Effect of nitrate addition on the diversity, activity and abundance of sulfate reducers in high-temperature oil production systems

Antje Gittel[1], Adam Wieczorek[1], Ketil Sørensen[2], Torben L. Skovhus[2] , Kjeld Ingvorsen[1] and Andreas Schramm[1]

[1] Section of Microbiology, Department of Biological Sciences, University of Aarhus, Aarhus, Denmark; [2] Chemistry and Water Technology, Danish Technological Institute, Aarhus, Denmark

Contact: antje.gittel@biology.au.dk

Introduction

Sulfate-reducing prokaryotes (SRP) cause severe problems like **microbially induced corrosion (MIC) and reservoir souring** in seawater-injected oil production systems by producing highly reactive and toxic hydrogen sulfide. Adding nitrate to the injection water is a possible strategy to control the activity of SRP by favoring the growth of heterotrophic, nitrate-reducing bacteria that outcompete SRP for substrates, or nitrate-reducing, sulfide-oxidizing bacteria. To assess the effects of nitrate addition, microbial diversity and community structure of *Bacteria*, *Archaea* & SRP and SRP activity were studied in the production waters of a nitrate-treated (HALFDAN) and a non-treated (DAN) high-temperature oil production system in the Danish sector of the North Sea.

Community analyses: Cloning and sequencing of bacterial and archael 16S rRNA and *dsr*AB genes

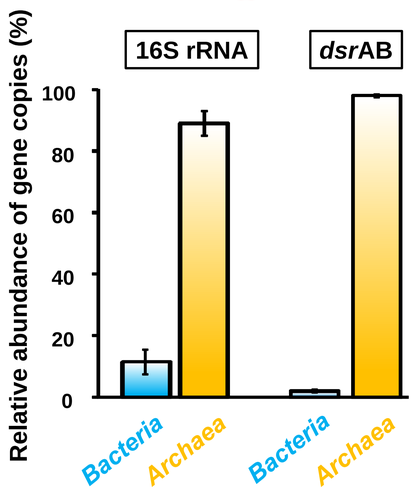
Activity: Determination of sulfate reduction rates (**SRR**) from annoxic time-series incubations of production water with radioactive sulfate and a substrate mixture

Quantification: quantitative PCR for bacterial and archael 16S rRNA and deltaproteobacterial and archaeal *dsr*AB genes

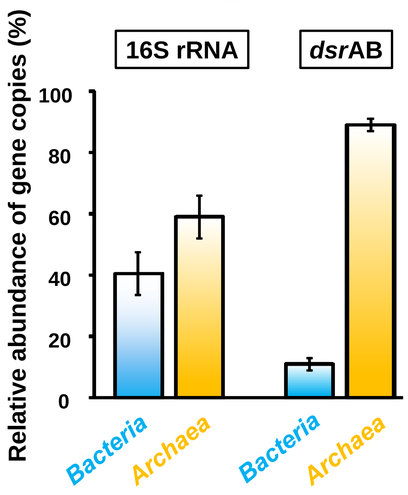
Results

Community structure and microbial diversity in production water

DAN (Nitrate)

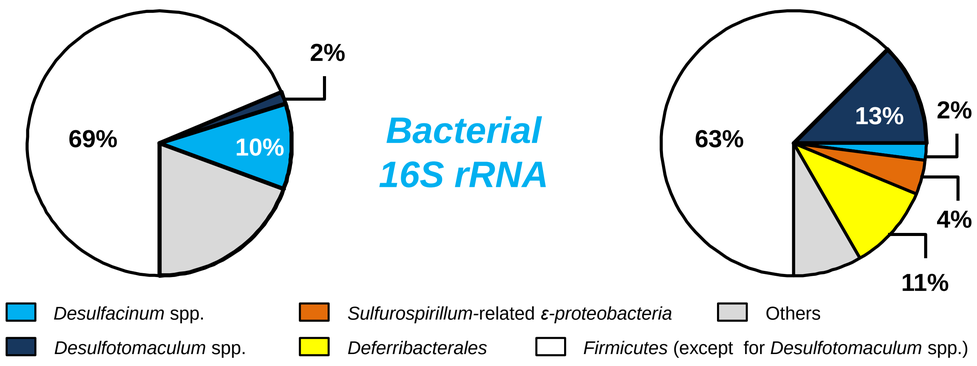


HALFDAN (Nitrate)

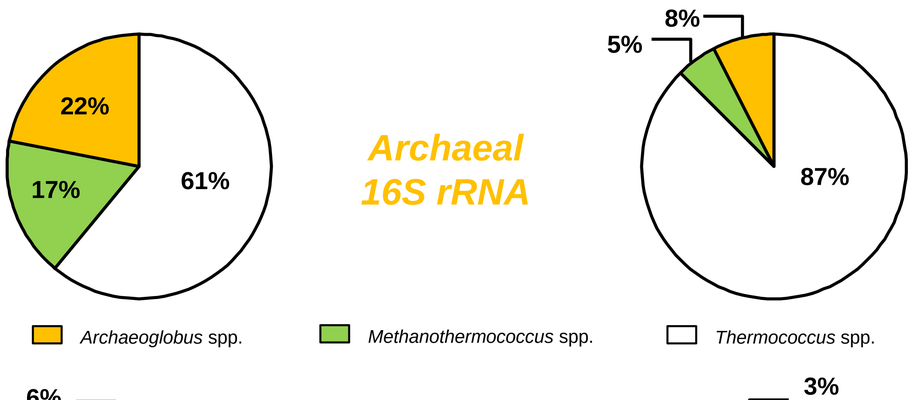


At both sites ***Archaea*** and ***Archaeoglobus-related SRP*** dominated the total prokaryotic and the sulfate-reducing community, respectively .

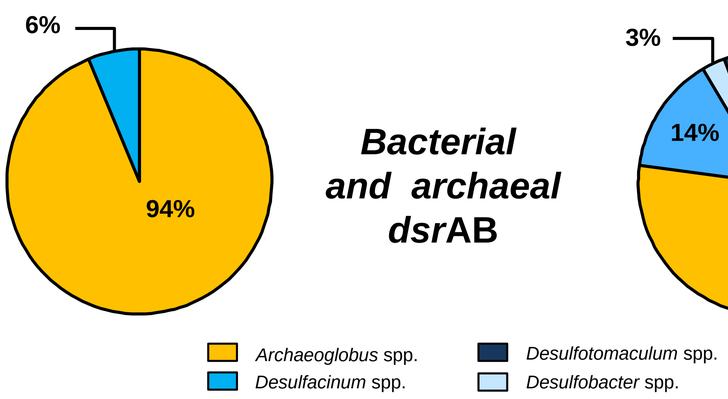
A — ***Bacterial 16S rRNA***



B — ***Archaeal 16S rRNA***



C — ***Bacterial and archaeal dsrAB***



It is indicated that **nitrate additon** results in

1) a **repression** of both thermophilic, deltaproteobacterial as well as archael sulfate reducers (A, B),
2) a **decrease** in *dsr*AB gene diversity (C) and
3) a **stimulation** of potential nitrate-reducing competitors (*Sulfurospirillum* spp., nitrate-reducing *Deferribacterales*)

Identification and quantification of potentially active SRP in production water

DAN (Nitrate)

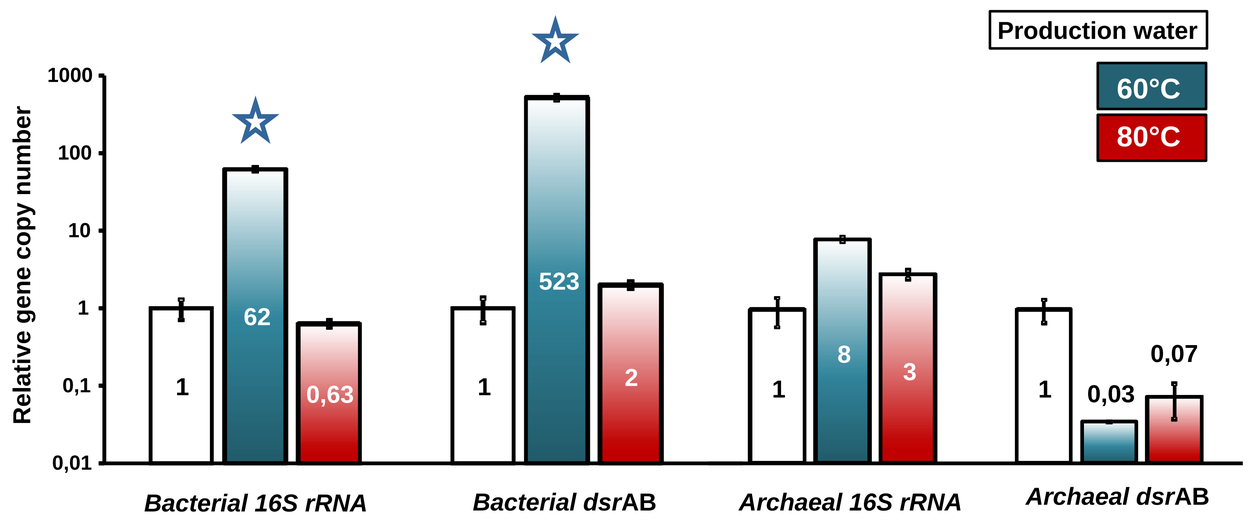
60°C (Pipeline temp.) — **~35 nmol mL^{-1} d^{-1}**

80°C (Reservoir temp.) — Below detection limit

HALFDAN (Nitrate)

No activity was observed for both temperatures even after 100 days of incubation

☆ Bacterial 16S rRNA gene and deltaproteobacterial *dsr*AB gene copy numbers significantly increased in the **60°C incubation**.

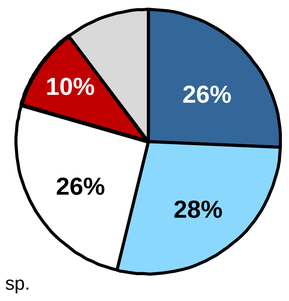


Cloning of full-length bacterial and archael 16S rRNA genes and deltaproteobacterial *dsr*AB genes

***Desulfacinum*-related SRP**

Methanosaeta*- related *Archaea

Enrichment of **thermophilic, deltaproteobacterial SRP** as well as thermophilic, sulfur-reducing *Synergistes* (*Anaerobaculum* spp.) and *Thermotogales* (*Petrotoga* spp.)



Conclusions & Perspectives

- Despite their low abundance in production water, **thermophilic, deltaproteobacterial sulfate reducers** might contribute significantly to the production of sulfide and therefore severe economical problems in oil production systems
- Abundance & diversity of SRP were shown to decrease in a nitrate-treated system and activity could not be stimulated, neither under pipeline nor reservoir conditions
- The remarkable high abundance of **archaeal sulfate reducers** enforces future studies on their physiological state, metabolic capacities and contribution to sulfide production in both systems
- Abundance and activity of promising nitrate-reducing competitors in the system will be assessed via functional gene detection and the ^{15}N isotope pairing technique

Acknowledgements We thank Britta Poulsen, Trine S. Thomsen and Tove Wiegers for assistance in molecular work and sulfate measurements. We are grateful to Jan Larsen (Maersk Oil, Denmark) who provided access to the sampling site.