



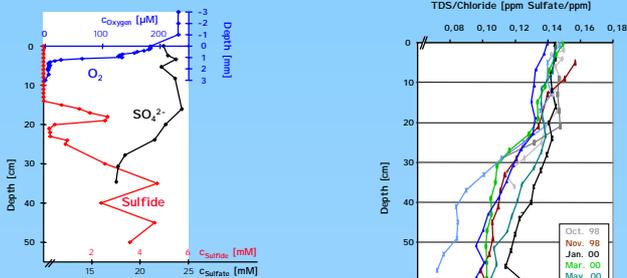
The influence of seasonal changes on microbial communities in the Wadden Sea



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AIM Investigation of microbial communities in intertidal sediments by a combined microbiological and geochemical Approach.

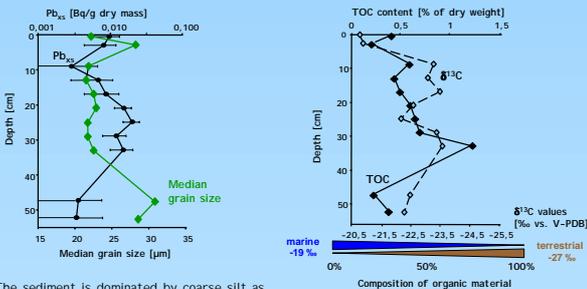
Pore Water Profiles



Oxygen could be detected in the uppermost 2-3 mm of the sediment (note different scales). Free sulfide could be detected below 10 to 15 cm depth.

Sulfate concentrations decreased with depth. Pore water-sulfate/chloride ratios were more or less constant along the upper 15-20 cm of the sediment. During winter a weak sulfate gradient was observed, while during summer (September '99) sulfate concentrations strongly declined with depth.

Sediment parameters

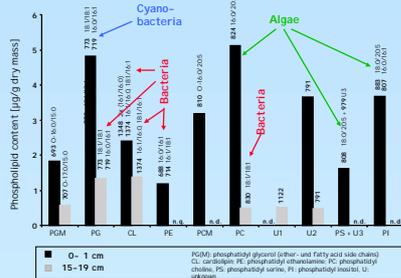


The sediment is dominated by coarse silt as can be seen from the median grain size.

The highly variable depth distribution of ²¹⁰Pb indicates that sedimentation is neither constant nor continuous and that the sediment is intensely reworked by bioturbation. The content of organic carbon is generally low and showed no correlation with the median grain size. The $\delta^{13}C$ values indicate a predominance of material of marine origin at the sediment surface, while in the deeper layers the content of terrestrial organic matter ranges between 38 and 56%.

Phospholipids in the sediment

Phospholipid contents in the sediment strongly decreased within the uppermost 5 cm. At the sediment surface phospholipids (PC, PI, PS) substituted with a C_{20:5} fatty acid dominated, indicating a strong contribution of algal biomass. In deeper Sediment layers bacterial biomass was dominating as deduced from the phospholipids and substituents detected. Surprisingly, in all depths only low contents of PE, the main lipid of sulfate reducers and most Gram-negative bacteria, were detected. Even from 50 cm depth phospholipids were retrieved, indicating the presence of intact microbial cells.



Viable counts obtained with different substrates

Substrate	Sediment depth [cm]	Sept. 1999	Jan. 2000	Mar. 2000	May 2000
Amino acids ¹⁾	0.5	1.3 · 10 ⁶	4.0 · 10 ⁶	1.9 · 10 ⁷	7.1 · 10 ⁷
	5	1.4 · 10 ⁴	6.2 · 10 ⁴	2.1 · 10 ⁵	2.4 · 10 ⁵
Short-chain fatty acids ²⁾	0.5	6.9 · 10 ³	1.2 · 10 ⁴	1.0 · 10 ⁴	9.4 · 10 ⁴
	5	4.0 · 10 ⁶	2.0 · 10 ⁶	1.3 · 10 ⁶	3.9 · 10 ⁵
Long-chain fatty acids ³⁾	0.5	6.9 · 10 ⁴	4.8 · 10 ⁴	3.8 · 10 ⁴	2.4 · 10 ⁴
	5	3.4 · 10 ⁴	3.7 · 10 ⁴	2.7 · 10 ⁴	4.0 · 10 ⁴
Cellulose	0.5	1.1 · 10 ⁷	7.7 · 10 ⁷	1.3 · 10 ⁸	1.4 · 10 ⁸
	5	2.2 · 10 ⁴	6.2 · 10 ³	1.6 · 10 ³	3.4 · 10 ³
	0.5	8.1 · 10 ³	7.4 · 10 ³	1.0 · 10 ⁴	2.8 · 10 ³
	5	n.d.	1.1 · 10 ³	1.4 · 10 ³	1.2 · 10 ³
	50	n.d.	2.4 · 10 ³	5.1 · 10 ³	9.1 · 10 ³
	50	n.d.	1.8 · 10 ⁵	3.0 · 10 ⁴	1.4 · 10 ⁴

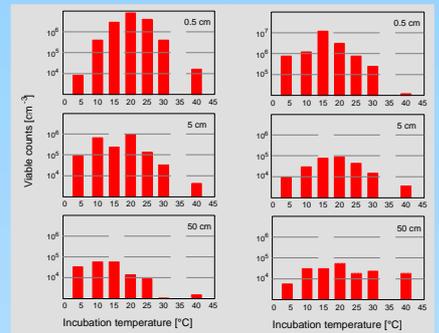
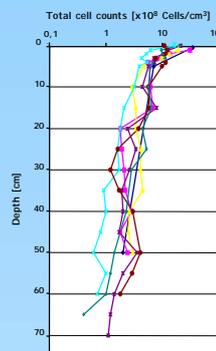
Anaerobic viable counts using single substrates showed seasonal changes (Tab. 3). This indicates that seasonal changes in substrate supply might affect microbial communities even in the deeper layers. MPN counts were quite similar, irrespective of the substrate used. At the sediment surface 0.1-1% of the total cell count could be grown in MPN series

Sediment temperature gradients

Sediment temperatures in the upper 50 cm of an intertidal sediment close to the village of Neuharlingsriehl (NW-Germany). Measurements were performed shortly after sunrise at low tide.

Sediment depth [cm]	Sept. 1999	Jan. 2000	Mar. 2000	May 2000	Aug. 2000	Jul. 2001
-2	19.3	3.3	7.0	15.6	19.1	15.2
0	18.6	3.4	7.0	14.4	17.4	15.2
2	18.2	3.4	6.8	14.6	17.3	15.1
5	18.9	3.4	6.6	14.4	17.2	15.8
10	17.5	3.4	6.5	14.4	17.5	16.0
15	18.0	3.4	6.5	14.4	17.7	16.5
20	18.2	3.4	6.4	14.6	17.8	17.3
30	17.9	3.4	6.3	14.5	17.2	17.0
40	17.9	3.4	6.1	14.4	17.2	16.5
45	17.8	3.4	6.0	14.4	17.1	17.0
50	17.7	3.4	5.8	14.2	17.0	17.4

Viable counts and presence of spores



Total cell counts („Acridin Orange Direct Counts“, AODC) decreased with depth, but showed only minor seasonal variation. Even in 50 cm sediment depth significant cell counts were detected.

Dependence of viable counts (MPN method) on the incubation temperature (left column: September 1999, right column: January 2000). Sediment was taken from three different depths. Viable counts showed similar patterns at all three depths and declined on a logarithmic scale with depth.

Temperature adaptation of pure cultures

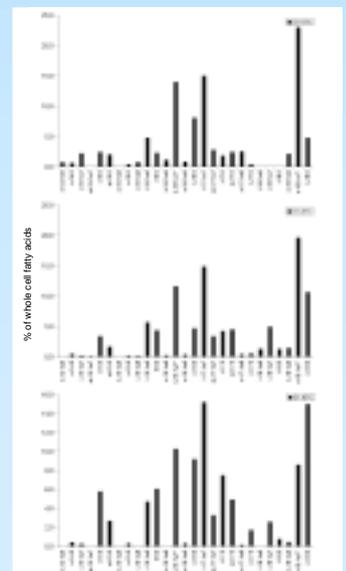
Isolation temperature	Depth [cm]	Strain	Growth range	Classification
10°C	0.5	<i>Desulfovibrio acrylicus</i> D1	4-30°C	Mesophilic
20°C	0.5	<i>Desulfotomaculum</i> sp. NA201	10-33°C	Mesophilic
	0.5	<i>Desulfovibrio acrylicus</i> NA202	10-30°C	Mesophilic
	5	<i>Desulfotomaculum</i> sp. NB81	4-30°C	Mesophilic
	5	<i>Desulfovibrio acrylicus</i> NB62	4-35°C	Mesophilic
30°C	0.5	<i>Desulfovibrio acrylicus</i> NA302	4-35°C	Mesophilic
	50	<i>Desulfovibrio acrylicus</i> NC301	4-35°C	Mesophilic
40°C	0.5	<i>Desulfotomaculum</i> sp. NA401	35-50°C	Slightly thermophilic
	5	<i>Desulfotomaculum</i> sp. NB401	30-55°C	Slightly thermophilic
	50	<i>Desulfotomaculum</i> sp. NC402	30-55°C	Slightly thermophilic

At 40°C only spore-forming bacteria were isolated. The growth temperatures of these organisms are in the range of 30-55°C what is clearly above the *in situ* temperatures. These strains were most likely detected as dormant spores.

At 30°C or below, only mesophilic, but psychrotolerant strains were isolated. At 10°C all isolates were still growing. Only few of the isolates grew at 4°C. This corresponds well with the findings of the MPN series.

Q₁₀ values for growth were in the range of 3.2 to 4.5.

Cells grown at 10°C showed a higher content of unsaturated fatty acids and shorter median chain length (Fig. 4). This result could be expected, since unsaturated fatty acids are known to increase membrane fluidity. Interestingly, the fatty acid patterns of a single strain, e.g. at 10°C and at 30°C, are more similar to each other, than to those of other strains, even of the same species.



SUMMARY Seasonal temperature fluctuations in intertidal sediments had only minor effect on the presence of bacterial temperature groups, but strongly affected microbial activities, as can be seen in sulfate depletion in deeper sediment layers.