Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.
This journal is © The Royal Society of Chemistry 2023

Impact of dissolved sulfide on a hybrid membrane bioreactor treating the effluent of a mainstream up-flow anaerobic sludge blanket

Tomás Allegue, Adrián Arias, Alberto Liñares, Francisco Omil and Juan Manuel Garrido.

CRETUS. Department of Chemical Engineering, Universidade de Santiago de Compostela, 15782, Santiago de Compostela, Galicia, Spain

tomas.martinez@ku.ac.ae, adrian.arias.bano@gmail.com, albertolinhareslamas@gmail.com, francisco.omil@usc.es, juanmanuel.garrido@usc.es

1. DNA extraction, sequencing, and analysis

To characterize the microbial community, total genomic DNA was extracted using the Biofilm DNA Isolation Kit (Norgen, Thorold, Canada) following the manufacturer instractions. Then, total DNA concentrations were determined using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA size and integrity was tested by standard electrophoresis. The V3V4 region of the bacterial 16S rRNA gene was amplified with the primer pair S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A [1]. The V2V3 region of the archaeal 16S rRNA gene was amplified with the primer set Arch1F and Arch1R [2]. For that, an initial amplification was carried out in 25 µL volumes containing 3 ng of total DNA, 100 nM of bacterial primers or 200 nM of archaeal primers, and 1X Q5® High Fidelity Master Mix (New England BioLabs) which contains the DNA polymerase, 2mM $MgCl_2$ and 200 µM dNTPs. PCR conditions were: initial denaturation at 98 °C for 30 s followed by 20 (*Bacteria*) or 22 (*Archaea*) cycles (denaturation: 98°C for 10 s, annealing: 50°C for *Bacteria* or 48°C for *Archaea* during 20 s, extension: 72 °C for 20 s); followed by an extension step of 2 min at 72°C.

A second PCR was used to add the Illumina adapters and barcodes to the amplicons. The conditions were similar to the first PCR but in this case, only 15 cycles were applied and the annealing temperature was 60°C. Library DNA concentration was then determined in a Bioanalyzer (Bioanalyzer, Agilent Technologies, Santa Clara, CA, USA). Libraires were pooled in equimolar amounts and sequenced in a MiSeq Illumina sequencer (Illumina, Inc.) at Unidad de Genómica, Parque Científico de Madrid. Paired-end reads (2x300) were generated following manufacturer protocols (Illumina, Inc.).

Obtained sequences were de-multiplexed and trimmed to remove Illumina adapters, barcodes. In addition, the last 50 pb of the 5' ends were removed due to the low-quality scores (Q<30). Next, paired-end reads were merged as previously described [3] filtering sequences with quality scores below Q30 and a minimum overlap of 50 bp. In addition, all sequences containing indeterminations were removed from further analysis. The high-quality sequences obtained, were analyzed with VSEARCH in de novo mode for chimera removal [4] and clustered into Operational Taxonomic Units (OTUs) using the 97% cutoff for sequences similarity using QIIME [5]. OTUs taxonomic affiliation was determined with USEARCH [4] with the Greengenes database version 13 8 [6].

2. Supplementary Table

Table S1. Relative abundances of the most predominant bacteria found on days 70 (Period I) and 154 (Period II) in three different types of biomass samples: i) suspended biomass in the MBR, ii) biofilm attached to the Biochip carriers located in the anoxic compartment of the MBR, and iii) biofilm attached to the biochip carriers located in the aerobic compartment of the MBR. Other includes minor and non-classified families. When the taxonomic classification of a family (f) was not possible, the best taxonomic classification for that microbial group is shown at the best taxonomic resolution achieved as follows: (p) phylum and (o) order.

	Period I (day 70)			Period II (day 154)		
	Suspended (MBR)	Biofilm (Anoxic)	Biofilm (Aerobic)	Suspended (MBR)	Biofilm (Anoxic)	Biofilm (Aerobic)
f_Ellin6075	1.5%	2.0%	1.5%	4.3%	4.7%	6.5%
f_Fimbriimonadaceae	1.0%	1.2%	1.0%	2.1%	1.1%	1.1%
f_Cryomorphaceae	1.4%	1.2%	1.6%	0.9%	1.7%	1.4%
f_Sphingobacteriaceae	1.7%	1.2%	2.1%	0.6%	1.1%	0.5%
f_Chitinophagaceae	15.9%	13.5%	17.7%	9.8%	11.8%	9.7%
f_Saprospiraceae	3.0%	2.9%	2.9%	16.2%	8.2%	7.7%
f_Carnobacteriaceae	3.2%	4.8%	3.1%	2.1%	2.4%	2.1%
f_Nitrospiraceae	0.7%	1.0%	0.7%	0.5%	0.5%	6.7%
f_Brocadiaceae	0.3%	0.0%	0.4%	0.2%	3.7%	4.6%
f_Caulobacteraceae	4.6%	5.5%	4.4%	3.6%	2.3%	1.9%
f_Comamonadaceae	4.3%	4.1%	3.8%	3.8%	4.6%	4.6%
f_Methylophilaceae	3.2%	3.2%	2.6%	1.1%	2.3%	2.5%
f_Nitrosomonadaceae	2.5%	2.8%	1.8%	1.2%	1.5%	3.4%
f_Rhodocyclaceae	2.0%	1.2%	1.8%	2.8%	4.3%	2.7%
f_Methylococcaceae	0.9%	0.7%	1.0%	0.7%	1.1%	5.6%
f_Verrucomicrobiaceae	0.3%	0.3%	0.3%	1.4%	0.7%	0.9%
o_Thiobacterales	0.3%	0.4%	0.5%	8.5%	4.6%	3.6%
p_Chlorobi	9.0%	10.2%	8.7%	1.8%	4.8%	3.6%
Other	44.3%	43.8%	44.2%	38.4%	38.6%	30.9%
Total	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

3. Supplementary Figure

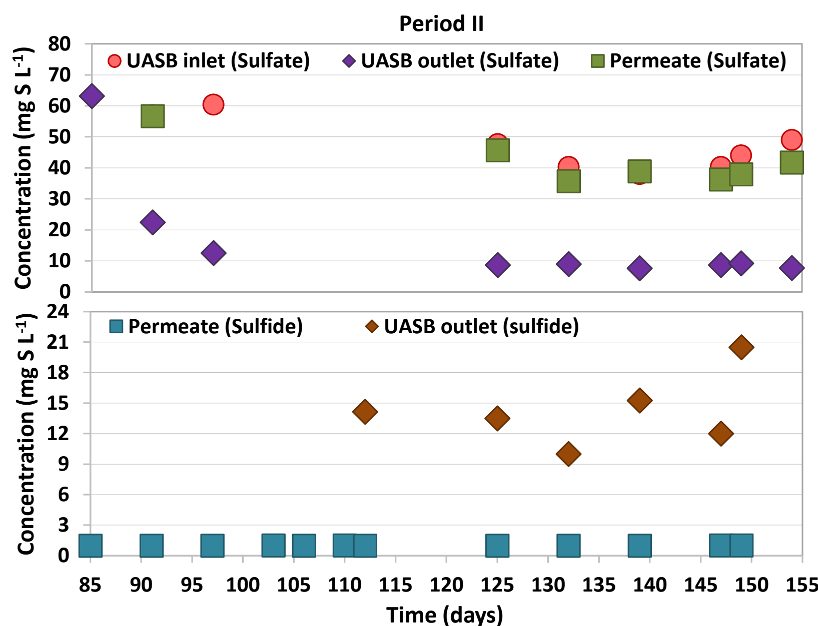

Figure S1. Changes in sulfur species (sulfate and sulfide) concentrations in different compartments of the UASB-MBR system throughout Period II.

4. References

1. A. Klindworth, E. Pruesse, T. Schweer, J. Peplies, C. Quast, M. Horn and F. O. Glöckner, Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies, *Nucleic Acids Res.*, 2013, **41**, 1–11.
2. P. Cruaud, A. Vigneron, C. Lucchetti-Miganeh, P. E. Ciron, A. Godfroy and M. A. Cambon-Bonavita, Influence of DNA extraction method, 16S rRNA targeted hypervariable regions, and sample origin on microbial diversity detected by 454 pyrosequencing in marine chemosynthetic ecosystems, *Appl. Environ. Microbiol.*, 2014, **80**, 4626–4639.
3. M. Eren, J. H. Vineis, H. G. Morrison and M. L. Sogin, A filtering method to generate high quality short reads using illumina paired-end technology., *PLoS One*, 2013, **8**, e66643.
4. T. Rognes, T. Flouri, B. Nichols, C. Quince and F. Mahé, VSEARCH: a versatile open source tool for metagenomics, *PeerJ*, 2016, **4**, e2584.
5. J. G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. Mcdonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld and R. Knight, QIIME allows analysis of high- throughput community sequencing data, *Nat. Methods*, 2010, **7**, 335–336.
6. T. Z. DeSantis, P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu and G. L. Andersen, Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB., *Appl. Environ. Microbiol.*, 2006, **72**, 5069–5072.