Supplementary Information

Enhanced hydrocarbons biodegradation at deep-sea hydrostatic pressure with microbial electrochemical snorkels

Federico Aulenta[1*], Enza Palma[1], Ugo Marzocchi[2,3], Carolina Cruz Viggi[1], Simona Rossetti[1], Alberto Scoma[4,5*]

[1] Water Research Institute (IRSA), National Research Council (CNR), Monterotondo, Italy

[2] Center for Electromicrobiology, Section for Microbiology, Department of Bioscience, Aarhus University, Aarhus, Denmark

[3] Integrative Marine Ecology Department, Stazione Zoologica Anton Dohrn, National Institute of Marine Biology, Ecology and Biotechnology, Napoli, Italy

[4] Section of Microbiology, Department of Biology, Aarhus University, Aarhus, Denmark

[5] Engineered Microbial Systems (EMS) Lab, Department of Biological and Chemical Engineering (BCE), Aarhus University, Aarhus, Denmark

*Correspondence to:
Assoc. Prof. Alberto Scoma
Head of the Engineered Microbial Systems (EMS) Lab,
Section of Biological and Chemical Engineering (BCE), Department of Engineering
Hangøvej 2, 8200, Aarhus University, Aarhus, Denmark
email: as@bce.au.dk

Dr. Federico Aulenta
Head of the Laboratory for Microbial Bioprocesses
Water Research Institute (IRSA), National Research Council (CNR)
Via Salaria km 29,300 - 00015, Monterotondo (RM), Italy
email: aulenta@irsa.cnr.it

Estimates on the respiration of O_2 in seawater owing to the electrochemical oxidation of H_2S in sediments through snorkels

Sediments were placed in cylinders with the following dimensions: internal diameter 3.8 cm; height, 33.5 cm. The internal volume was therefore: 379.74 cm^3 (or mL) This volume was occupied by sediment, water and rubber stoppers (on top and at bottom). In particular, the sediment had a volume of 110 mL and was about 9.7 cm high. The water had a volume of 200 mL, and was about 17.6 cm high. These dimensions were used to estimate the total amount of O_2 dissolved in water, and the total amount of H_2S accumulated in sediments.
In all experiments carried out with Duc oil, H_2S concentration was equal to zero at a specific depth below sediment surface level (bssl) (namely, between 0.1 and 0.9 cm, according to experimental condition; Table S2) and always increased linearly until 2 cm bssl. As H_2S increase was linear, a homogeneous average concentration along the whole section of sediment rich in H_2S can be calculated as:

Eq. 1 (Max H_2S concentration - Min H_2S concentration) / 2

The exact volume of sediment rich in H_2S is calculated by the specific height (in cm) multiplied by the internal surface area of the glass cylinder (in cm^2), which is equal in all conditions to 11.335 cm^2:

Eq. 2 (Onset H_2S accumulation - End of H_2S accumulation) · 11.335

The average concentration of H_2S (in μM or nmoles mL^{-1}) multiplied for the volume of sediment section (in cm^3 or mL) gives the amount of H_2S in the sediment section (in nmoles). A summary of all the data for the experiments with Duc oil is reported in Table S2. For experiments conducted with Statfjord oil the same procedure was followed, with the only exception of glass control experiments at both 10 and 0.1 MPa (HPC and APC, respectively). Here, the H_2S concentration pattern followed a first linear increase (to a depth of 1.5 cm bssl) followed by a somewhat linear decrease (to a depth of 3.5 cm bssl). As such, the sediment section rich in H_2S in HPC and APC with Statfjord oil was divided in two, and two average H_2S concentrations were assessed (all data in Table S2).
The total amount of H_2S accumulated in all sediments varied between 14 to 1596 nmoles.
H_2S microprofiles in sediments were not assessed throughout the whole sediment depth. This

means that the total H_2S accumulation (therefore, the O_2 respiration owing to its electrochemical oxidation) may have been underestimated. Such underestimation is not expected to be substantial as: 1) in Duc oil, H_2S concentrations at the deepest level analyzed (2 cm bssl) were never higher than 60 μM; 2) in Statfjord oil, H_2S levels at the deepest point analyzed were higher than in Duc only when testing glass controls at both 10 and 0.1 MPa (HPC and APC; above 80 μM); however, their concentration pattern was consistently decreasing at that depth, indicating that the bulk of H_2S accumulation had been detected.

To determine the net amount of H_2S oxidized electrochemically (Table S3), the difference between sediments incubated with snorkels and their respective glass controls was assessed as:

Eq. 3 H_2S concentration in HPS - H_2S concentration in HPC, or
H_2S concentration in APS - H_2S concentration in APC.

The stoichiometric electrochemical oxidation of H_2S (in sediments) using O_2 (in seawater) as terminal acceptor follows the equation:

Eq. 4 $2\ H_2S + 5\ O_2 = 2\ H_2O + 2\ SO_4$

As it takes 2.5 moles of O_2 to completely oxidize 1 mole of H_2S, the net amount of H_2S oxidized electrochemically (in nmoles) was multiplied by 2.5 to yield the amount of O_2 oxidized (in nmoles). Provided that O_2 molecular weight is about 32 g per mole, the net amount of O_2 respired electrochemically by snorkels (in μg) was determined. The volume of seawater in glass cylinders was always 200 mL. Thus, the concentration of O_2 (in mg/L) respired electrochemically could be estimated.
The total O_2 consumption in seawater had been assessed in all experiments. The difference between O_2 respiration in cylinders incubated with snorkels and their respective glass control was calculated using the same approach for Eq. 3. As such, the relative amount of O_2 respiration due to H_2S electrochemical oxidation could be estimated. This ranged between 42 and 7% across all experiments (Table S3).

Figure S1. Cell number in the water column at the end of 7 weeks of incubation in sediments contaminated with either Duc (A) or Statfjord (B) oil (n=3; bars represent standard errors). Cell numbers at time zero are reported as horizontal dotted lines, with the grey areas representing the standard error (n=3). Keys reported in the graph are: HPS, high pressure with snorkels; HPC, high pressure with glass controls; APS, ambient pressure with snorkels; APC, ambient pressure with glass controls. No statistical difference was observed between the different reactors contaminated with the same crude oil (p>0.05).

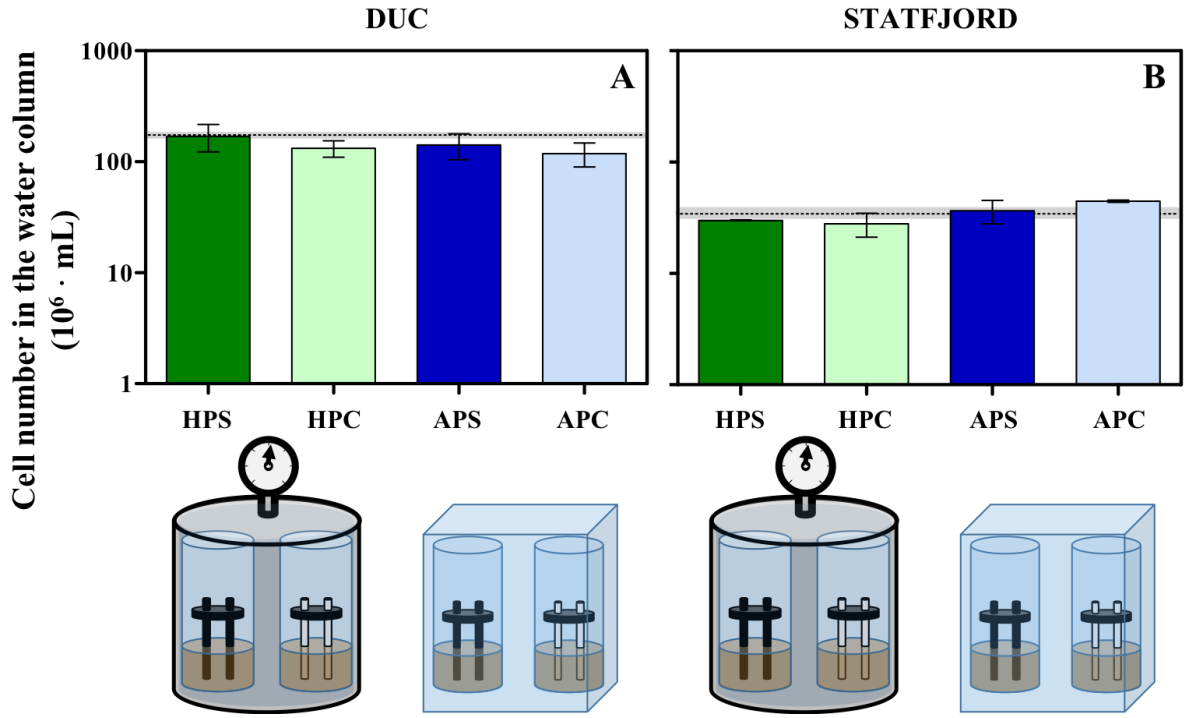

Table S1. Duc and Statfjord crude oil *n*-alkanes profiles in contaminated sediments at Time zero. Keys: DW, dry weight; s.d., standard deviation.

n-alkane chain length	DUC crude oil					Statfjord crude oil				
	µg g^{-1}_{DW}			µg g^{-1}_{DW}		µg g^{-1}_{DW}			µg g^{-1}_{DW}	
	Replicate 1	Replicate 2	Replicate 3	mean	s.d. error	Replicate 1	Replicate 2	Replicate 3	mean	s.d. error
C8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C10	0.0	0.0	0.0	0.0	0.0	1.2	1.0	1.3	1.2	0.1
C11	0.0	0.0	0.0	0.0	0.0	2.5	0.3	2.5	1.8	0.7
C12	0.0	0.0	0.0	0.0	0.0	1.7	1.7	1.7	1.7	0.0
C13	0.0	0.0	0.0	0.0	0.0	2.7	2.5	2.3	2.5	0.1
C14	1.7	0.5	0.9	1.0	0.3	7.7	6.8	6.6	7.0	0.3
C15	9.9	6.4	4.1	6.8	1.7	10.1	13.0	11.3	11.5	0.8
C16	5.8	3.8	3.2	4.2	0.8	18.1	13.7	13.2	15.0	1.5
C17	8.7	6.5	4.5	6.6	1.2	12.8	10.7	11.2	11.6	0.6
Pristane	25.5	20.8	16.9	21.1	2.5	9.2	8.9	9.1	9.1	0.1
C18	8.5	7.0	4.6	6.7	1.1	18.1	15.8	17.8	17.2	0.7
Phytane	14.6	22.3	14.4	17.1	2.6	9.3	7.7	8.4	8.5	0.5
C19	6.5	7.2	5.5	6.4	0.5	9.9	9.2	9.3	9.5	0.2
C20	6.3	9.4	9.7	8.4	1.1	14.1	13.5	14.4	14.0	0.3
C21	7.0	4.5	4.3	5.3	0.9	9.2	8.4	8.8	8.8	0.2
C22	6.7	4.3	7.6	6.2	1.0	18.6	14.6	16.2	16.5	1.2
C23	8.0	6.6	14.5	9.7	2.4	14.5	15.1	22.6	17.4	2.6
C24	6.2	11.7	3.3	7.1	2.5	13.0	8.6	10.3	10.6	1.3
C25	12.4	14.9	14.6	14.0	0.8	16.9	14.9	18.8	16.9	1.1
C26	10.7	11.1	9.4	10.4	0.5	21.8	22.3	23.8	22.6	0.6
C27	18.5	9.6	17.1	15.1	2.8	16.5	18.1	15.3	16.6	0.8
C28	18.5	11.9	11.2	13.9	2.3	27.7	25.0	25.9	26.2	0.8
C29	18.2	20.5	12.9	17.2	2.3	26.2	22.5	21.6	23.4	1.4
C30	0.0	0.0	0.0	0.0	0.0	39.8	35.1	30.2	35.0	2.8

C31	0.0	0.0	0.0	0.0	0.0		29.7	26.3	27.4	27.8	1.0
C32	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
C33	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	Totals			**mean**	**s.d. error**		**Totals**			**mean**	**s.d. error**
C14-C19	31.3	41.1	22.8	31.8	5.3	**C10-C19**	84.8	74.5	77.3	78.8	3.1
C20-C29	104.5	112.6	104.5	107.2	2.7	**C20-C31**	248.0	224.4	235.4	235.9	6.8
Total *n*-alkanes	135.9	153.7	127.4	139.0	7.8	**Total *n*-alkanes**	332.8	298.9	312.7	314.8	9.8
				mean	**s.d. error**					**mean**	**s.d. error**
Total Petroleum Hydrocarbons	3064	3531	3057	3217	157	**Total Petroleum Hydrocarbons**	2612	1861	1960	2144	236
Relative abundance of *n*-alkanes in crude oil (%)	4.43	4.35	4.17	4.32	0.08	**Relative abundance of *n*-alkanes in crude oil (%)**	12.74	16.06	15.96	14.92	1.09
	DUC crude oil						**Statfjord crude oil**				

Table S2. Estimation of the total H_2S (nmoles) accumulated in sediments, which were incubated with either Duc or Statfjord crude oil, with snorkels (S) or glass controls (C), at 10 (HP) or 0.1 MPa (AP).

Parameter	Unit of measurement	Duc oil				Statfjord oil			
		HPS	HPC	APS	APC	HPS	HPC	APS	APC
Onset of H_2S increase	cm	0.9	0.3	0.1	0.1	0.0	0.0	0.0	0.0
End of H_2S linear increase	cm	2.0	2.0	2.0	2.0	3.5	1.5	3.5	1.5
Net depth of the sediment rich in H_2S	cm	1.1	1.7	1.9	1.9	3.5	1.5	3.5	1.5
Internal surface area of the glass cylinder	cm^2	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3
Volume of sediment rich in H_2S	cm^3 or mL	12.5	19.3	21.5	21.5	39.7	17.0	39.7	17.0
Minimum H_2S concentration	μM or nmoles/mL	0.0	0.0	0.0	0.0	5.7	8.3	15.4	28.7
Maximum H_2S concentration	μM or nmoles/mL	2.3	15.6	19.4	57.7	15.3	83.8	52.6	136.2
Average H_2S concentration in the sediment section	μM or nmoles/mL	1.2	7.8	9.7	28.8	4.8	37.7	18.6	53.7
Amount of H_2S in the sediment section	nmoles	14.3	150.6	208.6	621.1	191.2	641.4	738.3	913.5
Onset of H_2S increase	cm						1.5		1.5
End of H_2S linear increase	cm						3.5		3.5
Net depth of the sediment rich in H_2S	cm						2.0		2.0
Internal surface area of the glass cylinder	cm^2						11.3		11.3
Volume of sediment rich in H_2S	cm^3 or mL						22.7		22.7
Minimum H_2S concentration	μM or nmoles/mL						38.4		75.9
Maximum H_2S concentration	μM or nmoles/mL						83.8		136.2
Average H_2S concentration in the sediment section	μM or nmoles/mL						22.7		30.1
Amount of H_2S in the sediment section	nmoles						514.9		682.6
Estimated total amount of H_2S accumulated in the sediment	nmoles	14.3	150.6	208.6	621.1	191.2	1156.3	738.3	1596.1

Table S3. Estimates of the total (mg L^{-1}) O_2 respiration in seawater due to electrochemical oxidation of H_2S in sediments, and of the relative contribution (%) to the electrochemical O_2 respiration to the total, in marine sediments incubated with either Duc or Statfjord crude oil, with snorkels (S) or glass controls (C), at 10 (HP) or 0.1 MPa (AP).

Parameter	Unit	Duc oil				Statfjord oil			
		HPS	HPC	APS	APC	HPS	HPC	APS	APC
Total amount of H_2S in the sediment	nmoles	14.3	150.6	208.6	621.1	191.2	1156.3	738.3	1596.1
Net amount of H_2S oxidized electrochemically	nmoles	136.3		412.6		965.1		857.8	
Net stoichiometric amount of O_2 respired electrochemically	μmoles	0.34		1.03		2.41		2.14	
Net stoichiometric amount of O_2 respired electrochemically	μg	10.9		33.0		77.2		68.6	
Volume of seawater in glass cylinders	mL	200		200		200		200	
Net stoichiometric O_2 concentration respired electrochemically	μg/mL or mg/L	0.05		0.17		0.39		0.34	
Total O_2 consumption in seawater	mg/L	40.5	40.4	39.6	38.1	33.1	29.4	37.2	32.4
Net amount of O_2 respired electrochemically	mg/L	0.13		1.50		3.71		4.84	
Relative amount of O_2 respired due to snorkels	%	41.9		11.0		10.4		7.1	