Bioremediation of steel plant wastewater and enhanced electricity generation in microbial desalination cell
Omkar A. Shinde, Ankita Bansal, Angela Banerjee and Supriya Sarkar

ABSTRACT
Microbial desalination cell (MDC) is a propitious technology towards water desalination by utilizing wastewater as an energy source. In this study, a multi-chambered MDC was used to bioremediate steel plant wastewater using the same wastewater as a fuel for anodic bacteria. A pure culture of *Pseudomonas putida* MTCC 1194 was isolated and inoculated to remove toxic phenol. Three different inoculum conditions, namely *P. putida* (INC-A), a mixture of *P. putida* and activated sludge (INC-B), and activated sludge alone (INC-C) were employed in an anodic chamber to mainly compare the electricity generation and phenol degradation in MDCs. The study revealed the maximum phenol removal of 82 ± 2.4%, total dissolved solids (TDS) removal of 68 ± 1.5%, and power generation of 10.2 mW/m² using INC-B. The synergistic interactions between microorganisms, can enhance the toxic phenol degradation and also electricity generation in MDC for onsite wastewater application.

INTRODUCTION
In India, the steel manufacturing process produces a huge amount of wastewater which contains several toxic organic and inorganic pollutants, such as phenol, cresol, cyanide, chloride, fluoride, calcium, magnesium and sodium. Phenol has been considered as a serious pollutant due to its toxic effect on human health (Weidemann et al. 2016). Phenol concentration >400 mg/L can adversely affect the effluent’s physical parameters such as colour and chemical parameters like chemical oxygen demand (COD) and nutrients (nitrogen and phosphorus) (Luo et al. 2009; Song et al. 2014). Several electrochemical methods have been explored to remove phenol from the industrial discharges. However, these methods being expensive and harmful to the environment has motivated researchers to search other treatment methods such as bioremediation (El-Sheekh & Mahmoud 2011). Several electrochemical methods have been explored to remove phenol from the industrial discharges. However, these methods being expensive and harmful to the environment has motivated researchers to search other treatment methods such as bioremediation (El-Sheekh & Mahmoud 2011). In bioremediation, microbes like, *Cupriavidus basilensis* and *Pseudomonas putida* have been used successfully to degrade toxic phenol to less toxic end products (Gonzalez et al. 2000; Friman et al. 2013).

Along with phenol, the dissolved inorganic matters (Ca²⁺, Mg²⁺, Na⁺, Cl⁻, SO₄²⁻) persist in the steel plant. These inorganic matters tend to develop scaling on wastewater carrier pipes which increases by recycling of the process wastewater. Generally, removal of dissolved solids is achieved through chemical and physicochemical processes, namely, thermal distillation, ion exchange, reverse osmosis (RO) and electrodialysis (ED) (Subramani & Jacangelo 2014; Loganathan et al. 2015). The high energy requirement involved in these conventional methods makes water desalination an expensive process (Chen et al. 2016). Microbial fuel cells (MFCs) have gained attention as a low cost and environmentally friendly method towards the conversion of organic waste directly into electricity (Singh & Suresh 2016). It has been reported that a dual chamber MFC has removed 93% phenol and generated power of 362 mW/m² from retting wastewater (Jayashree et al. 2014a). The MFC technology has shown promising results towards the conversion of organic waste material into useful energy.

Recently, another emerging technology called microbial desalination cell (MDC) has developed, which integrates the MFC technology and ED process to desalinate the saline water using bioenergy generated from wastewater (Brastad & He 2013). In MDC, the chemical energy stored in the organic matter is converted into electrical energy by exoelectrogens and this generates a potential gradient across the
cell. The potential gradient acts as a driving force for the desalination of saline water present in desalination chamber through ion selective membranes (Cao et al. 2009). Domestic wastewater, dewatered sludge, waste engine oil and dye house effluent have been used as substrates for practical applications of MDC (Luo et al. 2012b; Kalleary et al. 2014; Meng et al. 2014). Bacterial species, Bacillus subtilis moh3 and Aeromonas hydrophila have been used to degrade the complex organic matter from waste engine oil and dye house effluent (Affandi et al. 2014; Pradhan et al. 2015). With this background, it can be suggested that steel plant wastewater can be used as a substrate in MDC to degrade phenol and generate electricity employing microbial species.

In the present study, the removal of recalcitrant phenol and total dissolved solids (TDS) was observed and compared in an anodic chamber of multi-chambered MDC under different inoculum conditions. This is the first study that showed the recovery of energy from phenol containing steel plant wastewater in MDC along with desalination. The results of this study will be helpful in the removal of phenol and desalination from steel plant wastewater to facilitate the reuse of wastewater along with electricity generation.

**MATERIALS AND METHODS**

**Steel plant wastewater characterization**

The coke oven process effluents were collected from Tata Steel plant, Jamshedpur, India and analyzed for the constituents present in it (Table 1). The filtered raw wastewater was used as the sole anolyte in multi-chambered MDCs. The effluents were collected from each MDC’s port for their analysis after each batch run. The pH was found to be alkaline in nature.

**Microorganism and culture conditions**

A bacterial strain was isolated from the soil of an automobile workshop in Jamshedpur, Jharkhand, India. The strain used as an inoculum in INC-A was a pure culture of Pseudomonas putida. The strain was grown on nutrient agar and its composition was (per L of deionized water): peptone, 5.0 g; NaCl, 5.0 g; beef extract, 1.5 g; yeast extract, 1.5 g; agar, 20.0 g at 37 °C (Bandhyopadhyay et al. 2001). The media slants were preserved at 4 °C. The isolated strain was identified as Pseudomonas putida MTCC 1194 based on 16S rRNA sequencing from Microbial Culture Collection and Gene Bank, IMTECH, Chandigarh, India. The culture was grown in 250 mL Erlenmeyer flask containing 100 mL of mineral salt medium (MSM) consisting of (per L of deionized water): NH4NO3, 1.0 g; (NH4)2SO4, 0.5 g; NaCl, 0.5 g; MgSO4.7H2O, 0.5 g; K2HPO4, 1.5 g; KH2PO4, 0.5 g; CaCl2, 0.01 g; FeSO4.7H2O, 0.01 g. The pH of the medium was maintained at 7.0 ± 0.2 by using 0.1 N HCl and 0.1 N NaOH (Ehrhardt & Rehm 1989; Banerjee et al. 2001; Kumar et al. 2005). The organism was acclimatized to higher phenol concentration (400 mg/L) into the sterilized MSM. The inoculum was further grown in phenol containing sterilized MSM at 37 °C, at 150 revs/min for 16 h (Saravanan et al. 2008). The broth culture (optical density, OD600 of 0.15) of volume 5 mL was transferred to 250 mL of Erlenmeyer flask comprising 100 mL of MSM with phenol and was kept at 37 °C with shaking at 150 revs/min for 72 h.

**MDC construction**

Three identical multi-chambered MDCs using inoculums INC-A, INC-B and INC-C were constructed with polyacrylic sheets that contained five partitions and formed the anodic and cathodic chambers, desalination chamber in the middle, and two concentrate chambers placed between anodic/cathodic chambers and central desalination chamber. All polyacrylic sheets were separated by pairs of anion exchange membrane (AEM) and cation exchange membrane (CEM) (Figure 1). The membranes were soaked in

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.92 ± 0.15</td>
</tr>
<tr>
<td>TDS, g/L</td>
<td>3.76 ± 0.2</td>
</tr>
<tr>
<td>Conductivity, mS/cm</td>
<td>7.6 ± 0.24</td>
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<tr>
<td>COD, mg/L</td>
<td>2,800 ± 210</td>
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<tr>
<td>Total phenol, mg/L</td>
<td>450 ± 45</td>
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<tr>
<td>Alkalinity, mg/L</td>
<td>537.5</td>
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<tr>
<td>Hardness as CaCO3, mg/L</td>
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<td>Sulfide, mg/L</td>
<td>88 ±</td>
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<td>Turbidity, NTU</td>
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<td>NH3-N, mg/L</td>
<td>154</td>
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<td>Total Kjeldhal nitrogen (TKN), mg/L</td>
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<tr>
<td>Fe3+ mg/L</td>
<td>2.25 ± 0.11</td>
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<tr>
<td>Total volatile fatty acids (VFA), mg/L</td>
<td>2052</td>
</tr>
<tr>
<td>Total protein, mg/L</td>
<td>Negligible</td>
</tr>
</tbody>
</table>

**Table 1** Characteristics of process wastewater collected from Tata steel plant in the study

With this background, it can be suggested that steel plant wastewater can be used as a substrate in MDC to degrade phenol and generate electricity employing microbial species.
distilled water for 48 h before use in all the MDCs (Pradhan & Ghangrekar 2015). The effective volume of both anodic and cathodic chambers was 110 mL each with width of 3 cm, whereas desalination and concentrate chambers had volume of 25 mL each with width of 0.5 cm. Carbon felt was used as both anode and cathode with the surface area of 48 cm². The electrodes were linked through concealed copper wires with an external load resistance of 100 Ω. The MDCs were sterilized at the beginning of the experiment and the reference electrode was washed with 70% ethanol and sterile deionized water before introducing in the anodic chamber.

Microbial inoculums and MDC operation

The multi-chambered MDCs were operated in batch mode for more than 70 days at an ambient temperature of 25 ± 5 °C. The anodic chamber using INC-A was inoculated with 40 mL pure culture of *P. putida* having optical density OD₆₀₀ of 0.35. Activated sludge (20 g/L) was collected from biological oxygen treatment (BOT) plant from Tata Steel, Jamshedpur. The anodic chamber using INC-B was inoculated with a mixture of *P. putida* and activated sludge (1:1, v/v) of volume 40 mL. In the third MDC using INC-C, 40 mL of activated sludge alone was added to the anodic chamber as inoculum (Ghangrekar & Shinde 2007). In the anode chamber inoculum, 2-bromoethane sulfonate (2-BES) was added at 10 mM concentration, to inhibit the growth of initial methanogenic bacteria present in activated sludge (Capodaglio et al. 2015) (Molognoni et al. 2017). The functioning of all the MDCs with different inoculum conditions in the anodic chamber were compared.

The coke oven process wastewater with COD concentration of 2,800 ± 210 mg/L and total phenol of 450 ± 45 mg/L was fed in the anodic chamber. Since the pH of wastewater was alkaline (8.92 ± 0.9) in nature, pH of anolyte was maintained at 7.0 ± 0.2 by the addition of phosphate buffer solution. Tap water was used as catholyte in all MDCs (Behera et al. 2010; Zhang et al. 2010). Air was supplied to the cathode chamber by an aquarium pump (SOBO aquarium air pump, China) with a flow rate of 50 mL/min. The effluent from the anodic chamber was fed directly into the desalination chamber after removal of organic matter to prolong the life of the membrane. The TDS concentration of coke oven wastewater was 3.76 ± 0.2 g/L. The concentrate chambers of the MDCs were filled with deionized water.

Analyses and calculations

Samples were obtained from the anodic chamber of MDC at the time gap of 12 h for evaluating the phenol concentration by colorimetric method (APHA 2005). COD was determined using closed reflux procedure as described in *Standard Methods* (APHA 2005). The initial and final TDS of the liquid present inside the anodic, desalination and cathodic compartments were assessed by the conductivity probe. The desalination efficiency was calculated as the percentage of reduced TDS over 72 h of batch cycle.

A digital multi-meter (Agilent Technologies, Penang, Malaysia) was used to measure potential and current across the external resistor, R of 100 Ω. The anode and cathode potentials were measured using Ag/AgCl reference electrode (+197 mV vs. standard hydrogen electrode (SHE), Bioanalytical Systems Inc., West Lafayette, USA). The Coulombic efficiency (CE) is the ratio of electrons transported to the anode to the total electrons present in the substrate. CE of the MFC operated under batch mode over a time period ′t′ was calculated as per Equation (1) (Logan...
et al. 2008).

\[
CE = \frac{8}{F} \int I dt \quad \frac{F}{V_{an}} \Delta COD
\]  

(1)

where \( I \) is current, A; 8 is a constant used, based on \( O_2 \) molecular weight, \( F \) is Faraday’s constant = 96,485 C/mol; \( V_{an} \) is the volume of anolyte, L; and \( \Delta COD \) is the difference in the influent and effluent CODs, g/L.

Faradaic efficiency or charge transfer efficiency \( (\eta_F) \) of the MDC system was obtained as the ratio of the theoretical amount of coulombs \( (Q_{th}) \) required to remove NaCl to the total coulombs acquired through the electrical circuit \( (Q = \int I dt) \), assuming that removal of one mole of NaCl will require one mole of electron. The Faradaic efficiency of the system was calculated according to Equation (2) (Vaszilcsin & Nemes 2009).

\[
\eta_F = \frac{Q_{th}}{Q}
\]  

(2)

**Scanning electron microscopy**

Scanning electron microscopy (SEM) (Zeiss, EVO40 SEM) was performed to evaluate the morphological features of the bacteria proliferating on the anode. An incident electron beam energy of 10 keV and a working distance of 6 mm was retained. The sample for SEM analysis was prepared using the standard procedure of fixation, dehydration and drying. Cell fixation was done using glutaraldehyde solution and air dried. Sequential dehydration of the electrode was done in each solution – 30%, 50%, 70%, 80%, 90%, and 99% of alcohol for 5 min (Pandit et al. 2014).

**RESULTS AND DISCUSSION**

**Bacterial growth and pH optimization**

Bacterial growth pattern of *Pseudomonas putida* MTCC 1194 was observed and the growth curve plotted. The bacterial culture followed a sigmoidal curve with an initial lag phase of 20 h, a log phase of 100 h and afterwards reached a stationary phase where the growth remained constant.

pH was maintained at 7.0 ± 0.2 by the addition of phosphate buffer to create favourable conditions for microbial metabolism. Most of the literature suggests that pH 7.0 is appropriate for microbial activity of most of the microorganisms (Chen et al. 2012). pH had decreased to 6.0 at the end of 72 h and this happens normally due to bacterial metabolism that constantly produces weak acids and compounds to maintain intracellular bacterial pH (He et al. 2008).

**COD removal**

The coke oven process wastewater after BOT was used in multi-chambered MDCs, pH (7 ± 0.2) by addition of phosphate buffer in order to create a favourable condition for anodic microbial metabolism (Luo et al. 2012). The removal of COD was observed in all three multi-chambered MDC with an initial COD concentration of 2,800 ± 210 mg/L for 72 h of batch operation. The COD removal was observed under each inoculum condition in batch mode of operation to check the ability of all MDCs for organic matter removal. The highest average COD removal efficiency of 70 ± 1.8% was observed in the second MDC; whereas, COD removal efficiencies of 63 ± 2.4% and 67 ± 2.2% were observed in the first MDC and the third MDC, respectively (Figure 2(a)).
Higher COD removal efficiency in the second MDC was due to the mixture of pure culture of *P. putida* and activated sludge used as inoculum, indicating synergistic interaction between these two inoculums (Mukred *et al.* 2008; Cerqueira *et al.* 2011). The lowest COD removal efficiency was observed in the first MDC with a pure culture of *P. putida* as inoculum. It has been reported that a higher concentration of phenol (>400 mg/L) in the sample decreases the COD removal rate (Song *et al.* 2014). Bacterial growth is inhibited owing to the toxicity of phenol, thereby reducing the removal rate of COD. Using pure culture as an inoculum for real wastewater treatment might affect the metabolism of the microorganism. In addition to this, the pure culture may get contaminated and be unable to acclimatize in the presence of foreign organic substances. Hence, the performance of COD decreased in MDC using pure culture as a sole inoculum. Furthermore, thermo-chemical pretreatment of dairy waste activated sludge at optimized condition (70 °C for 24 h) was considered for evaluating its effect on COD removal efficiency wherein 29% increase in COD solubilization was observed as compared to the control (Jayashree *et al.* 2014b). Hence, pretreatment of activated sludge bacteria at optimum conditions may result in increasing its COD removal efficiency. The effluent from the anodic chamber of all the MDCs were collected and compared with the raw coke oven process wastewater for any change in their typical colour (Figure 2(b)). The anodic effluent collected from MDC using a mixture of *P. putida* and activated sludge as the inoculums was quite clear as compared to other MDCs using only *P. putida* and activated sludge as individual inoculum.

### Degradation of phenol

The process wastewater from steel plant is composed of phenol and several polyaromatic hydrocarbons (PAH) within it that makes the effluent complex in nature. Microbial degradation of this complex wastewater is difficult and it is impossible to degrade this efficiently using a pure culture of microbes. Several studies have reported the synergistic effect of microorganisms for effective removal of recalcitrant organics which affect the removal of COD and nitrogen from wastewater (Mukred *et al.* 2008; Cerqueira *et al.* 2011). The concentration of total phenol in coke oven process wastewater was 450 ± 45 mg/L. The removal of total phenol in different MDCs were compared. Efficiencies of phenol degradation such as 65 ± 2.1%, 82 ± 2.4% and 62 ± 1.6% were observed with inoculum INC-A, INC-B and INC-C, respectively, in 72 h of batch operation (Figure 3(a)). Highest phenol degradation observed with INC-B was due to the positive interaction between the pure culture of *P. putida* and activated sludge used as inoculum as compared to *P. putida* and activated sludge used solely as an inoculum. The mixing of two different microorganisms lead to increased phenol removal rate. This can be attributed to higher bacterial growth as well as synergistic effect of both the isolates (Mukred *et al.* 2008). Individual microbes can metabolize only few substrates but mixing different inoculums with pure culture of phenol degrading bacteria can efficiently degrade complex phenolic compounds (Walton & Anderson 1988). However, in some cases, single bacterial species like *Stenotrophomonas* is able to...
produce power efficiently from seafood processing wastewater rather than synergistic interaction of two or more different microbial consortia required for the same effect (Jayashree et al. 2016).

Phenol is a complex organic material which is degraded by P. putida (Hill & Robinson 1975). Phenol converts into acetate under anaerobic conditions by two possible pathways; one, through 4-hydroxybenzoate into the benzoyl-CoA path and another, through caproate in the presence of various microorganisms. Caproate is observed in thermophilic temperature (>50 °C) and the microbes involved in this process is unknown (Levén et al. 2012). The phenol degradation through microorganisms is a temperature sensitive process. Anaerobic degradation of phenol by microbes occurs at both mesophilic (37 °C) and thermophilic (55 °C) conditions (Limam et al. 2013). On comparison of the phenol degradation efficiency in both mesophilic and thermophilic conditions, it is found that better phenol degradation occurs at mesophilic conditions as the majority of the phenol degrading microbes function better at mesophilic conditions (Levén et al. 2012). Moreover, it is found that the degradation rate increases sharply when the temperature is reduced from thermophilic to mesophilic conditions (Hernon et al. 2006). The possible reason can be under different temperature conditions different growth factors are produced in microbes which may lead to differences in their phenol degradation ability (Levén et al. 2007). Moreover, some enzymes involved in the degradation of phenol to benzoate are temperature sensitive. Degradation of phenol at more than 48 °C is due to the thermal inactivation of enzymes at thermophilic conditions (Leven & Schnürer 2005). Thus, it is imperative to consider temperature when treating anaerobic phenol-degrading bacteria as phenol degradation occurs efficiently at mesophilic conditions.

TDS removal

Initial TDS concentration of 3.76 ± 0.2 g/L was observed in the anodic chamber of each MDC. The second MDC exhibited a highest TDS removal of 68 ± 1.5% as compared to the first MDC (58 ± 1.8%) and the third MDC (64 ± 1.2%) in 72 h of batch operation (Figure 3(b)). Improved ability of TDS removal of the second MDC was because of the high operating voltage (OV) of 128 mV with 100 Ω external resistance. High voltage generation in MDCs maintains high TDS removal (Pradhan & Ghangrekar 2014). The TDS removal seen in the second MDC was 68 ± 3% and higher as compared to 43 ± 6% with 5 g/L of NaCl in the desalination chamber (Mehanna et al. 2010a, 2010b). The lower internal resistance of 230 Ω in the second MDC supported highest TDS removal (Table 2). The desalination performance in MDCs is influenced by the initial solution conductivity in the desalination chamber (Kim & Logan 2013). The high desalination efficiency of the second MDC was due to mixed inoculum condition using INC-B.

Electricity generation

The generation of electricity in the MDCs were noted for 70 days from the start of the experiment. The OV and open circuit voltage (OCV) were assessed in all the MDCs. Maximum OV of 68 mV, 128 mV and 118 mV were observed with inoculums INC-A, INC-B and INC-C, respectively. Average OCVs of 513 ± 22 mV, 576 ± 18 mV and 556 ± 21 mV were observed with inoculums INC-A, INC-B and INC-C, respectively, in fed-batch operation. The second MDC showed higher OV than the first and third MDC. The results demonstrate that the mixture of P. putida and activated sludge interacted positively to facilitate the degradation of complex phenol-glucose mixture along with improved electricity generation. Hence, in the second MDC, the highest TDS removal was detected. The internal resistances of 1,000 Ω, 230 Ω and 400 Ω were observed during the polarization in MDCs with inoculums INC-A, INC-B and INC-C, respectively (Table 2). Lower internal resistance in the second MDC demonstrated better anodic biofilm growth and enhanced electricity generation employing mixed inoculums. The mixed inoculums also demonstrated its suitability for the optimum performance of MDC.

### Table 2 | Performance comparison of MDCs with different inoculum conditions in anodic chamber

<table>
<thead>
<tr>
<th>MDC types</th>
<th>COD removal (%)</th>
<th>Phenol removal (%)</th>
<th>TDS removal (%)</th>
<th>Faradaic efficiency (%)</th>
<th>PD (mW/m²)</th>
<th>CD (mA/m²)</th>
<th>IR (Ω)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INC-A</td>
<td>63 ± 2.4</td>
<td>65 ± 2.1</td>
<td>58 ± 1.8</td>
<td>23 ± 2.8</td>
<td>3.0</td>
<td>25.0</td>
<td>1,000</td>
</tr>
<tr>
<td>INC-B</td>
<td>70 ± 1.8</td>
<td>82 ± 2.4</td>
<td>68 ± 1.5</td>
<td>58 ± 2.4</td>
<td>10.2</td>
<td>96.0</td>
<td>230</td>
</tr>
<tr>
<td>INC-C</td>
<td>67 ± 2.2</td>
<td>62 ± 1.6</td>
<td>64 ± 1.2</td>
<td>27 ± 3.2</td>
<td>5.3</td>
<td>52.6</td>
<td>400</td>
</tr>
</tbody>
</table>

PD: power density; CD: current density; IR: internal resistance.
Polarization

The maximum power density of 10.2 mW/m² was generated in the second MDC during polarization as compared to 3.0 mW/m² and 5.3 mW/m² observed in the first MDC and third MDC, respectively (Figure 4(a)). Similarly, maximum current densities of 25.0, 96.01 and 52.6 mA/m² were observed in MDCs with inoculum INC-A, INC-B and INC-C, respectively, during polarization (Table 2). The power generation in the second MDC was three-fold and two-fold higher compared to the first MDC and the third MDC, respectively. The highest power density observed in the second MDC indicates better electron recovery of mixture of pure culture and activated sludge inoculum through oxidation of coke oven process wastewater.

The improved electricity generation along with low internal resistance of the second MDC using inoculum INC-B compared to the first and the third MDCs indicates a substantial role of the mixture P. putida and activated sludge for degradation of complex organic substances. It has been observed that a population of mixed microbial species functions better in MFCs where complex organics substances are used as the fuel (Luo et al. 2009). It is known that P. putida is a useful bacteria, capable of using a broad variety of substrates to generate electricity in MFCs (Majumder et al. 2014). The power production from different wastewater was compared with the power density obtained in the present study. The maximum power density 10.2 mW/m² obtained in the present study was lower compared to the power production obtained from retting wastewater (254 mW/m²) or from swine wastewater (261 mW/m²) (Kim et al. 2013; Jayashree et al. 2018).

Electrode potential

The initial anode potentials of −378 mV, −435 mV and −395 mV were observed at maximum current densities of 25.0 mA/m², 96.01 mA/m² and 52.6 mA/m², respectively. The anode potential was increased by 128 mV, 110 mV and 120 mV in MDCs with inoculum INC-A, INC-B and INC-C, respectively, during polarization (Table 2). The increase in anode potential was higher in INC-A and INC-C as compared to INC-B, reflecting a stable anode potential in the second MDC (Figure 4(b)). In the first MDC with a pure culture of P. putida as inoculum, the anode potential amplified from −378 mV to −250 mV. The higher anode potential in the first MDC was due to the unstable nature of the pure culture of P. putida with complex wastewater used as anolyte. The stable anode potential observed in the second MDC compared to the first MDC and the third MDC suggests favourable conditions for bacterial activity utilizing coke oven wastewater as substrate and electricity generation. During polarization, the cathode potential in all MDCs gradually decreased from their original values. Furthermore, considerable decrease in cathode potential of the first MDC and the third MDC as compared to the second MDC during polarization indicates a slow reduction kinetic at cathode because of limitations of proton availability.

Faradaic and coulombic efficiency

The contribution of electrical current generated in MDCs for TDS removal from desalination chamber can be
estimated by Faradaic efficiencies. The Faradaic efficiencies of MDCs with INC-A, INC-B, and INC-C were observed to be 23 ± 2.8%, 58 ± 2.4% and 27 ± 3.2%, respectively, during fed-batch operation. The Faradaic efficiencies were calculated by the formula given in Equation (2). Higher Faradaic efficiency was observed in the second MDC, indicating the current produced in this MDC contributed more effectively towards TDS removal in the desalination chamber. The higher OV of 128 mV and power generation of 10.2 mW/m² observed in the second MDC supported the higher Faradaic efficiency value (Table 2). It has been reported that with low TDS concentration liquid in desalination chamber, the noted Faradaic efficiency was lower (Pradhan & Ghangrekar 2014). The Faradaic efficiency is an important tool to determine the contribution of electrical current in ion separation processes through ion selective membranes. The Faradaic efficiency should be between 90-100% in well-controlled ED systems and Faradaic efficiencies lower than this value (<90%) indicate a significant loss of current in water feed route (Jones et al. 1995). In this study, the lower Faradaic efficiencies observed in all MDCs indicates further scope for enhancement in TDS removal in the desalination chamber by increasing the limited current generation in the system (Kim & Logan 2015). The MDC needs further modification in its design to recover more electrons for higher TDS removal.

The CE recovered from the coke oven process wastewater used as substrate was 3.5 ± 1.8%, 10.2 ± 2.1% and 5.0 ± 1.5% in MDC with inoculums INC-A, INC-B and INC-C, respectively. The high CE noted in the second MDC using inoculum INC-B specifies better electron recovery from the complex nutrient substances utilized by P. putida and activated sludge as inoculum. However, the low CE observed in the first MDC and the third MDC established the inability of pure culture and activated sludge consortia individually for recovery of electrons from complex organic substrates.

Figure 5 | SEM micrographs of (a) fresh carbon felt and biofilm growth on carbon felt using (b) INC-A, (c) INC-B and (d) INC-C.
Scanning electron microscopy of different inoculums

The biofilm developed on the anode surface was observed by the SEM analysis. The pictures obtained revealed the occurrence of different microbial species that formed uniform biofilm on the anode surface (Figure 5). These micrographs showed the morphology of biofilm formed on carbon felt, indicating good bacterial adhesion properties and biocompatibility of the anode material. The morphology of different inoculum attachment on the anode surface and the electrode before attachment of microbial growth was shown in Figure 5(a). The pure culture of P. putida in the first MDC shows rod-shaped attachment on the surface of the anode (Figure 5(b)). The microbial growth of activated sludge on the carbon felt shows abundant attachment of round shaped structures on the electrode surface (Figure 5(d)). The morphology of anode of the second MDC shows the mixed growth of both round and rod-shaped microorganisms, on the electrode surface (Figure 5(c)).

Comparison of the present MDC performance with other bioelectrochemical systems

The operation of multi-chambered MDCs reported in the present study was compared with other bioelectrochemical systems (Table 3). In the present study, the phenol removal was observed in the multi-chambered MDC using a mixture of pure culture of P. putida and activated sludge as anodic inoculum was lower compared to other reported MFCs (Luo et al. 2009; Friman et al. 2013; Song et al. 2014). In the present study, the phenol removal was achieved with the complex coke oven process wastewater as a substrate compared to synthetic phenol–glucose mixture which is in simpler form. Simultaneous phenol removal, energy recovery, along with desalination in a multi-chambered MDC was achieved for the first time. The removal of phenol in the second MDC (82 ± 2.4) using a mixture of P. putida and activated sludge demonstrated higher phenol removal compared to the phenol removal of 80% using Cupriavidus basilensis inoculum from a phenol–glucose mixture (Friman et al. 2013). The power generated by this MDC using a mixture of P. putida and activated sludge was 2.5 times higher than using coal tar wastewater as a substrate in a membrane-less tubular MFC (Park et al. 2012). The multi-chambered MDC using a mixture of P. putida and anaerobic sludge as inoculum can be employed for wastewater treatment containing complex organic substances. Since most of the MDCs were operated using easily biodegradable substrates like sodium acetate to generate current to achieve

Table 3 Overview of phenol degradation and power generation with other reported BESs

<table>
<thead>
<tr>
<th>Types of BES</th>
<th>Inoculum</th>
<th>Anodic substrate</th>
<th>COD removal, %</th>
<th>Phenol removal, %</th>
<th>Power/current density, mW/m²</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFC</td>
<td>Mixed aerobic and anaerobic sludge</td>
<td>Phenol</td>
<td>83.9–96.4</td>
<td>90</td>
<td>9.1 W/m²³</td>
<td>Luo et al. (2009)</td>
</tr>
<tr>
<td>MFC</td>
<td>Mixed aerobic and anaerobic sludge</td>
<td>Glucose-phenol mixture</td>
<td>83.9–96.4</td>
<td>95</td>
<td>28.5 W/m²³</td>
<td>Luo et al. (2009)</td>
</tr>
<tr>
<td>MFC</td>
<td>Membrane-less tubular</td>
<td>Activated sludge</td>
<td>88</td>
<td>&gt; 90</td>
<td>4.5</td>
<td>Park et al. (2002)</td>
</tr>
<tr>
<td>BFC</td>
<td>Cupriavidus basilensis</td>
<td>Phenol-glucose mixture</td>
<td>100, 200</td>
<td>88</td>
<td>478 mA/m²²</td>
<td>Friman et al. (2003)</td>
</tr>
<tr>
<td>MFC</td>
<td>Anaerobic sludge</td>
<td>Phenol</td>
<td>82, 6</td>
<td>88</td>
<td>9</td>
<td>93</td>
</tr>
<tr>
<td>MFC</td>
<td>Municipal sewage sludge</td>
<td>Coconut husk retting wastewater</td>
<td>450 ± 45</td>
<td>65 ± 2.4</td>
<td>5</td>
<td>102</td>
</tr>
<tr>
<td>Five chambered MDC</td>
<td>Pseudomonas aeruginosa</td>
<td>Steel plant wastewater</td>
<td>450 ± 45</td>
<td>67 ± 2.2</td>
<td>10.2</td>
<td>This study</td>
</tr>
<tr>
<td>Five chambered MDC</td>
<td>Mixed of P. aeruginosa and mixed anaerobic sludge</td>
<td>Steel plant wastewater</td>
<td>450 ± 45</td>
<td>67 ± 2.2</td>
<td>10.2</td>
<td>This study</td>
</tr>
<tr>
<td>Five chambered MDC</td>
<td>Mixed anaerobic sludge</td>
<td>Steel plant wastewater</td>
<td>450 ± 45</td>
<td>62 ± 1.6</td>
<td>5.3</td>
<td>This study</td>
</tr>
</tbody>
</table>

PD: power density.
desalination, it is essential to operate MDC with real wastewater for practical application. The MDCs offer an exciting technology for bioelectrochemical desalination compared to other physicochemical desalination processes. The industrial wastewater rich in complex organic substances can be successfully used in multi-chambered MDC. Substantial toxic phenol degradation, power generation along with desalination offers a sustainable and economical solution for the treatment of phenolic wastewater generated by industries.

CONCLUSIONS

The present study establishes the ability of MDC for simultaneous removal of phenol and TDS from coke oven process wastewater along with electricity generation. Highest phenol degradation, $82 \pm 2.4\%$, was observed with INC-B due to the positive interaction between the pure culture of \textit{P. putida} and activated sludge, indicating synergistic interaction between these two inoculums. The second MDC exhibited a highest TDS removal of $68 \pm 1.5\%$ due to high OV of $128$ mV and the low internal resistance of $230$ $\Omega$. Lower internal resistance in the second MDC also demonstrated enhanced electricity generation employing mixed inoculums. The CE, $10.2 \pm 2.1\%$, recovered from the coke oven process wastewater used as substrate with inoculum INC-B, specifies better electron recovery from the complex substances utilized by \textit{P. putida} and activated sludge mix inoculum. Conclusively, mixed inoculum condition in MDC is preferred for effective removal of organic and inorganic dissolved solids from steel plant wastewater. Alternatively, it is possible to introduce MDC as a primary treatment method for treating and generating electricity from steel plant wastewater.

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