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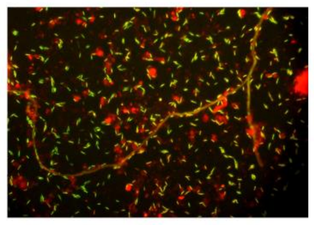

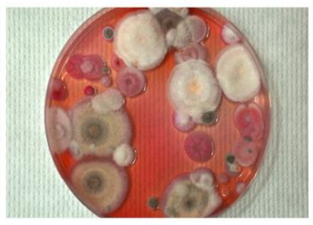

Effect of sulfate addition on the microbial community during anaerobic degradation of cellulose

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Introduction

The anaerobic digestion of organic matter involves a range of different microorganisms with characteristic metabolic potentials, which cooperate to finally produce biogas. High sulfate concentration levels enable sulfate reducing Bacteria (SRB) to compete with methane producing Archaea (MPA) for substrates, including hydrogen and acetate. This shifts the carbon flow in the system away from methane toward other fermentation products, and leads as a consequent to a decreased gas yield and quality.

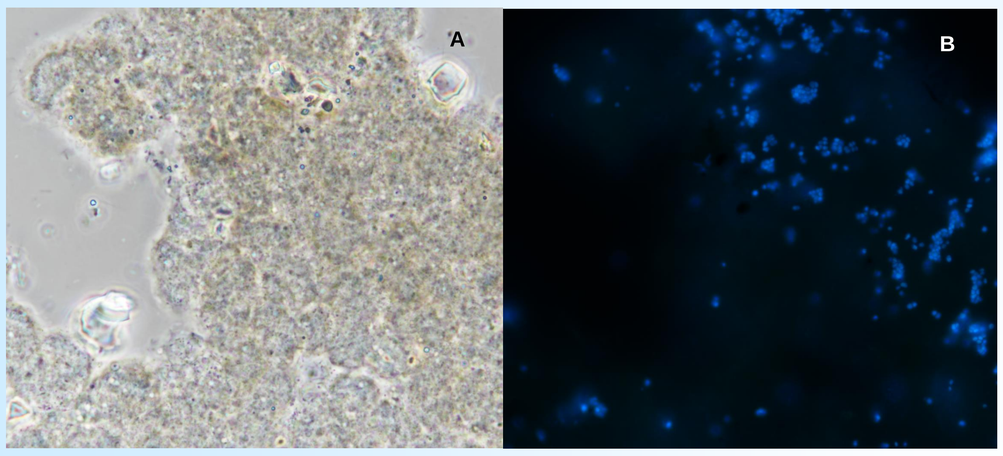

Fig. 1: Phase contrast (A) and fluorescence (B) microscopy of a microbial floc during the uninhibited anaerobic digestion of cellulose in the absence of additional sulfate. Blue fluorescence derives from the F_{420} factor of active methanogenic Archaea.

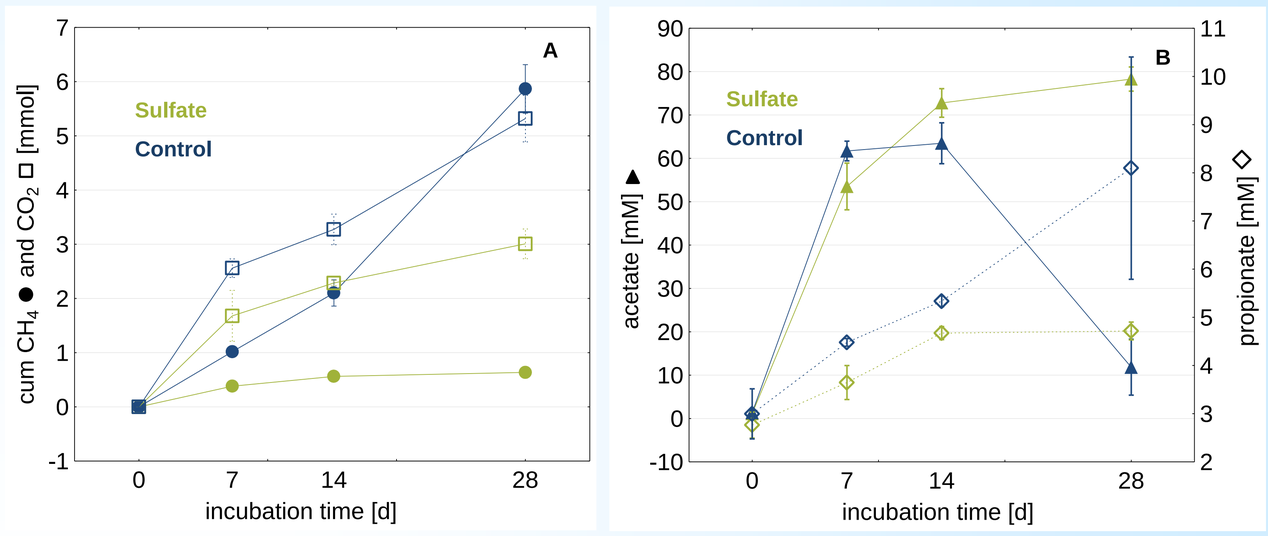

Fig. 2: Cumulative CH_4 and CO_2 production (A) and propionate and acetate concentrations (B) during the anaerobic degradation of a cellulose rich medium in the presence and absence of sulfate. Means of triplicates +/-standard deviation.

Material und Methods

A medium containing cellulose as main carbon source was anaerobically filled in serum flasks and partially amended with 3 g SO_4^{2-}/L. The medium was inoculated with diluted fermenter sludge from a thermophilic plug flow biogas reactor in Roppen (Tyrol) and incubated in batch reactions over a period of 4 weeks. Gas quantity and quality were examined via pressure and gas chromatography measurements. Further the production and consumption of volatile fatty acids (VFAs) was determined with high pressure liquid chromatography. To investigate patterns in the microbial community, samples were withdrawn on day 14 and 28 and the total genomic DNA extracted. Subsequently, PCR reactions with specific primers for Bacteria and Archaea were performed and the resulting products were analyzed using denaturing gradient gel electrophoresis (DGGE) .

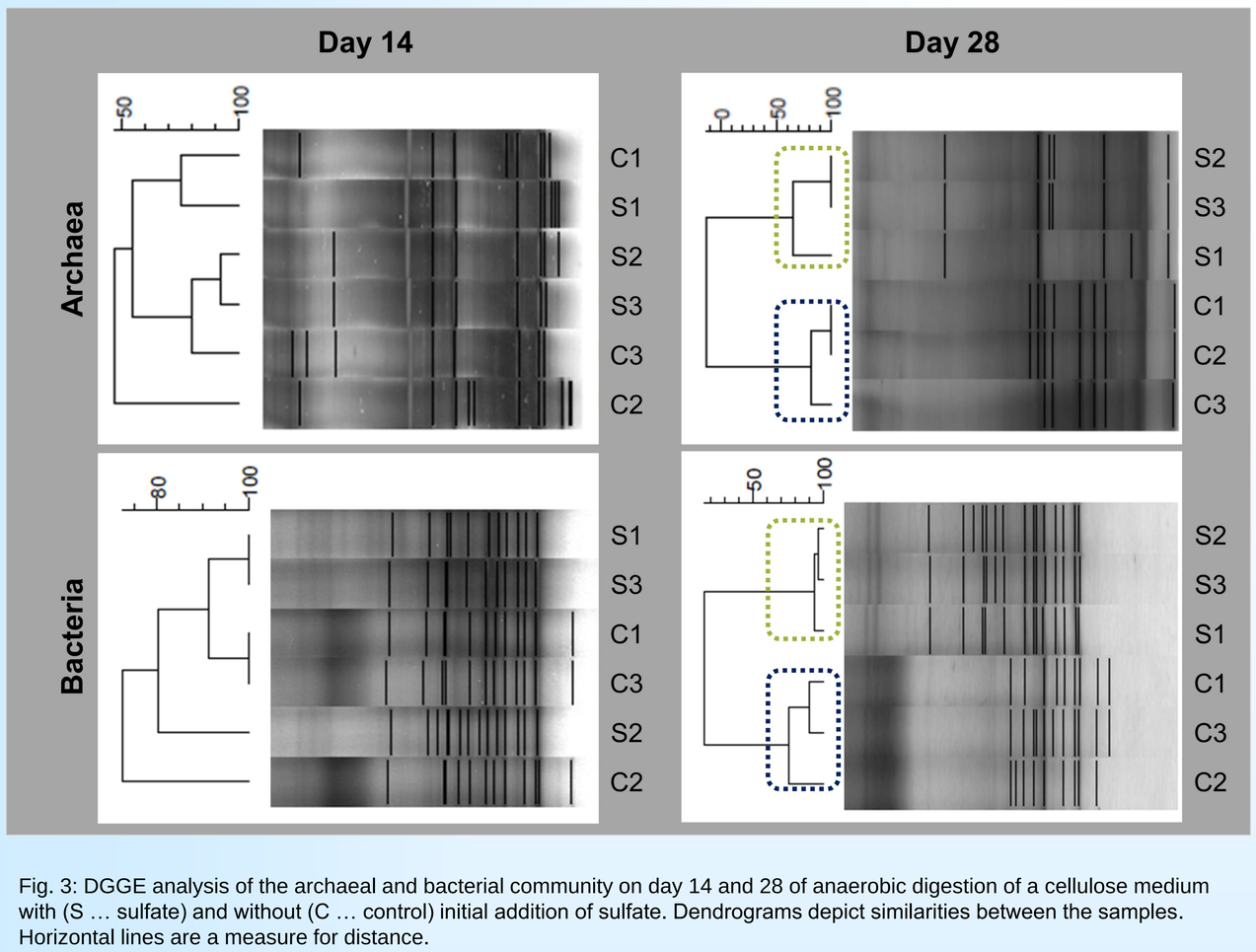

Fig. 3: DGGE analysis of the archaeal and bacterial community on day 14 and 28 of anaerobic digestion of a cellulose medium with (S … sulfate) and without (C … control) initial addition of sulfate. Dendrograms depict similarities between the samples. Horizontal lines are a measure for distance.

Results and Discussion

- Sulfate addition had a significant negative effect on the methane, carbon dioxide, and overall gas production (**Fig. 2A**).
- Hydrogen was not detected in any of the samples at any sampling time
- Acetate levels rose in all samples at the beginning of the digestion, the produced acetate was subsequently degraded in the controls, whereas it accumulated in the sulfate containing samples (**Fig. 2B**).
- Propionate concentrations in the sulfate containing samples were generally lower and stayed at a constant level in the second half of the incubation, while they steadily increased throughout the entire incubation in the controls (**Fig. 2B**).
- After 2 weeks no distinct differences in the composition of the microbial consortium could be observed (**Fig. 3**).
- At the end of the incubation period of 4 weeks, however, changes in the archaeal and bacterial community composition were found, when the triplicates clearly clustered together and differentiated from the control samples (**Fig. 3**).

Methanogenesis was strongly inhibited in the presence of high sulfate concentrations. As the acetate levels in the sulfate variants were very high, whereas hydrogen could not be measured, it can be concluded that competition between the SRB and the MPA focused on hydrogen not acetate. Propionate accumulation was reduced in the presence of sulfate, so it probably served as substrate for SRB. The molecular analysis showed, that visible changes in the microbial community lag distinctly behind changes in the gas and VFA data. Therefore it can be deduced, that the degrading consortium reacts to an altered sulfate level primarily by changing the activity of different groups, whereas changes in the abundances are a long-term adaption.

Summary

The addition of sulfate to the anaerobic digestion of cellulose had a significant effect on the gas production and composition as well as the VFA levels and the microbial community. Changes on the organism-level were, however, detectable weeks after changes in the other process parameters. This fact constrains the applicability of the microbial community as a short-term predicator for process disturbances. However, the present study also shows that a microbial community reacts very specifically and reproducibly to a newly introduced environmental change.

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