

Simultaneous Removal of Uranium (VI) and Nitrate in a Microbial Fuel Cell Cathode and Applicability on Real Nuclear Fuel Recycle Effluents

Nuclear wastes which are emerging from nuclear power plants, nuclear industries, uranium mining are generally rich in heavy metals particularly radionuclides like uranium [Lloyd and Macaskie, 2000]. Uranium is generally found in two states, one is soluble hexavalent state U (VI) and another one is insoluble tetravalent state U (IV). Uranium predominates in industrial effluents as a salt of U (VI) and can leach into ground water. The conventional methods for the uranium remediation from waste water such as chemical precipitation, liquid-liquid extraction, evaporation, membrane techniques, and ion exchange are highly expensive and not effective [Gavrilescu *et al.*, 2009]. Bioremediation of Uranium contaminated ground water is a propitious strategy for reduction of uranium in its insoluble form [William *et al.*, 2013]. A plethora of research papers have focused on in-situ removal of uranium by micro-organisms but no report of uranium removal by MFC has been published so far.

In this study, we have attempted U (VI) bioremediation at MFC cathode carrying out simultaneous nitrate and uranium removal. The simultaneous removal of uranium and nitrate was carried out using denitrifying microbial consortia in the presence of glycerol-3-phosphate as a carbon and phosphorous source in the cathode. The nitrate acted as terminal electron acceptor at the biocathode supporting power output from MFC. This process was accompanied with a release of phosphatase enzyme by the microbes which released phosphate from glycerol-3-phosphate for uranium precipitation. Also, microbial community prevailing in the cathode chamber was identified.

5.1 MFC OPERATION AND INOCULATION

Double chamber MFC was constructed using transparent poly-acrylic sheets. The MFC schematic is depicted in Figure 5.1. The volume of each chamber was 100 ml. Graphite felt (dimension- 4×3×0.58 cm, Nickunj Eximp Pvt. Ltd.) was used as a cathode and anode. Both chambers were physically separated by a proton exchange membrane (Nafion- 117). Anode and cathode were connected using copper wire (Gauge-2mm) and the circuit was operated under a fixed external resistance of 1000 Ohm. The anode and cathode consisted of synthetic wastewater. It contained (per liter: 4.4 g KH_2PO_4 , 3.4 g K_2HPO_4 , 2 g NaHCO_3 , 0.5 g NaCl , 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0146 g CaCl_2 , pH- 7.0). The initial nitrate nitrogen concentration was 680mg/l (5g/l KNO_3) in cathode. Cathode chamber was inoculated with denitrifying bacterial consortia originally obtained from cow manure and soil. To check for the presence of phosphatase activity, the microbial consortium was grown in synthetic wastewater with 10mM glycerol-3-phosphate (Sigma) as the sole carbon and phosphorous source. The consortium showed the phosphatase activity. Uranyl acetate at 200 μM served as the source of U (VI). Both the chambers were thoroughly flushed with oxygen-free nitrogen gas. Sodium acetate served as the anodic substrate. The anodic and cathodic reactions are depicted in Figure 5.2. The experiment involved two types of test conditions. In one of the condition, nitrate and U (VI) were added together (Test 1 MFC). In another, the cathode contained U (VI) without nitrate (Test 2 MFC).

This set up was initiated to see the effect of U (VI) alone on power generation. The control set up involved abiotic cathode (control MFC). The MFC's were operated at 31 °C in the fed-batch mode for up to 7 cycles wherein each cycle consisted of 5 days. After each cycle, the fresh substrate was supplemented to regain voltage and current. All the electrochemical and microbial community analysis was done in a 6th or 7th batch cycle.

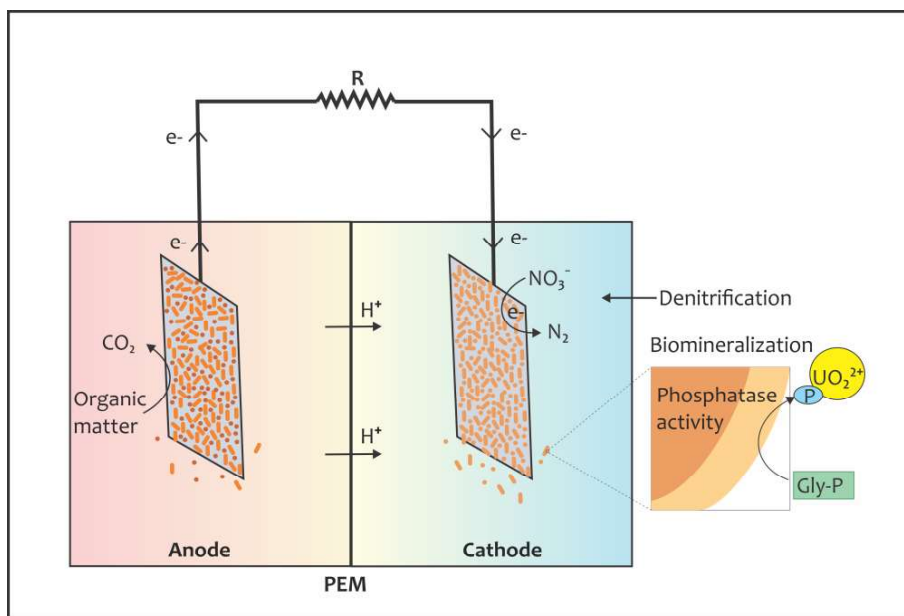


Figure 5.1: Schematic of MFC system used in the study

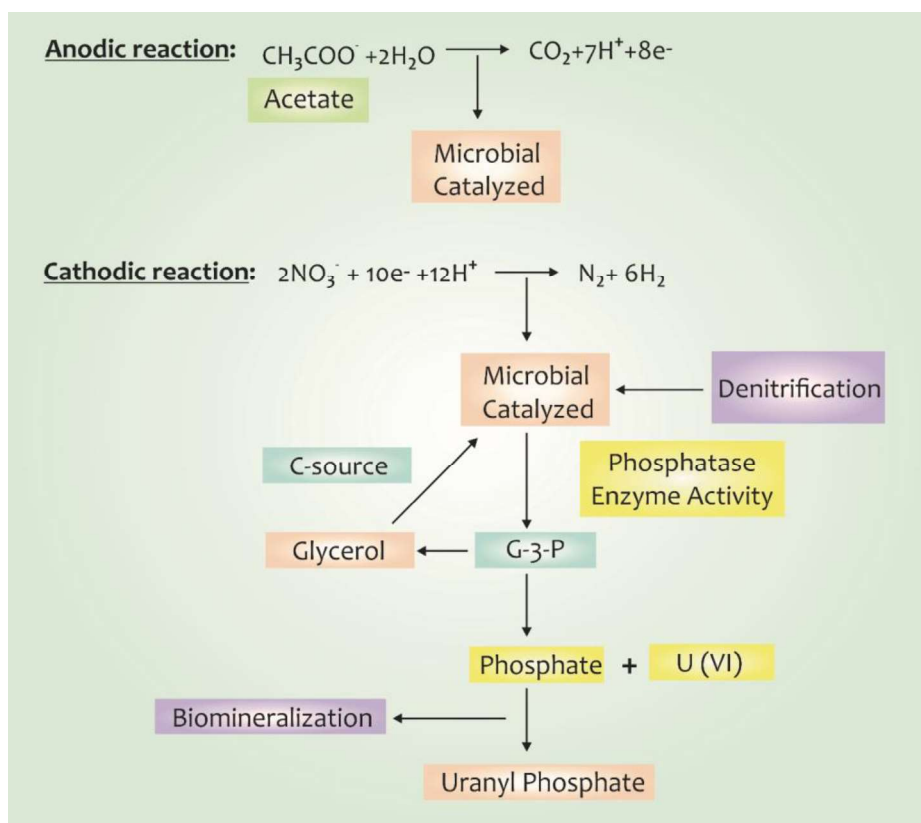


Figure 5.2: The mechanism of anodic and cathodic reactions in MFC

5.2 RESULTS AND DISCUSSION

5.2.1 Power and Voltage Generation in MFC

The MFC's were initially operated in open circuit mode for 4-5 days to allow the development of a potential difference between anode and cathode. Later, the MFC's were operated at an external load of $1000\ \Omega$. The voltage generation started in initial batch cycles. The differences in the voltage generation between Test and control were evident from initial batch cycles. The voltage values increased in the subsequent batch cycle due to the probable build-up of biofilm on the electrode surface. The peak voltage of 700mV and 65mV were observed in the eighth batch cycle in Test 1 and Test 2 MFC respectively. The low operating voltage in Test 2 MFC clearly indicated the high overpotential associated with U (VI) electroreduction. Test 2 also had low microbial growth linking the low rate U (VI) reduction with low microbial growth rate. A drop in voltage with each batch cycle was accompanied by medium replacement and nitrogen purging. The OCV value of 830 mV, 290mV, and 110mV was observed in Test 1, Test 2 and control MFC respectively. Polarization studies were performed on the MFCs operating in their last batch cycle wherein all the MFC's were exhibiting peak operating voltages and steady power output. Polarization results (Figure 5.3) were useful in depicting the peak power and current density obtainable in these conditions. In the Test 1 MFC, the highest power density of $2.91\ \text{W m}^{-3}$ and a current density of $29.7\ \text{A m}^{-3}$ were achieved, and in Test 2 MFC, the highest power density of $0.37\ \text{W m}^{-3}$ and a current density of $3.89\ \text{A m}^{-3}$ were observed. At $5\ \Omega$, the highest current density of $35.6\ \text{A m}^{-3}$ and $9.11\ \text{A m}^{-3}$ was achieved in Test 1 and Test 2 MFC respectively.

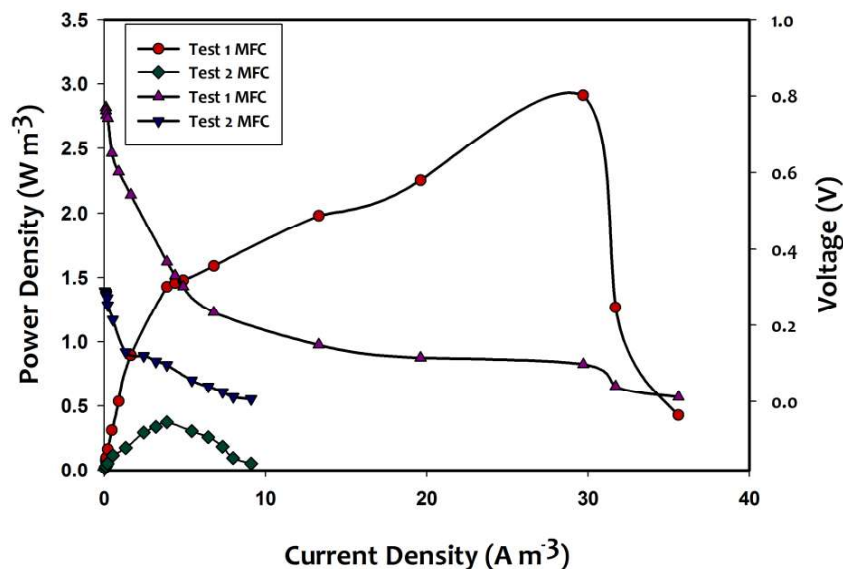


Figure 5.3: Polarization curve obtained for Test 1 and Test 2 MFC

5.2.2 Nitrate Nitrogen Removal Rate

The initial nitrate nitrogen concentration was 680mg/l (5g/l KNO₃). The profile of nitrate with time is shown in Figure 5.4. The control MFC with abiotic cathode did not show any nitrate removal which points to the fact that activation energy required to carry out electrochemical nitrate reduction is very high and a catalyst is required to mediate the nitrate reduction. In Test 1 MFC, the nitrate removal rate increased with the increasing batch cycle. This is associated with the biomass build-up and the higher magnitude of voltage and current supported during the later batch cycles. Also, Current flow through the electrode promotes biofilm formation [Gregoire *et al.*, 2014]. The nitrate removal rate was 0.130 NO₃⁻-N m⁻³ d⁻¹. A total of 96% removal was achieved at the completion of the batch cycle.

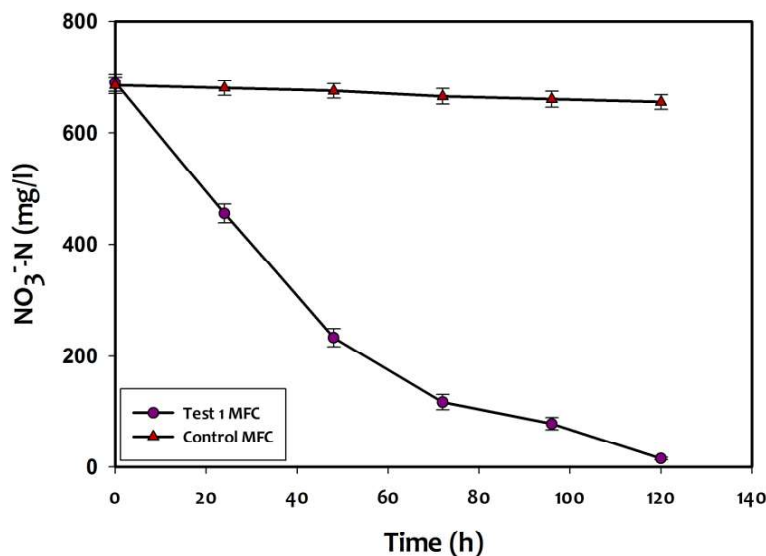


Figure 5.4: Profile of NO₃⁻-N (mg/l) concentration with time in Test 1 and Control MFC

5.2.3 Uranium Removal and Phosphatase Activity in MFC

The experiments were conducted to determine whether the consortium possess phosphatase activity and has a potential to bio precipitate soluble U (VI). Whole cell phosphatase activity in Test 1 and Test 2 MFC was 6.7 nmol min⁻¹ and 2.5 nmol min⁻¹ respectively. The profile of enzyme activity with time is shown in Figure 5.5. The maximum phosphatase activity was observed at 96 h in both the conditions (Figure 5.5). Test 1 conditions supported high phosphatase activity due to the presence of nitrate which supported the growth and metabolism of denitrifying bacteria.

U (VI) was added after 24 h of incubation at biocathode. The Uranium removal with time is depicted in Figure 5.6. It was found that only 32% uranium was removed in the first 24 h in Test 1 MFC. Uranium removal reached 90% at the end of 120h. Abiotic control did not support uranium removal. Thus, the removal of U (VI) cannot be attributed to electro adsorption or abiotic chemical interaction (Figure-5). A total of 40% U (VI) was removed at the end of the 120h in Test 2. The removal of U (VI) in Test 2 is most likely a bioreduction/biosorption process. While a removal of 40% was achieved, the reoxidation/desorption made U (VI) appear back in the catholyte just after air exposure. In test 1 MFC, XRD analysis confirmed the nature of precipitate as uranyl phosphate. The results obtained in this study were similar to the observations made by researchers in the previous studies. Beazley *et al* performed uranium biomineralization in the presence of high nitrate (15 mM) and found that 95% of U precipitated after 120h [Beazley *et al.*, 2009]. Similarly, in aerobic

conditions *Bacillus* and *Rahnella* spp. Y9-2 and Y9602 precipitated 73% and 95% of total soluble U (200 μ M) after 120 h [Beazley *et al.*, 2007].

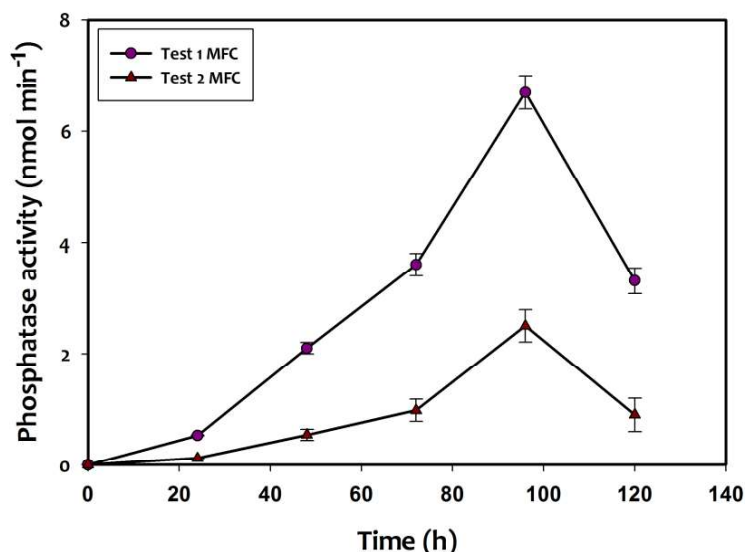


Figure 5.5: Phosphatase enzyme activity with time in cells amended with 10mM G3P in Test 1 and Test 2 MFC

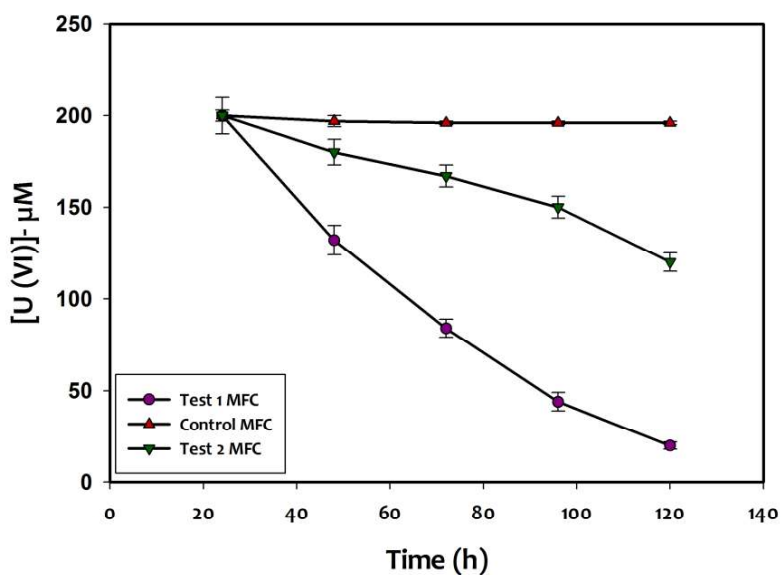


Figure 5.6: Profile of U (VI) concentration with time in Test 1 and Test 2 MFC

5.2.4 XRD Analysis

XRD analysis was used to determine the crystallinity and identification of U (VI) phosphate precipitates. Only precipitates produced in the Test 1 MFC generated a diffraction pattern characteristic of crystalline material. Samples from Test 2 and abiotic control did not produce any precipitate. The XRD spectrum of the Test 1 MFC precipitate confirmed the presence of uranyl phosphate species in the U (VI) oxidation state in the form of potassium uranyl phosphate [$K_4UO_2(PO_4)_2$]. (Figure 5.7) The three prominent peaks matched with the standard PDF 084-0225 (specific for uranyl phosphate).

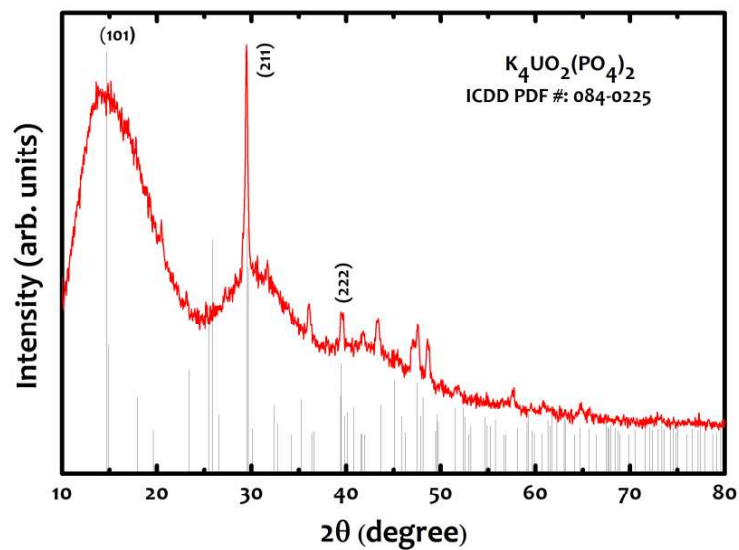


Figure 5.7: XRD analysis of precipitate generated during uranium biomineralization process

5.2.5 SEM and EDX Analysis

The biofilm morphology was seen on the electrode surface using SEM. Figure 5.8 shows the SEM micrographs. The bioprecipitate was also subjected to SEM analysis. The Energy Dispersive X-ray (EDX) spectrum displayed peaks attributed to phosphorous and uranium confirming the nature of the precipitate (Figure 5.9).

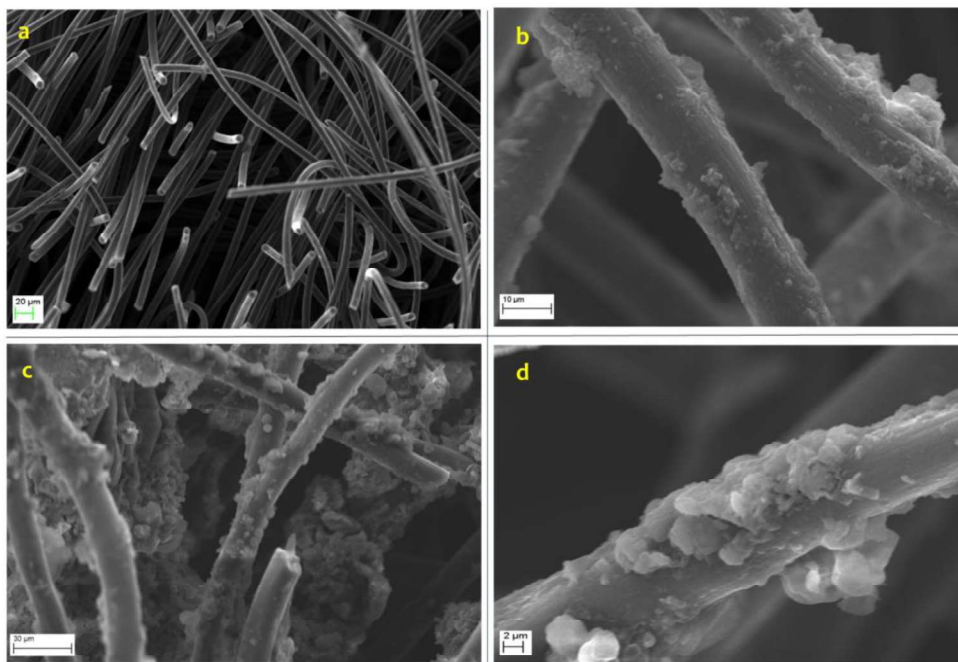


Figure 5.8: SEM analysis of graphite electrode biofilm

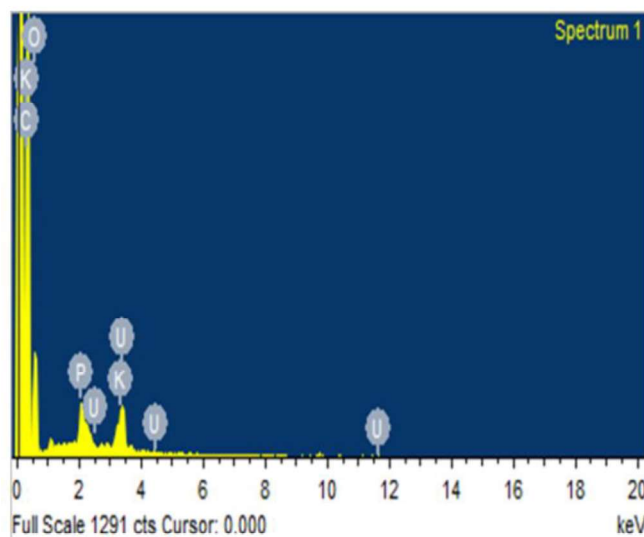


Figure 5.9: EDX analysis of precipitate formed after Uranium Biomineralization

5.2.6 Cyclic Voltammetry Analysis

Cyclic voltammetry (CV) was used to analyze the redox reactions occurring at the electrode surface. CV analysis was done during the last batch cycle where the highest operating voltage was observed. CV curve in all the three conditions is depicted in Figure 5.10. CV curves of Test and control MFC indicated the variation in the redox peaks. In Test 1 MFC the oxidative peak current value of 7.7mA was observed at -86mV whereas in the Test 2 MFC oxidative peak current value of 4.9mA at -138mV was observed. The high intensity oxidative peak which is observed in Test 1 MFC is completely absent in the Test 2 MFC. In Test 1 MFC, the two reductive peak current values of 6.5mA at -156mV and 7.5mA at -552mV was observed. On the other hand, in the Test 2 MFC reductive peak current value of 4.9mA at -72mV was observed. It is evident that in the Test 1 MFC high peak current value was observed in the CV curve. The differences in the peak current output between Test 1 MFC and Test 2 MFC are manifested in the form of differences in peak power outputs. Also, the peaks appear at different voltages, are indicative of different electroactive agents in Test 1 and Test 2. This is apparently due to the absence of nitrate in Test 2 condition.

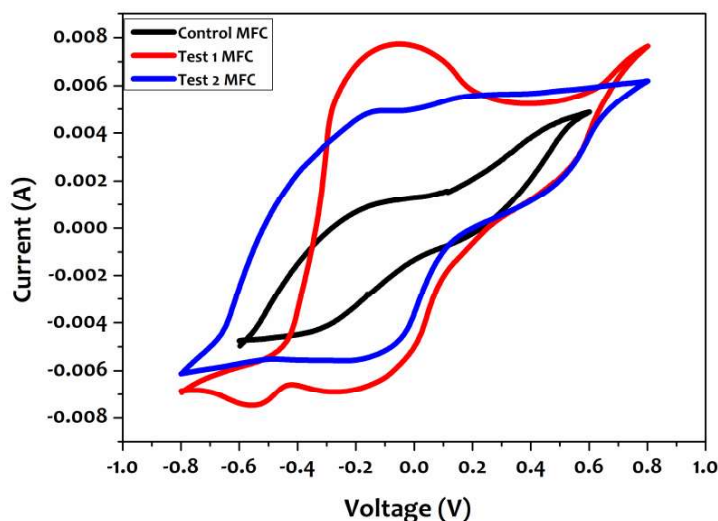


Figure 5.10: Cyclic voltammetry profile of Test 1, Test 2, and Control MFC

5.2.7 Richness and Diversity Analysis of Bacterial Community

Bacterial diversity analysis using V3-V4 region sequencing was performed to assess the communities prevailing in the biofilm for Test 1 MFC cathode. In total, 10, 90,106 raw reads were obtained for cathode sample. OTUs for cathodic biofilm sample were observed as 877. Diversity metric calculation and taxonomic assignments were done according to the OTU's distribution. It was observed that Shannon index value was 1.55 and chao 1 was 877. The value of both the Shannon index and chao1 is low, indicating the lower richness and diversity of the bacterial community. The lower diversity can be attributed to the presence of U (VI) which was possibly exerting an inhibitory effect on the microbial populations. Rarefaction curve was also plotted which shows the relationship between the number of species (OTU's) as a function of the number of sequences per samples. The OTU number increased with the increasing number of sequences initially and further it reached a plateau. This plateau indicated that the sequences representing the microbial community have been saturated and further sequencing can only reveal slightly more details on the microbial diversity.

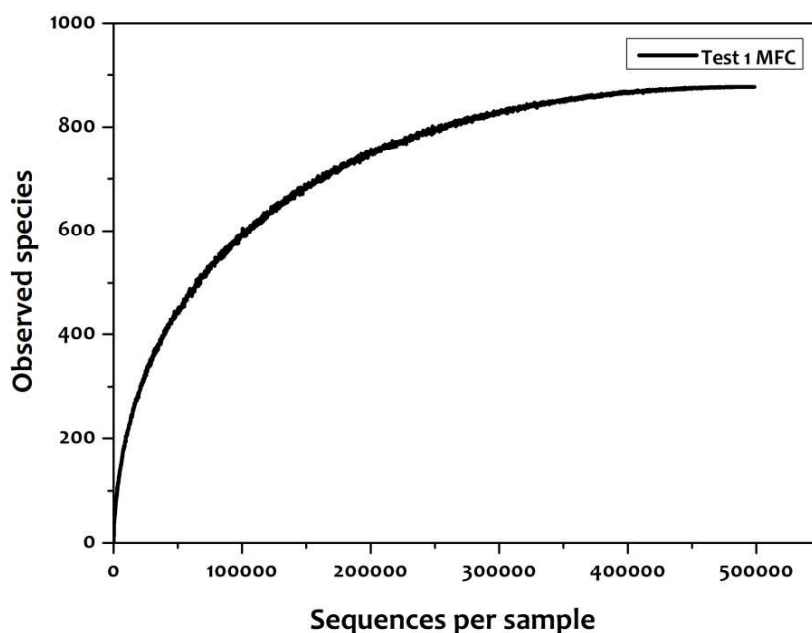


Figure 5.11: Rarefaction plot obtained for Test 1 MFC

5.2.8 Bacterial Community Analysis of Denitrifying Biocathode

Taxonomic distribution of samples was done from phylum level to genus level. The abundance at phylum, class and family level is depicted in Figure 5.12. At the phylum level taxonomic distribution, the relative abundance of Test 1 MFC was Proteobacteria (96.22%), Bacteroidetes (1.48%), Firmicutes (1.60%) and Synergistetes (0.25%), Actinobacteria (0.089%), and Tenericutes (0.069%). It is interesting to note that Proteobacteria, Bacteroidetes, and Firmicutes are the representative phylum of denitrifying bacteria [Demaneche *et al.*, 2009; Wrighton *et al.*, 2010]. Proteobacteria have been found to be abundant phylum in other denitrifying MFCs microbial community's too [Xiao *et al.*, 2015; Chen *et al.*, 2018]. The presence of Proteobacteria was found in the site contaminated with both uranium and nitrate [North *et al.*, 2004]. The predominance of this phylum was observed in the majority of the studies which investigated the microbial communities in the presence/exposure of uranium [Mondani *et al.*, 2011; Suriya *et al.*, 2017; Zeng *et al.*, 2018]. Similarly, Proteobacteria, Bacteroidetes, and Firmicutes were the major phyla found in the sediment sample (Cauvery River) contaminated with uranium [Suriya *et al.*, 2017]. Similarly, a study involving investigation of microbial community dynamics upon exposure to increasing uranium concentrations found that the abundance of Proteobacteria increased in the bacterial community of the granular sludge with

an increase in the uranium concentration [Zeng *et al.*, 2018]. In the present study, Proteobacterial predominance, growth, and production of phosphatase enzyme in the presence of U (VI) (while carrying out denitrification) are all observed together.

According to the taxonomic distribution at class level the relative abundance of Test 1 MFC is Gammaproteobacteria (85.93%), Alphaproteobacteria (6.66%), Betaproteobacteria (3.13%), Clostridia (1.54%), Bacteroidia (1.14%), Deltaproteobacteria (0.47%), and Synergistia (0.25%). The results are consistent with the previous studies reporting denitrification [Chen *et al.*, 2018] and the uranium presence [Mondani *et al.*, 2011; Suriya *et al.*, 2017].

According to the taxonomic distribution at family level the relative abundance of test 1 MFC is, Pseudomonadaceae (84.91%), Rhodobacteraceae (4.99%), Comamonadaceae (1.35%), Phyllobacteriaceae (0.90%), Tissierellaceae (0.77%), Porphyromonadaceae (0.76%), Aeromonadaceae (0.49%), Desulfobulbaceae (0.45%), Xanthomonadaceae (0.45%), Rhodospirillaceae (0.43%), Oxalobacteraceae (0.39%), Rhodocyclaceae (0.20%).

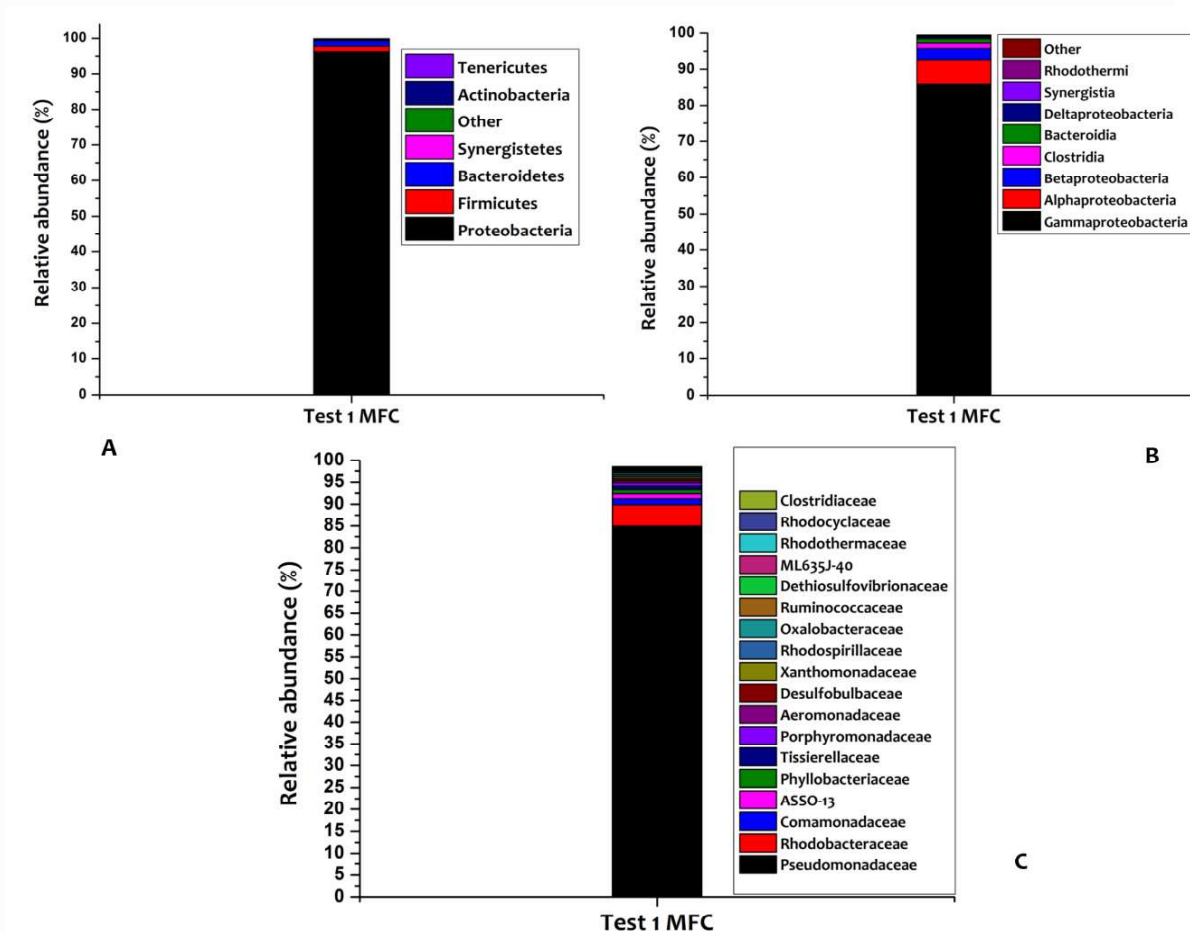


Figure 5.12: Bacterial community analysis of Test 1 MFC at (A) Phylum level (B) Class Level (C) Family Level

To gain the deeper insight, an OTU table is visualized as a heatmap where each row corresponds to particular taxonomy and each column corresponds to a sample (Figure-5.13). Amongst the abundant genera, *Pseudomonas* (84.60%) and *Paracoccus* (4.99%) were the most prominent. Zeng et al reported *Pseudomonas* as the predominant genus prevailing in the system treating uranium at 50 μ M [Zeng *et al.*, 2018].

The uranium biomineralization process was previously observed with *Pseudomonas* sp. J007 strain isolated from uranium mine water tank of Uranium Corporation of India Ltd., Jaduguda, India [Choudhary and Sar, 2011]. *Pseudomonas* sp. is also known to carry out U (VI) reduction [Akob *et al.*, 2008] and sequestration [Kazy *et al.*, 2009]. In addition, this species was detected in the soils contaminated with high uranium concentrations (Limousin, France) [Mondani *et al.*, 2011].

Considering the consistency obtained with previous studies and the additional diversity of bacteria obtained in this study, it can be stated that effective microbial consortium was developed from cow manure and soil. The consortium carried out denitrification, phosphatase production, and uranium precipitation while producing electrical energy in a MFC. The scope of improving the consortium exists. The microbial consortia from uranium and nitrate contaminated sites may show better diversity and enhanced removal rates.

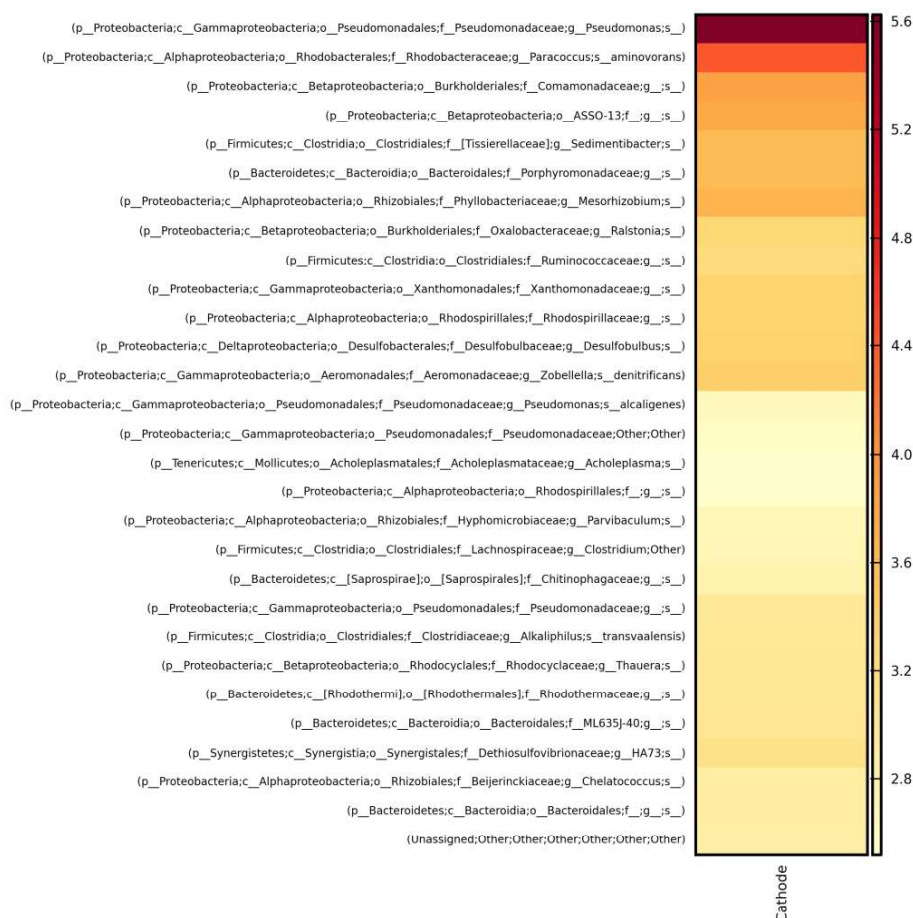


Figure 5.13: A heat map showing the taxonomy assignment for each OTU. [Intense colour (Red) represents OTU having relatively higher abundance whereas light color (yellow) represents OTUs having relatively lower abundance]

5.2.9 Testing the MFC Efficiency on the Real Effluents

In order to test the efficiency of MFC system on real effluents, the experiments were carried out at Bhabha Atomic Research Centre (BARC, Mumbai) at the Process Development Division (PSDD) facility. The MFC operation was done exactly as described above. Except that the cathodic medium contained low-level waste (LLW) obtained from BARC nuclear fuel recycle plant. Active waste was directly added into the cathode chamber without any modification. The experiments were performed in the active lab (lab handling radioactive wastes) (Figure-1). The MFC reactors were operated in quadruplets. The basic characterization of waste can be seen in Table 5.1. Cathode chamber was inoculated with acclimatized denitrifying microbial consortia. The initial nitrate concentration was 560mg/l in the LLW. The initial pH of LLW was 9. The MFCs were operated in the fed-batch mode. In the cathode chamber, 85±5% of nitrate was removed in 12h. The operating voltage of 390±10 mV was achieved at 1000Ω resistance. The power density of MFC was 1.092 Wm⁻³. All the results are summarized in Table 5.2.

Table-5.1: Characterization of low-level waste collected from BARC (The characterization data was provided by BARC Personnel)

Parameters	Low-Level Waste
pH	9
β activity (Bq/ml)	11.1
α activity (Bq/ml)	<0.02
TDS (ppm)	5.67
Nitrate (mg/l)	560

Table-5.2: Summary of the MFC performance

Parameters	MFC
Nitrate removal (%)	85±5
Operating voltage (mV)	390±10
Operating power density (Wm ⁻³)	1.092
Resistance used (Ω)	1000



Figure 5.14: MFC reactors in the active lab (Process Development Division)

5.3 CONCLUSIONS

The study describes the simultaneous removal of nitrate and uranium from simulated and real nuclear fuel recycles effluent in a MFC while generating power. The U (VI) removal is facilitated by the electrochemical reactions happening at MFC cathode. Also, a stable precipitate is formed. The process is promising as it is accompanied by power output. The study has been conducted at the scale of 100 ml and requires scaling up for large scale application. This would involve MFC design modification, continuous processing, and harvesting of the electrical energy produced during the process.

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