Supplementary Information

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

Federico Aulenta¹*, Enza Palma¹, Ugo Marzocchi²,³, Carolina Cruz Viggi¹, Simona Rossetti¹, Alberto Scoma⁴,⁵*

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\textsubscript{2} in seawater owing to the electrochemical oxidation of H\textsubscript{2}S 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\textsubscript{2} dissolved in water, and the total amount of H\textsubscript{2}S accumulated in sediments.

In all experiments carried out with Duc oil, H\textsubscript{2}S 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\textsubscript{2}S increase was linear, a homogeneous average concentration along the whole section of sediment rich in H\textsubscript{2}S can be calculated as:

\textbf{Eq. 1} \quad (\text{Max H}_2\text{S concentration} - \text{Min H}_2\text{S concentration}) / 2

The exact volume of sediment rich in H\textsubscript{2}S 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\(^2\):

\textbf{Eq. 2} \quad (\text{Onset H}_2\text{S accumulation} - \text{End of H}_2\text{S accumulation}) \cdot 11.335

The average concentration of H\textsubscript{2}S (in $\mu$M or nmoles mL\(^{-1}\)) multiplied for the volume of sediment section (in cm\(^3\) or mL) gives the amount of H\textsubscript{2}S 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\textsubscript{2}S 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\textsubscript{2}S in HPC and APC with Statfjord oil was divided in two, and two average H\textsubscript{2}S concentrations were assessed (all data in Table S2).

The total amount of H\textsubscript{2}S accumulated in all sediments varied between 14 to 1596 nmoles. H\textsubscript{2}S microprofiles in sediments were not assessed throughout the whole sediment depth. This
means that the total H₂S accumulation (therefore, the O₂ respiration owing to its electrochemical oxidation) may have been underestimated. Such underestimation is not expected to be substantial as: 1) in Duc oil, H₂S concentrations at the deepest level analyzed (2 cm bssl) were never higher than 60 μM; 2) in Statfjord oil, H₂S 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 H₂S accumulation had been detected.

To determine the net amount of H₂S oxidized electrochemically (Table S3), the difference between sediments incubated with snorkels and their respective glass controls was assessed as:

\textbf{Eq. 3} \quad \text{H}_2\text{S concentration in HPS} - \text{H}_2\text{S concentration in HPC, or} \quad \text{H}_2\text{S concentration in APS} - \text{H}_2\text{S concentration in APC.}

The stoichiometric electrochemical oxidation of H₂S (in sediments) using O₂ (in seawater) as terminal acceptor follows the equation:

\textbf{Eq. 4} \quad 2 \text{H}_2\text{S} + 5 \text{O}_2 = 2 \text{H}_2\text{O} + 2 \text{SO}_4

As it takes 2.5 moles of O₂ to completely oxidize 1 mole of H₂S, the net amount of H₂S oxidized electrochemically (in nmoles) was multiplied by 2.5 to yield the amount of O₂ oxidized (in nmoles). Provided that O₂ molecular weight is about 32 g per mole, the net amount of O₂ respired electrochemically by snorkels (in μg) was determined. The volume of seawater in glass cylinders was always 200 mL. Thus, the concentration of O₂ (in mg/L) respired electrochemically could be estimated. The total O₂ consumption in seawater had been assessed in all experiments. The difference between O₂ 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 O₂ respiration due to H₂S 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 | | |
|-------------------------|---------------|---------------|---------------|-------------------------|---------------|
|                         | mean | s.d. error | mean | s.d. error | mean | s.d. error | mean | s.d. error |
| C8                      | 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          |
| C10                     | 0.0  | 0.0          | 0.0  | 0.0          | 1.2  | 1.3          |
| C11                     | 0.0  | 0.0          | 0.0  | 0.0          | 2.5  | 2.5          |
| C12                     | 0.0  | 0.0          | 0.0  | 0.0          | 1.7  | 1.7          |
| C13                     | 0.0  | 0.0          | 0.0  | 0.0          | 2.7  | 2.3          |
| C14                     | 1.7  | 0.5          | 0.9  | 1.0          | 7.7  | 6.8          |
| C15                     | 9.9  | 6.4          | 4.1  | 6.8          | 10.1 | 13.0         |
| C16                     | 5.8  | 3.8          | 3.2  | 4.2          | 18.1 | 13.7         |
| C17                     | 8.7  | 6.5          | 4.5  | 6.6          | 12.8 | 10.7         |
| Pristane                | 25.5 | 20.8         | 16.9 | 21.1         | 9.2  | 8.9          |
| C18                     | 8.5  | 7.0          | 4.6  | 6.7          | 18.1 | 15.8         |
| Phytane                 | 14.6 | 22.3         | 14.4 | 17.1         | 9.3  | 7.7          |
| C19                     | 6.5  | 7.2          | 5.5  | 6.4          | 9.9  | 9.2          |
| C20                     | 6.3  | 9.4          | 9.7  | 8.4          | 14.1 | 13.5         |
| C21                     | 7.0  | 4.5          | 4.3  | 5.3          | 9.2  | 8.4          |
| C22                     | 6.7  | 4.3          | 7.6  | 6.2          | 18.6 | 14.6         |
| C23                     | 8.0  | 6.6          | 14.5 | 9.7          | 14.5 | 15.1         |
| C24                     | 6.2  | 11.7         | 3.3  | 7.1          | 13.0 | 8.6          |
| C25                     | 12.4 | 14.9         | 14.6 | 14.0         | 16.9 | 14.9         |
| C26                     | 10.7 | 11.1         | 9.4  | 10.4         | 21.8 | 22.3         |
| C27                     | 18.5 | 9.6          | 17.1 | 15.1         | 16.5 | 18.1         |
| C28                     | 18.5 | 11.9         | 11.2 | 13.9         | 27.7 | 25.0         |
| C29                     | 18.2 | 20.5         | 12.9 | 17.2         | 26.2 | 22.5         |
| C30                     | 0.0  | 0.0          | 0.0  | 0.0          | 39.8 | 35.1         | 30.2 | 35.0 | 2.8 |

| C8                      | 0.0  | 0.0          | 0.0  | 0.0          | 39.8 | 35.1         | 30.2 | 35.0 | 2.8 |
| C9                      | 0.0  | 0.0          | 0.0  | 0.0          | 0.0  | 0.0          |
| C10                     | 0.0  | 0.0          | 0.0  | 0.0          | 1.2  | 1.3          |
| C11                     | 0.0  | 0.0          | 0.0  | 0.0          | 2.5  | 2.5          |
| C12                     | 0.0  | 0.0          | 0.0  | 0.0          | 1.7  | 1.7          |
| C13                     | 0.0  | 0.0          | 0.0  | 0.0          | 2.7  | 2.3          |
| C14                     | 1.7  | 0.5          | 0.9  | 1.0          | 7.7  | 6.8          |
| C15                     | 9.9  | 6.4          | 4.1  | 6.8          | 10.1 | 13.0         |
| C16                     | 5.8  | 3.8          | 3.2  | 4.2          | 18.1 | 13.7         |
| C17                     | 8.7  | 6.5          | 4.5  | 6.6          | 12.8 | 10.7         |
| Pristane                | 25.5 | 20.8         | 16.9 | 21.1         | 9.2  | 8.9          |
| C18                     | 8.5  | 7.0          | 4.6  | 6.7          | 18.1 | 15.8         |
| Phytane                 | 14.6 | 22.3         | 14.4 | 17.1         | 9.3  | 7.7          |
| C19                     | 6.5  | 7.2          | 5.5  | 6.4          | 9.9  | 9.2          |
| C20                     | 6.3  | 9.4          | 9.7  | 8.4          | 14.1 | 13.5         |
| C21                     | 7.0  | 4.5          | 4.3  | 5.3          | 9.2  | 8.4          |
| C22                     | 6.7  | 4.3          | 7.6  | 6.2          | 18.6 | 14.6         |
| C23                     | 8.0  | 6.6          | 14.5 | 9.7          | 14.5 | 15.1         |
| C24                     | 6.2  | 11.7         | 3.3  | 7.1          | 13.0 | 8.6          |
| C25                     | 12.4 | 14.9         | 14.6 | 14.0         | 16.9 | 14.9         |
| C26                     | 10.7 | 11.1         | 9.4  | 10.4         | 21.8 | 22.3         |
| C27                     | 18.5 | 9.6          | 17.1 | 15.1         | 16.5 | 18.1         |
| C28                     | 18.5 | 11.9         | 11.2 | 13.9         | 27.7 | 25.0         |
| C29                     | 18.2 | 20.5         | 12.9 | 17.2         | 26.2 | 22.5         |
| C30                     | 0.0  | 0.0          | 0.0  | 0.0          | 39.8 | 35.1         | 30.2 | 35.0 | 2.8 |
|       | C14-C19 | C20-C29 | Totals | mean  | s.d. | error |
|-------|---------|---------|--------|-------|------|-------|
| C31   | 0.0     | 0.0     | 0.0    | 0.0   | 0.0  | 0.0   |
| C32   | 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   |
|       | 31.3    | 41.1    | 22.8   | 31.8  | 5.3  | 0.0   |
|       | 104.5   | 112.6   | 104.5  | 107.2 | 2.7  | 0.0   |
|       | 135.9   | 153.7   | 127.4  | 139.0 | 7.8  | 0.0   |
| C10-C19 |       |         |        |       |      |       |
| C20-C31 |       |         |        |       |      |       |
|       | 84.8    | 74.5    | 77.3   | 78.8  | 3.1  | 0.0   |
|       | 248.0   | 224.4   | 235.4  | 235.9 | 6.8  | 0.0   |
|       | 332.8   | 298.9   | 312.7  | 314.8 | 9.8  | 0.0   |
| Total n-alkanes | 135.9 | 153.7 | 127.4 | 139.0 | 7.8 | 0.0 |
| Total Petroleum Hydrocarbons | 3064 | 3531 | 3057 | 3217 | 157 |
| Relative abundance of n-alkanes in crude oil (%) | 4.43 | 4.35 | 4.17 | 4.32 | 0.08 | 0.0 |

|       | C10-C19 | C20-C31 | Totals | mean  | s.d. | error |
|-------|---------|---------|--------|-------|------|-------|
|       | 29.7    | 26.3    | 27.4   | 27.8  | 1.0  | 0.0   |
|       | 0.0     | 0.0     | 0.0    | 0.0   | 0.0  | 0.0   |
|       | 0.0     | 0.0     | 0.0    | 0.0   | 0.0  | 0.0   |
|       | 31.3    | 41.1    | 22.8   | 31.8  | 5.3  | 0.0   |
|       | 104.5   | 112.6   | 104.5  | 107.2 | 2.7  | 0.0   |
|       | 139.0   | 7.8     | 0.0    | 0.0   | 0.0  | 0.0   |
| C10-C19 |       |         |        |       |      |       |
| C20-C31 |       |         |        |       |      |       |
|       | 84.8    | 74.5    | 77.3   | 78.8  | 3.1  | 0.0   |
|       | 248.0   | 224.4   | 235.4  | 235.9 | 6.8  | 0.0   |
|       | 332.8   | 298.9   | 312.7  | 314.8 | 9.8  | 0.0   |
| Total n-alkanes | 31.3 | 41.1 | 22.8 | 31.8 | 5.3 | 0.0 |
| Total Petroleum Hydrocarbons | 2612 | 1861 | 1960 | 2144 | 236 |
| Relative abundance of n-alkanes in crude oil (%) | 12.74 | 16.06 | 15.96 | 14.92 | 1.09 | 0.0 |

DUC crude oil

Statfjord crude oil
Table S2. Estimation of the total H$_2$S (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 HPS | Duc oil HPC | Duc oil APS | Duc oil APC | Statfjord oil HPS | Statfjord oil HPC | Statfjord oil APS | Statfjord oil APC |
|----------------------------------------------|---------------------|------------|------------|------------|------------|-------------------|------------------|------------------|------------------|
| Onset of H$_2$S increase                     | cm                  | 0.9        | 0.3        | 0.1        | 0.1        | 0.0               | 0.0              | 0.0              | 0.0              |
| End of H$_2$S linear increase                | cm                  | 2.0        | 2.0        | 2.0        | 2.0        | 3.5               | 3.5              | 1.5              | 1.5              |
| Net depth of the sediment rich in H$_2$S    | 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$_2$S           | cm$^3$ or mL        | 12.5       | 19.3       | 21.5       | 21.5       | 39.7              | 17.0             | 39.7             | 17.0             |
| Minimum H$_2$S concentration                | μM or nmoles/mL     | 0.0        | 0.0        | 0.0        | 0.0        | 5.7               | 8.3              | 15.4             | 28.7             |
| Maximum H$_2$S concentration                | μM or nmoles/mL     | 2.3        | 15.6       | 19.4       | 57.7       | 15.3              | 83.8             | 52.6             | 136.2            |
| Average H$_2$S 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$_2$S in the sediment section    | nmoles              | 14.3       | 150.6      | 208.6      | 621.1      | 191.2             | 641.4            | 738.3            | 913.5            |
| Onset of H$_2$S increase                     | cm                  | 1.5        | 1.5        |            |            |                   |                  |                  |                  |
| End of H$_2$S linear increase                | cm                  | 3.5        | 3.5        |            |            |                   |                  |                  |                  |
| Net depth of the sediment rich in H$_2$S    | cm                  | 2.0        | 2.0        |            |            |                   |                  |                  |                  |
| Internal surface area of the glass cylinder  | cm$^2$              | 11.3       |            |            |            |                   |                  |                  |                  |
| Volume of sediment rich in H$_2$S           | cm$^3$ or mL        | 22.7       |            |            |            |                   |                  |                  |                  |
| Minimum H$_2$S concentration                | μM or nmoles/mL     | 38.4       |            |            |            |                   |                  |                  |                  |
| Maximum H$_2$S concentration                | μM or nmoles/mL     | 83.8       |            |            |            |                   |                  |                  |                  |
| Average H$_2$S concentration in the sediment section | μM or nmoles/mL  | 22.7       |            |            |            |                   |                  |                  |                  |
| Amount of H$_2$S in the sediment section    | nmoles              | 514.9      |            |            |            |                   |                  |                  |                  |
| Estimated total amount of H$_2$S 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\(_2\)S 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\(_2\)S in the sediment | nmoles | 14.3 | 150.6 | 208.6 | 621.1 | 191.2 | 1156.3 | 738.3 | 1596.1 |
| Net amount of H\(_2\)S 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 |
