Microbial ecology of marine sediments

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Course script

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Introduction

Primary production of organic matter in the oceans is mainly performed by phototrophic organisms. The formation of complex organic matter occurs in surface-near waters where enough light for photosynthesis is available. Organic compounds that are composed in the euphotic zone serve as an energy- and carbon-source for heterotrophic microorganisms. During sedimentation, organic matter is already degraded to different degrees by those organisms and only a small proportion is deposited. While the most important electron acceptor in the water column is oxygen, the subsequent microbial mineralization within the sediments happens under oxic- and anoxic conditions.

Transport of oxygen into the seafloor is mainly caused by diffusion. In tidal-flat sediments, for instance, the availability of dissolved oxygen is limited to the very upper millimeters due to microbial respiration and the (abiotic) reaction with sulfide (Revsbech et al. 1980). Underneath, the degradation of organic matter, that is still available within these layers, is performed stepwise by anaerobic microorganisms using different terminal electron acceptors. The first step of hydrolizing the residual complex organic polymers to monomers is catalyzed by fermentative bacteria. The following oxidation of fermentation products is again mediated by bacteria via the reduction of nitrate, $iron^{III}$, $manganese^{IV}$ and sulfate (Canfield 1993). As sulfate concentrations in the ocean are much higher than nitrate and as the hardly soluble $iron^{III}$ is often not available, dissimilatory sulfate reduction becomes the most important process for mineralizing organic carbon within marine sediments (Jørgensen 1982).

Energetically, sulfate-reducing bacteria have an advantage over methanogenic archaea in competing for degradation products of fermentative bacteria and acetogens (e.g. formate and acetate). The exception are layers in which sulfate is completely respired by sulfate reducers. Here, methane is produced and typically higher amounts of methanogens are found. However, some methanogenic archaea are able to utilize methylated compounds (methanol, methylamine or methylsulfide) that are unfavourable substrates for sulfate reducers. This can lead to a coexistence of methylotrophic methanogens and sulfate-reducing bacteria in sulfate containing horizons of marine sediments (Oremland 1982).

Aim of the course

The physiological diversity of microorganisms and their spatial distribution within marine sediments will be demonstrated according to chemical and physical parameters. Different physiological groups will be analysed along the sediment column of an intertidal sandflat and a beach. Sediment sampling will be preformed at site “Janssand”, a back barrier tidal-flat of the island “Spiekeroog” and the southern beach of the island in the beginning of the course.

Oxygen penetration, porewater sulfate and methane concentrations will be measured down to a depth of app. 5 meters (depending on sediment recovery). As microbiological parameters, total cell numbers will be counted after SYBR-green staining and the numbers of archaea and bacteria will be calculated after quantitative PCR. More specifically, the relative amount of sulfate reducers and methanogens will also be determined by quantitative PCR targeting key-genes for sulfate reduction and methanogenesis. Furthermore, every single group of students will specifically enrich one physiological type of microorganisms from distinctive sediment layers of site Janssand. Microbial growth and activity will be monitored over the whole period of the course.

Accompanying the course, all participants will give a 30-45 minute talk to introduce “their” physiological group concerning its ecology, physiology, and strategies for a specific enrichment. All the data and observations of the single groups will be combined at the end of the course to draw an overall picture of microbial diversity and the occurrence of the different physiological groups corresponding to relevant geochemical gradients.

Group O
Aerobic bacteria in Wadden Sea sediment

Tasks:

- Oxygen profile and determination oxygen penetration depth
- Quantification of the total cell number
- Selective enrichment of heterotrophic aerobes
  a) Comparison of two selected depths (0-2cm, 9-10cm)
  b) Influence of different ratio between glucose and oxygen
- Estimation of aerobic heterotrophe numbers by dilution series and deep agar technique
- Selective enrichment of aerobic chemolithotrophes
- Quantification of total archaeal 16S copies by q-PCR

Seminar:

A) Background
- Oxygen availability in marine environments
- Respiration and fermentation

B) Halftime report (17.06.)
- Introduction into the methods applied
- Aims, expectations and preliminary results

Group N

Nitrate-reducing bacteria in Wadden Sea sediment

Tasks:

- Porewater profile of ammonia, nitrite and nitrate
- Quantification of the total cell number
- Selective enrichment of nitrate-reducing bateria
    - c) Comparison of two selected depths (0-2cm, 9-10cm)
    - d) Influence of different electron donors (formate, acetate, propionate and glucose) on nitrate consumption (IC) and nitrite (IC) and nitrogen gas production
- Estimation of nitrate reducer numbers by dilution series and deep agar technique
- Quantification of total bacterial 16S copies by q-PCR

Seminar:

A) Background

- The marine nitrogen cycle
- Nitrate reducers: Physiology and Ecology

B) Halftime report (17.06.)

- Introduction into the methods applied
- Aims, expectations and preliminary results

Group S

Sulfate-reducing bacteria in Wadden Sea sediment

Tasks:

- Porewater profile of sulfate and chloride
- Quantification of the total cell number
- Abundance of SRB determined by q-PCR analysis of dsrAB genes
- Selective enrichment of SRB
    - e) Comparison of two selected depths (10 and 50 cm)
    - f) Influence of different electron donors (formate, acetate, propionate and glucose) on sulfate consumption (IC) and sulfide production (photometric)
- Estimation of SRB numbers by dilution series and deep agar technique

Seminar:

A) Background

- The marine sulfur cycle
- Sulfate-reducing bacteria: Physiology and Ecology

B) Halftime report (18.06.)

- Introduction into the methods applied
- Aims, expectations and preliminary results

Group C

Methanogens in Wadden Sea sediment

Tasks:

- Methane profile
- Quantification of the total cell number
- Selective enrichment of methonegens
    - g) Comparison of two selected depths (50 and 250 cm)
    - h) Comparison of two sampling sites
    - i) Influence of different electron donors (formate, acetate, propionate, glucose) on the production of methane
- Detection of enriched methanogenes via autofluorescence microscopy
- Determination of sediment porosity and density
- Abundance of methanogenes determined by q-PCR analysis of mcrA genes

Seminar:

A) Background

- The marine carbon cycle
- Methanogens: Physiology and Ecology

B) Halftime report (18.06)

- Introduction into the methods applied
- Aims, expectations and preliminary results

Schedule of the very first day: Mon, 7. June 2010

11:00, Rom W15-0-023:

a) Introduction

b) Briefing for both sampling trips and master plan for sampling

Group O

- Media preparation: 1 liter basic media (SCM, HEPES buffered)
- Preparation of stock solutions:
  1.) Glucose (1 M, 100 ml)
  2.) Thiosulfate (1M, 100 ml)
  3.) Ammonia (1 M, 100 ml)
- Preparation and washing (3x) of agar (3.3g in 100 ml final), portion to 3 ml per tube
- Autoclaving of media, stock solutions, glass ware (+ stoppers) and agar
- Cooling, finishing and filling of media in serum bottles (18x) and Pfennig bottles (3-4x)
- Packing for sampling (eg. Short push tubes and stoppers)

Group N

- Media preparation: 1 liter anoxic basic media for nitrate reducers (w/o sulfide)
- Preparation of stock solutions:
  1.) Sodium nitrate (1 M, 100 ml)
  2.) Sodium formate (1M, 100 ml)
  3.) Ascorbic acid (1 M, 100 ml)
- Preparation and washing (3x) of agar (3.3g in 100 ml final), portion to 3 ml per tube
- Autoclaving of media, stock solutions, glass ware (+ stoppers) and agar
- Cooling, finishing and filling of media in serum bottles (18x) and Pfennig bottles (3-4x)
- Packing for sampling (eg. Short push tubes and stoppers)

Groups S und C

- Media preparation: 2 liter anoxic basic media for SRB and methanogens
- Preparation of stock solutions:
  1.) Sulfate (2 M, 100 ml)
  2.) Sodiumacetate (1M, 100 ml)
  3.) Propionate (1M, 100 ml)
- Autoclaving of media, stock solutions, glass ware (+ stoppers)
- Group S: Cooling, finishing and filling of media in serum bottles (18x) and Pfennig bottles (3-4x)
- Group C: Preparation of NaOH solution for sediment fixation and portioning in tubes
- Group C: Cooling, finishing and filling of media in serum bottles (18x) and Pfennig bottles (3-4x)
- Group S: Preparation of fixing agents for total cell counts
- Packing for sampling

Schedule for the entire course

	Group 1 (O)	**Group 2 (N)**	**Group 3 (S)**	**Group 4 (C)**
Mon (07.06)	9:00 Written test Microbial Ecol 11:00 Intro Preparation of media and sampling (see detailed schedule)	9:00 Written test Microbial Ecol 11:00 Intro Preparation of media and sampling (see detailed schedule)	9:00 Written test Microbial Ecol 11:00 Intro Preparation of media and sampling (see detailed schedule)	9:00 Written test Microbial Ecol 11:00 Intro Preparation of media and sampling (see detailed schedule)
Tue (08.06)	9:00 preparation of sampling 12:30 Departure to **Janssand** (recovery of a 5m core) 17:00 Sampling for pore water, cell counts, DNA Inoculation of media (enrichments, dilution series)	9:00 preparation of sampling 17:00 Sampling for pore water, cell counts, DNA Inoculation of media (enrichments, dilution series)	9:00 preparation of sampling 12:00 Departure to **Janssand** (recovery of a 5m core) 17:00 Sampling for pore water, cell counts, DNA Inoculation of media (enrichments, dilution series)	9:00 preparation of sampling 12:00 17:00 Sampling for pore water, cell counts, DNA Inoculation of media (enrichments, dilution series)
Wed (09.06)	9:00 O_2 profile Janssand 18:00 Sampling for pore water, cell counts, DNA	9:00 Agar shakes 13:00 Departure to **Spiekeroog** (recovery of a 5m core) Pore water 18:00 Sampling for pore water, cell counts, DNA	9:00 Agar shakes 18:00 Sampling for pore water, cell counts, DNA	9:00 Agar shakes 13:00 Departure to **Spiekeroog** (recovery of a 5m core) Pore water 18:00 Sampling for pore water, cell counts, DNA
Thu (10.06)	12:00 O_2 profile Spiekeroog	12:00 Pore water Ammonia	12:00 Pore water Sulfate IC	12:00 Pore water Methane GC
Fri (11.06)	9:00 Enrichment cultures Microscopy,OD	9:00 Enrichments (Nitrate, nitrite, ammonia, etc	9:00 Enrichments (Sulfate, sulfide)	9:00 Enrichments (Methan) Porosity/density

	Group 1 (O)	Group 2 (N)	Group 3 (S)	Group 4 (C)
Mon (14.06)	9:00 Enrichment Isolation Microscopy,OD Cell counts	9:00 Enrichments (Nitrate,nitrite, ammonia) N-Profiles	9:00 Enrichments (Sulfate, sulfide) Sulfate, chlorid	9:00 Enrichments (Methan) Porosity/density Methan-Profile
Tue (15.06)	9:00 Enrichment Isolation Microscopy,OD Cell counts	9:00 Enrichments (Nitrate,nitrite, ammonia) N-Profiles	9:00 Enrichments (Sulfate, sulfide) Sulfate, chlorid	9:00 Enrichments (Methan) Methan-Profile
Wed (16.06)	9:00 Enrichment Isolation 17:00 Jed Fuhrman	9:00 Enrichments 17:00 Jed Fuhrman	9:00 Enrichments 17:00 Jed Fuhrman	9:00 Enrichments Cell counts 17:00 Jed Fuhrman
Thu (17.06)	9:00 Seminar O+N Enrichment Isolation	9:00 Seminar O+N Enrichment Cell counts	9:00 Seminar O+N Enrichment	9:00 Seminar O+N Enrichment
Fri (18.06)	9:00 Seminar S+C Enrichment	9:00 Seminar S+C Enrichment	9:00 Seminar S+C Enrichment Cell counts	9:00 Seminar S+C Enrichment

	Group 1 (O)	Group 2 (N)	Group 3 (S)	Group 4 (C)
Mon (21.06)	9:00 VL Microscopy 10:30 Enrichment Isolation	9:00 VL Microscopy 10:30 Enrichment Isolation	9:00 VL Microscopy 10:30 Enrichment Isolation	9:00 VL Microscopy 10:30 Enrichment
Tue (22.06)	9:00 VL Wadden Sea 10:30 Enrichment Isolation DNA-extraction	9:00 VL Wadden Sea 10:30 Enrichment Isolation DNA-extraction	9:00 VL Wadden Sea 10:30 Enrichment Isolation HPLC	9:00 VL Wadden Sea 10:30 Enrichment HPLC
Wed (23.06)	9:00 VL Spectroscopy DNA-extraction HPLC	9:00 VL Spectroscopy DNA-extraction HPLC	9:00 VL Spectroscopy DNA-extraction	9:00 VL Spectroscopy DNA-extraction
Thu (24.06)	9:00 VL Chromatography	9:00 VL Chromatography	9:00 VL Chromatography DNA-extraction	9:00 VL Chromatography DNA-extraction
Fri (25.06)	9:00 Enrichment q-pcr	9:00 Enrichment	9:00 Enrichment	9:00 Enrichment

	Group 1 (O)	**Group 2 (N)**	**Group 3 (S)**	**Group 4 (C)**
Mon (28.06)	9:00 Enrichment Isolation	9:00 Enrichment Isolation q-pcr	9:00 Enrichment	9:00 Enrichment
Tue (29.06)	9:00 Enrichment	9:00 Enrichment	9:00 Enrichment q-pcr	9:00 Enrichment
Wed (30.06)	9:00 Enrichment	9:00 Enrichment	9:00 Enrichment	9:00 Enrichment q-pcr
Thu (01.07)	Data aquisition 17:00 Barbecue and Volta	Data aquisition 17:00 Barbecue and Volta	Data aquisition 17:00 Barbecue and Volta	Data aquisition 17:00 Barbecue and Volta
Fri (02.07)	Final reports 9:00 Goup O 9:45 Group N 10:45 Group S 11:30 Group C	Final reports 9:00 Goup O 9:45 Group N 10:45 Group S 11:30 Group C	Final reports 9:00 Goup O 9:45 Group N 10:45 Group S 11:30 Group C	Final reports 9:00 Goup O 9:45 Group N 10:45 Group S 11:30 Group C