (Behera and Ghangrekar, 2009). The inoculum that have been obtained from pre acclimatized MFC would have resulted in rapid acclimatization and power generation from the wastewater. In case of anaerobic sludge used as inoculum (mixed culture), there are electrophiles as well as natural mediators which contribute for higher power densities (Du et al., 2007). Further increase in OLR to 4.5 g d⁻¹ lead to significant reduction in power density to 32 mW m⁻² (0.56 W m⁻³). The VSS/SS ratio was maintained constantly in the range of 80% at OLR ranging from 0.6 g d⁻¹ to 4.5 g d⁻¹. This emphasizes that the organic loading of the seafood processing wastewater was beyond the oxidation ability of electrogenic micro-organisms present in the anode biofilm and the substrates seem to be utilized by the other anaerobic micro-organisms in the anode chamber (Behera and Ghangrekar, 2009).

Earlier, MFC with modified anoxic/oxic architecture (A/O–MFC) used in the treatment of the saline seafood wastewater obtained a

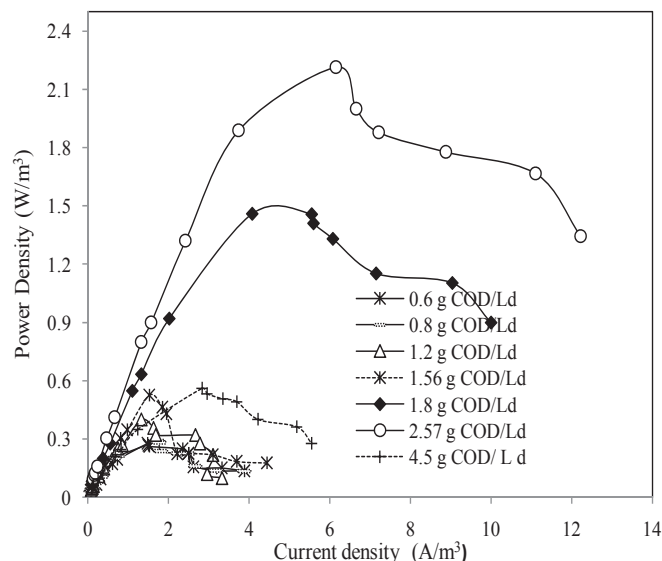


Fig. 4. Polarization curve (Power density Vs Current density).

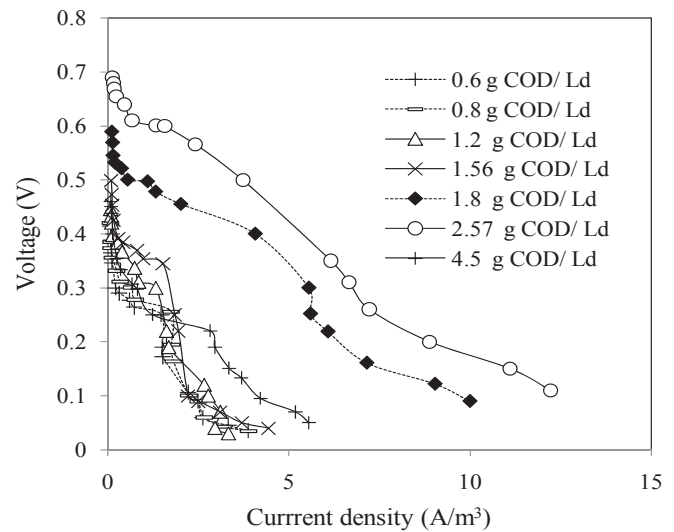


Fig. 5. Polarization curve (Voltage Vs Current density).

maximum power density of 16.2 W m⁻³ at HRT of 4.2 h and in addition FISH analysis revealed the presence of nitrifying microbes at the cathode (You et al., 2010). Sukkasem and Laehlah (2013), investigated the treatment of seafood wastewater at OLR of 30 g d⁻¹ using upflow bio-filter circuit (UBFC) achieved a COD removal efficiency of 94.37% and maximum power density of 9.47 mW m⁻³. Single chamber MFC (SCMFC) utilizing seafood processing wastewater, reported a power density of 390 mW m⁻² (Sun, 2012). This study employing treatment of the seafood processing wastewater using upflow MFC achieved a maximum power density of 105 mW m⁻². The variation in the power densities achieved among the studies utilizing similar kind of wastewater is mainly contributed by the difference in substrate concentration (soluble or particulate) of the wastewater, complexity of the microbial consortium in the anode biofilm (Rabaey and Verstraete, 2005), salinity of the wastewater and reactor configuration (single chamber MFC or upflow MFC) as it can alter the internal resistance (Zhang et al., 2014).

At the initial loading of 0.6 g d⁻¹, the highest internal resistance of 603 Ω was recorded. Internal resistance of 252 Ω at OLR of 2.57 g d⁻¹ was the least resistance recorded in comparison with other loading rate of the seafood processing wastewater. The internal resistance of MFC decreased with high loading rate due to increased catalytic activity of anodophilic microorganism and higher volatile fatty acid (VFA) concentration that lead to enhancement in the ionic strength of the anode (Martin et al., 2010). Use of appropriate electrodes like ACFF with high surface area to favour biofilm formation and standardized reactor configuration are obligatory to obtain ample amount of energy from the wastewater.

3.3. Influence of phosphate buffer as catholyte in power generation

The internal resistance of the MFC should be reduced to derive maximum power generation. High salinity in anode chamber of MFC results in increased conductivity of the system which leads to more power generation. Since the salinity of the seafood processing wastewater utilized in this study was normal, it was decided to enhance the performance of MFC through the addition of salts (Gil et al., 2003) or using higher buffer concentrations (Fan et al., 2007) which reduces the ohmic resistance. MFC performance is influenced by the buffer owing to its chemical composition and

interaction with the membrane, electrodes and bacteria (Nam et al., 2010a,b). The OLR of seafood processing wastewater at anode chamber was maintained at 2.57 g d^{-1} , since maximum power generation occurred at this OLR and concentration of the phosphate buffer was increased gradually from 50 mol m^{-3} , 100 mol m^{-3} and 150 mol m^{-3} in the cathode chamber. The tubular upflow MFC was operated for a total of 120 days containing phosphate buffer as catholyte. Each mol m^{-3} phosphate concentration was run for 30 days. For every 50 mol m^{-3} increase in the phosphate buffer concentration, an enhancement of $35 \pm 10 \text{ mW m}^{-2}$ in power generation was achieved. The power density of the MFC utilizing seafood processing wastewater with 50 mol m^{-3} and 100 mol m^{-3} of phosphate buffer as catholyte was 137 mW m^{-2} (5.49 W m^{-3}) and 179 mW m^{-2} (7.16 W m^{-3}), respectively. At 150 mol m^{-3} concentration of phosphate buffer, the highest power density of 222 mW m^{-2} (8.86 W m^{-3}) was achieved and the internal resistance of the upflow MFC was reduced to 200Ω . The increase of phosphate concentration can lead to increase in proton transfer rate of MFC which simultaneously lowers the internal resistance and results in enhanced power generation (Gil et al., 2003; Fan et al., 2007).

The power density of 2011 mW m^{-2} was achieved in a cube reactor MFC with 200 mM phosphate buffer and acetate as the substrate (Yu et al., 2007). Feng et al. (2008) obtained an increased power production of 136%, after the addition of phosphate buffer (50 mol m^{-3}) into the brewery wastewater. In this study, the added advantage is that the salinity of the seafood processing wastewater used in MFC was within the normal range (209 g m^{-3}) and the phosphate buffer added to increase the conductivity of the system was employed only as catholyte and it was not added into the anode chamber. Although phosphate buffer improves power generation when employed in the treatment of real wastewater, it is not a suitable option for long term operation of MFC since it has to be periodically replenished and also forms precipitation in the electrode and membrane. For further development and commercialization of MFC the alternative would be open to air cathode.

3.4. Coulombic efficiency and cyclic voltammetry analysis of the MFC

Coulombic efficiency (CE) of 26.56% was obtained at the loading rate of 0.6 g d^{-1} . CE of 28.03% and 8.13% was observed at an OLR of 2.57 g d^{-1} and 4.5 g d^{-1} , respectively in the treatment of seafood processing wastewater in the upflow MFC (Fig. 3). Competition between exo electrogenic micro-organisms and other types of bacteria with respect to anode surface at high OLR can lead to decrease in coulombic efficiency (Lorenzo et al., 2010). Similar type of decrease in CE with the increase in OLR of the wastewater was reported in fermented wastewater treatment (Nam et al., 2010a,b). In addition, coulombic efficiency of the system can be decreased by diffusion of oxygen into the anode chamber through PEM (Min and Logan, 2004). Real time application of MFC is feasible only after considerable reduction of internal resistance with simultaneous increase in coulombic efficiency.

3.5. Effect of OLR on suspended solids removal

The suspended solids concentration of the seafood processing wastewater was diluted to the range of $1200 \pm 200 \text{ g m}^{-3}$ before it was fed to the MFC. The removal of suspended solids from the seafood processing wastewater varied in the range of 45%–55% at various OLR (Fig. 6). Initially, there was an enhancement in SS removal (%) as the loading rate of the seafood processing wastewater in the upflow MFC was increased. At the highest applied OLR, SS removal might be attributed to the accumulation of the

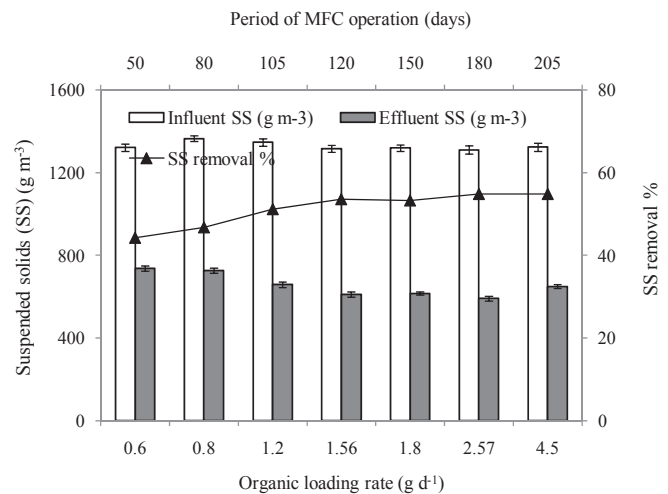


Fig. 6. SS removal efficiency of the MFC at different OLR of the seafood processing wastewater.

fermentation products, such as VFA (volatile fatty acids) which reduces the pH in the anode chamber and thereby inhibiting the anodophilic activity.

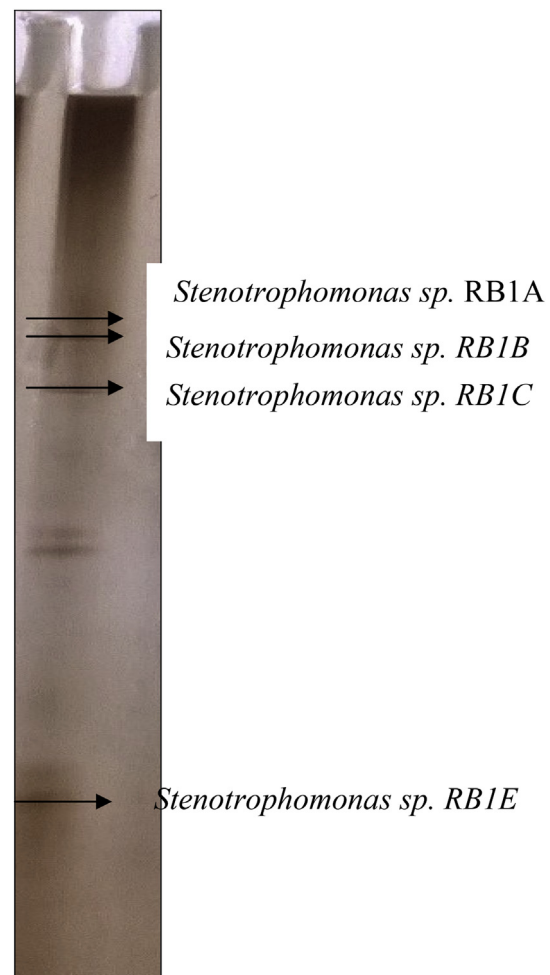


Fig. 7. DGGE profile of the micro-organisms isolated from the anode biofilm of MFC treating seafood processing wastewater.

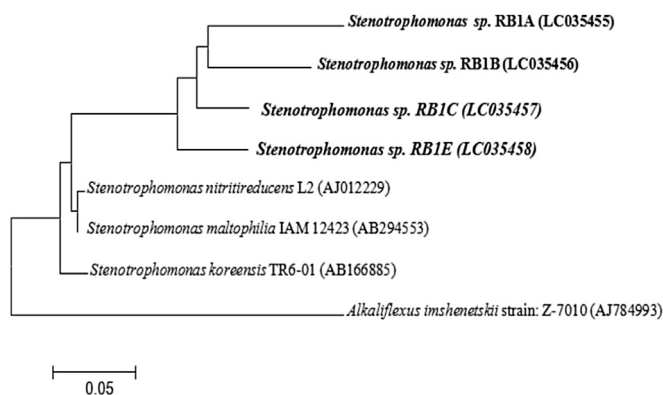


Fig. 8. Phylogenetic tree based on 16S rRNA sequences from *Stenotrophomonas* sp. RB1A (LC035455), *Stenotrophomonas* sp. RB1B (LC035456), *Stenotrophomonas* sp. RB1C (LC035457) and *Stenotrophomonas* sp. RB1E (LC035458).

3.6. Identification of microflora from anode biofilm through 16S rRNA sequencing

After 205 days of treatment of sea food processing wastewater using upflow MFC, the anode biofilm was scrapped along with the suspension in the anode chamber, identified using DGGE and 16S rRNA sequencing. The DGGE analysis revealed four bands and the strains were identified as *Stenotrophomonas* sp. RB1A (LC035455), *Stenotrophomonas* sp. RB1B (LC035456), *Stenotrophomonas* sp. RB1C (LC035457) and *Stenotrophomonas* sp. RB1E (LC035458) (Figs. 7 and 8). Most of the exoelectrogenic or anode respiring bacteria corresponds to five classes of Proteobacteria (Alpha, Beta, Delta, Epsilon and Gamma), as well as to the phylum Acidobacteria, Bacteroidetes, and Firmicutes (Zhi et al., 2014). All the isolated strains in this study belonged to single genus *Stenotrophomonas* which is classified under γ – Proteobacteria. The maximum potential of 689 mV was achieved in the treatment of the seafood processing wastewater in upflow MFC using a consortium of micro – organism belonging to *Stenotrophomonas* and in addition, the power generation was continuous for 205 days. *Stenotrophomonas* sp. is gram negative, non fermentative, exo – electrogenic and denitrifying bacteria. *Stenotrophomonas* sp. isolated in this study exhibits highest similarity with that of *Stenotrophomonas maltophilia* (Fig. 8). *Stenotrophomonas maltophilia* are gram negative, opportunistic pathogen that are capable of biofilm formation and are found to co exist with *Pseudomonas aeruginosa* (Brooke, 2012). Han et al. (2011) has isolated *Stenotrophomonas maltophilia* NIU- X2 in his study using single chamber MFC for dye decolorization using activated sludge from wine bearing wastewater treatment and reported them as one of the predominant electrochemically active organisms. Arulazhagan and Vasudevan (2009) reported that the *Stenotrophomonas maltophilia* present in the halotolerant consortium was able to degrade different hydrocarbons under saline condition. *Stenotrophomonas* sp. has also been isolated in a study using MFC for power generation from dye industrial effluent soil (Raj et al., 2013). *Stenotrophomonas* sp. is a filamentous bacterium capable of forming biofilm on the anode, which forms the basis of mediator-less EET. Bacterial biofilms are rich in complex extracellular proteins, exopolysaccharides and uronic acids. The matrix polymer enables bacteria to form biofilms on the anode, thereby facilitating electron transport. The entire biofilm acts like a living electrode and complex ecosystem, where bacteria are living within a self-generated matrix that conducts the electrons (Liu et al., 2010). Single chamber air cathode MFC containing single strain of *Stenotrophomonas* sp. depicted almost complete decolorization of diazoic dye reactive black 5 (RB5) and simultaneous electricity

production (Galai et al., 2015).

In majority of the MFC studies concerned with wastewater treatment, 16S rRNA sequencing analysis of anodic biofilm exhibits exo electrogenic micro – organisms belonging to gram negative, opportunistic pathogen and γ – Proteobacteria (Wesley et al., 2011). However in this study, electrochemical activity of single bacterium *Stenotrophomonas* sp. played an imperative role in the power production from the treatment of the seafood processing wastewater, rather than the synergistic interaction among different microbial consortium. Further research is needed to elucidate the selective dominance of single genera of anodic micro- organism contributing for power generation.

4. Conclusion

The Upflow MFC operated with seafood processing wastewater was able to generate power continuously for almost 205 days. It achieved a maximum power density of 105 mW m^{-2} (2.21 W m^{-3}) at an OLR of 2.75 g d^{-1} .

- 1) The high TCOD and SCOD removal, accompanied with power generation using MFC offers an efficient alternative for treatment of seafood processing wastewater.
- 2) The highest power density of 222 mW m^{-2} was obtained using phosphate buffer as catholyte.
- 3) The predominant micro floras in the anode biofilm of MFC belonged to genus *Stenotrophomonas*.

This study demonstrated that the upflow MFC technology was able to treat seafood processing wastewater successfully, and the bacterial strains found in the anode biofilm are responsible for power generation and organics removal.

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