Nutrient recovery and microbial diversity in human urine fed microbial fuel cell
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ABSTRACT

Presence of urine in municipal wastewater is a major problem faced by wastewater treatment plants. The adverse effects are noticeable as crystallization in equipment and pipelines due to high concentration of nitrogen and phosphorus. Therefore, improved technologies are required that can treat urine separately at the source of their origin and then discharge it in the main wastewater stream. In this study, the performance of the microbial fuel cell (MFC) was evaluated with mixed consortia and isolated pure cultures (Firmicutes and Proteobacter species) from biofilm for electricity generation and nutrient recovery.Microbes utilize less than 10% of total phosphorus for their growth, while 90% is recovered as struvite. The amount of struvite recovered was similar for pure and mixed culture (12 ± 5 g/L). The microbial characterization also shows that not all the biofilm-forming bacterial isolates are very much efficient in power generation and, hence, they can be further exploited to study their individual role in operating MFC. The different organic loading rates experiment shows that the performance of MFC in terms of power generation is the same for undiluted and five times diluted urine while the recovery of nutrients is better with undiluted urine, implying its direct use of urine in operating fuel cell.

Key words: bioenergy, biofilm, microbial diversity, microbial fuel cell, struvite, urine

INTRODUCTION

Human urine constitutes 50–60% of total phosphorus (P) and 70–80% of total nitrogen (N) present in the municipal wastewater (Wilsenach & Van Loosdrecht 2005). An individual person contributes about 2.5 L of urine per day, which ends up in municipal wastewater treatment plants (WWTPs) (Ieropoulos et al. 2010). Globally, 2–3 trillion L of urine is accounted per day and discharged via municipal sewage for treatment in WWTPs, without any possible recovery. Nutrient recovery from such a municipal sewage, primarily urine in its highly diluted form with added organics, is costly due to high-energy demands. Hence, diverting the urine at the source and exploring its treatment and nutrient recovery separately would be more holistic and sustainable. Recovering nutrients reduces their load on the water bodies where the treated wastewater is discharged. The separate treatment of urine can prove to be a cost-effective method for reducing the toxic effects of pharmaceuticals and high concentration of nutrients (ammonia toxicity, eutrophication) on the environment (Maurer et al. 2006).

Several technologies are available to recover P and N from urine such as biological uptake, nanofiltration, ozonation or adsorption and struvite precipitation. Among these, struvite precipitation is most preferable due to its agricultural use, as reported earlier (Zhang et al. 2011). Struvite is a crystalline structured phosphate mineral, precipitated when magnesium, ammonium, and phosphorus (MgNH4PO4.6H2O) are present in equal molar ratios (Ronteltap et al. 2010). This kind of precipitation is seen on the surfaces of pipelines and other equipment in WWTPs (Fattah 2012), which leads to the blockage and further increases the operating costs. Although this seems to be a problem in WWTPs, struvite holds the potential to be used as fertilizer as there is a continuous demand of these nutrients in agriculture (Ronteltap et al. 2010). The conventional method of struvite precipitation involved dosing magnesium into the urine at high pH (addition of alkali). The method is efficient in recovering nutrients (N, P); however, it does not help in the reduction of the organics
present in wastewater. Therefore, a technology is needed that can recover nutrients along with treatment of urine.

Microbial fuel cell (MFC) is one such system that can be employed for struvite precipitation along with generating electrical energy. MFC can be incorporated as treatment assembly to efficiently reduce organics in urine and recover N and P, providing a potential solution to the problems faced by WWTPs. MFC working relies on the activity of microbes (microbial metabolism) known as exoelectrogens, anchored to the anode surface as a biofilm. The dual chamber MFC consists of anodic and cathodic chamber where oxidation and reduction reactions occur respectively. The chambers are separated by cation exchange membrane. The exoelectrogens decompose the organic matter and release electrons, which are transferred to anode (Hameilers et al. 2010). The electrons move from anode to cathode via the external load resulting in electric current (Kelly & He 2014). In cathode chamber, conversion of nitrogen into ammonia facilitates the pH increase required for struvite precipitation. Therefore, MFC can transform chemical energy stored in urine into electrical energy with significant treatment of urine and struvite recovery. The use of other substrates in MFC have been reported earlier, but urine has recently gained interest due to its abundance and high conductivity.

The efficiency of urine treatment by MFC depends upon the factors like organic loading rate, pH of the sample and the ability of microorganisms to degrade organic matter. The anolyte pH is important for bacterial growth (optimal growth at neutral pH) and at cathode, oxygen reduction resulted in alkaline pH (Rozendal et al. 2006). The other important parameter is substrate concentration that affects the power generation which must be optimized, as no changes are observed in power at high concentrations (Aghababaie et al. 2015). Microorganisms play a vital role in oxidation of organic waste and electron transfer to the electrode. There are different species of microorganisms that vary in mechanism of voltage generation in MFC due to varied electron transfer. Most successful pure cultures used in MFCs that produce high current densities are from Shewanella and Geobacteraceae family (Bond & Lovley 2005). Firmicutes and Bacillus species have also produced higher current densities. Thus, pure bacterial cultures hold the potential to be used for MFC application (Freguia et al. 2007). Besides pure cultures, mixed cultures have also been used to inoculate MFCs as pure cultures have certain practical implications (economic factors). The individual power output of each microorganism in mixed biofilm is limited by microbial competition for substrate usage and is less than the power output when the microorganism is inoculated as pure culture. However, the combined electrochemical activity of electrogenic bacteria in mixed cultures can be more than pure cultures (Fatemi 2012). Therefore, mixed inoculum from anaerobic digestate was used to inoculate the MFC initially and further, after characterizing biofilm, the performance of MFC was compared for mixed culture with pure cultures.

In view of these issues, the objective of the present work was to analyze the effect of substrate (human urine) on MFC performance and studying the relationship between substrate concentrations and generated electric current and power densities. The biofilm formed on anode surface was characterized to identify the microbial community contributing in treatment (organic matter degradation) and pure cultures were isolated to study individual exoelectrogenic activity. The performance of MFC with mixed and pure isolates was compared with respect to their substrate degradation ability and power generation.

**MATERIALS AND METHODS**

**Sample collection**

Urine was collected from healthy individual volunteers with informed consent. It was stored at 4 °C and used within 2–3 days from collection.

**Chemical analysis and measurements**

The samples were analyzed for chemical oxygen demand (COD) by closed reflux colorimetric method, total ammoniacal nitrogen (NH4+/N) by titrimetric method and ortho-phosphate (Ortho-P) by vanadomolybdophosphoric acid colorimetric method as mentioned in Standard Methods (APHA/AWWA/WEF 2012). Nitrites (NO2−) were determined by using a nitrate test kit based on photometric method (Merck, Germany). Other parameters such as pH were measured using a bench pH meter (Oakton, ION 700) while conductivity was measured using conductivity meter (Contech).

**Operating conditions of MFC**

**MFC setup**

A dual chamber plate and frame type MFC (inner dimensions: 8 × 8 × 2 cm3, total working volume: 230 mL)
separated by a cation exchange membrane (Ultrex CM17000, Membranes International Inc., USA) was used to conduct the study. Both the electrodes were made up of stainless steel mesh (8 × 8 cm²) (SS 304) (Ashoka & Bhat 2022).

Inoculation

The digestate obtained from anaerobic digester (running on food waste in BITS Pilani KK Birla Goa Campus) was heat treated at 100 °C for 15–20 min to inhibit the methanogens. After heating, it was diluted (1:10, v/v) with the media containing CH₃COONa (1 g/L), KH₂PO₄ (0.6 g/L), K₂HPO₄ (0.6 g/L), KCl (0.7 g/L), NaCl (0.6 g/L), NH₄Cl (0.4 g/L), MgSO₄.7H₂O (0.1 g/L), CaCl₂.2H₂O (0.1 g/L) and 1 mL of macronutrients and micronutrients. This microbial suspension (500 mL) was used as anolyte to inoculate the anode chamber. The catholyte in the cathode chamber was phosphate buffer (100 mM, pH = 6.8), aerated continuously.

Both the samples (anolyte and catholyte) were replenished thrice a week. The inoculation was carried for 2 months to allow biofilm formation on the anode.

Batch experiment

After the maturation of the biofilm (extracellular polymeric substance formation on the mesh surface), urine was fed to the reactor. The initial experiments were performed to optimize the hydraulic retention time (HRT) of the reactor, to achieve high pH at the cathode and maximum substrate degradation. The MFC was operated in batch mode at room temperature (25 °C). The urine (500 mL) was recirculated (anode-cathode) through a reservoir at a flow rate of 10 mL/min using a peristaltic pump (Flowtech-NFP01, India). Sampling was done from the reservoir every hour to observe the maximum change in the values of the parameters like COD, NH₄⁺-N, NO₃⁻ and Ortho-P. Magnesium chloride (MgCl₂, Merck, Mumbai, India) was added at equimolar concentration to NH₄⁺-N and Ortho-P to the final effluent of the batch. The precipitates obtained from cathode effluent were filter-dried at room temperature.

Effect of organic loading on the electrochemical performance of MFC and struvite precipitation

The urine sample was diluted 5, 10, 15 and 20 times with normal tap water. The reactor acclimatized to raw urine for a month was fed with 5X (2.4 g/L COD) diluted urine. During the run, urine was first passed through anode chamber then through cathode chamber via reservoir continuously. The effluent was sampled and analyzed for different parameters as mentioned above. Similar to the experiments above, MgCl₂ (2.5 mL/h saturated solution) was added to the effluent to precipitate the nutrients. The polarization and power curve were recorded by connecting the MFC to an external resistance of 100 Ω and operating until three cycles of stabilized current was obtained. The experiment was repeated for 10X (1.2 g/L COD), 15X (0.8 g/L COD), 20X (0.6 g/L COD) diluted urine and finally with raw urine (11.8 g/L COD) only. MFC was monitored for stable current density and COD removal efficiency for all the concentrations. The precipitates obtained from cathode effluent on MgCl₂ addition in all the dilutions were collected, weighed and characterized.

Biofilm development

After optimization studies, MFC was operated continuously for 6 months with urine fed at a flow rate of 10 mL/h (anode to cathode, Figure 1). The biofilm development was monitored by analyzing piece of anode using scanning electron microscopy. A saturated solution of MgCl₂ was added into the effluent of cathode chamber at a rate of 2.5 mL/h for precipitating the struvite.

![Figure 1 | Schematic diagram of laboratory-scale MFC reactor showing flow path of urine (feed).](https://iwaponline.com/wst/article-pdf/79/4/718/562380/wst079040718.pdf)
Biofilm characterization

The biofilm developed at the anode post the continuous operation of the reactor was collected and stored in sterile 50 mM phosphate-buffered saline (PBS) at −20 °C before characterization. The genomic DNA of the biofilm, anaerobic digestate and blank urine were extracted as described in literature (Zhou et al. 1996). For sequencing, DNA was amplified using the PCR reaction. Universal primer pairs 27F (5’-AGAGTTTGATCMTGGCTCAG-3’) and 1401R (5’-CGGTGTGTACAAGACCC-3’) were used for the amplification of the 16S rDNA variable regions V1-V3. The PCR products were amplified with the primers 968F containing GC clamp (5’-CGCCCGCCGCGCCCCGCCCCAAGCGGAA CGCTTAC-3’) and 1401R. The PCR amplification was carried out using Mastercycler AG (Eppendorf, Hamburg). The resulted amplicons were separated using polyacrylamide gel. Denaturing gradient gel electrophoresis (DGGE) was performed using DGGE unit-1001 (CBS Scientific Company, Inc., CA, USA) with a gradient ranging from 40 to 75% (40% acrylamide, TAE buffer, urea and formamide). The gel was casted with the mentioned gradient and the electrophoresis was performed for 16–18 h at 80 V and 70–80 mA current at 60 °C. The gel was silver stained after the run (Ren et al. 2007). The predominant bands in the gel were aseptically excised, suspended in 50 μL distilled water and stored overnight. These bands were amplified with primers 968F and 1401R and was sequenced to identify the microbial communities. The sequences were deposited in the GenBank (NCBI). A neighbor-joining phylogenetic tree was constructed using the Molecular Evolutionary Genetics Analysis package (MEGA, Version 3) (Kumar et al. 2004).

Isolation of pure cultures

Pure cultures were isolated from the biofilm developed during continuous operation of MFC. The biofilm was suspended in 0.9% saline. This sample suspension was inoculated onto MacConkey agar (MAC) followed by 18–24 h incubation at 37 °C. The confirmation of the obtained isolates were done by biochemical method, such as IMViC test (Indole, Methyl red, Voges-Proskauer, and citrate utilization tests) (O’Hara et al. 2000; Zinnah et al. 2007; Tariq et al. 2016). The isolated colonies were inoculated separately in a media (glucose, 4 g/L; NH₄Cl, 0.465 g/L; yeast extract, 3 g/L; KH₂PO₄, 0.7 g/L; K₂HPO₄, 1 g/L; peptone, 1 g/L) and were allowed to grow till the exponential phase (absorbance at 600 nm). Pellet for each culture was obtained by centrifuging the culture media for 10 min at 5,000 g. The pellets were suspended in same fresh media separately and used to inoculate the MFC. Phosphate buffer (100 mM) with continuous aeration was recirculated in the cathode chambers during the MFC runs. Phosphate buffer was added to maintain optimum pH conditions and participate in oxygen reduction. This will also help in increased conductivity to enhance power generation. Aeration was provided for proton reduction to water.

Performance comparison of mixed and isolated cultures

During these experiments, the chemical and electrochemical performance of MFC inoculated with mixed culture and isolated pure cultures were compared. For MFC run with one such isolated culture, the anode chamber and cathode chamber were recirculated (10 mL/h) with fresh media inoculated with the culture and phosphate buffer (100 mM), respectively. The setup with recirculation was incubated for a week to allow the isolate growth and biofilm formation. After the biofilm formation, raw urine was fed continuously (10 mL/h) into the MFC (anode-reservoir-cathode), replacing the culture media and phosphate buffer. MFC was run for a month. Also, the effluent from the cathode was fed continuously with MgCl₂ (2.5 mL/h) for struvite precipitation. Effluent sample was collected and analyzed once in 3 days for COD, NH₄-N, NO₃ and Ortho-P. Polarization study was conducted by decreasing an external resistance from 5,000–50 Ω and leaving the MFC for 10 min at each resistance to stabilize the voltage. The electrodes were detached from the external load and the open circuit voltage (V_oc) was measured once the reactor stabilized. The amount of precipitate formed was determined. Similar runs were conducted for all the other isolated cultures and the mixed culture.

Struvite characterization

Scanning electron microscopy and energy dispersive spectroscopy (SEM-EDAX) was performed to identify the shape, morphology and original elemental composition of the precipitate (obtained in experiments above) at 10 keV (QUANTA FEG 250) (Prabhu & Mutnuri 2014). The powdered precipitate was deposited on a carbon tape stuck to
an aluminum stub. It was coated with a conductive layer (5 nm) of gold-palladium in LEICA EM ACE200 (sputter coater). Fourier transform infrared spectroscopy (FTIR) was performed (IRAffinity-1 FTIR spectrophotometer, Shimadzu) by mixing 1% precipitate with 99% KBr to obtain the spectra showing vibrational bands with respect to wave numbers.

RESULTS

Optimization of HRT for COD reduction and struvite precipitation

The chemical composition of the raw urine is shown in Table 1. The conductivity of the urine was 11.14 ± 0.12 mS/cm with a pH of 6.3 ± 0.6. The collected sample was pale yellow in color and turbid in appearance due to phosphates (Jadhav et al. 2016). During the initial laboratory-scale experiments, the change in the COD concentration as a function of the reaction time in the reactor operated in batch mode was measured. The maximum COD removal was seen after 20–22 h of HRT (trend not shown) with the pH change from 6.3–9.1 (achieved in 6–8 h) in cathode chamber. The increased pH favors the struvite precipitation (Kuntke 2015). Hence, precipitation was seen on addition of MgCl2 in the final batch effluent. The variation of pH and Ortho-P precipitation of catholyte with time is shown in Figure 2(a). Thus, 90% of the Ortho-P was recovered as struvite, which was confirmed as described below. After precipitation, the concentration of NH4⁺-N was reduced by only 50% as compared to Ortho-P due to its high concentration in the initial sample. For subsequent experiments, a reaction time of 22 h was thus adopted.

The precipitate obtained was analyzed by SEM analysis. The morphological observations, showed that the particles were elongated, needle shaped with flat irregular surfaces similar to the structure mentioned in the literature for struvite (Prabhu & Mutnuri 2014). The arrangement of the crystals and the elemental analysis of the precipitate is shown in Figure 2(b). It is evident from EDAX data that the precipitate contains N, Mg and P, as found in struvite. The FTIR spectrum for the precipitate was in the range of 400 to 4,500 cm⁻¹. The robust and broad peak in the range between 3,508 and 2,853 cm⁻¹ is consigned to the H-O-H stretching vibrations of the water in struvite crystals. The medium bands appeared at 1,608.42 cm⁻¹, and 2,047.50 cm⁻¹ indicate the H-O-H bending modes of vibrations or due to H-N-H effect. The band close to 1,440.48 cm⁻¹ is due to NH4 group. The band at 1,022.31 cm⁻¹ corresponded to the ν1 symmetric stretching vibration of PO4³⁻.
vibrations of the precipitate. Another band at 889.748 cm\(^{-1}\) indicates the corresponding water and metal–oxygen bond in the compound. The peak around 570 cm\(^{-1}\) matches the \(\nu_2\) symmetric bending vibrations and \(\nu_4\) asymmetric bending vibrations of PO\(_4\) units. The comparison of obtained FTIR spectrum with the standard FTIR spectrum for struvite, confirmed that the precipitate analyzed was struvite.

### Struvite precipitation and electrochemical performance from raw and diluted urine

The change in different parameters for diluted and undiluted urine sample is shown in Table 2. After acclimatization of the reactor, MFC was operated at different organic loading rates starting from 0.6 g/L and was monitored for reproducible current densities for three consecutive batches for each dilution. The replacement of the fresh anolyte of same concentration does not affect the electrochemical performance of MFC. Figure 3(a) shows the current generation as a function of decreasing urine dilution. As the COD concentration increases from 0.6 g/L to 2.4 g/L, an increase in current density from 0.126 ± 0.12 to 0.461 ± 0.14 A/m\(^2\) was observed. Further, when feed was changed to raw urine, in the first cycle, there was an increase in current density to 0.56 ± 0.04 A/m\(^2\), which was unstable and dropped down to 0.12 ± 0.06 A/m\(^2\). The second cycle did not show the same behavior on fresh anolyte addition and attained a maximum value of 0.46 ± 0.08 A/m\(^2\) that remained stable during further cycles as well. Hence, a stable current density of 0.461 ± 0.10 A/m\(^2\) (0.24 ± 0.15 W/m\(^2\)) at 0.48 V voltage was obtained for undiluted urine sample, which is equivalent to data, obtained for 2.4 g/L COD concentration. The MFC performance with undiluted urine was further evaluated by recording the polarization curve at stable current generation using different external resistances (5 kΩ–50 Ω). The open circuit voltage measured was 0.645 V with the highest power density of 0.68 W/m\(^2\) at 100 Ω (Figure 3(b)). The power density or power output from MFC depends on the type of substrate used, its loading rate and its biodegradability. The same type of reactor can result in

<table>
<thead>
<tr>
<th>Different dilutions of urine sample</th>
<th>20x</th>
<th>15x</th>
<th>10x</th>
<th>5x</th>
<th>Undiluted</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>82.25 ± 3.1</td>
<td>80.54 ± 4.8</td>
<td>77 ± 3.6</td>
<td>71.42 ± 3.4</td>
<td>69.97 ± 2.2</td>
</tr>
<tr>
<td>NH(_4)(^+)-N</td>
<td>64.35 ± 5.1</td>
<td>58.32 ± 2.4</td>
<td>54 ± 2.8</td>
<td>51.60 ± 1.2</td>
<td>49.96 ± 0.9</td>
</tr>
<tr>
<td>NO(_3)</td>
<td>74.65 ± 1.6</td>
<td>72.20 ± 1.9</td>
<td>67 ± 7.2</td>
<td>61.48 ± 5.3</td>
<td>54.28 ± 3.3</td>
</tr>
<tr>
<td>Ortho-P</td>
<td>&gt;99</td>
<td>&gt;99</td>
<td>&gt;99</td>
<td>97.5 ± 1.4</td>
<td>94.2 ± 2.3</td>
</tr>
</tbody>
</table>

Table 2: MFC performance in treating urine at different substrate concentrations
different power densities, as mentioned in previous studies. The power density obtained using swine wastewater was 0.261 W/m², while for paper-recycling wastewater it was 0.672 W/m² (Min et al. 2005; Huang & Logan 2008). One of the MFC study conducted with urine generates very low power density of 2.25 mW/m² (Winkler 2009). However, it was 0.68 W/m² in our study.

From Table 2, it can be seen that the percentage removal efficiency of different parameters increases with dilution. COD, NH₄⁻N, NO₃₋ and Ortho-P removal was highest for 20 times diluted urine. However, recovery of nutrients in the form of struvite decreases with dilution. The maximum struvite precipitation of 13 g/L was obtained for undiluted urine as compared to 2.5 g/L for 5 times diluted urine and no precipitates were recovered in further dilutions. The high organics and nutrients removal in diluted urine can be attributed to their low concentration in the influent. Microorganisms utilized the available phosphorus for their growth. Hence, the effluent shows complete removal of Ortho-P and high removal of other nutrients. The precipitated struvite contained 0.2 ± 0.08 mg N/mg struvite and 0.54 ± 0.02 mg P/mg of struvite.

**DGGE analysis of biofilm and isolation of pure cultures**

The bands corresponding to each sample were excised from the DGGE gel for sequencing. In total, 17 bands were sequenced. The sequences are available under the accession number of MG980312–MG980328. The biofilm formed at anode showed higher number of communities from *Firmicutes* phylum (80%) with 20% *Proteobacter* species. The 73% of initial seed inoculum was comprised of *Proteobacter* species with single species of methanogenic group while the urine sample showed equal distribution of both the bacterial taxonomies with no species of archaeal group. *Proteobacter* species found in the communities were of both the types- Gammaproteobacter (*Pseudomonadales*, *Enterobacterales*) and Betaproteobacter (*Burkholderiales*) along with some unknown and uncultured species. *Firmicutes* were allocated to the orders *Clostridiales* and *Bacillales*, *Negativicutes*, *Tissierellales* and some uncultured *Firmicutes*. The distribution of different microbes is shown in Figure 4(a). The DNA sequences were compared to the GenBank database using a BLASTn search. Table 3 shows the sequence similarities to known sequences, most of which were uncultured bacterial clones isolated from environmental samples. Phylogenetic analysis of 16S rRNA gene sequences of the biofilm and closely related organisms in the GenBank database is shown in Figure 5. Based on the data obtained from GenBank, we found that the microbial community in the biofilm was dominated by *Escherichia coli* and *Pseudomonas* followed by *Bacillus* and *Proteus* species.

The MacConkey agar is a selective media used to isolate Gram-negative or enteric bacteria. On incubating the biofilm suspension on MacConkey agar, round pink colonies grown due to lactose fermentation were selected as *E. coli*, while non-lactose fermented colorless colonies were suspected as *Proteus vulgaris*. The colonies of *Bacillus* and *Pseudomonas* were picked from nutrient agar plates. The confirmatory IMViC test results are shown in Table 4. In the first test (Indole), indole-positive bacteria shows the formation of a pink colored ring at the top during the test.

![Figure 4](https://iwaponline.com/wst/article-pdf/79/4/718/562380/wst079040718.pdf)
The basis of the second test (methyl red test) is the ability of bacterial isolate to ferment glucose and to neutralize the phosphate buffer. The development of red color indicates the production of acid and is a positive reaction. The third test (Voges-Proskauer) shows the red coloration due to the presence of acetoin (positive test). The last test (citrate) is based on the utilization of citrate as carbon source and contains pH indicator. Positive test shows blue color formation, whereas green color is a feature for negative test.

**Struvite precipitation and electrochemical performance comparison using pure cultures and mixed culture**

The MFC was inoculated with each of the isolated culture individually (*Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Proteus vulgaris*) and was monitored for power generation and the efficiency of each isolate in changing or reducing COD, NH$_4^+$-N, NO$_3^-$ and Ortho-P. The MFC inoculated with anaerobic digestate produced the higher power density of 123 mW/m$^2$ at a maximum $V_{oc}$ of 645 mV. Among the pure cultures, *Proteus vulgaris* showed a maximum voltage of 482 mV with the power density of 99 mW/m$^2$ followed by *Pseudomonas aeruginosa* with the maximum voltage of 455 mV. The maximum $V_{oc}$ and power density obtained with mixed culture, along with the reduction in COD, NH$_4^+$-N and NO$_3^-$ is given in Table 5. The reduction in Ortho-P was very less and was in the range of 5–10% but increased when recovered as struvite by adding MgCl$_2$. Approximately, similar quantity of precipitate was obtained with all the cultures (12 g/L).

The variation of $V_{oc}$ and power density with the current density is shown in Figure 4(b) for all the cultures. From the results (Table 5, Figure 4(b)), it is evident that the performance of MFC differs for each bacteria (individual microorganism used for inoculation) when operated with urine as feed. Mixed culture showed better performance in energy recovery and COD removal than pure cultures; however, co-cultures can also be used to increase the electrochemical performance of MFC as reported earlier (Jothinathan & Wilson 2017).

**DISCUSSION**

The source-separated urinals have gained popularity towards sustainable management of wastewater as it offers

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Table 3 | DGGE 16S rRNA gene band identifications (U-blank urine; B-biofilm; D-anaerobic digestate; similar sequences: U1 – B6, U3 – B7, U4 – U2, U7 – B4 – U6, U8-B5, D5 – B6)

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Band name</th>
<th>Closest match (accession number)</th>
<th>Similarity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>U2</td>
<td>Uncultured organism clone ELU0045-T454-S-NIPCRAMgANb_000470 small subunit ribosomal RNA gene, partial sequence (HQ760185.1)</td>
<td>94</td>
</tr>
<tr>
<td>2</td>
<td>U5</td>
<td><em>Pseudomonas</em> sp. strain OB0511_220 – 1 16S ribosomal RNA gene, partial sequence (KY020313.1)</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td>U6</td>
<td>Uncultured <em>Thermohalobacter</em> sp. isolate DGGE gel band C5 16S ribosomal RNA gene, partial sequence (MF071503.1)</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>D1</td>
<td><em>Bacillus</em> sp. JCM 28687 gene for 16S ribosomal RNA, partial sequence (LC133718.2)</td>
<td>99</td>
</tr>
<tr>
<td>5</td>
<td>D2</td>
<td>Methanogenic archaeon DCM1 16S ribosomal RNA gene, partial sequence (GQ339876.1)</td>
<td>98</td>
</tr>
<tr>
<td>6</td>
<td>D3</td>
<td><em>Proteus</em> sp. K107 16S ribosomal RNA gene, partial sequence (EU710747.1)</td>
<td>99</td>
</tr>
<tr>
<td>7</td>
<td>D4</td>
<td>Uncultured <em>Acetobacterium</em> sp.</td>
<td>99</td>
</tr>
<tr>
<td>8</td>
<td>D6</td>
<td><em>Pseudomonas</em> sp. strain TRB160 16S ribosomal RNA gene, partial sequence (KX981371.1)</td>
<td>94</td>
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<tr>
<td>9</td>
<td>D7</td>
<td>Uncultured <em>Arocobacter</em> sp. clone 63 16S ribosomal RNA gene, partial sequence (KJ862122.1)</td>
<td>90</td>
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<tr>
<td>10</td>
<td>D8</td>
<td><em>Wohlhafteniimonas</em> sp. strain 22912 16S ribosomal RNA gene, partial sequence (KY713623.1)</td>
<td>97</td>
</tr>
<tr>
<td>11</td>
<td>B1</td>
<td>Uncultured <em>Tissierella</em> bacterium isolate DGGE gel band C4 16S ribosomal RNA gene, partial sequence (MF071502.1)</td>
<td>95</td>
</tr>
<tr>
<td>12</td>
<td>B2</td>
<td><em>jeotgalibaca danhookeonis</em> strain EX – 07, complete genome (CP019728.1)</td>
<td>95</td>
</tr>
<tr>
<td>13</td>
<td>B3</td>
<td><em>Atopostipes</em> sp. strain ZH16 16S ribosomal RNA gene, partial sequence (MG752880.1)</td>
<td>93</td>
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<tr>
<td>14</td>
<td>B5</td>
<td>Uncultured <em>Vagococcus</em> sp. isolate DGGE gel band A6 16S ribosomal RNA gene, partial sequence (MF071498.1)</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>B6</td>
<td><em>Escherichia coli</em> strain MSV5 – 4a2 16S ribosomal RNA gene, partial sequence (MF661780.1)</td>
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<td>16</td>
<td>B7</td>
<td><em>Pseudomonas</em> sp. strain TRB175 16S ribosomal RNA gene, partial sequence (KX981386.1)</td>
<td>98</td>
</tr>
<tr>
<td>17</td>
<td>B8</td>
<td><em>Alcaligenes</em> sp. strain <em>aquatilis</em> 16S ribosomal RNA gene, partial sequence (KJ862122.1)</td>
<td>98</td>
</tr>
</tbody>
</table>

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recovery of nutrients (N and P) present in urine before entering the main wastewater stream and WWTPs (Maurer et al. 2006). The regulatory bodies have imposed strict regulations on the discharge of treated effluent in water bodies. Hence, the recovery of nutrients simplifies the processes in treatment plants by reducing the nutrients load and helps to
Table 4 | IMViC test results of three isolates of biofilm

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Indole</th>
<th>Methyl red</th>
<th>Voges-Proskauer</th>
<th>Citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

achieve better quality effluent. There are various techniques reported for the efficient treatment of urine, MFCs being the most promising one (Maurer et al. 2006).

In our work, by using a double-chamber MFC, it was demonstrated that certain anode respiring bacteria are capable of generating electricity by using urine as substrate for their metabolism. Such microorganisms belong to group of exoelectrogens (extracellular transfer of electrons). In MFC, these microorganisms adhere to the surface of the anode acting as electron acceptor. They further grow and divide to form biofilm. These bacterial species are capable of oxidizing the organic matter in urine and transferring the electrons (external electron transfer) to anode under anaerobic conditions. These electrons then move towards cathode through an external circuit and combine with protons. The electron transfer can be either by using direct (membrane cytochromes) or by indirect transfer mechanism (reduction on the cell surface and diffusion on anode), as reported earlier (Read et al. 2010). Hence, at anode, in case of urine, the urea present is oxidized to N2 and CO2, with release of electrons and protons. The cation exchange membrane placed in between the two chambers, leads to the influx of protons and NH4⁺-N (urea decomposition) to cathode chamber. Increase in pH of the catholyte is observed which is due to ammonia volatilization in the cathode chamber and the production of hydroxide ions from water due to oxygen reduction (Fornero et al. 2010).

Struvite precipitation is the most efficient method to recover N and P simultaneously. As struvite crystallization is directly influenced by pH, the cost associated in pH adjustment is a limiting factor in struvite precipitation. Previous work stated that more than 90% chemical costs are involved in optimizing the pH for struvite recovery (Le Corre et al. 2007). Urine is rich in N and P but deficient in Mg. Therefore, by adding Mg externally at alkaline pH (8–9) in the catholyte, N and P can be recovered as struvite. In the present study, the pH change in both anolyte and catholyte was observed during HRT optimization. The optimal HRT in the MFC to achieve maximum COD reduction (80 ± 5%) was found to be 22 h. The COD reduction observed was due to anodic oxidation of the organics. Simultaneous increase in the catholyte pH was also observed. The pH was 9.1 after 8 h. The pH achieved was optimal for precipitation, hence on addition of MgCl2, 90% Ortho-P was recovered as struvite. Struvite contains 5.7% N and 12.6% P (N: P ratio 1:2) (Etter et al. 2011). However, in the present study, we found a comparatively higher N: P ratio of 1.5 as observed from EDAX data (8.9% N and 26.4% P) and confirmed by the chemical analysis of struvite (N: P ratio 1.27), as mentioned earlier. This shows that MFC helps in enhancing the phosphorus precipitation. Therefore, for simultaneous COD removal and nutrient recovery from raw urine the minimum HRT was 22 h.

The concentration of organics affects the microbial diversity at anode and is critical for their electron transfer mechanisms. Therefore, the effect of COD on the performance of MFC was tested. Earlier it has been reported that low substrate concentration is required for the efficient growth of exoelectrogens (Sleutels et al. 2016), and hence the metabolic activity of these microbes slows down at very high substrate concentration. However, the electrochemical results obtained for undiluted urine (11.87 g/L COD) was similar to that obtained for diluted feed (2.4 g/L COD). From the observations, it can be inferred that the electron transfer mechanism of the exoelectrogens is not affected at high COD loading, supporting the direct use of undiluted urine as a feed to MFC. Also, significant recovery of nutrients (N and P) from undiluted urine can be achieved.

Table 5 | Power generation and reduction in different wastewater defining parameters in MFC with different inoculums

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Open circuit voltage (mV)</th>
<th>Power density (mW/m²)</th>
<th>Current density (mA/m²)</th>
<th>COD removal (%)</th>
<th>NH₄⁺-N reduction (%)</th>
<th>NO₃⁻ reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>356 ± 22</td>
<td>31.11 ± 1.22</td>
<td>193 ± 1.76</td>
<td>46 ± 4</td>
<td>24 ± 4</td>
<td>21 ± 3</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>405 ± 25</td>
<td>42.04 ± 1.63</td>
<td>270 ± 4.23</td>
<td>57 ± 5</td>
<td>18 ± 2</td>
<td>15 ± 4</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>455 ± 17</td>
<td>54.82 ± 1.17</td>
<td>312 ± 7.21</td>
<td>66 ± 9</td>
<td>35 ± 6</td>
<td>32 ± 5</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>482 ± 18</td>
<td>98.57 ± 2.13</td>
<td>347 ± 3.32</td>
<td>55 ± 4.28</td>
<td>15 ± 7</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>Mixed culture</td>
<td>645 ± 12</td>
<td>123.09 ± 1.87</td>
<td>450 ± 4.27</td>
<td>75 ± 2.42</td>
<td>57 ± 4</td>
<td>60 ± 6</td>
</tr>
</tbody>
</table>
which is considerably more than that recovered from diluted urine. Hence, integration of MFC directly to waterless urinals to treat urine by organic matter degradation and simultaneous recovery of N and P is possible. The resultant effluent with lower COD and other nutrients can then be discharged to WWTPs for treatment.

The operation of MFC depends on the microorganisms residing on the anode. The microbial community formed at anode surface, characterized by DGGE profiling, showed diversity in the 16S rDNA molecular fingerprinting of the samples. Variations were seen among the original inoculum (anaerobic digestate), the feed (undiluted urine) and the biofilm formed on the anode. The gene libraries of bacterial 16S rDNA were examined separately, to get better understanding of the microbial diversity. The initial inoculum showed more of Proteobacter species (Pseudomonas, E. coli and Proteus vulgaris) while the biofilm microbial community analysis showed that the Firmicutes (Tissierella, Atopostipes, Jeotgalibacca, Wohlhritimonas and Bacillus) was the most predominant phylum developed in the anodic biofilm although Proteobacter species were also present in less number. Among all the observed Firmicutes, Tissierella was found to be the dominant species in the biofilm analyzed. Tissierella is involved in creatinine degradation while Atopostipes is involved in carbohydrates fermentation present in urine (Barbosa et al. 2015). Firmicutes act as oxygen scavengers but shows limited power production when compared to Proteobacter species. Hence, the observations are significantly different from the Zhang et al.’s work showing that the anodic biofilm included mainly Proteobacter species (Zhang et al. 2016). The underlying reason for this change in microbial community (reduction in Proteobacter species) could be the concentration of NH$_4^+$-N in the anolyte (Zhang et al. 2016).

Based on DGGE profiling, E. coli, Bacillus subtilis, Pseudomonas aeruginosa and Proteus vulgaris were isolated from the biofilm to study their individual effect on MFC performance. Their role as exoelectrogens and their efficiency in electron transfer have been well-reported (Pham et al. 2008; Nimje et al. 2009).

MFC operated under similar conditions, produced low current when inoculated with individual isolate than mixed culture. Proteus vulgaris among the four isolated cultures generated the maximum $V_{oc}$ of 482 ± 18 mV. However, the experiment conducted with Pseudomonas aeruginosa gave more stable data. The $V_{oc}$ and power density output by Pseudomonas aeruginosa were 455 ± 17 mV and 54.82 ± 1.1 mW/m$^2$, respectively, which were lower when compared to the output of mixed culture (645 ± 12 mV), showing that the mixed culture is more electrochemically active and effective in the studied MFC. The energy output achieved is low compared to other MFCs previously studied, possibly due to high internal resistance offered by the membrane. Some earlier studies have mentioned that low power densities from pure cultures is directly associated with the type of substrate and electron transfer mechanism used by organisms (Pham et al. 2008). It was also observed that the $V_{oc}$ increases at the beginning of the experiment and attains the maximum value, after which there is decrease in both the voltage and current. This decrease was initially due to the time taken by the microbes to consume the organics and later due to low availability of the organic matter. Mixed cultures are generally preferred over pure isolates due to favorable biofilm formation. However, not all the microorganisms in biofilm are involved in power production creating a competition among exoelectrogens and non-exoelectrogens for substrate use and, hence, limit the power production. Therefore, single bacterial species are also valuable for MFC when the major application is power production (Kiely et al. 2010).

With respect to reduction in various wastewater parameters analyzed, the performance of mixed consortia to treat urine is better than isolated individual cultures. Also, only 5–10% reduction in Ortho-P was seen (utilized by bacteria for its growth) during all the MFC runs with individual microbial cultures and the mixed culture, implying that an almost similar amount of remaining phosphate is available for struvite precipitate, hence the amount of struvite precipitated was similar in all cases (12 ± 5 g/L). Based on all the results obtained with respect to urine treatment, COD reduction and nutrient recovery, mixed culture is preferred over pure cultures.

**CONCLUSION**

The results presented in this paper showed MFC can be used as a viable technology to recover nutrients from urine along with power generation and partial treatment. The high pH at cathode seems to be optimal for struvite recovery from urine. The results obtained with different dilutions showed that using raw urine (undiluted) is beneficial in terms of struvite precipitation as there is no precipitation observed when the experiment was conducted with diluted urine. Also, there is no negative effect of using raw urine on energy production and microbial community of biofilm. Although the percentage reduction in different wastewater defining parameters was comparatively more with mixed
culture than with individual cultures, the amount of struvite obtained was similar in both cases. Hence, use of mixed inoculum is preferred in running MFC. The reported work on MFC run with isolated pure cultures can assist to get an insight of organic matter degradation metabolic pathways followed by each culture. Further, individual isolates can be exploited for antibiotic degradation studies. This could be advantageous, as the treatment of urine in MFC inoculated with isolated and enriched culture would result in micro-pollutant free urine, which could then result in an uncontaminated struvite when precipitated. Therefore, the next study will be focused on using isolated cultures in MFC as a potential system for antibiotic/antibiotic metabolite degradation.

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