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Introduction

Water pollution is the main concern in the recent era due to industrialization and urbanization. It is estimated that approximately 1.1 billion people drink unsafe water globally [Rajasulochana and Preethy, 2016]. The different sources of water pollution include wastes, generated from domestic and industrial activities like nuclear operations. Nuclear wastes are among them. Nuclear wastes emerging from nuclear power plants, nuclear industries, and uranium mining are rich in heavy metals particularly radionuclides like uranium and have high level of nitrate [Lloyd and Macaskie, 2000]. Uranium exerts toxic effects to the environment. Nitrate is also a common contaminant present in wastewater interfering with the healthy functioning of the environment. World Health Organization (WHO) has set the permissible limit of nitrate and uranium at 50mg/l and 30ug/l respectively [WHO, 1996]. The major sources of nitrate pollution include industrial, agricultural activities, and nuclear wastes. The physicochemical methods adopted to treat these wastes are unsustainable from an economic and environmental point of view. Biological denitrification, on the other hand, is an economical and effective strategy to remove nitrate from waste water [Mohseni-Bandpi *et al.,* 2013]. Biological denitrification is catalyzed by anaerobic bacteria and is mediated by a set of four enzymes sequentially carrying out 5 electron reduction of nitrate to molecular nitrogen. While the process of conventional biological denitrification is actively utilized for the treatment of water contaminated with nitrate, the process can be performed in a Microbial Fuel Cell (MFC) with concomitant energy production. Similarly, U (VI) removal by biological methods is advantagous over physicochemical methods.

MFCs are emerging wastewater treatment systems [Logan *et al.,* 2006] and a source of renewable energy. MFCs can convert chemical energy stored in the wastes into the electrical energy. The bacteria as a catalysts carry out extracellular electron transfer (EET) in the MFC. These bacteria are called as exo-electrogenic bacteria [Logan, 2009]. MFCs utilize the redox potential differences between the electron donors and electron acceptors for the production of energy. Nitrate ion holds a high reduction potential (+0.74 V) and therefore supports significant redox potential difference and power output in a MFC [He and Angenent, 2006]. The process appears promising in contrast to the conventional activated sludge process as the energy recovered from MFC can offset the total energy requirements of the process.

Nitrate and uranium commonly co-exist in nuclear fuel recycle wastes [Finneran *et al.*, 2002]. The present work focuses on the treatment of wastewater contaminated with nitrate alone or nitrate + uranium (VI) in MFC with simultaneous power generation.

1.1 PURPOSE AND OBJECTIVES OF THE STUDY

A number of studies reported MFC mediated denitrification in the literature [Kelly and He, 2014]. The denitrification studies are done in two different conditions namely autotrophic (AD) and heterotrophic (HD). These two processes differ in the nature of electron donor for nitrate reduction [Park and Yoo, 2009]. HD always gives higher denitrification rate as compared to the AD as heterotrophic conditions support higher bacterial growth rate [Ghafari *et al.,* 2008]. Very few studies attempted HD in the MFC as it supports low power output. However,

wastewaters are heterogeneous and often contaminated with organic matter. Also, wastewater originating from nuclear fuel operations contains very high nitrate content. Therefore, MFCs capable of high rate denitrification is needed to avoid long operations times. Since HD supports high denitrification rate, the system becomes capable of treating nitrate wastewater co-contaminated with organic carbon. The power output is bound to be low in such conditions yet it should not be considerably less. All these requirements can be met to a very large extent by identifying the effective source of Microbial consortia. The microbes are the key players carrying out both nutrient removal and power output in a MFC.

Identifying the microbial populations prevailing in the active consortia is equally important to help understand the system and devise tunability procedures to treat water with variable C/N ratios. In addition, wastewater originating from nuclear fuel recycle operations is often contaminated by both nitrate and U (VI). Therefore, a MFC based process for simultaneous removal of the two contaminants is developed. Also, no report on MFC mediated U (VI) removal can be seen in literature.

Keeping all these points in consideration and after reviewing the state of the art, the following objectives are defined for this study:

1. Identification of the effective microbial consortia capable of high rate cathodic denitrification at MFC.
2. Testing the adaptability of the consortia under autotrophic and heterotrophic conditions while analyzing denitrification rate and electrochemical performance.
3. Identifying the electro-active denitrifying microbial populations under autotrophic and heterotrophic conditions.
4. Simultaneous removal of nitrate and U (VI) in simulated and real nuclear fuel recycle effluent and identification of microbial populations.

1.2 BRIEF RESULTS, SCOPE AND FUTURE PROSPECTS OF THE WORK

This thesis is divided into six chapters. The second chapter gives the detailed literature review on the nitrate contamination, denitrification process, MFC mechanism/applications, and U (VI) removal studies. The limitations and drawbacks of the existing procedures are also discussed. Next three chapters discuss the detailed experimental outcomes which are summarized as follows:

Microbial fuel cell assisted nitrate-nitrogen removal using cow manure and soil (Chapter 3: published as Vijay et al., 2016; *Environmental Science and Pollution Research*, 23; 7744–7756)

In this study, a high-rate denitrifying MFC was developed. The anode consisted of cow manure and fruit waste and the cathode consisted of cow manure and soil. The initial chemical oxygen demand (COD)/nitrate nitrogen (NO_3^--N) was varied from 2 to 40 at the cathode while keeping the anode ratio fixed at 100. NO_3^--N removal rate of 7.1 ± 0.9 kg NO_3^--N m^{-3} net cathodic compartment (NCC) day^{-1} was achieved at cathode COD/NO_3^--N ratio 7.31 with the current density of 190 ± 9.1 mA m^{-2} and power density of 31.92 ± 4 mW m^{-2} of electrode surface area. An open-circuit voltage (OCV) of 410 ± 20 mV was achieved at initial cathodic NO_3^--N of 0.345 g/l. The cathode COD/ NO_3^--N ratio had a significant influence on MFC's OCV and nitrate removal rate. Lower OCV (<150 mV) and NO_3^--N removal rates were observed at COD/ NO_3^--N ratio >12 and <7. Experiments done at different cathode pH values indicated that the optimum pH for denitrification was 7. The present study thus demonstrates the utility of MFCs for the treatment of high nitrate wastes.

Microbial community modulates electrochemical performance and denitrification rate in a biocathodic autotrophic and heterotrophic denitrifying microbial fuel cell (Chapter 4: published as Vijay et al., 2019; *Bioresource technology*, 272; 217-225)

A comparison of autotrophic (AD) and heterotrophic (HD) cathodic denitrification in a Microbial Fuel Cell (MFC) was made in this study. Denitrifying microbial consortia were developed from cow manure and soil and acclimatized under AD and HD conditions. The AD MFC supported the power output of 4.45 Wm^{-3} while removing nitrate nitrogen (NO_3-N) at the rate of 0.118 kg NO_3-Nm^{-3} d^{-1}. Significant power output (3.02 Wm^{-3}) and nitrate removal rate (2.06 kg NO_3-Nm^{-3} d^{-1}) were achieved in HD MFC. Further, 16S rDNA based community analysis revealed higher diversity in HD MFC. The genus *Thauera* and *Pseudomonas* were predominant in AD MFC while genus *Klebsiella* and *Alkaliphilus* were abundant in HD MFC. The abundance of the denitrifying genes namely *narG*, *nirS*, and *nosZ* were assessed with the help of quantitative PCR and presence of all the genes in both the conditions ensured the necessary molecular requirements for complete denitrification.

Simultaneous removal of uranium (VI) and nitrate in a microbial fuel cell cathode and applicability on real nuclear fuel recycle effluents

This study demonstrates the successful removal of nitrate and U (VI) using MFC for the first time in the literature. The denitrifying bacterial consortia developed from cow manure and soil produced phosphatase enzyme which catalyzed the controlled release of phosphate from organic compounds. In this case, nitrate acted as an electron acceptor at cathode thereby allowing simultaneous nitrate and U (VI) removal. Since the process was performed in the MFC; the remediation was accompanied by power output. Glycerol-3-phosphate was used as a carbon and phosphate source in the cathode. In this study, the power density of 2.91 Wm^{-3} and nitrate removal rate of 0.130 kg NO_3-N m^{-3} d^{-1} was achieved. Further, the bacterial consortia were able to remove 90% of U (VI) in biocathode. XRD analysis confirmed the presence of uranyl phosphate precipitating at the cathode. The process was applied to the effluents generated from BARC (Bhabha Atomic Research Center, Mumbai) nuclear fuel recycling process. The effluent was contaminated with nitrate at the concentration of 560mg/l. This led to nearly 85% nitrate removal accompanied with a power output of 1.092 Wm^{-3}

This study demonstrates the applicability of MFC for simultaneous nitrate and U (VI) removal while producing power. The process was carried out on a small scale and needs to be scaled up for large scale application. Also, the consortia can be improved further to enhance U (VI) tolerance followed by removal.

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