Supplementary material to: “A laboratory experiment of intact polar lipid degradation in sandy sediments”

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1 Introduction

To provide background information on the microbial activity and community structure, the fermentation products as well as the bacterial and archaeal community profiles were determined.

2 Materials and methods

2.1 Fermentation products

Concentrations of fermentation products in the pore water were analyzed by high-performance liquid chromatography (Sykam, Fürstenfeldbruck, Germany) using an Aminex HPX-87H column (Biorad, München, Germany) at 60 °C. The eluent was 5 mM H_2SO_4 at 0.5 ml min^{-1}. Organic acids were detected by an UV-VIS detector (UVIS 204; Linear Instruments, Reno, USA) at 210 nm. Alcohols were detected by a refractive-index detector (Knauer, Berlin, Germany). Prior to injection, the pore water was filtered through a 2 µm filter.

2.2 Denaturing gradient gel electrophoresis (DGGE) analysis

Partial 16S rRNA were amplified using the OneStep RT-PCR Kit (Qiagen, Hilden, Germany) with bacterial primers GC-357f and 907r as given in Muyzer et al. (1995) and archaeal primers S D Arch-GC-0344-a-S-20 und 907r as described previously

(Wilms et al., 2006). Each forward primer contained a GC-clamp (Muyzer et al., 1993). Thermal cycling included a reverse transcription step for 30 min at 50 °C, followed by an initial denaturation step for 15 min at 95 °C, 30 cycles of bacterial RNA amplification and 35 cycles of archaeal RNA amplification (30 s at 94 °C, 45 s at 57 °C for bacterial RNA amplification or 48 °C for archaeal RNA amplification, 60 s at 72 °C), and a terminal step (10 min at 72 °C). The PCR amplicons and loading buffer (40% [wt/vol] glycerol, 60% [wt/vol] 1× tris acetate-EDTA [TAE], and bromphenol blue) were mixed in a ratio of 1:2. DGGE was carried out as described by Süß et al. (2004) using an INGENYphorU-2 system (Ingeny, Leiden, Netherlands) and a 6% (wt/vol) polyacrylamide gel containing denaturant gradients of 50 to 70% for separation of bacterial PCR products. For separation of archaeal PCR products a denaturant gradient of 30 to 80% was prepared. The gels were stained for 2 h with 1×SYBR Gold (Molecular Probes, Leiden, Netherlands) in 1× tris-acetate-EDTA buffer and washed for 20 min in distilled water prior to UV transillumination.

2.3 Sequence analysis

Individual DNA bands were excised from the gel with sterile scalpels, and the DNA was eluted into 50 µl molecular-grade water (Eppendorf, Hamburg, Germany) by incubation at 4 °C. For subsequent sequence analysis, PCR products of DGGE bands were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and commercially sequenced by GATC Biotech (Konstanz, Germany). The partial 16S rRNA sequences of the DGGE bands were compared to those in GenBank using the BLAST function (Altschul et al., 1997). All partial 16S rRNA gene sequences obtained in this study have been deposited in the EMBL database under accession numbers HE585651 – HE585692.

3 Results and Discussion

3.1 Fermentation products

Fermentation products were analyzed to monitor the utilization of organic matter. The data show the net balance of fermentation products since they are produced and consumed simultaneously (Fig. S1). In the beginning of the experiment the short-chain fatty acids were produced faster than consumed resulting in increasing concentrations.

The main fermentation product was acetate followed by propionate, butyrate and valerate. During the final phase of the experiment, the fermentation products were utilized at the same rate as they were produced or the consumption was faster than the formation. Ethanol was also produced but could not be quantified due to signal overlapping with those of other unknown organic compounds from the sediment matrix. The graphs for short-chain fatty acids in the degradation experiment and the untreated control shared the same characteristics. The accumulation of short-chain fatty acids tended to be slightly faster in the degradation experiment but overall the added cell material had only a minor influence on the shape of the production curves of short-chain fatty acids. This indicates that the experiment was not disturbed by the addition of biomass as external substrate.

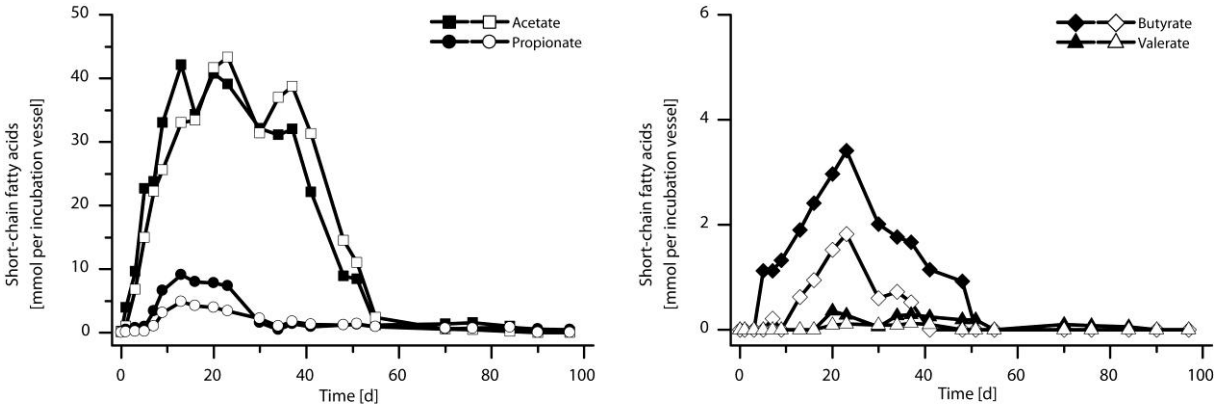

Figure S1: Pore water concentrations of acetate, propionate, butyrate and valerate in mmol per incubation vessel vs. time in days. Closed symbols: degradation experiment; open symbols: untreated control.

3.2 Community profiles

Sequencing of representative bands indicated the presence of different fermenting and sulfate-reducing bacteria (Fig. S2). The predominant organisms were relatives of *Pelobacter* sp. and *Dethiosulfovibrio acidaminovorans*. These bacteria were highly abundant during the end phase of the experiment, when acetate and propionate were absent, indicating an enhanced activity in this period. A sequence of a *Cytophaga fermentans*-related organism was detected after one week, also hinting toward an increased activity of that organism from that time on. Additionally, this bacterium showed a higher abundance in samples from the degradation experiment. The

abundance of other organisms, most of them being sulfate-reducing bacteria like a *Desulfobacter psychrotolerans*-related organism, appeared to be relatively constant over time, since sulfate was available almost during the entire experiment. Sulfur-oxidizing bacteria were only detected at the beginning of the experiment.

Surprisingly, chloroplast RNA of diatoms was found over the whole course of the experiment. Since no oxygen or light for algal growth were available in the experiment, the diatoms may have been inactive in a resting state. On the other hand, the RNA of chloroplasts may be exceptionally stable, because the RNA in this cell organelle is surrounded by four membranes (Kroth and Strotmann, 1999). In addition to this, the RNA of diatoms is protected by their rigid silica shell.

For both, the degradation experiment and the untreated control six bands of *Archaea* were detected throughout the whole experiment (Fig. S3). All sequences were closely related to either *Methanogenium frigidum* or *Methanogenium marinum*. The community patterns reflect the quantification of Archaea by qPCR: In the first week when the number of archaeal 16S rRNA gene targets was lowest, only very faint bands were detected in the DGGE profile.

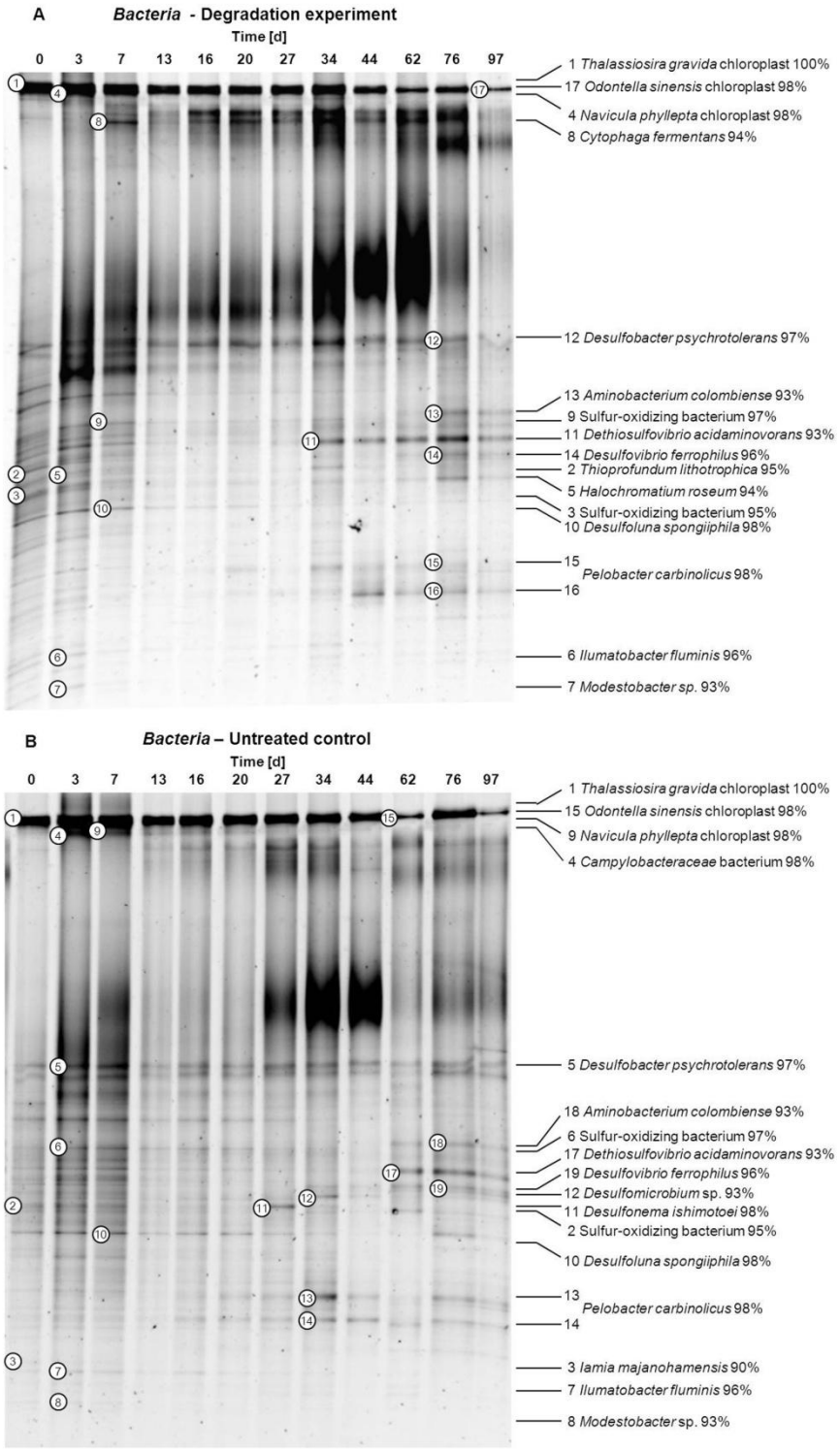

Figure S2: DGGE community profiles of *Bacteria* and closest cultivated relatives of the sequenced DGGE bands with similarity in % compared to data from the EMBL database. A: degradation experiment B: untreated control.

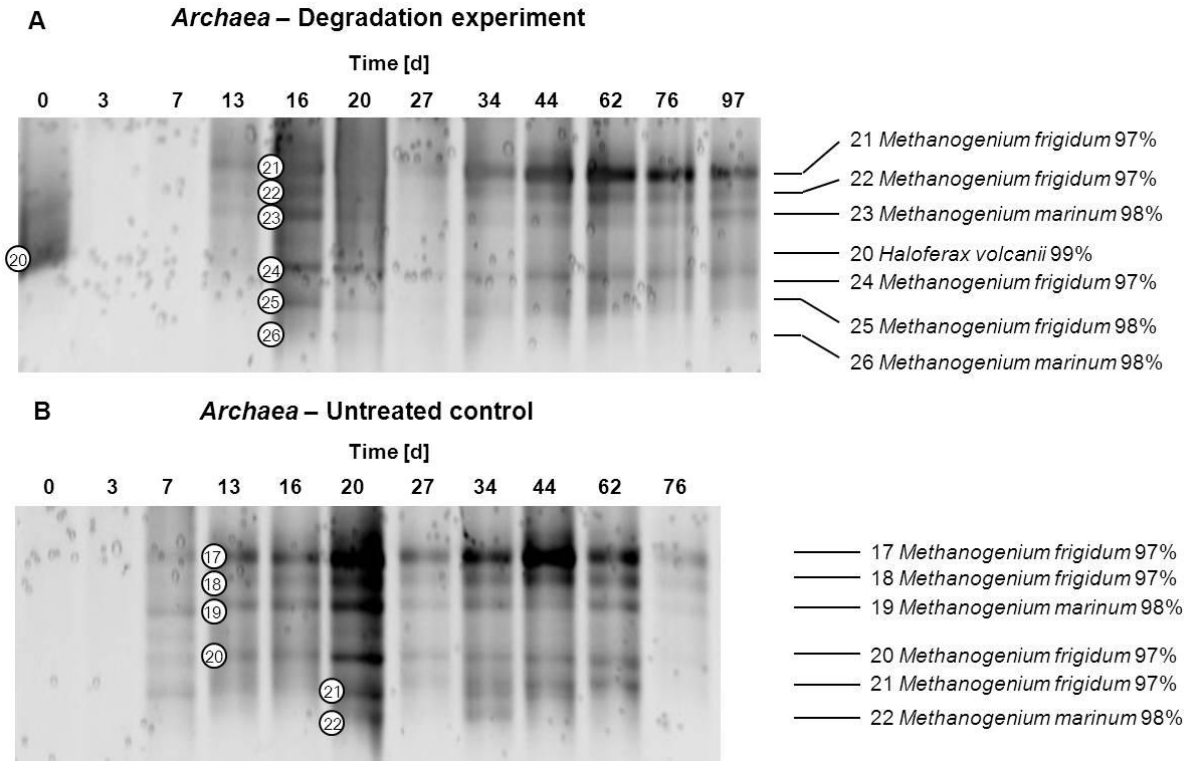

Figure S3: DGGE community profiles of *Archaea* and closest cultivated relatives of the sequenced DGGE bands with similarity in % compared to data from the EMBL database. A: degradation experiment B: untreated control.

4 Conclusion

The results of fermentation product and community analyses lead to the assumption that the added biomass did not significantly influence the microbial degradation and community structure in this study.

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