

Microbial Community Modulates Electrochemical Performance and Denitrification Rate in a Biocathodic Autotrophic and Heterotrophic Denitrifying Microbial Fuel Cell

Direct use of cow manure and soil at MFC cathode supported high nitrate removal rate as described in the previous chapter. Development of denitrifying microbial consortia from cow manure and soil was done in this study. The consortia were acclimatized under autotrophic and heterotrophic conditions. Heterotrophic denitrification at the cathode is rarely attempted and only a few reports exist in the literature. The presence of organic matter at cathode has been found to lower the power output [Vilar-Sanz *et al.*, 2013; Ding *et al.*, 2018]. Since the wastewater has variable characteristics and different C/N ratios, a robust MFC system should be able to treat water contaminated with both nitrate and organic matter while also not compromising too much on the power output. Since, the microbial consortia are the key players catalyzing oxidation and reduction reactions in MFC, search for better microbial communities must continue. Most of the studies use activated sludge as a source of microbial consortia and the active microbial populations in such consortia are prevailing based on the wastewater it has been treating and toxicants therein. The source of microbial consortia is very important when carrying out denitrification in MFC because the microbial populations also have to be electro-active. The soil is a rich source of electroactive microbes [Cercado *et al.*, 2013] and both soil and cow manure is rich in denitrifying bacterial populations [Enwall *et al.*, 2005].

This study was performed to compare nitrate removal rates and power output during denitrification of waste water with organic carbon (HD) and without organic carbon (AD), to check the adaptability of the microbial consortia and the changes in the microbial community in the two systems by 16S rDNA based metagenomic analysis and to analyze the abundance of *narG*, *nirS*, *nosZ* genes in the consortia growing under these two conditions.

4.1 MFC OPERATION AND INOCULATION

Double-chambered MFC was fabricated using transparent poly-acrylic sheets. Graphite felt (Nickunj Eximp Pvt. Ltd., dimensions- 3×4×0.58 cm) was used as anode and cathode electrode respectively. The distance between anode and cathode electrode was 2.5cm. The Nafion-117 membrane was used as a proton exchange membrane between the anode and cathode. A copper wire was used to connect the anode and cathode and a fixed external resistance of 100Ω was plugged between both of the electrodes. The volume of the total cathodic and anodic compartment was 100ml. The anodic inoculum was taken from previously operated MFC anode in laboratory originally inoculated from cow manure. The anodic medium contained (per liter) 4.4 g KH₂PO₄, 3.4 g K₂HPO₄, 0.5 g NaCl, 0.2 g MgSO₄·7H₂O, 0.0146 g CaCl₂, 0.4g NH₄Cl, pH- 7.0±0.2. Sodium acetate was added to a concentration of 3.5g/l which served as a sole electron donor for anodic oxidation. The initial influent COD of the anode was 2975 ±47 mg/l.

Cathode chamber was inoculated with acclimatized denitrifying microbial consortia from previously operating MFC reactors in the lab originally inoculated with cow manure and soil. The cathodic media contained (per liter) 4.4 g KH_2PO_4 , 3.4 g K_2HPO_4 , 0.5 g NaCl , 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0146 g CaCl_2 [Clauwaert *et al.*, 2007]. Sodium acetate (2.0g/l, COD/ NO_3^- -N ratio- 4.96) was supplemented at cathode for HD MFC and sodium bicarbonate (2.0 g/l) was added as a source of inorganic carbon in AD MFC. Potassium nitrate (2.5 g/l) was added in the cathode chamber of both AD MFC and HD MFC. The MFC's were operated in the fed-batch mode at 30 °C for up to 8 cycles wherein each cycle consisted of 4 days. The fresh substrate was supplemented after each cycle, and biochemical parameters were adjusted.

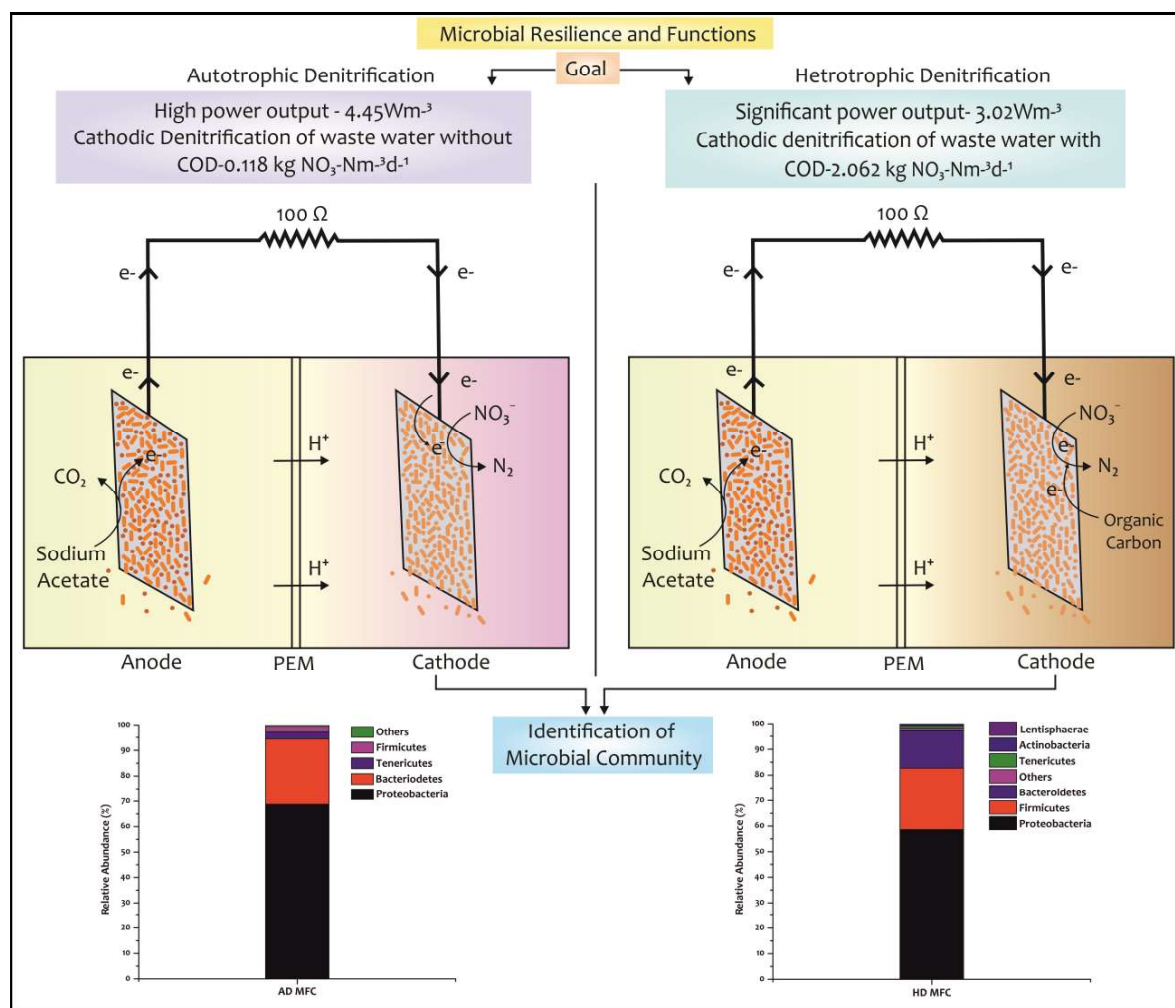


Figure 4.1 The schematic of MFC system used in the study

4.2 RESULTS AND DISCUSSION

4.2.1 Profile of Voltage with Time and Polarization Studies

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 100Ω . The voltage profile during eight consecutive batch cycles is shown in Figure 4.2. The differences in the AD and HD MFC experiments were evident right from the first batch cycle. The first batch cycle supported the operating voltage of 75 mV and 30

mV in the AD and HD MFC respectively. It should be noted that during the early cycles, the biomass (or catalyst) is building and electron donor substrate is channeled for biomass synthesis. The magnitude of voltage increased in the subsequent cycles with each feeding event. In the second batch cycle, the voltage increased up to 110mV and 70mV in the AD and HD MFC respectively. After 4 cycles of operation, the peak voltage values stabilized and sustained for a longer time as compared to previous batch cycles. The peak voltage of 585 mV and 310 mV were observed in the eighth batch cycle in the AD and HD MFC respectively (Figure 4.1). The lower operating voltage in HD MFC can be attributed to the presence of organic carbon which affects the open circuit potential of the cathode. These observations were in line with what was reported earlier [Ding *et al.*, 2018]. A drop in voltage with each batch cycle was accompanied by medium replacement and nitrogen purging.

Polarization studies were performed on the MFCs operating in last batch cycle wherein both the modes were exhibiting peak operating voltages and steady power output. Polarization results (Figure 4.3) were useful in depicting the peak power and current density obtainable in the two modes. In the AD MFC, the highest power density of 4.45 W m^{-3} and a current density of 40.5 A m^{-3} were achieved, and in HD MFC, the highest power density of 3.02 W m^{-3} and a current density of 29.7 A m^{-3} were observed. The highest power density in both the conditions was achieved at 100Ω resistance (Figure 4.3). At 5Ω , the highest current density of 43.4 A m^{-3} and 37.6 A m^{-3} was achieved in the AD and HD MFC respectively. It can be concluded that power density was 1.5 times higher in AD MFC as compared to HD MFC. The results of the comparative study are summarized in Table 4.1. Graphite felt was used in our study and the electrode material play a very important role in MFC performance. Ding *et al.* obtained a power output of 24 mW m^{-2} using carbon felt electrode in denitrifying MFC [Ding *et al.*, 2018]. A power density of 7 W m^{-3} was achieved using granular graphite in sandwiched denitrifying MFC [Al-Mamun *et al.*, 2016]. Therefore, the system can be further optimized by choosing electrodes which offer high reaction rates.

It is interesting to note here that while HD MFC supported lower power and current output than the AD MFC, the value was significant and competed with values obtained for AD MFC in literature. Oon *et al.* achieved a power density of 669 mW m^{-3} in a double-chambered microbial fuel cell using activated carbon flakes with $88 \pm 4\%$ of nitrate removal efficiency during cathodic AD [Oon *et al.*, 2016]. The reason for the significant power output in the HD MFC can be attributed to the microbial consortia prevailing in this system. Therefore, the effectiveness of the microbial consortia was evident and this necessitated the microbial community analysis and its comparison with the literature.

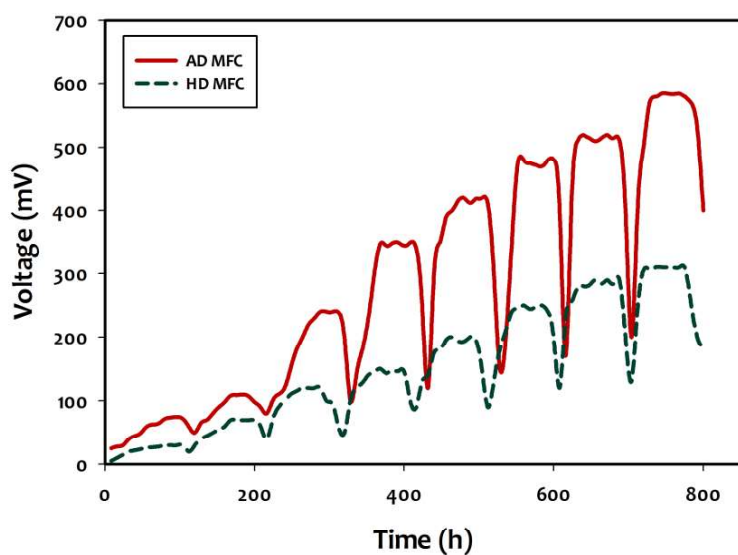


Figure 4.2: Voltage profile with time obtained for AD MFC and HD MFC

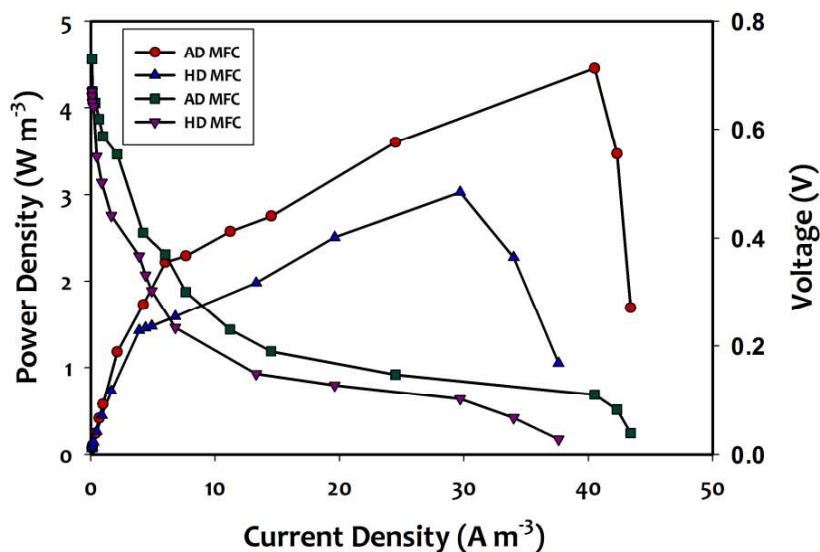


Figure 4.3: Polarization curve obtained for AD MFC and HD MFC

Table 4.1: Comparison between the performance of AD and HD MFC

Parameters	AD MFC	HD MFC
Carbon Source	Sodium bicarbonate	Sodium Acetate
Cathodic COD/ NO_3^- -N	NA	4.96
Open Circuit Voltage (mV)	810 $\pm$ 18	670 $\pm$ 16
Voltage at 100 Ω (mV)	585 $\pm$ 12	310 $\pm$ 9
Power Density (W m^{-3})	4.45 $\pm$ 0.42	3.02 $\pm$ 0.3
Current Density (A m^{-3})	43.4 $\pm$ 5	37.6 $\pm$ 4
Nitrate-nitrogen removal rate (kg NO_3^- -N $\text{m}^{-3} \text{d}^{-1}$)	0.118 $\pm$ 0.007	2.062 $\pm$ 0.5

4.2.2 Biocathodic Denitrification in AD MFC and HD MFC

The nitrate-nitrogen removal rate was influenced by the mode of microbial metabolism. The variation of nitrate concentration with time in both the conditions is shown in Figure 4.4. The nitrate removal rate was higher in HD MFC with a value of $2.062 \pm 0.15 \text{ kg NO}_3\text{-N m}^{-3} \text{ d}^{-1}$. This value was achieved during the eighth batch cycle. The values of nitrate removal rates were low during the initial batch cycles. But with the buildup of the biomass and the magnitude of voltage and current, the removal rates increased. Current flow through the electrode promotes biofilm formation [Gregoire *et al.*, 2014] and biofilm stratification is inevitable. With the presence of organic carbon in HD MFC, a synergy between electro-catalytic nitrate reduction and heterotrophic denitrification is achieved thereby giving high denitrification rates. The phenomenon of simultaneous autotrophic and heterotrophic denitrification cannot be ruled out in such a process.

In the AD MFC, nitrate removal rate was $0.118 \pm 0.010 \text{ kg NO}_3\text{-N m}^{-3} \text{ d}^{-1}$. In this mode, the nitrate removal rate increased with increasing cycles and raised from $0.06 \text{ kg NO}_3\text{-N m}^{-3} \text{ d}^{-1}$ to $0.118 \pm 0.010 \text{ kg NO}_3\text{-N m}^{-3} \text{ d}^{-1}$. The AD MFC and HD MFC supported 86.08% and 96% of total nitrate removal respectively. This was achieved at the initial nitrate loading of 2.5 g/l KNO_3 ($345 \text{ mg/l NO}_3\text{-N}$).

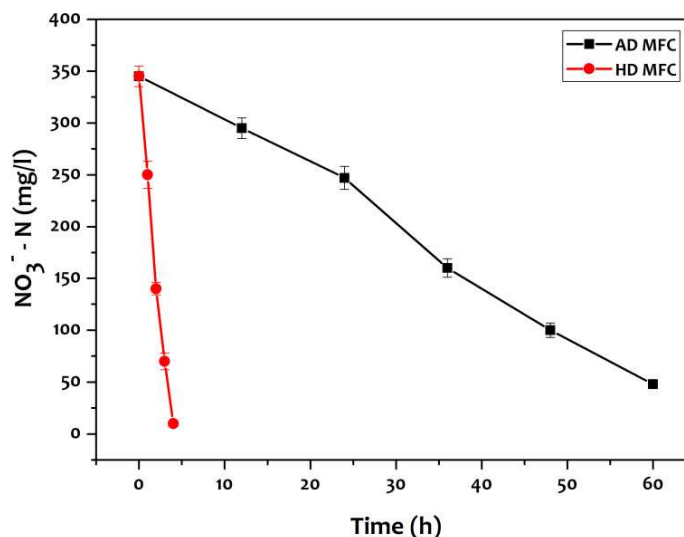


Figure 4.4: Profile of $\text{NO}_3\text{-N}$ (mg/l) concentration with time in AD MFC and HD MFC in the last batch cycle

4.2.3 Electrochemical Analysis

Cyclic voltammetry is an important technique to analyze the redox reaction occurring at the electrode surface [Rabaey *et al.*, 2004]. CV analysis was done during the last batch cycle of the experiment where the highest operating voltage was observed. CV analysis for both of the conditions is depicted in Figure 4.5. CV curve of both AD and HD MFC indicated variation in the redox peaks. In the AD MFC the oxidative peak current value of 6.7 mA was obtained at -140 mV whereas in the HD MFC, the oxidative peak current value was 5.1mA observed at -70 mV. In the AD MFC, the reductive peak current value of 5.6 mA was observed at -121 mV and in HD MFC the reductive peak current value of 5mA was observed at -182 mV. It is evident that autotrophic conditions supported high peak current values as observed in the CV curve. The same phenomenon was observed in an earlier study also [Ding *et al.*, 2018]. This demonstrates

that addition of organic carbon (electron donor substrate) in the HD MFC decreases the potential difference between the anode and cathode thereby lowering of the power output and current generation in MFC.

EIS analysis revealed that the total internal resistance of AD MFC was considerably lower than that of HD MFC (Figure 4.6). The Nyquist plots were obtained and the resistance values were obtained by fitting the equivalent circuit. The charge transfer resistance and ohmic resistance for AD MFC were $1.30\ \Omega$ and $0.961\ \Omega$ respectively. The HD MFC exhibited $3.23\ \Omega$ and $4.36\ \Omega$ of charge transfer and ohmic resistance respectively.

The chronoamperometry analysis showed a high current value at the initial phase which then decreased after a few seconds to a sustainable value (Figure 4.7). The stabilized current value (also called sustainable current) was 9 mA and 3.4 mA in AD MFC and HD MFC respectively. The results were consistent with the previously reported studies [Kondaveeti and Min, 2013].

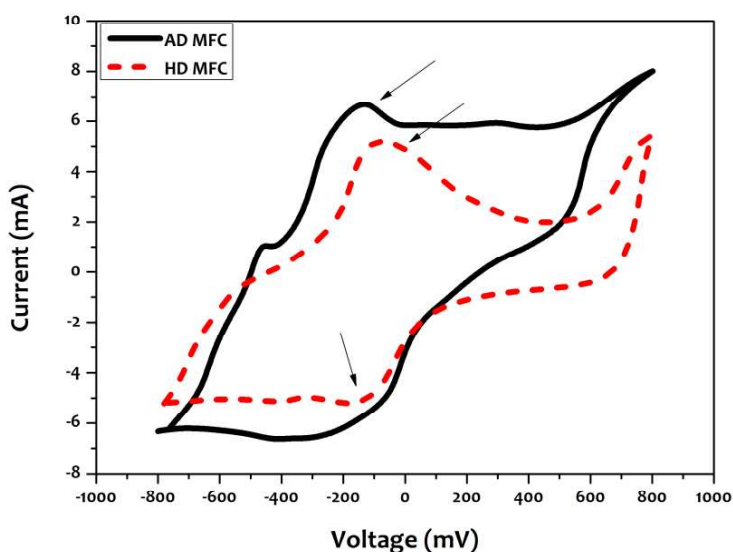


Figure 4.5: Cyclic Voltammetry profile of AD MFC and HD MFC (Arrows indicate the position of oxidative and reductive peaks)

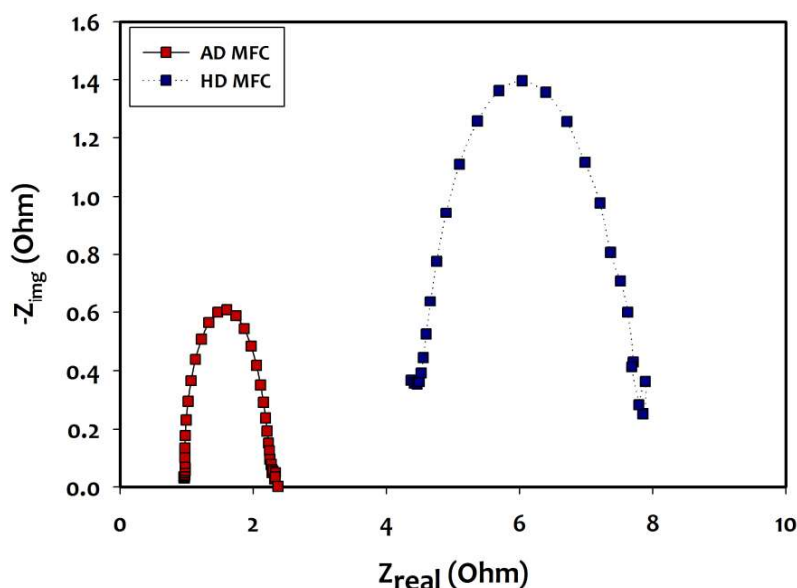


Figure 4.6: Nyquist plot obtained for AD MFC and HD MFC (No Warburg region was obtained)

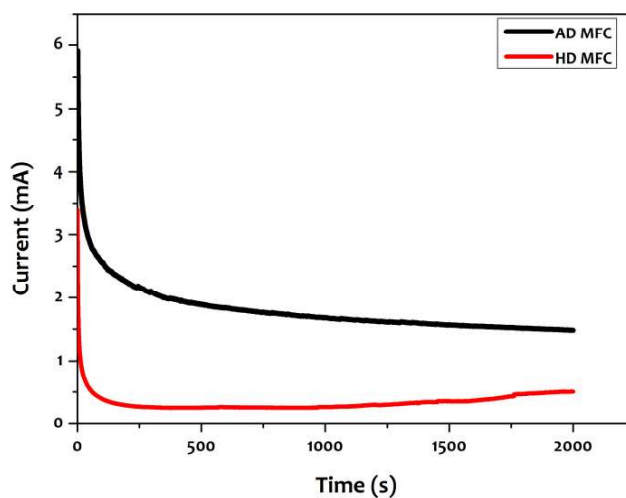


Figure 4.7: Chronoamperometric analysis of AD MFC and HD MFC at constant applied voltage

4.2.4 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 both AD and HD MFC. In total, 9, 37,712 and 8, 87,824 raw reads were obtained for autotrophic and heterotrophic samples. Different distribution of Operational Taxonomic units (OTUs) was observed for both the samples with autotrophic sample having 1083 and heterotrophic sample having 2365 OTUs. Diversity metric calculation and taxonomic assignments were done according to the OTU's distribution. It was observed that Shannon index value was 4.17 and chao 1 was 1083 for AD MFC and in HD MFC, Shannon index value was 6.15 and chao 1 value was 2365. In the HD MFC, the value of both the Shannon index and chao1 is high, indicating the higher richness and diversity of the bacterial community. Rarefaction curve was also plotted for both of the samples which shows the relationship between the number of species (OTU's) as a function of the number of sequences

per samples (Figure 4.8). The OTU number increased with the increasing number of sequences initially in both AD and HD MFC 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.

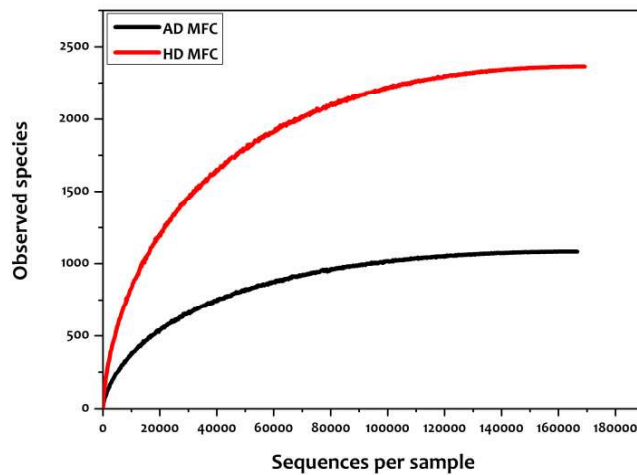


Figure 4.8: Rarefaction plots for AD MFC and HD MFC

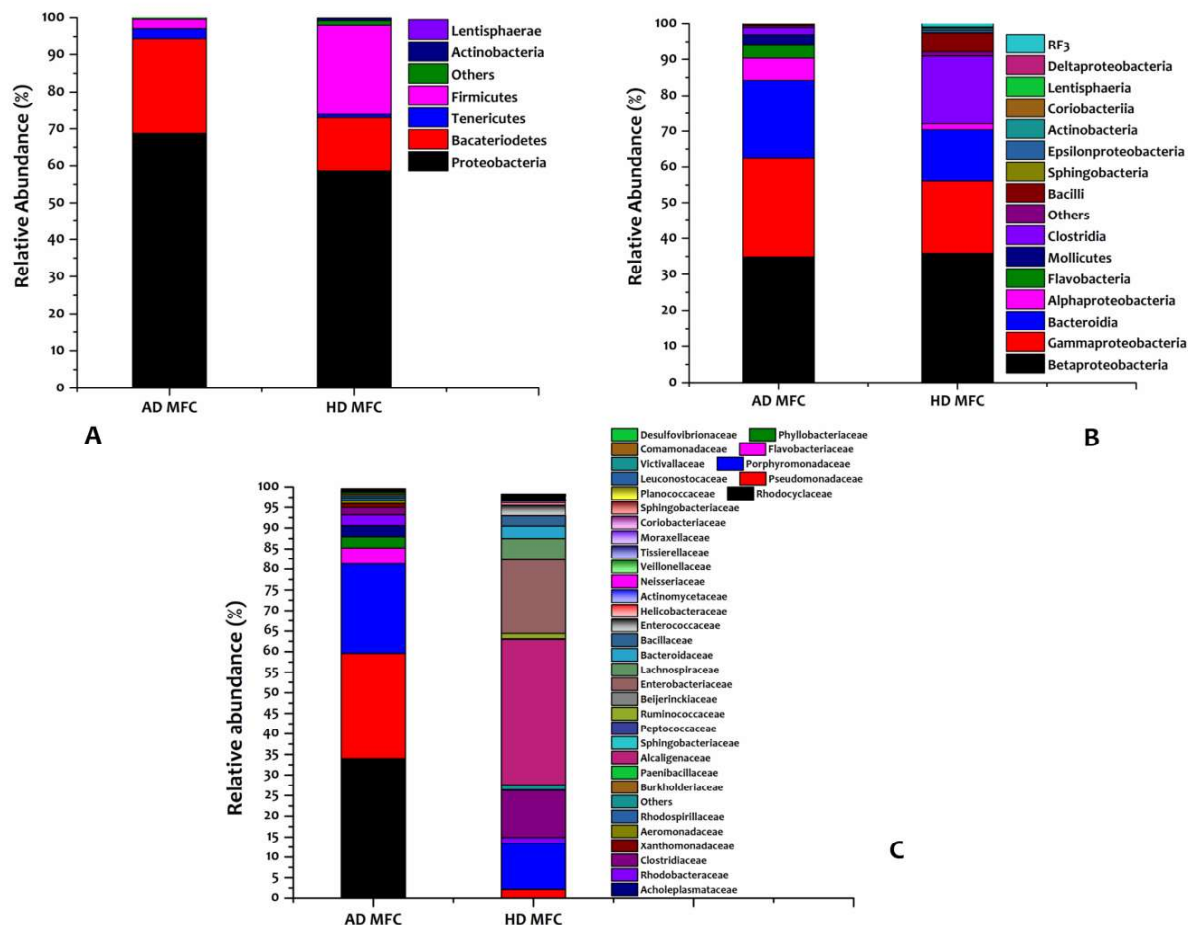


Figure 4.9: The relative abundance of microbial community in Autotrophic and Heterotrophic denitrification at (A) Phylum level, (B) class level, (C) Family Level

4.2.5 Microbial Community Analysis

Taxonomic distribution of samples was done from phylum level to genus level. The abundance at phylum, class and family level in both AD and HD conditions is depicted in Figure 4.9. At the phylum level taxonomic distribution, the relative abundance of AD MFC were Proteobacteria (68.67%), Bacteroidetes (25.70%), Tenericutes (2.70%) and Firmicutes (2.50%). The relative abundance in HD MFC were Proteobacteria (58.60%), Firmicutes (24.10%), Bacteroidetes (14.40%), Tenericutes (0.88%), and Actinobacteria (0.68%). The differences at the phylum level are quite significant in ADMFC and HDMFC. Reduction in the relative abundance of Proteobacteria in HDMFC can be attributed to the presence of organic matter which in turn lower the electrode potential and current. Bacteroidetes are also predominantly known for their autotrophic denitrification with few members capable of HD and their reduction in HD MFC is understandable in the context of their physiology. A marked increase in the Firmicutes from 2.50 % in ADMFC to 24.10% in HD MFC was noted since Firmicutes generally denitrifies heterotrophically [Xiao *et al.*, 2015]. The members of the phylum Tenericutes are also normally associated with the autotrophic denitrification processes which also explains their lowered abundance in HD MFC [Chen *et al.*, 2018]. The lower abundance of the phyla Actinobacteria is also in line with the observations made by previous researchers. According to the taxonomic distribution at class level, Betaproteobacteria (34.90%), Gammaproteobacteria (27.60%), Bacteroidia (21.70%), Alphaproteobacteria (6.10%) and Flavobacteria (3.90%), Mollicutes (2.70%), Clostridia (2%) were observed in AD MFC and Betaproteobacteria (35.90%), Gammaproteobacteria (20.20%), Clostridia (18.80%), Bacteroidia (14.30%), and Bacilli (5.30%), Alphaproteobacteria (1.7%) were observed in HD MFC. Betaproteobacteria was dominant in both the condition but the relative abundance was higher in HD as compared to the AD. It is interesting to note that Alphaproteobacteria have been reported to be sensitive towards current and potential therefore their lowered abundance in HD MFC can be attributed to relatively lower current and potential in HDMFC cathode. To gain the deeper insight into the differences between the two conditions the OTU table is visualized as a heatmap where each row corresponds to particular taxonomy and each column corresponds to a sample (Figure 4.10). The detailed study of bacteria involved in the AD and HD MFC and their role is described below:

In AD MFC, Proteobacteria turned out to be the predominant phylum and similar observations have been made earlier [Wrighton *et al.*, 2010]. The most abundant families in AD MFC were Rhodocyclaceae (34%), Pseudomonadaceae (25.60%), Porphyromonadaceae (21.70%), Flavobacteriaceae (3.90%), Phyllobacteriaceae (2.70%), Acholeplasmataceae (2.67%), Rhodobacteriaceae (2.60%). Amongst the abundant genera, *Pseudomonas* (24.90%) and *Thauera* (30.80%) were the most prominent. Genus *Thauera* is already known as an autotrophic denitrifier and is also associated with the electricity generation in the MFC studies [Xiao *et al.*, 2015; Yang *et al.*, 2018]. In this study, the presence of *Thauera* in high abundance can be correlated to the high power output in the ADMFC. *Pseudomonas stutzeri* is also identified as an important denitrifier as seen in the heat map and it is capable of carrying out both autotrophic and heterotrophic denitrification [Szekeres *et al.*, 2002]. *Pseudomonas stutzeri* was found to be the key electrotrophic denitrifier in the bio-cathode of the MFC [Xu *et al.*, 2017; Ding *et al.*, 2018]. In addition, Genus *Acholeplasma* (2.70%) observed in this study is also known for the electrotrophic denitrification in dual-chambered MFC [Ding *et al.*, 2017]. Further *Paracoccus aminovorans* (2.60%) and *Alkaliphilus transvaalensis* (1.80%) were also observed in the ADMFC. It can be concluded that the consortia in the autotrophic mode comprised of the well-known denitrifiers and electroactive microbes. The best of all the known autotrophic denitrifiers were present in this consortia and the synergy between them was evident in terms of MFC performance.

In HD MFC, the most abundant families included Alcaligenaceae (35.40%), Enterobacteriaceae (17.90%), Clostridiaceae (11.40%), Porphyromonadaceae (11.30%), Lachnospiraceae (5.20%), Bacteroidaceae (3.0%), Bacilliaceae (2.50%), Enterococcaceae (2.50%), Pseudomonadaceae (2.10%). Heat map of HD MFC is also showing the most abundant bacteria

present in the community. Alcaligenaceae was the most abundant family and is known to cover diverse heterotrophic denitrifiers [Sotres *et al.*, 2016]. Similarly, the second most abundant family Enterobacteriaceae is also known for its nitrate removal ability in the presence of organic carbon such as glucose and acetate [Srinandan *et al.*, 2012]. Adav *et al.* found Porphyromonadaceae and Alcaligenaceae in the denitrifying sludge growing on acetate [Adav *et al.*, 2010]. Also, the bacteria *Alcaligenes faecalis* has been shown to accept electrons from the electrode and carry out nitrate reduction in a MFC [Wang *et al.*, 2015]. The enhanced nitrate removal rate and simultaneous power output observed in the HD MFC can be attributed to the presence of this bacteria. At the genus level, *Klebsiella* (10.40%) was abundant and its presence in activated sludge during heterotrophic denitrification has been reported earlier [Drysedale *et al.*, 1999]. *Klebsiella* sp. has also been found to carry out simultaneous heterotrophic nitrification and aerobic denitrification (SND) process for high rate nitrogen removal [Padhi *et al.*, 2013]. Also, this bacteria has been identified as an electrochemically active bacterium which can contribute to the electricity production in the MFC system [Zhang *et al.*, 2008]. The bacteria *Alkaliphilus transvaalensis* (8.70%) was also abundant in this study. This bacterium is an alkaliphilic anaerobic chemoorganotroph capable of growing on acetate [Takai *et al.*, 2001]. *Bacteroides* (3.0%) and *Parabactroides* (2.90%) were the other two important genera which were observed in HD MFC. *Bacteroides* have been found to denitrify heterotrophically with ethanol as the electron donor by previous researchers [Gusmao *et al.*, 2006].

Additionally, the significant abundance of *Pseudomonas stutzeri* (2.0%) was also observed in the HD MFC as visualized in the heatmap. This bacteria was identified as a heterotrophic denitrifier capable of carrying out complete denitrification [Drysedale *et al.*, 1999]. Similarly, *Coprococcus* (3.20%) is generally found in the cattle manure [Kim *et al.*, 2014] but has not been reported as a denitrifier or exoelectrogen. However, their significant numbers indicate important role these bacteria must be playing in the consortia. Considering the diversity of bacteria obtained in HD MFC, it can be stated that effective microbial consortia were developed from cow manure and soil. While the reports of individual genera, family or class as denitrifiers are reported in the literature, the consortia developed in this study brought together the best of the denitrifiers enabling an efficient process for nitrate removal and power generation.

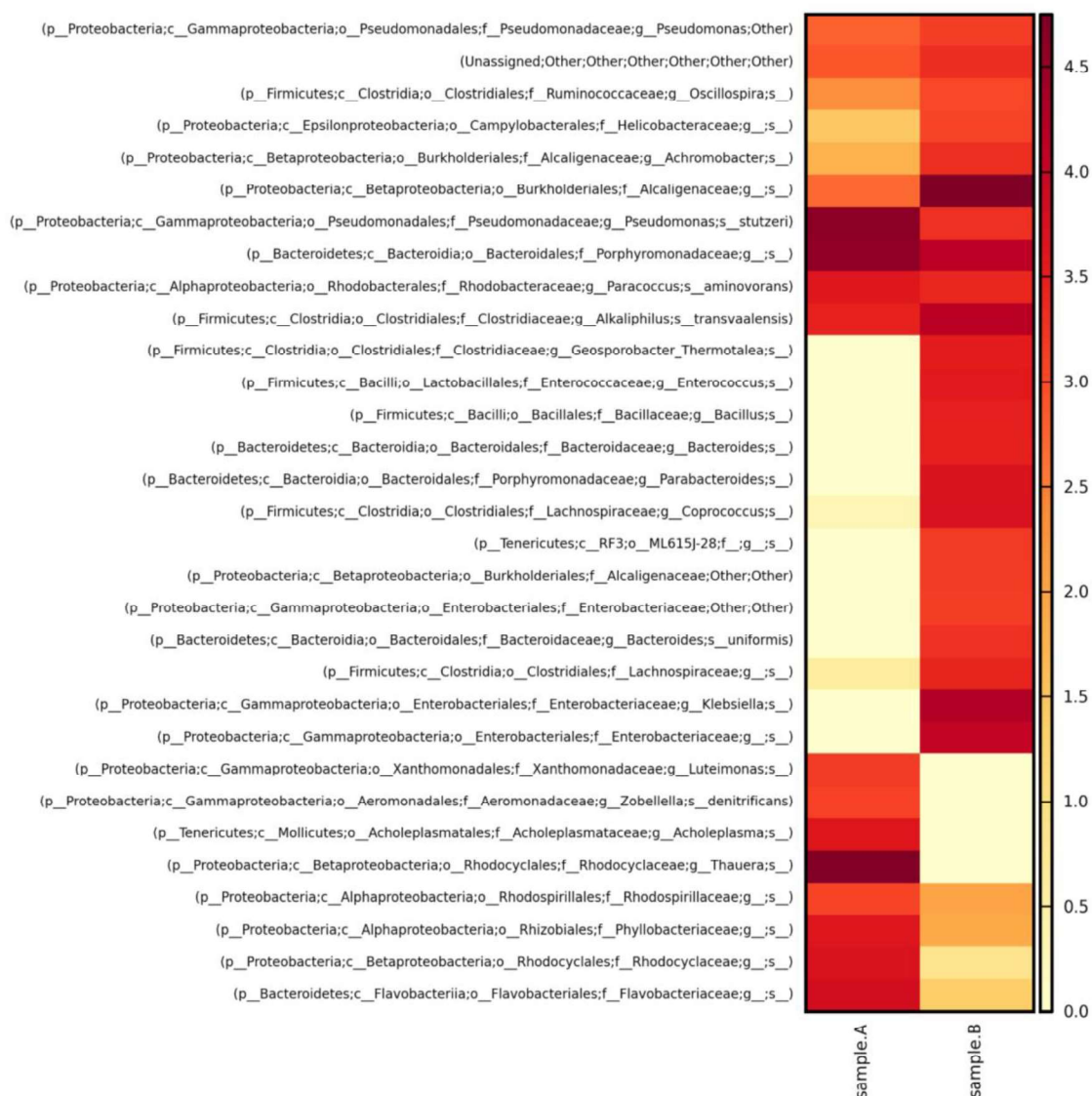


Figure 4.10: A heat map showing the taxonomy assignment for each OTU. [Intense colour (Red) represents OTU having relatively higher abundance whereas light color (yellow) represents OTUs having relatively lower abundance. The OTUs (rows) were log transformed. (Sample A– AD MFC, Sample B– HD MFC)]

4.2.6 Quantification of Denitrifying Genes

The enzymatic pathway used by denitrifying bacteria in order to carry out denitrification is comprised of four steps catalyzed by nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase. These four genes are the key molecular markers for denitrification pathway [Philippot, 2002]. Among all these genes, nitrous oxide reductase (*nosZ*) plays a very important role because this is the enzyme which is responsible for converting dinitrous oxide into N_2 gas. Nitrous oxide is a potent greenhouse gas and its accumulation is harmful. A successful denitrification process, therefore, ensures complete reduction of nitrate to molecular nitrogen. Identification of molecular markers is, therefore, an important step towards ensuring the successful denitrification process. The agarose gel image of the amplicons of denitrifying genes from the AD and HD consortia can be seen in Figure 4.11.

The 16S rDNA gene quantification was done first as this gene is possessed by all the bacteria and this was expected to exhibit the highest copy number. The abundance of 16S rDNA gene was 1.74×10^8 and 2.6×10^8 copies/ng of DNA for the AD and HD MFC respectively. Gene copy number associated with all the genes are summarized in Table 4.2. The abundance of the *narG* gene was 1.12×10^7 and 1.6×10^7 copies/ng of DNA in the AD and HD MFC respectively. The abundance of the *narG* gene was the highest among all three denitrifying genes in both the conditions. The abundance of *nirS* gene was 2.5×10^5 and 4.95×10^5 copies/ng of DNA in the AD and HD MFC respectively. The abundance of the *nosZ* gene was 1.02×10^5 and 2.1×10^5 copies/ng of DNA in the AD and HD MFC respectively. The abundance of the *nosZ* gene was less as compared to other genes in both the conditions. However, the presence of all three genes ensured that the complete denitrification is achievable in this system. The relative gene abundance (ratio to 16s rDNA) was also calculated (Table 3). The ratio of *narG*/16s rDNA was the highest among all the gene ratios and ratio of *nosZ*/16s rDNA was the lowest in both the conditions. Similar results were obtained in previous studies [Kandeler *et al.*, 2006]. Further, the ratio of *nirS*/16S rDNA and *nosZ*/16S rDNA was higher in HD MFC as compared to ADMFC. The abundant genera in AD MFC such as *Pseudomonas stutzeri* is known to possess all the denitrifying genes [Throback *et al.*, 2004]. Similarly, the other abundant genera *Thauera* has been reported for the presence of the *nirS* gene [Etchebehere and Tiedje, 2005]. In HD MFC, *Klebsiella* and *Alcaligenes* were the most abundant genera and they too are known to possess all denitrifying genes [Pal *et al.*, 2015; Throback *et al.*, 2004]. The results of RT PCR, therefore, can be understandably correlated with the bacteria present in an AD and HD MFC.

The observed functional gene ratios are in accordance with previously reported studies [Henry *et al.*, 2006]. The high abundance of all genes in HD MFC correlated well with the high nitrate removal rate achieved in this mode. Determining the presence of the genes and their copy numbers is an important aspect of any study as it implies the molecular level confirmation of the proposed process.

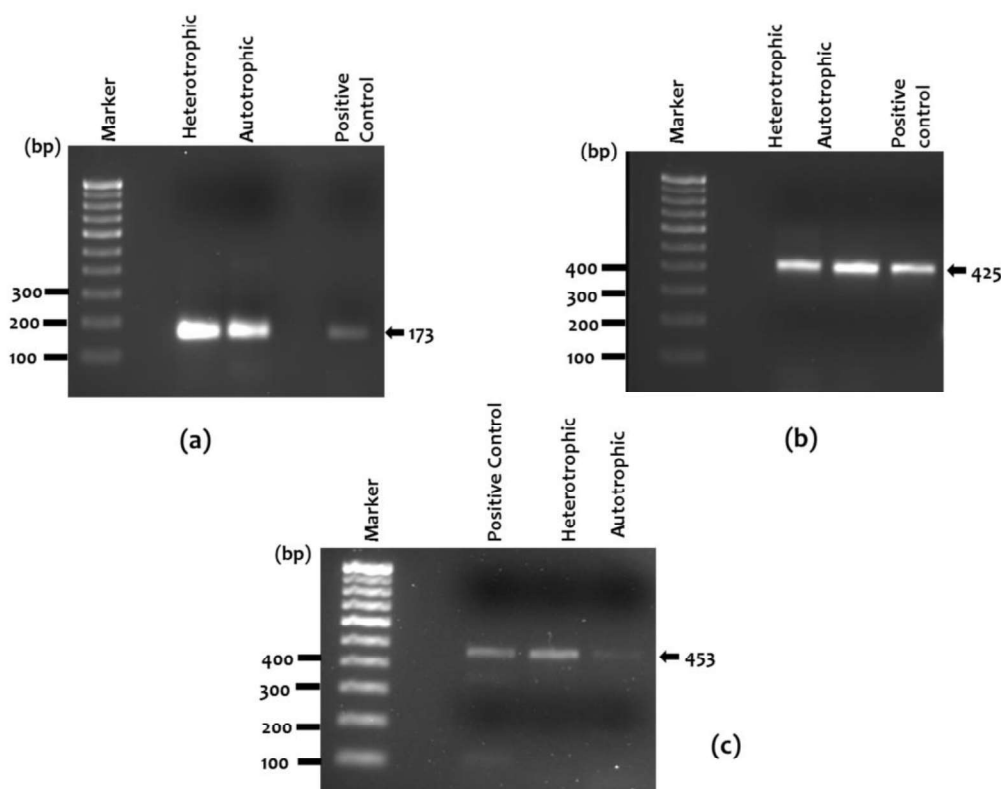


Figure 4.11: Amplicons obtained for *narG* (a), *nirS* (b) and *nosZ* (c) genes

Table 4.2: Copy numbers of the 16SrDNA, *narG*, *nirS*, and *nosZ* genes in autotrophic and heterotrophic denitrification conditions

Gene copy numbers and Functional Gene/16S rDNA ratio	AD MFC	HD MFC
16S rDNA (gene copies/ng of DNA)	1.74×10^8	2.6×10^8
<i>narG</i> (gene copies/ng of DNA)	1.12×10^7	1.6×10^7
<i>nirS</i> (gene copies/ng of DNA)	2.5×10^5	4.95×10^5
<i>nosZ</i> (gene copies/ng of DNA)	1.02×10^5	2.1×10^5
<i>narG</i> /16S rDNA ($\times 100$)	6.4	6.03
<i>nirS</i> /16S rDNA ($\times 100$)	0.14	0.19
<i>nosZ</i> /16S rDNA ($\times 100$)	0.058	0.081

4.3 CONCLUSIONS

The study demonstrates that effective microbial consortia are obtainable from cow manure and soil. The HDMFC supported higher microbial diversity and higher abundance of denitrifying genes. The presence of common species in both conditions indicated microbial resilience. The microbial consortia were found to adapt itself based on the availability or non-availability of organic carbon supporting power output and nitrate removal in both conditions. The system is thus tunable based on the wastewater characteristics with an AD supporting high power and HD supporting high nitrate removal. Further research is needed to increase process efficiency by optimizing MFC configuration for continuous operation.

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