Development of an electroactive aerobic biocathode for microbial fuel cell applications

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Summary

Microbial biocathodes are gaining interest due to their low cost, environmental friendliness, and sustainable nature. In this study, a microbial consortium was enriched from activated sludge obtained from a common textile effluent treatment plant in the absence of organic carbon source to produce an electroactive biofilm. Chronoamperometry method of enrichment was carried out for over 70 days to select for electroactive bacteria that could be used as a cathode catalyst in microbial fuel cells (MFC). The resultant biofilm produced an average peak current of -0.7 mA during the enrichment and produced a maximum power density of 64.6±3.5 mW m^{-2} compared to platinum (72.7±1.2 mW m^{-2}) in a *Shewanella*-based MFC. Microbial community analysis of the initial sludge sample and enriched samples, based on 16S rRNA gene sequencing, revealed the selection of chemolithotrophs with the most dominant phylum being Bacteriodetes, Proteobacteria, Firmicutes, Actinobacteria and Acidobacteria in the enriched samples. A variety of CO_2 fixing and nitrate reducing bacteria were present in the resultant biofilm on the cathode. This study suggests that microbial consortia are capable of replacing expensive platinum as a cathode catalyst in MFCs.

Keywords: Microbial fuel cell, biocathode, electroactive bacteria, community analysis, 16s rRNA.

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1. Introduction

To make microbial fuel cell technology cost-effective, there is currently a lot of research to develop microbial communities from various habitats that can form biofilms and can accept electrons from electrodes. This would eliminate the need to use large quantities of ferricyanide and noble metals as cathode catalysts. The bacteria in the mixed community are usually identified through metagenomic analysis by 16S rRNA sequencing and they mainly belong to the phyla Proteobacteria, Firmicutes, Bacteroidetes, Planctomycetes etc.(Milner *et al.*, 2016; Lam *et al.*, 2019; Zafar *et al.*, 2019). The enrichment of electroactive bacteria takes place either in a three-electrode system known as a half-cell (electron supplied externally) or a MFC (electron supplied from the anode). In previous studies, Zhang *et al.*, 2012 developed a new biocathode from dairy manure waste to produce a maximum power density of 15.1 W m^{-3}. Microbial community analysis revealed presence of species from *Alcaligenaceae* family (38.3%), followed by *Xanthomonadaceae* (6.0%), *Brucellaceae* (5.1%), *Bradyrhizobiaceae* (4.2%), *Enterobacteriaceae* (4.0%) etc (Zhang *et al.*, 2012).

Rabaey and co-workers compared the efficiency of mixed microbial population with that of individual isolates for reducing oxygen in a MFC. The biofilm was obtained from a mixture of samples from a river, pond and an activated sludge plant after 212 days of incubation in MFC conditions. These biocathodes produced a maximum power density of 303 mW m^{-2}. Analysis of the cathode microbial community revealed *Sphingobacterium, Acinetobacter and Acidovorax* sp. as dominant species. These isolates, tested individually as cathode catalysts, obtained maximum power of only 49 mW m^{-2}. Therefore, mixed populations seemed to produce significantly higher power than pure cultures due to the population density and the co-metabolic activity (Rabaey *et al.*, 2008).

Although the start-up time is quite long compared to chemical or enzymatic catalysts, microbial cathodes can be regenerated and are suitable for long term usage. In a MFC, biocathodes enriched from a sewage treatment plant produced a power density of 62 μW cm^{-2} which was comparable to platinum at 70 μW cm^{-2} (Milner *et al.*, 2016). A decrease in activation overpotential was observed when the biocathodes were used in an MFC, suggesting that the bacteria act as true catalysts for cathodic reaction (Rabaey *et al.*, 2008). The source of inoculum, the enrichment method (half-cell, MFC) and the applied potential used in a bio-electrochemical system (BES) can affect the type of microbial community selected (Table 1).

As seen in Table 1 various inoculum sources and enrichment methods produce different microbial communities capable of carrying out reactions such as oxygen reduction and denitrification at the cathode of MFC. In this study, aerobic sludge from an activated sludge tank of a textile wastewater treatment plant in India was used to develop an electroactive cathode biofilm, in the absence of any carbon source, capable of catalyzing the oxygen reducing reaction (ORR). The catalytic efficiency of this biofilm was compared to Platinum in the cathode of a microbial fuel cell. The sequencing and bioinformatics analysis of the biofilm was carried out to identify the dominant species responsible for electrochemical activity by comparing it with other forms of growth (planktonic) observed in the study. The aim was to enrich from novel sources, and identify, microbes able to replace platinum as a biocatalyst in cathodes of microbial fuel cells.

2. Results and Discussion

2.1 Enrichment of electron-accepting microbes using chronoamperometry and their performance as biocathode

A stable current was obtained from the biofilm after a period of 70 days. A stable current is defined by a phase where there is no sharp increase in current on replacement of the catholyte. The average peak current produced was -0.7 mA at -0.1 V vs Ag/AgCl. (Figure 1).

Many studies utilized an organic carbon source (glucose, acetate) for the first few cycles to accelerate the formation of biofilm or a previously acclimated inoculum to decrease the start-up time (Zaybak *et al.*, 2013; Xu *et al.*, 2019). The activated sludge used in this study acclimated faster to the new media in the absence of organic carbon to form a biofilm. At day 60 there was an increase in current to a maximum of -0.9 mA after which it decreased to a steady state value of -0.7 mA. The sustained current production for a period of 70 days indicates the electroactive behavior of the biofilm.

A biofilm formation was also observed on the connection between the wire and the graphite rod which suggests that the microorganisms are electrophilic and depend on the electrons for their respiration and other metabolic activities. The average resistance of the system was 142 Ω, varying depending on the current produced. Apart from biofilms on the electrodes, there were planktonic cells in the media and on the glass walls of the electrochemical set-up. The characteristics of the biofilm and planktonic cells were analysed by sequencing the microbial community.

The maximum voltage (OCV) obtained was highest for Platinum MFC with 950 mV, 890 mV for biocathode and 400 mV for plain graphite. The acclimated biocathode achieved a cell potential equivalent to Platinum MFC in 4-5 hours. The prior acclimation eliminates the start-up lag in MFC as observed by (Clauwaert *et al.*, 2007; Mao *et al.*, 2010). A steady voltage was maintained for 7 days after which the media was replenished.

Conventionally the efficiency of MFCs is affected mainly because of three losses, namely, activation losses (AL) caused by high overpotential at electrodes, ohmic losses (OL) due to reactor design and the mass transfer losses (ML) due to low substrate diffusion. The biocathode in this study decreased the activation overpotential at the cathode and performed at a rate comparable to platinum (Figure 2 inset). This was confirmed by voltage vs current density graph, where Pt MFC showed a steeper potential drop at lower current densities indicating a higher activation loss compared to the biocathode MFC. Similar results were observed by (Rabaey et al., 2008) suggesting that the bacteria act as true catalysts for cathode reduction reaction.

The polarization tests revealed a maximum power density of 72.7±1.2 mW m^{-2} for platinum MFC followed by 64.6±3.5 mW m^{-2} for biocathode MFC and 4.3±0.1 mW m^{-2} for plain graphite MFC (Figure 2). The internal resistance of the cell with biocathode MFC was 680 Ω and with platinum it was 655 Ω. The internal resistance depends on several factors such as reactor design, electrode configurations, type of catalyst etc. The distance between the electrodes was large (~13 cm) in the 'H' type reactor used in this study and this accounts for potential losses and high ohmic resistance. Zhang *et al.*, 2012 developed a novel tubular MFC with graphite brush electrodes (~2-3 cm distance between the electrodes) that produced a low internal resistance of only 30 Ω with a bioanode and biocathode. Therefore, with improvement in the reactor design the ohmic losses could be considerably reduced. The comparable power density and the internal resistance of the biofilm with platinum suggests that bacteria can perform as efficient cathode catalysts in a microbial fuel cell.

2.2 Microbial community analysis

Four different types of samples were analysed by Illumina-Next generation sequencing (detailed in supplementary S2) viz. Sludge- Initial sludge used for enrichment, EB- biofilm on the graphite electrode, EW- biofilm on the connecting wires and plank- planktonic cells formed on the walls of the enrichment set-up. Sludge had OTUs of 1044 and after enrichment EB had an OTUs of 889, whereas EW and Plank had 930 and 822 respectively (Figure 3 inset). The number of observed species in sludge was 1044, which on enrichment decreased to ~765 for EW, less than 710 for EB and 624 for plank (Figure 3). These results contradict Wang et al., 2013, who obtained a higher number of species in planktonic compared to biofilm (Wang *et al.*, 2013). The higher number of species near the electrode wire (EW) suggests these bacteria are electrophilic and survive by accepting electrons for their metabolism.

Shannon index provides an estimate of the diversity and variance among the species population within a sample. A high diversity in the sample is characterized by a higher Shannon index. Sludge had a Shannon index of 6.648 whereas it was significantly reduced for EB and plank with values 5.102 and 4.394. This indicates that the population within the enriched sample belonged to related groups carrying out similar metabolic function.

The dominant phylum for all the samples were Bacteriodetes, Proteobacteria, Firmicutes, Actinobacteria and Acidobacteria. There was variation in the relative amount of species between sludge and the enriched samples. The sludge sample had the following dominant class α-

Proteobacteria (24%), γ-Proteobacteria (15%) and Sphingobacteriia (12%). On enrichment α-Proteobacteria (Plank 32%, EW 31% and EB 29%) was further increased and Sphingobacteriia was replaced with Flavobacteriia as the dominant class in Bacteriodetes phylum (Figure 4). The phylum Bacteriodetes and Proteobacteria is dominant in majority of wastewater enriched biofilms of both the anode and cathode. The species present in the biofilms vary depending on the mechanism of electron transfer (Ishii *et al.*, 2017).

The dominant species present in the cathodic biofilm were *Nitrosomonas europaea*, *Sphingobium aminese, Nitratireductor indicus* and *Gordonia polyisoprenivorans* as observed in the ternary plot between EB, EW and Plank samples (Figure 5).

Nitrosomonas europaea was one of the prominent species that was enriched in all the biofilms, and hence it could be spotted at the center of the ternary plot (Figure 5). *N. europaea* is a chemolithoautotroph, deriving its energy from oxidation of ammonium ions to nitrite (Laanbroek *et al.*, 2002). The source of ammonia could be the ammonium chloride and ammonium sulphate present in the enrichment media. The nitrite produced by this species would have been utilised by *Nitrobacter sp*. as it obtains energy from oxidation of nitrite ions to nitrate ions (Grundmann *et al.*, 2000). *N. europaea* also has the ability to fix CO_2 through the Calvin cycle to form sugars that can be utilized by other organisms in the biofilm (Chain *et al.*, 2003). A species from phylum Actinomycetes, namely *Gordonia polyisoprenivorans* was seen to be enriched specifically in EB and plank biofilm. It was first isolated from automobile tyres and is one of the few available latex degrading microbes (Ding *et al.*, 2017). In addition, *G. polyisoprenivorans* coverts sugars to extracellular polysaccharides which is responsible for the formation of biofilms (Fusconi *et al.*, 2006). One of the few chemoheterotrophs enriched on biofilms were *Sphingobium aminese*, which was present specifically on EB and plank samples (Figure 5). This species can utilize only organic carbon sources for its metabolism (Ushiba *et al.*, 2003). Only subtle differences were observed between Plank and EB samples. Plank had a higher population of *Mesorhizhobium sp.* and *Paracoccus pantotrophus. P. pantotrophus* has nitrate reductase which can convert nitrate to nitrite (Sears *et al.*, 2000). In addition, *P. pantotrophus* has the ability to fix CO_2 through ribulose bi-phosphate pathway (Bardischewsky and Friedrich, 2001). The primary microbe that might be responsible for the cathodic current is *Nitratireductor indicus* through the process of denitrification. *Nitratireductor indicus* can degrade crude oil and was isolated from the deep-sea water of the Indian ocean and it reduces nitrate to nitrite (Lai *et al.*, 2011). A dominant genus observed in *Xanthomonadaceae* family was *Luteimonas* that was high in EB (11%) and EW (14%) compared to Plank (4%). Some species of this genus are capable of nitrate reduction to nitrite (Young *et al.*, 2007). The dominance of *Luteimonas* EB and EW indicates that it follows similar mechanism as *Nitratireductor indicus* to contribute to the current produced. *Luteimonas,* the nitrate reducing bacteria is known to utilize organic acids and certain amino acids as the organic substrates. These metabolites are the by-products of other types of bacterial metabolism. Thus, the overall consortia of the microbes established in the biofilm might have had complementary metabolic roles leading to a formation of a chemolithotrophic community. These microbial interactions are widely seen in biofilms and planktonic bacteria. Further meta-transcriptomics analysis such as gene expression studies are required to predict the possible metabolic interaction pathway within the community (Ishii *et al.*, 2015).

Nitrates have been used as terminal electron acceptors at the cathode of MFCs. Biocathodes equipped for reduction of nitrate from wastewater produced a low power density of 9.4 mW m^{-2} in a MFC (Lefebvre *et al.*, 2008). In this study, the power output of biocathode (64.6±3.5 mW m^{-2}) was comparable to platinum (72.7±1.2 mW m^{-2}) in a MFC. Therefore, it can be concluded that biocathodes utilising alternative terminal electron acceptors (nitrates) together with oxygen could be used to achieve a high-power output at the cathode of MFC.

Several species present in the samples (*Gordonia sp*., *S. aminese*, *Nitratireductor indicus*) are capable of degrading environmental pollutants. The role of the species in nitrate reduction, pollutant removal and power production in a MFC may be a perfect combination to develop MFC for bioremediation applications.

3. Conclusion

In this study, electroactive bacteria were enriched from textile wastewater to utilize them as a cathode catalyst in a MFC. The enriched biofilm produced an average peak current of 0.7 mA during the enrichment and produced a maximum power density of 64.6±3.5 mW m^{-2} comparable to platinum (72.7±1.2 mW m^{-2}) when employed in a MFC. The microbial community analysis of initial sludge sample and the enriched samples (plank, EB, EW) revealed the selection of chemolithotrophic organisms that fix CO_2 for their metabolism. The most dominant order of species was Flavobacteriales (Bacteriodetes) and Rhizhobiales (Alphaproteobacteria) in the enriched samples. The metabolic interaction between CO_2 fixers and reduction of nitrate to nitrite contributes to the biofilm formation and current production. As the bacterial biofilm was formed in the absence of any organic carbon this method of enrichment eliminates the need for carbon replenishment and can be used for wastewater containing low carbon content. Thus, the present study has established and identified a novel consortium of electro active bacteria that are capable of accepting electrons from the electrode and act as cathode catalyst in a MFC.

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5. Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6. References

Bardischewsky, F. and Friedrich, C.G. (2001) The shxVW locus is essential for oxidation of inorganic sulfur and molecular hydrogen by Paracoccus pantotrophus GB17: A novel function for lithotrophy. *FEMS Microbiol Lett* **202**: 215–220.

Blázquez, E., Gabriel, D., Baeza, J.A., and Guisasola, A. (2017) Evaluation of key parameters on simultaneous sulfate reduction and sulfide oxidation in an autotrophic biocathode. *Water Res* **123**: 301–310.

Chain, P., Lamerdin, J., Larimer, F., Regala, W., Lao, V., Land, M., et al. (2003) Complete Genome Sequence of the Ammonia-Oxidizing Bacterium and Obligate Chemolithoautotroph Nitrosomonas europaea Complete Genome Sequence of the

Ammonia-Oxidizing Bacterium and Obligate Chemolithoautotroph Nitrosomonas europaea †. *J Bacteriol* **185**: 2759–2773.

Ding, X., Yu, Y., Chen, M., Wang, C., Kang, Y., Li, H., and Lou, J. (2017) Bacteremia due to Gordonia polyisoprenivorans: Case report and review of literature. *BMC Infect Dis* **17**: 1–4.

Fusconi, R., Leal Godinho, M.J., Cruz Hernández, I.L., and Segnini Bossolan, N.R. (2006) Gordonia polyisoprenivorans from groundwater contaminated with landfill leachate in a subtropical area: Characterization of the isolate and exopolysaccharide production. *Brazilian J Microbiol* **37**: 168–174.

Grundmann, G.L., Neyra, M., and Normand, P. (2000) High-resolution phylogenetic analysis of NO2--oxidizing Nitrobacter species using the rrs-rrl IGS sequence and rrl genes. *Int J Syst Evol Microbiol* **50**: 1893–1898.

Hu, J., Zeng, C., Liu, G., Luo, H., Qu, L., and Zhang, R. (2018) Magnetite nanoparticles accelerate the autotrophic sulfate reduction in biocathode microbial electrolysis cells. *Biochem Eng J* **133**: 96–105.

Ishii, S., Ishii, S., Suzuki, S., Wu, A., Wu, A., Bretschger, O., et al. (2017) Population dynamics of electrogenic microbial communities in microbial fuel cells started with three different inoculum sources. *Bioelectrochemistry* **117**: 74–82.

Ishii, S., Suzuki, S., Tenney, A., Norden-Krichmar, T.M., Nealson, K.H., and Bretschger, O. (2015) Microbial metabolic networks in a complex electrogenic biofilm recovered from a stimulus-induced metatranscriptomics approach. *Sci Rep* **5**: 1–14.

Laanbroek, H.J., Bär-Gilissen, M.J., and Hoogveld, H.L. (2002) Nitrite as a stimulus for ammonia-starved Nitrosomonas europaea. *Appl Environ Microbiol* **68**: 1454–1457.

Lai, Q., Yu, Z., Yuan, J., Sun, F., and Shao, Z. (2011) Nitratireductor indicus sp. nov., isolated from deep-sea water. *Int J Syst Evol Microbiol* **61**: 295–298.

Lam, B.R., Barr, C.R., Rowe, A.R., Nealson, K.H., and Torres, C.I. (2019) Differences in Applied Redox Potential on Cathodes Enrich for Diverse Electrochemically Active Microbial Isolates From a Marine Sediment. **10**: 1–17.

Lefebvre, O., Al-Mamun, A., and Ng, H.Y. (2008) A microbial fuel cell equipped with a biocathode for organic removal and denitrification. *Water Sci Technol* **58**: 881–885.

Mani, P., Fidal, V.T., Bowman, K., Breheny, M., Chandra, T.S., Keshavarz, T., and Kyazze, G. (2019) Degradation of Azo Dye (Acid Orange 7) in a Microbial Fuel Cell: Comparison Between Anodic Microbial-Mediated Reduction and Cathodic Laccase-Mediated Oxidation. *Front Energy Res* **7**: 101.

Milner, E.M., Popescu, D., Curtis, T., Head, I.M., Scott, K., and Yu, E.H. (2016) Microbial fuel cells with highly active aerobic biocathodes. *J Power Sources* **324**: 8–16.

Rabaey, K., Read, S.T., Clauwaert, P., Freguia, S., Bond, P.L., Blackall, L.L., and Keller, J. (2008) Cathodic oxygen reduction catalyzed by bacteria in microbial fuel cells. *ISME J* **2**: 519–527.

Sears, H.J., Sawers, G., Berks, B.C., Ferguson, S.J., and Richardson, D.J. (2000) Control of periplasmic nitrate reductase gene expression (napEDABC) from Paracoccus pantotrophus in response to oxygen and carbon substrates. *Microbiology* **146**: 2977–2985.

Ushiba, Y., Takahara, Y., and Ohta, H. (2003) Sphingobium amiense sp . nov ., a novel nonylphenol-degrading bacterium isolated from a river sediment. *Int J Syst Evol Microbiol* **53**: 2045–2048.

Wang, Z., Zheng, Y., Xiao, Y., Wu, S., Wu, Y., Yang, Z., and Zhao, F. (2013) Analysis of oxygen reduction and microbial community of air-diffusion biocathode in microbial fuel cells. *Bioresour Technol* **144**: 74–79.

Xia, X., Sun, Y., Liang, P., and Huang, X. (2012) Long-term effect of set potential on biocathodes in microbial fuel cells: Electrochemical and phylogenetic characterization. *Bioresour Technol* **120**: 26–33.

Yang, N., Zhan, G., Li, D., Wang, X., He, X., and Liu, H. (2019) Complete nitrogen removal and electricity production in Thauera-dominated air-cathode single chambered microbial fuel cell. *Chem Eng J* **356**: 506–515.

Young, C.-C., Kampfer, P., Chen, W.-M., Yen, W.-S., Arun, A.B., Lai, W.-A., et al. (2007) Luteimonas composti sp. nov., a moderately thermophilic bacterium isolated from food waste. *Int J Syst Evol Microbiol* **57**: 741–744.

Zafar, Z., Ayaz, K., Nasir, M.H., Yousaf, S., Sharafat, I., and Ali, N. (2019) Electrochemical performance of biocathode microbial fuel cells using petroleum-contaminated soil and hot water spring. *Int J Environ Sci Technol* **16**: 1487–1500.

Zhang, G., Zhao, Q., Jiao, Y., Wang, K., Lee, D.-J., and Ren, N. (2012) Biocathode microbial fuel cell for efficient electricity recovery from dairy manure. *Biosens Bioelectron* **31**: 537–543.

Zhao, J., Zhang, C., Sun, C., Li, W., Zhang, S., Li, S., and Zhang, D. (2018) Electron transfer mechanism of biocathode in a bioelectrochemical system coupled with chemical absorption for NO removal. *Bioresour Technol* **254**: 16–22.

7. Figure Legends

Figure 1: Chronoamperometry of biofilm showing the peak current for each cycle observed through half-cell microbial enrichment method (Refer Supplementary S1).

Figure 2: Maximum power density for each cathode catalysts in MFC measured using polarization tests as in (Mani *et al.*, 2019). Inset: Voltage/current graph for the cathodes indicating the losses.

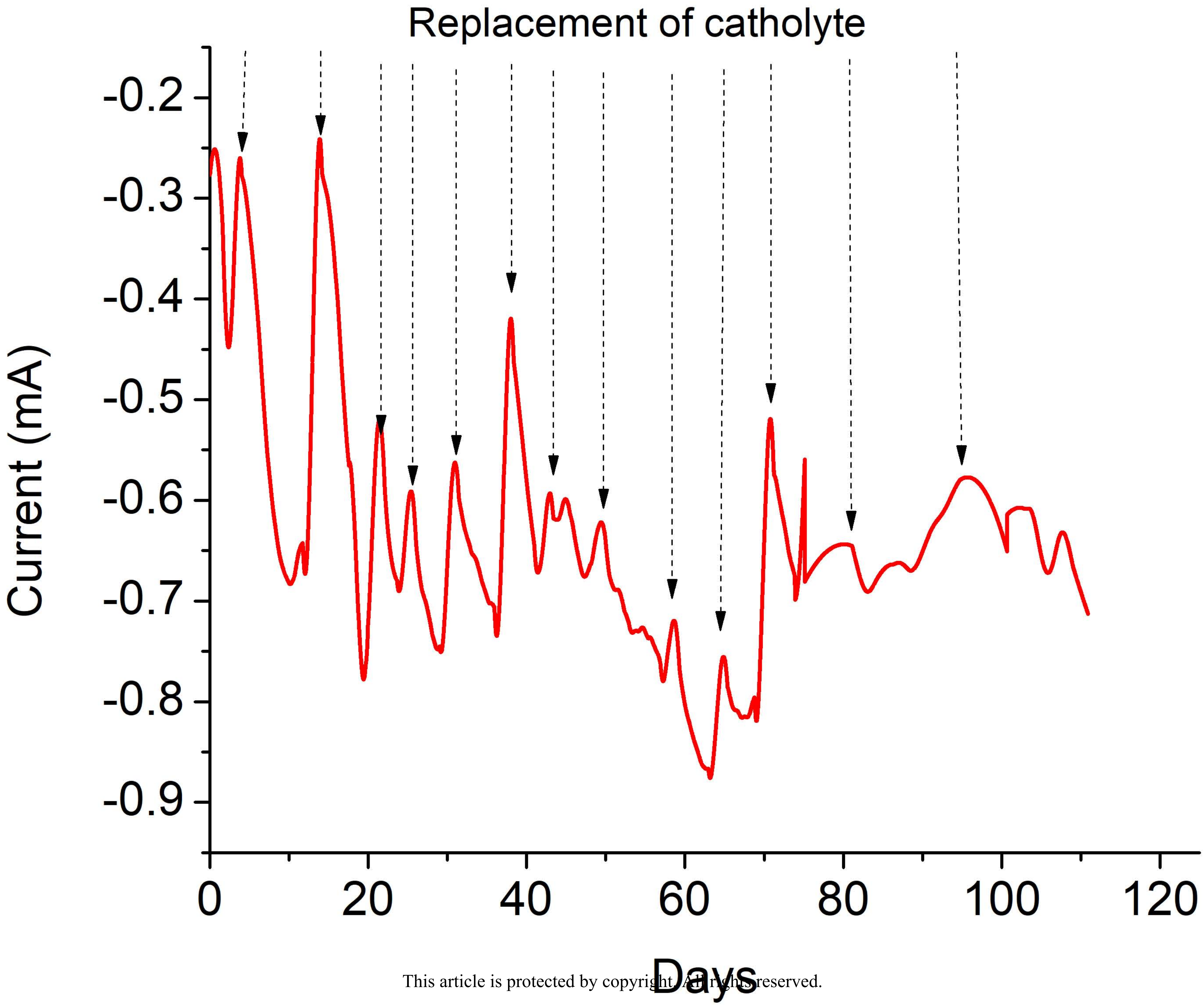
Figure 3: Observed number of species in each sample. Inset: OTUs obtained from each of the samples.

Figure 4: The relative abundance for the dominant class in each phylum for all samples.

Figure 5: Ternary plot indicating the dominant species in the enriched samples

8. Table

Table 1: Bio-electrochemical system employed in development of microbial biocathode and the dominant microbial communities identified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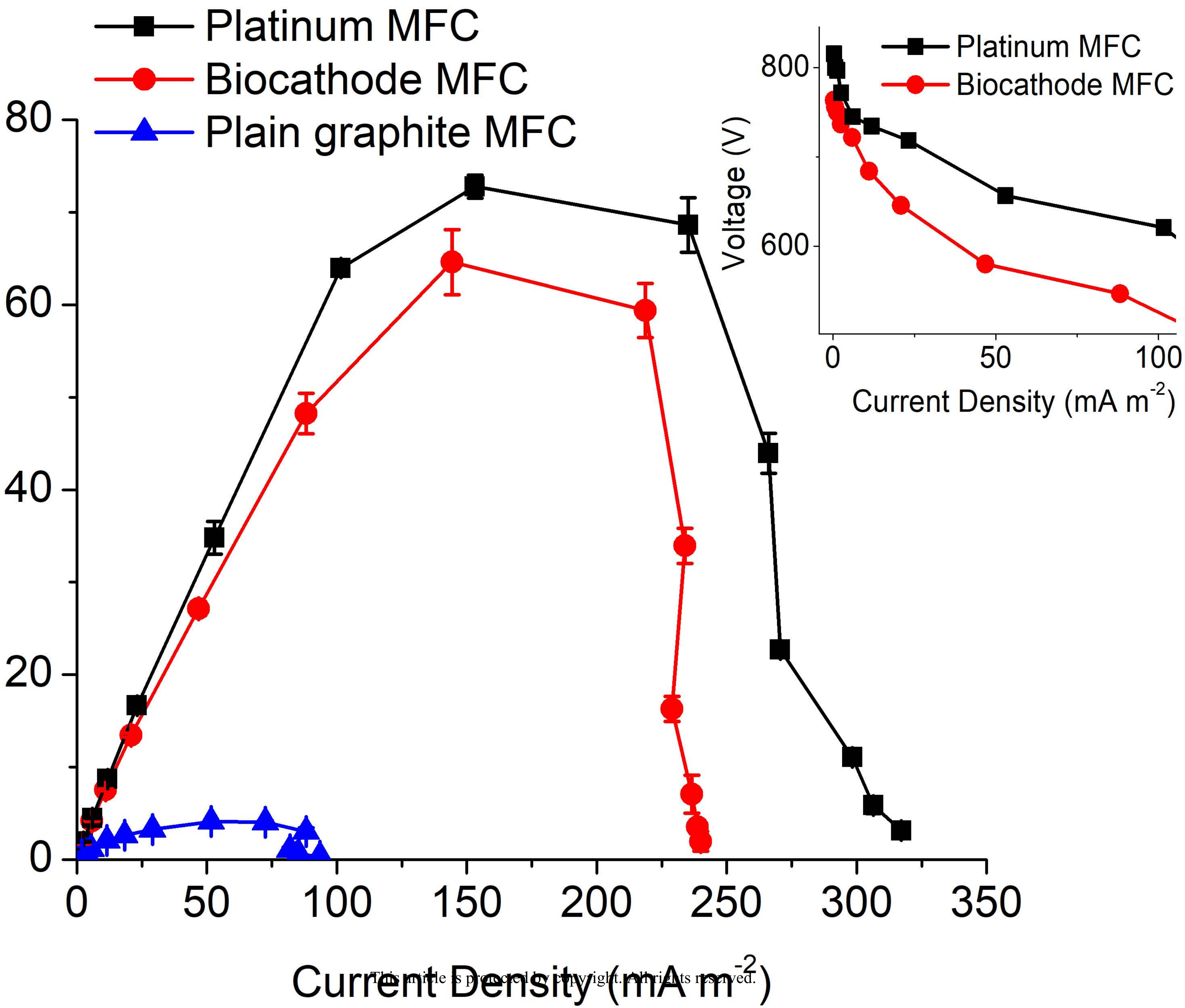
-0.7
-0.8
-0.9
0
20
40
60
80
100
120
Days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Biocathode MFC
Plain graphite MFC
Power Density (mW m^{-2})
Current Density (mA m^{-2})
0
20
40
60
80
0
50
100
150
200
250
300
350
Platinum MFC
Biocathode MFC
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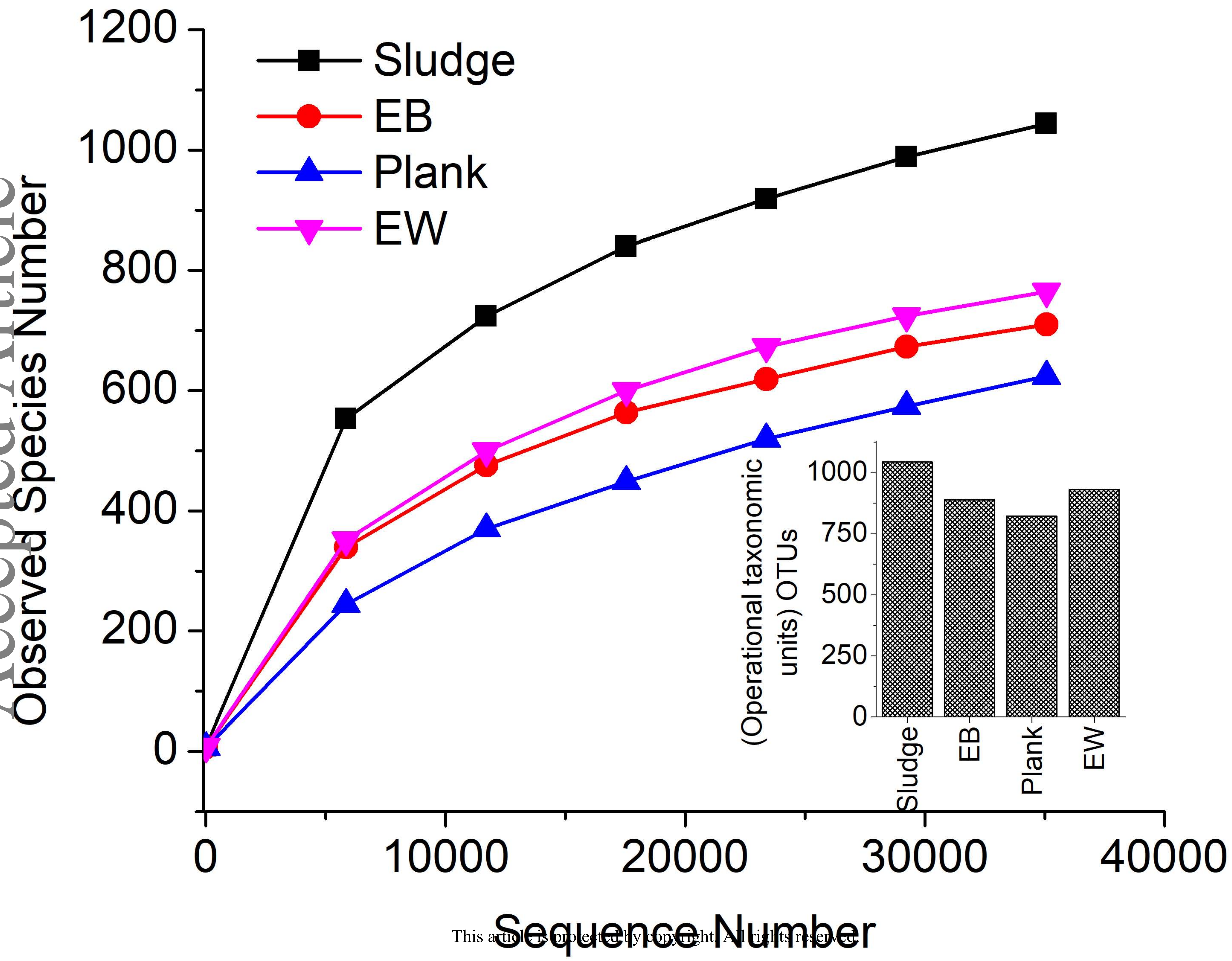
600
800
0
50
100
Current Density (mA m^{-2})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EB
Plank
EW
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Sequence Number
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800
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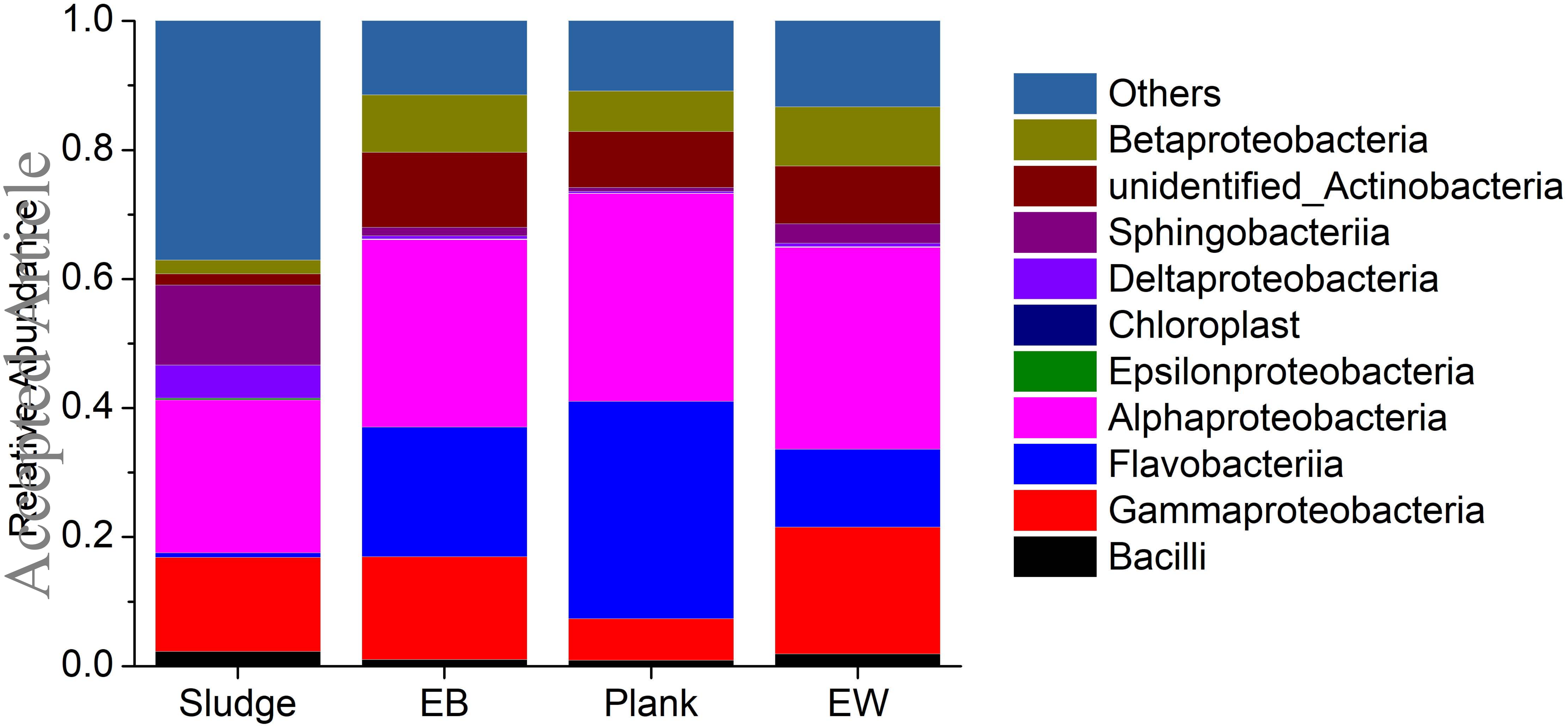
500
750
1000
Sludge
EB
Plank
EW


1.0
0.8
0.6
0.4
0.2
0.0
Relative Abundance
Sludge
EB
Plank
EW
Others
Betaproteobacteria
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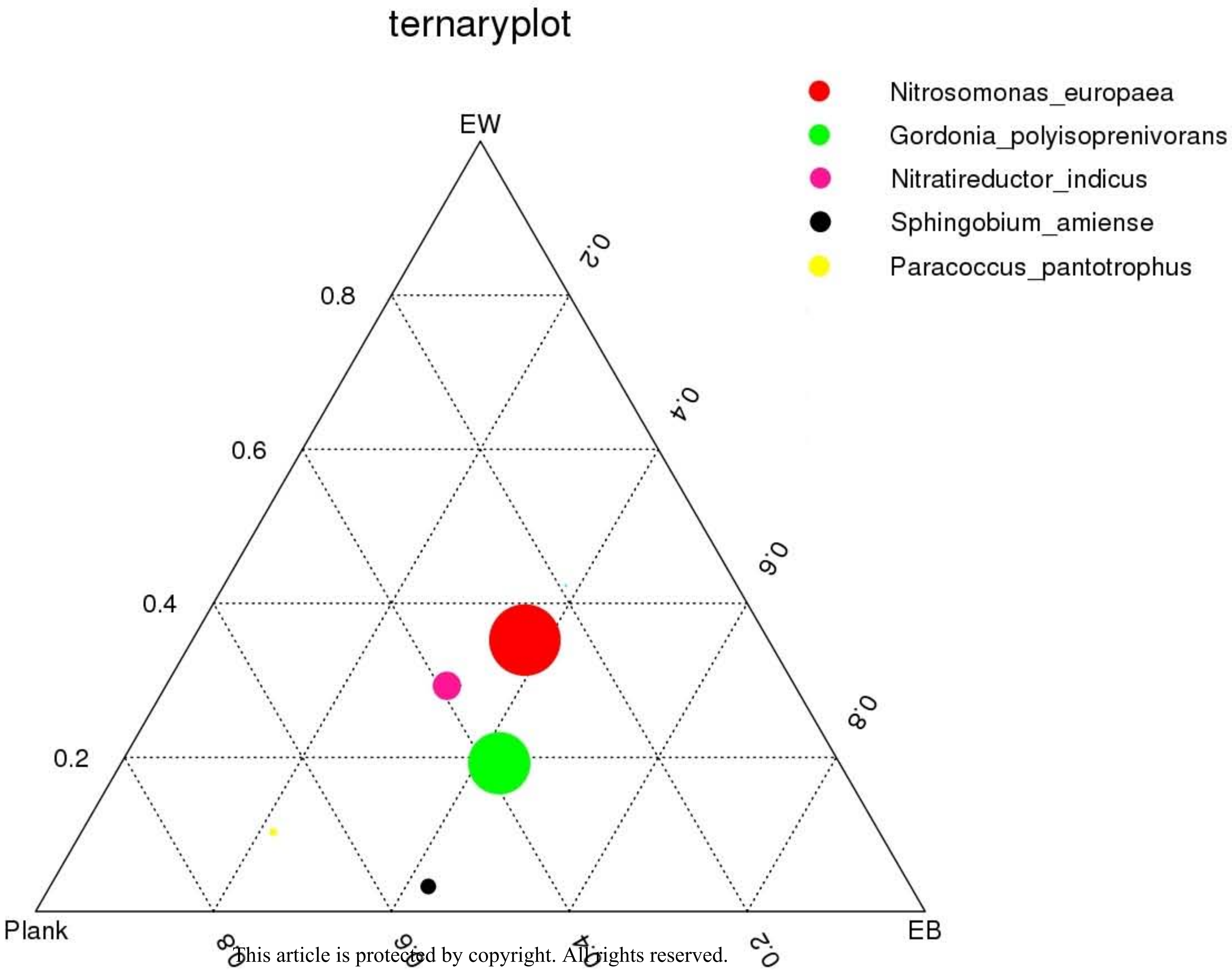
Sphingobacteriia
Deltaproteobacteria
Chloroplast
Epsilonproteobacteria
Alphaproteobacteria
Flavobacteriia
Gammaproteobacteria
Bacilli


ternaryplot
EW
Plank
EB
0.8
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0.4
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0.2
● Nitrosomonas_europaea
● Gordonia_polyisoprenivorans
● Nitratireductor_indicus
● Sphingobium_amiense
● Paracoccus_pantotrophus

Table 1: Bio-electrochemical systems employed in development of microbial biocathode and the dominant microbial communities identified

Cathode Material	**Source of inoculum**	**Enrichment method/Potential**	**Microbial community**	**References**
Carbon paper	Aerobic sludge	MFC (-0.1 V vs SCE)	80% Uncultured Bacteriodetes 13% *Thiorhodospira* sp. (γ-Proteobacteria)	(Xia et al., 2012)
Mn based catalyzed Carbon	Denitrifying sludge	Singe chambered MFC (1000 Ω)	*Thauera sp. (43–74%), Nitrosomonas sp. (3–8%), Desulfomicrobium sp. (1–8%) and Thiobacillus sp. (3–5%)*	(Yang *et al.*, 2019)
Graphite fibre brush	Sulphur reducing bacteria sludge (SRB)	MFC (-0.8 V vs SHE)	*Desulfovibrio* sp., *Thiomonas* sp., *Sulfuricurvum* sp. and *Thiobacillus* sp.	(Blázquez *et al.*, 2017)
Graphite plate	Acclimated SRB+ Magnetite particles	MEC (0.8 V)	72.2% *Desulfovibrio* sp., 14.2% *Acetobacterium* sp.	(Hu *et al.*, 2018)
Graphite granules	Denitrifying sludge	MEC	*Escherichia/Shigella* spp., *Actinotalea* sp., *Desulfitobacterium* sp. (Fe reducing bacteria);	(Zhao *et al.*, 2018)
Carbon cloth	Aerobic sludge	MFC (-0.3 V vs Ag/AgCl)	39.9% Proteobacteria, 29.9% Planctomycetes, 13.3% Bacteroidetes	(Wang *et al.*, 2013)
Carbon felt	Activated sludge	Half cell (0.1 V vs Ag/AgCl)	23.3%-44.3% Unidentified γ- Proteobacteria	(Milner *et al.*, 2016)