

Supplemental Materials

Oxidation of Ammonium by Feammox *Acidimicr:ae* sp. A6 in Anaerobic Microbial Electrolysis Cells

Melany Ruiz-Urigüen, Daniel Steingart, Peter R. Jaffé

Corresponding author: P.R. Jaffé: jaffe@princeton.edu

Reduction potential calculation for anaerobic ammonium oxidation to nitrite

The anaerobic ammonium (NH_4^+) oxidation reaction that takes place in the absence of iron oxides, in MECs is $\text{NH}_4^+ + 2\text{H}_2\text{O} \rightarrow \text{NO}_2^- + 3\text{H}_2 + 2\text{H}^+$, where the anode functions as the electron acceptor. The reduction potential (ΔE°) difference between two half reactions measured in volts (V) ($\Delta E^\circ = E_{\text{anode}} - E_{\text{substrate}}^\circ$) determines the feasibility of such reaction. Therefore, to make the reaction feasible, E_{anode} needs to be above $E_{\text{substrate}}^\circ$ which is equal to 0.07 V as shown in the calculations below based on equation S1.

$$E^\circ = E_{\text{acceptor}}^\circ - E_{\text{donor}}^\circ \quad (\text{Eq. S1})$$

Anodic half reaction in MEC	E°	Reference
$\text{NO}_3^- + 10\text{H}^+ + 8\text{e}^- \rightleftharpoons \text{NH}_4^+ + 3\text{H}_2\text{O}$	0.36 V	(Schwarzenbach et al. 2003)
$\text{NO}_3^- + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{NO}_2^- + \text{H}_2\text{O}$	0.43 V	(Schwarzenbach et al. 2003)
$\text{NO}_2^- + 8\text{H}^+ + 6\text{e}^- \rightleftharpoons \text{NH}_4^+ + 2\text{H}_2\text{O}$	- 0.07 V	
or		
$\text{NH}_4^+ + 2\text{H}_2\text{O} \rightleftharpoons \text{NO}_2^- + 8\text{H}^+ + 6\text{e}^-$	0.07 V	

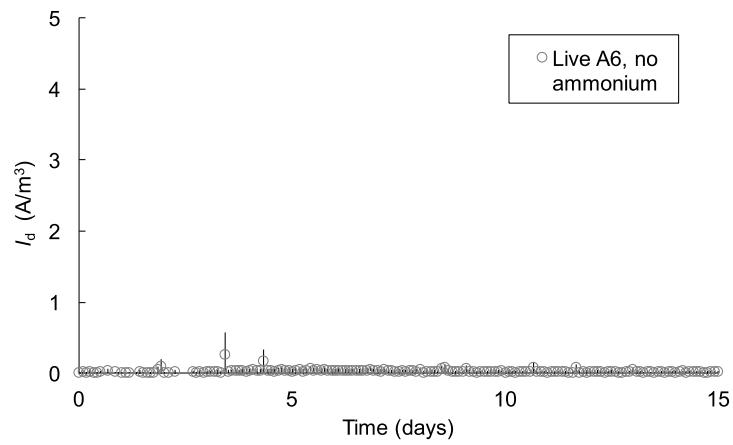


Figure S1. Average current density measured in MECs with pure live A6 in Feammox medium without NH_4^+ under stirring conditions. Marks show the mean and lines the standard error ($n=3$).

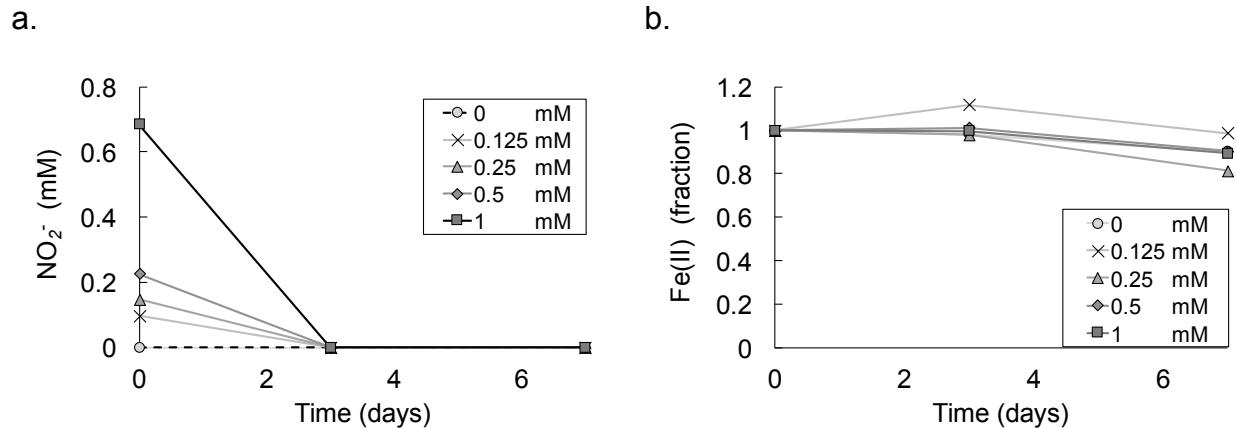


Figure S2. NO_2^- incubations with Fe(II). **a)** NO_2^- measurements over time. **b)** Fe(II) measurements over time.

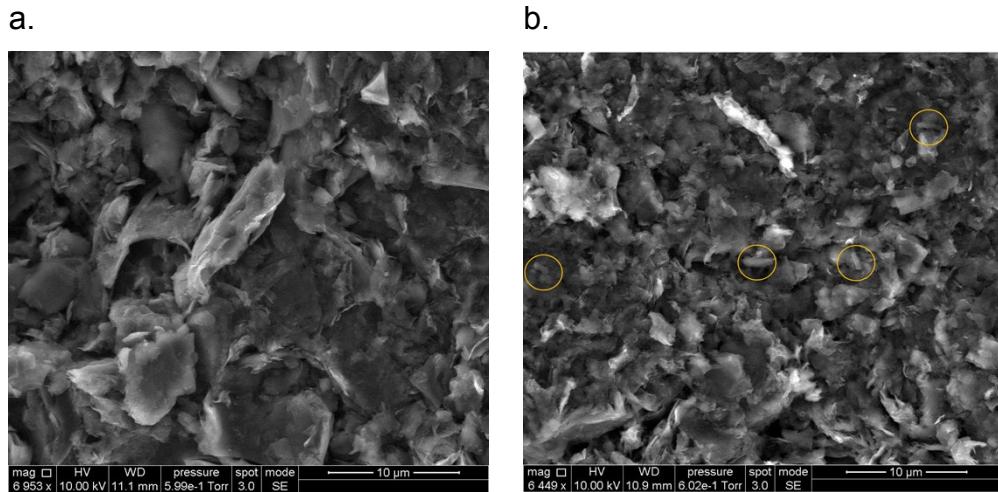


Figure S3. **a.** E-SEM images of the graphite anode of a control MEC operated with dead A6, no bacterial cells were found. **b.** Graphite anode of MEC operated with live A6, bacterial cells are encircled.

Table S1. Initial Fe transferred to MECs from the bacterial seed which is grown in the presence of Fe (III) as the electron acceptor.

MEC	Fe (II) (mM)	Fe (III) (mM)	Amount of NH ₄ ⁺ (mM) that could be removed by Fe (III) available
Pure A6 under stirring conditions	1.94 ± 0.1	0.59 ± 0.035	0.1± 0.006
Pure A6 mixed on shaker	0.46 ± 0.1	0.59 ± 0.025	0.1 ± 0.004
A6 enrichment mixed on shaker	0.5	0.0	0.0

Table S2. Taxonomy of the microbial community composition of the top 100 most abundant genus from a MEC with an A6 enrichment culture. Kingdom (k), phylum (p), class (c), order (o), family (f) and genus (g). Feammax bacteria A6 shown in bold.

Relative abundance	Taxonomy
0.21684	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Burkholderiales; f: Burkholderiaceae; g: Ralstonia
0.21111	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Rhodospirillaceae; g: Telmatospirillum
0.06897	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Hyphomicrobiaceae; g: Hyphomicrobium
0.03672	k: Bacteria; p: Proteobacteria; c: Gammaproteobacteria; o: Xanthomonadales; f: Xanthomonadaceae; g: Lysobacter
0.02925	k: Bacteria; p: Bacteroidetes; c: Sphingobacteriia; o: Sphingobacteriales; f: Chitinophagaceae; g: Sediminibacterium
0.02515	k: Bacteria; p: Actinobacteria; c: Acidimicrobii; o: Acidimicrobiales; f: unidentified Acidimicrobiales; g: Feammax bacterium A6
0.02258	k: Bacteria; p: Proteobacteria; c: Gammaproteobacteria; o: Pseudomonadales; f: Pseudomonadaceae; g: Pseudomonas
0.02124	k: Bacteria; p: Bacteroidetes; c: Bacteroidia; o: Bacteroidales; f: Prevotellaceae; g: Prevotellaceae:UCG-00
0.01720	k: Bacteria; p: Proteobacteria; c: Gammaproteobacteria; o: Xanthomonadales; f: Xanthomonadaceae; g: Thermomonas
0.00801	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Bradyrhizobiaceae; g: Bradyrhizobium
0.00588	k: Bacteria; p: Proteobacteria; c: Gammaproteobacteria; o: Xanthomonadales; f: unidentified Xanthomonadales; g: Acidibacter
0.00538	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Burkholderiales; f: Burkholderiaceae; g: Burkholderia
0.00510	k: Bacteria; p: Acidobacteria; c: unidentified Acidobacteria; o: Subgroup 3; f: unidentified Acidobacteria; g: Bryobacter
0.00435	k: Bacteria; p: Acidobacteria; c: unidentified Acidobacteria; o: Subgroup 3; f: unidentified Acidobacteria; g: Candidatus Solibacter
0.00397	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: unidentified Rhizobiales; g: Rhizomicrobium
0.00394	k: Bacteria; p: Proteobacteria; c: Gammaproteobacteria; o: Xanthomonadales; f: Xanthomonadaceae; g: Rhodanobacter
0.00360	k: Bacteria; p: Actinobacteria; c: unidentified Actinobacteria; o: Frankiales; f: Acidothermaceae; g: Acidothermus
0.00341	k: Bacteria; p: Firmicutes; c: Bacilli; o: Bacillales; f: Alicyclobacillaceae; g: Alicyclobacillus
0.00338	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Nitrosomonadales; f: Gallionellaceae; g: Sideroxydans
0.00322	k: Bacteria; p: Proteobacteria; c: Deltaproteobacteria; o: Desulfuromonadales; f: Geobacteraceae; g: Geobacter
0.00297	k: Bacteria; p: Acidobacteria; c: Holophagae; o: Holophagales; f: Holophagaceae; g: Geothrix
0.00291	k: Bacteria; p: Proteobacteria; c: Deltaproteobacteria; o: Desulfovibrionales; f: Desulfovibrionaceae; g: Desulfovibrio
0.00288	k: Bacteria; p: Proteobacteria; c: Deltaproteobacteria; o: Myxococcales; f: Polyangiaceae; g: Sorangium

0.00260	k: Bacteria; p: Proteobacteria; c: Deltaproteobacteria; o: Desulfovibrionales; f: Desulfomicrobiaceae; g: Desulfomicrobium
0.00228	k: Bacteria; p: Firmicutes; c: Clostridia; o: Clostridiales; f: Clostridiaceae:1; g: Clostridium sensu stricto 12
0.00225	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Burkholderiales; f: Burkholderiaceae; g: Cupriavidus
0.00222	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Phyllobacteriaceae; g: Mesorhizobium
0.00210	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Rhodocyclales; f: Rhodocyclaceae; g: Azospira
0.00191	k: Bacteria; p: Proteobacteria; c: Deltaproteobacteria; o: Desulfobacterales; f: Desulfobulbaceae; g: Desulfobulbus
0.00181	k: Bacteria; p: Bacteroidetes; c: Bacteroidia; o: Bacteroidales; f: Bacteroidaceae; g: Bacteroides
0.00178	k: Bacteria; p: Firmicutes; c: Bacilli; o: Bacillales; f: Bacillaceae; g: Bacillus
0.00141	k: Bacteria; p: Proteobacteria; c: Gammaproteobacteria; o: Enterobacteriales; f: Enterobacteriaceae; g: Escherichia-Shigella
0.00135	k: Bacteria; p: Firmicutes; c: Clostridia; o: Clostridiales; f: Family XI; g: Sedimentibacter
0.00131	k: Bacteria; p: Acidobacteria; c: unidentified Acidobacteria; o: Acidobacteriales; f: Acidobacteriaceae Subgroup 1.; g: Telmatobacter
0.00125	k: Bacteria; p: Acidobacteria; c:unidentified Acidobacteria; o: Acidobacteriales; f: Acidobacteriaceae Subgroup 1; g: Ca. Koribacter
0.00122	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Rhodocyclales; f: Rhodocyclaceae; g: Dechloromonas
0.00116	k: Bacteria; p: Proteobacteria; c: Gammaproteobacteria; o: Xanthomonadales; f: Xanthomonadaceae; g: Dyella
0.00109	k: Bacteria; p: Bacteroidetes; c: Bacteroidia; o: Bacteroidales; f: Rikenellaceae; g: vadimBC27 wastewater-sludge group
0.00106	k: Bacteria; p: Proteobacteria; c: Gammaproteobacteria; o: Xanthomonadales; f: Xanthomonadaceae; g: Dokdonella
0.00100	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Caulobacterales; f: Caulobacteraceae; g: Phenyllobacterium
0.00097	k: Bacteria; p: Proteobacteria; c: Gammaproteobacteria; o: Chromatiales; f: Ectothiorhodospiraceae; g: Acidiferrobacter
0.00091	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Burkholderiales; f: Comamonadaceae; g: Simplicispira
0.00091	k: Bacteria; p: Firmicutes; c: Clostridia; o: Clostridiales; f: Clostridiaceae:1; g: Clostridium sensu stricto 1
0.00088	k: Bacteria; p: Verrucomicrobia; c: Opitutae; o: Opitutales; f: Opitutaceae; g: Opitutus
0.00081	k: Bacteria; p: Firmicutes; c: Clostridia; o: Clostridiales; f: Christensenellaceae; g: Christensenellaceae R-7 group
0.00081	k: Bacteria; p: Proteobacteria; c: Deltaproteobacteria; o: Syntrophobacterales; f: Syntrophaceae; g: Syntrophus
0.00078	k: Bacteria; p: Firmicutes; c: Clostridia; o: Clostridiales; f: Peptococcaceae; g: Desulfosporosinus
0.00075	k: Bacteria; p: Chloroflexi; c: Anaerolineae; o: Anaerolineales; f: Anaerolineaceae; g: Leptolinea
0.00072	k: Bacteria; p: Proteobacteria; c: Deltaproteobacteria; o: Myxococcales; f: Cystobacteraceae; g: Anaeromyxobacter
0.00066	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Nitrosomonadales; f: Gallionellaceae; g: Candidatus Nitrotoga
0.00066	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Acetobacteraceae; g: Acidocella
0.00053	k: Bacteria; p: Chloroflexi; c: Anaerolineae; o: Anaerolineales; f: Anaerolineaceae; g: Longilinea

0.00053	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Xanthobacteraceae; g: Pseudolabrys
0.00050	k: Bacteria; p: Firmicutes; c: Clostridia; o: Clostridiales; f: Peptococcaceae; g: Desulfitobacterium
0.00050	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Burkholderiales; f: Oxalobacteraceae; g: Collimonas
0.00047	k: Bacteria; p: Proteobacteria; c: Deltaproteobacteria; o: Desulfobacterales; f: Desulfobulbaceae; g: Desulfocapsa
0.00047	k: Bacteria; p: Thermotogae; c: unidentified:Thermotogae; o: Thermotogales; f: Thermotogaceae; g: GAL15
0.00044	k: Bacteria; p: Firmicutes; c: Clostridia; o: Clostridiales; f: Clostridiaceae:1; g: Clostridium sensu stricto 9
0.00041	k: Bacteria; p: Firmicutes; c: Clostridia; o: Clostridiales; f: Clostridiaceae:1; g: Clostridium sensu stricto 10
0.00041	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Burkholderiales; f: Comamonadaceae; g: Aquabacterium
0.00041	k: Bacteria; p: Bacteroidetes; c: Sphingobacterii; o: Sphingobacteriales; f: Sphingobacteriaceae; g: Muciluginibacter
0.00038	k: Bacteria; p: Proteobacteria; c: Deltaproteobacteria; o: Myxococcales; f: Haliangiaceae; g: Haliangium
0.00034	k: Bacteria; p: Proteobacteria; c: Epsilonproteobacteria; o: Campylobacterales; f: Helicobacteraceae; g: Sulfurovum
0.00034	k: Bacteria; p: Firmicutes; c: Erysipelotrichia; o: Erysipelotrichales; f: Erysipelotrichaceae; g: Erysipellothrix
0.00034	k: Bacteria; p: Proteobacteria; c: Deltaproteobacteria; o: Desulfobacterales; f: Desulfobulbaceae; g: Desulfurivibrio
0.00034	k: Bacteria; p: Spirochaetes; c: unidentified:Spirochaetes; o: Spirochaetales; f: Spirochaetaceae; g: Spirochaeta:2
0.00034	k: Bacteria; p: Proteobacteria; c: Deltaproteobacteria; o: Syntrophobacterales; f: Syntrophobacteraceae; g: Syntrophobacter
0.00031	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Burkholderiales; f: Burkholderiaceae; g: Chitinimonas
0.00031	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Burkholderiales; f: Oxalobacteraceae; g: Pseudoduganella
0.00028	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Rhodospirillaceae; g: Inquilinus
0.00028	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Bradyrhizobiaceae; g: Rhodoblastus
0.00025	k: Bacteria; p: Proteobacteria; c: Deltaproteobacteria; o: Desulfobacterales; f: Desulfobacteraceae; g: Desulfatirhabdium
0.00022	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Rhodospirillaceae; g: Elstera
0.00022	k: Bacteria; p: Acidobacteria; c: unidentified Acidobacteria; o: Acidobacterales; f: Acidobacteriaceae Subgroup 1.; g: Granulicella
0.00019	k: Bacteria; p: Planctomycetes; c: Planctomycetacia; o: Planctomycetales; f: Planctomycetaceae; g: Planctomyces
0.00016	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Burkholderiales; f: Comamonadaceae; g: Polaromonas
0.00016	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Hydrogenophilales; f: Hydrogenophilaceae; g: Thiobacillus
0.00016	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Sphingomonadales; f: Sphingomonadaceae; g: Novosphingobium
0.00016	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Acetobacteraceae; g: Rhodovastum
0.00016	k: Bacteria; p: Chloroflexi; c: Anaerolineae; o: Anaerolineales; f: Anaerolineaceae; g: Anaerolinea
0.00016	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Burkholderiales; f: unidentified Burkholderiales; g: Thiomonas

0.00016 k: Bacteria; p: Chloroflexi; c: Anaerolineae; o: Anaerolineales; f: Anaerolineaceae; g: unidentified Anaerolineaceae
0.00016 k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Rhodocyclales; f: Rhodocyclaceae; g: Denitratisoma
0.00013 k: Bacteria; p: Chloroflexi; c: Ktedonobacteria; o: Ktedonobacterales; f: Ktedonobacteraceae; g: Ktedonobacter
0.00013 k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Nitrosomonadales; f: Gallionellaceae; g: Gallionella
0.00013 k: Bacteria; p: Bacteroidetes; c: Bacteroidia; o: Bacteroidales; f: Porphyromonadaceae; g: Paludibacter
0.00013 k: Bacteria; p: Planctomycetes; c: Planctomycetacia; o: Planctomycetales; f: Planctomycetaceae; g: Isosphaera
0.00013 k: Bacteria; p: Firmicutes; c: Bacilli; o: Bacillales; f: Alicyclobacillaceae; g: Tumebacillus
0.00009 k: Bacteria; p: Proteobacteria; c: Epsilonproteobacteria; o: Campylobacterales; f: Helicobacteraceae; g: Sulfuricurvum
0.00009 k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Rhodocyclales; f: Rhodocyclaceae; g: Candidatus Accumulibacter
0.00006 k: Bacteria; p: Firmicutes; c: Clostridia; o: Clostridiales; f: Clostridiaceae:1; g: Clostridium sensu stricto 3
0.00006 k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Hydrogenophilales; f: Hydrogenophilaceae; g: Ferritrophicum
0.00006 k: Bacteria; p: Verrucomicrobia; c:OPB35 soil group; o:unident.OPB35 soil g.; f:unident.OPB35 soil g.; g:unident.OPB35 soil group
0.00003 k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Rhodocyclales; f: Rhodocyclaceae; g: Georgfuchsia
0.00003 k: Bacteria; p: Verrucomicrobia; c:OPB35 soil group; o:unidentified OPB35 soil group; f:unident OPB35 soil group; g: Pedosphaera
0.00003 k: Bacteria; p: Bacteroidetes; c: Bacteroidia; o: Bacteroidales; f: Rikenellaceae; g: Blvii28 wastewater-sludge group
0.00003 k: Bacteria; p: Acidobacteria; c: unidentified Acidobacteria; o: Acidobacteriales; f: Acidobacteriaceae Subgroup 1; g: Acidicapsa
0.24345 Others

Reference

Schwarzenbach, R.P., Gschwend, P.M. and Imboden, D.M. (2003) Environmental Organic Chemistry, Wiley Interscience.