ISSM2011 – microbial life below our feet

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Who is doing the job during anaerobic degradation in a tidal-flat sediment?

Sulfate reducers and methanogens

- **easily detectable by ‘functional gene markers‘**
- **make ~10% of anaerobic communities**

Little known about the inital steps

Hamster

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ORIGINAL ARTICLE

Degradation of cyanobacterial biomass in anoxic tidal-flat sediments: a microcosm study of metabolic processes and community changes

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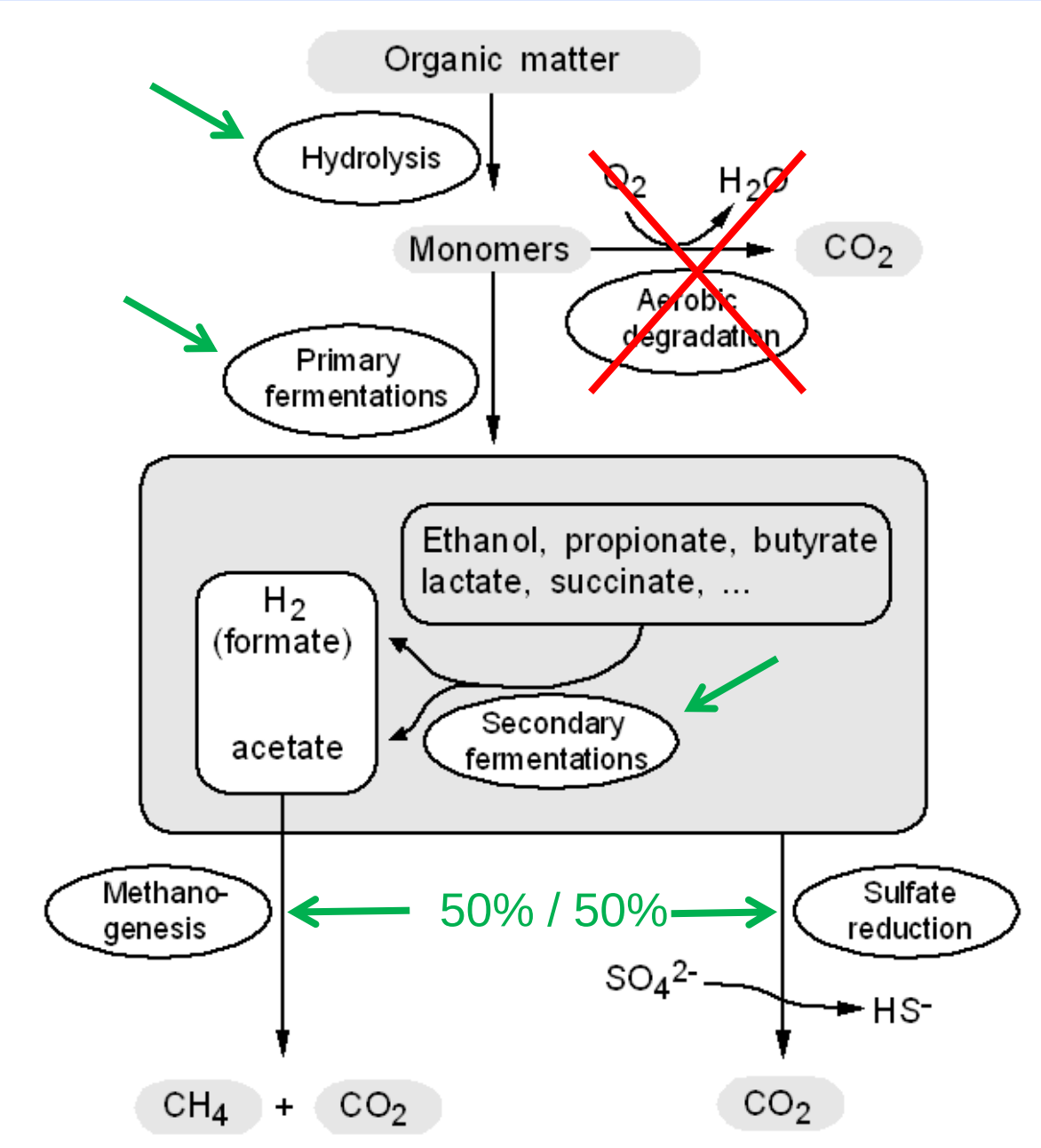
Degradation of organic matter



Sampling tidal-flat sediment

Vials used in the microcalorimeter

Microcalorimeter

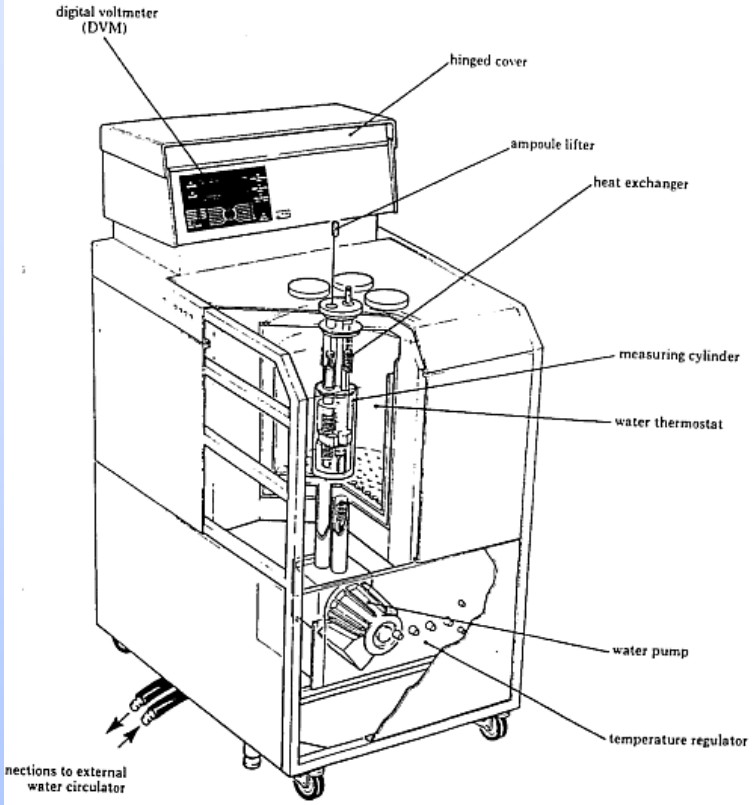


Fig.2 Thermal Activity Monitor

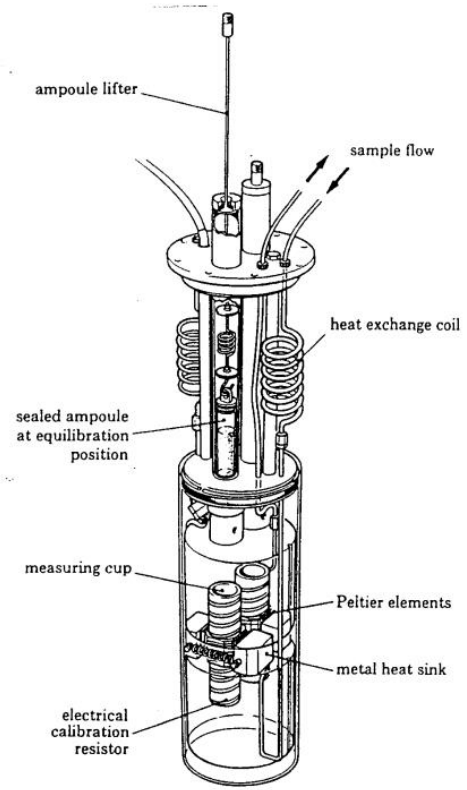


Fig.4 Combination Measuring Cylinder

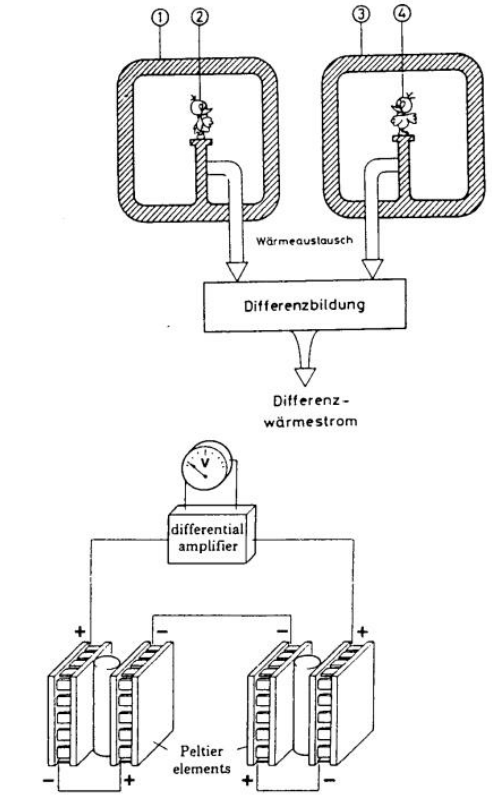
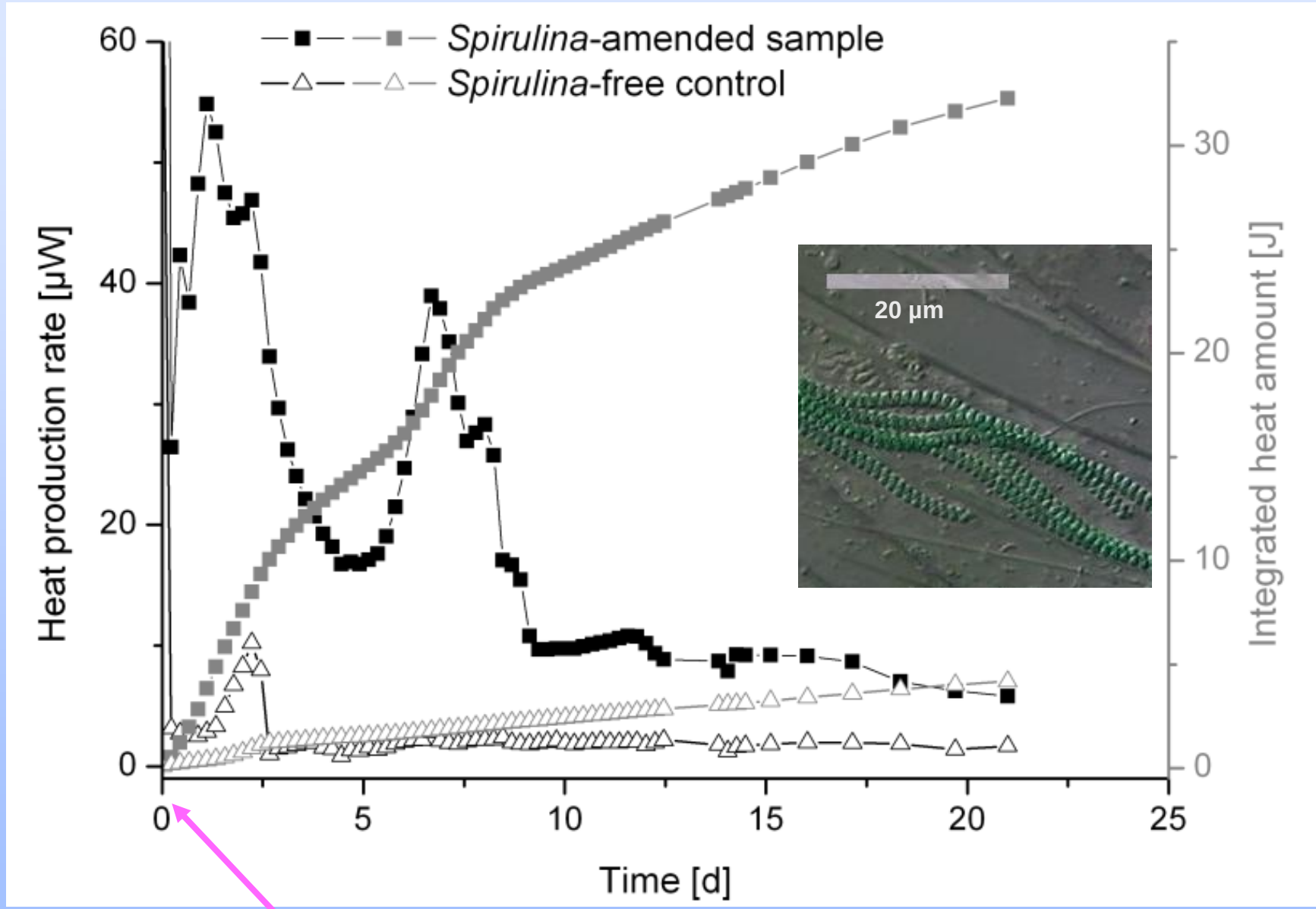


Fig.5 Twin Measuring Principle

$$\Delta G = \Delta H - T\,\Delta S$$

Detection limit ~1 µW

Stimulation of microbial activity by addition of ^{13}C-labelled biomass



130 mg *Spirulina* in 22 ml sediment slurry

Overall reaction (after 3 weeks)

$2530.5 <CH_2O> + 183\ SO_4^{2-} \longrightarrow$

366 acetate + 102 propionate + 123 butyrate + 5 valerate

$+ 183\ HS^- + 214\ CH_4 + 761.5\ CO_2 + 547.5\ H_2O + 413\ H^+$

- Maximum activity after 2 days, 2nd maximum after 7 days
- 90 % of the activity due to *Spirulina* addition
- Degradation incomplete
- Sulfate reduction ≅ methanogenesis
- Acidification (pH 7.3 → 6.3)
- Heat release (-33 J) < $\Delta G°'$ of equation (-104 J)

Process analyses (GC, HPLC, IC)

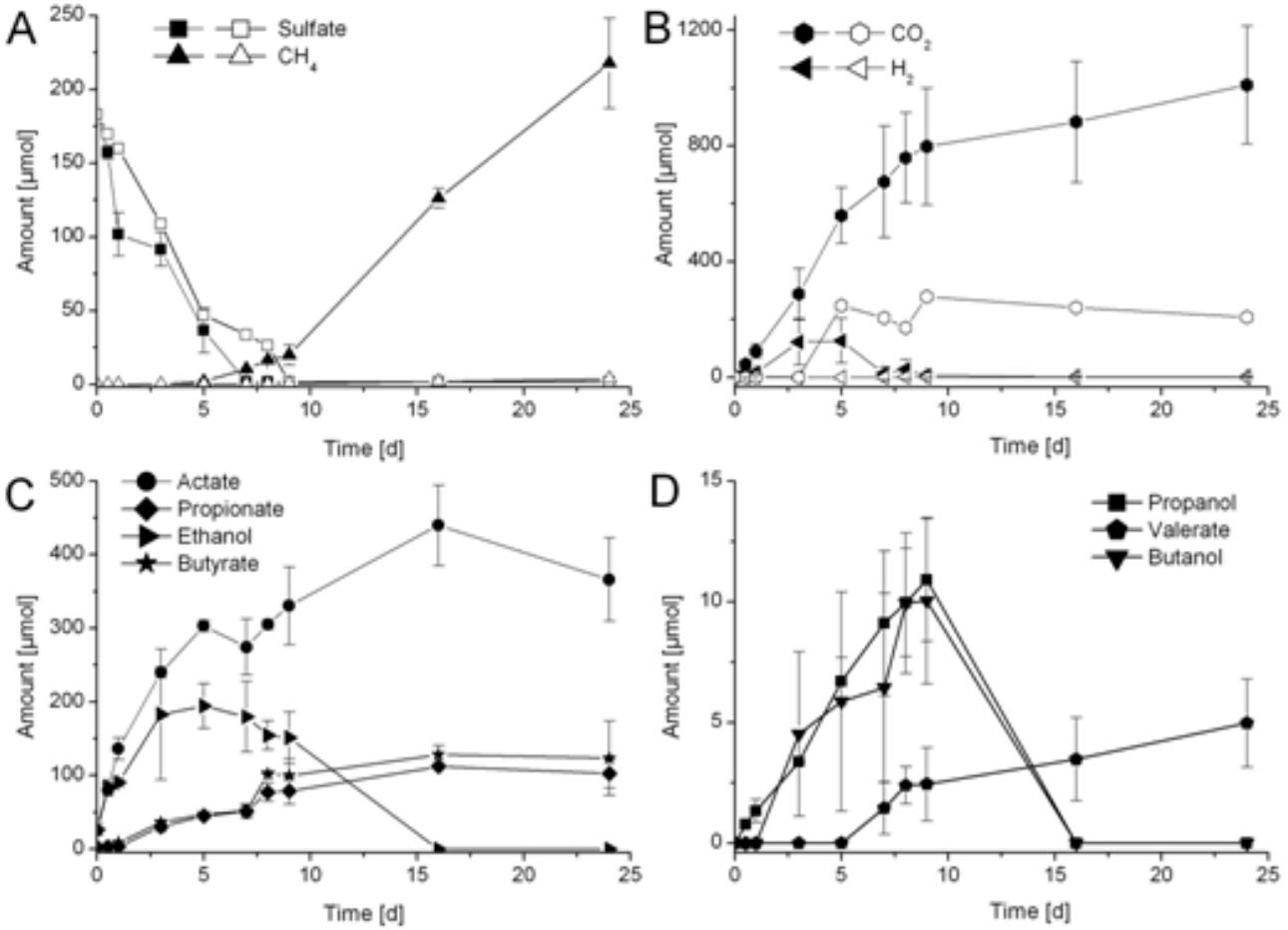


Figure 2 Chemical analyses of ^{13}C-*Spirulina* amended samples (filled symbols, mean values of triplicates) and *Spirulina*-free controls (open symbols, single measurements). A: CH_4 production and sulfate consumption, B: CO_2 and H_2, C: Major fermentation products, D: minor fermentation products. Error bars are given for the *Spirulina*-amended samples.

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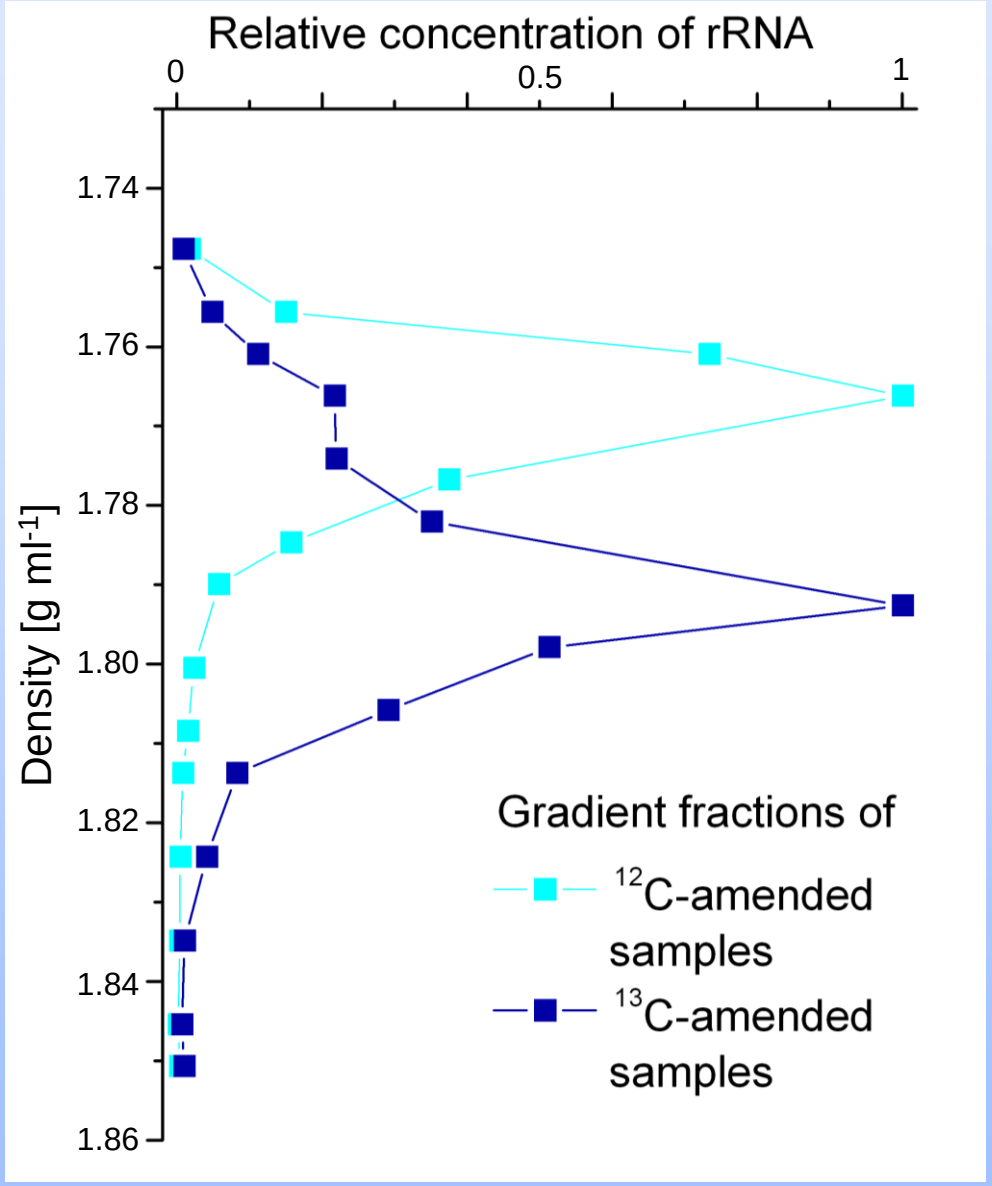
Processes

- Sulfate consumed after 6 days (control 2 days later)
- Methanogenesis only with *Spirulina* amendment
- H_2 rapidly consumed, main visible intermediate: acetate
- Ethanol, propanol, butanol consumed after 2 weeks
- Acetate, propionate, butyrate slowly consumed
- Secondary fermentations dominant after sulfate depletion

Stable-Isotope Probing

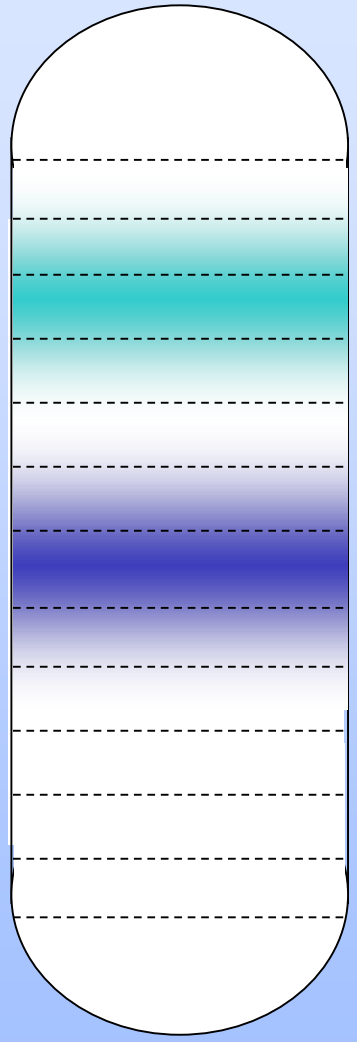
- Provide excess of ^{13}C-labeled substrate
- Growing cells incorporate heavy carbon
- Ribosome numbers and rRNA contents increase in active cells
- RNA separated by density gradient centrifugation
- Reverse transcriptase used to get DNA for further analyses
- DGGE and sequencing …

RNA-SIP



^{12}C-RNA

^{13}C-RNA



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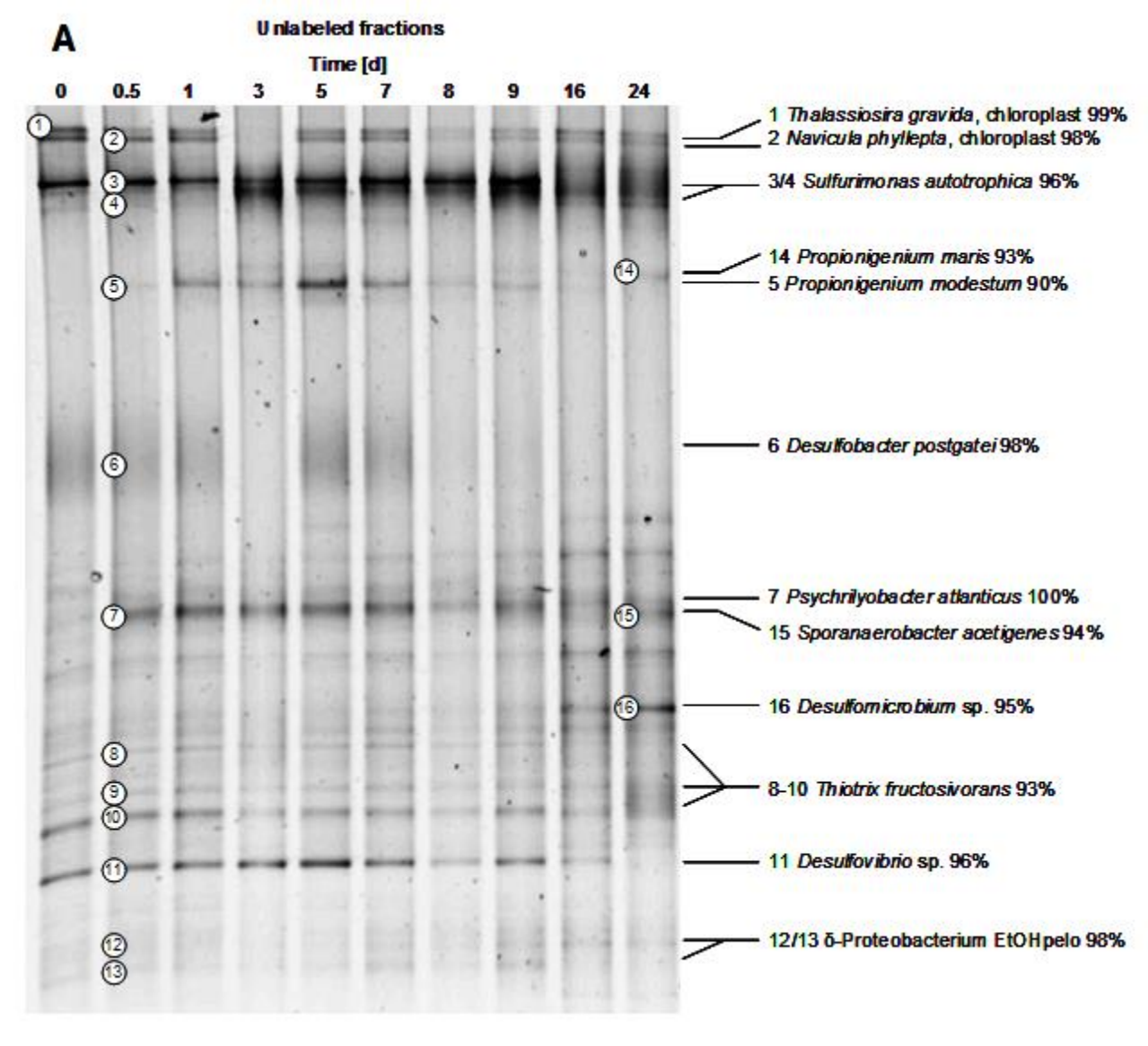
Distribution of RNA after gradient centrifugation

SIP – non-labeled community



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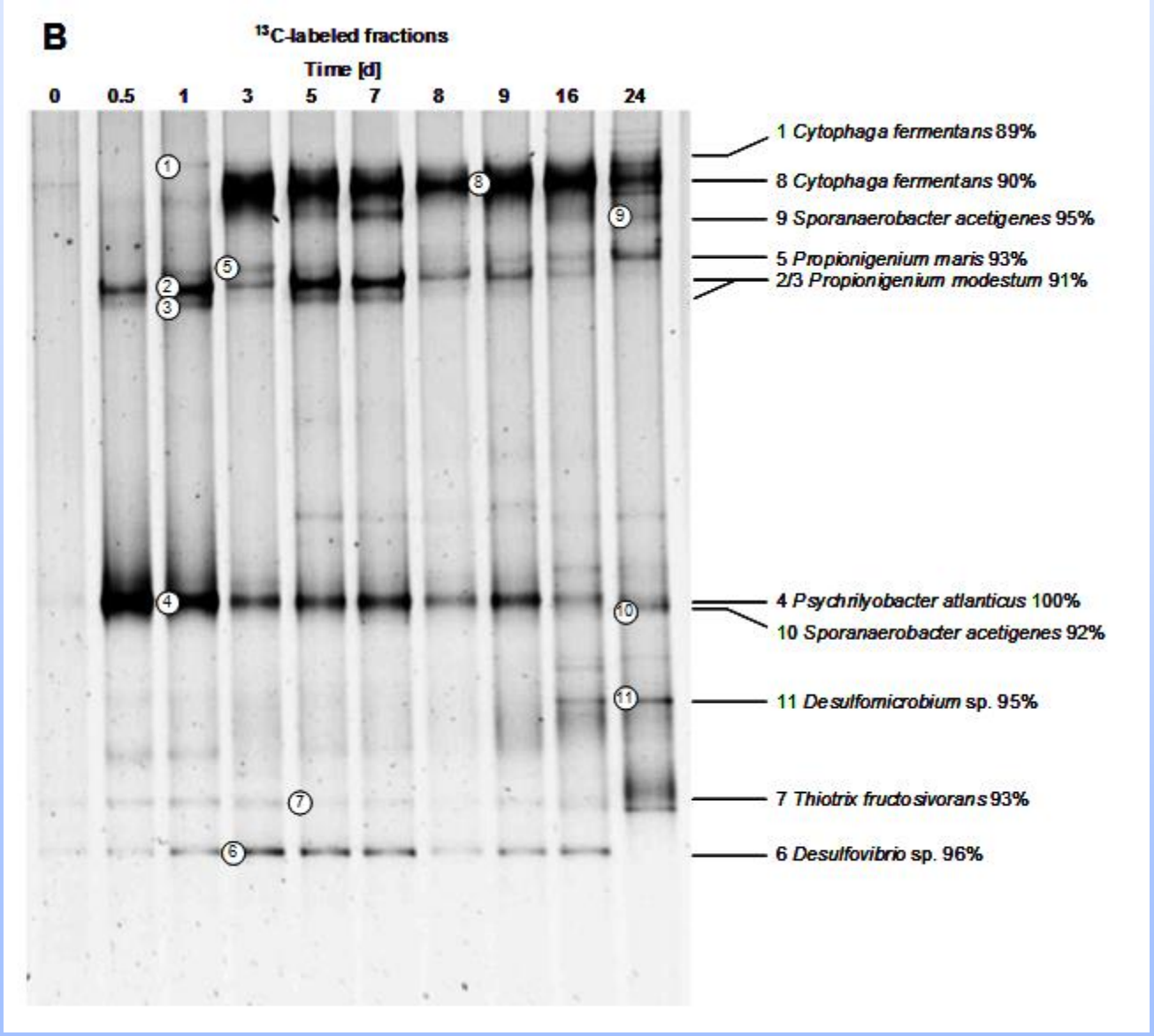
SIP – ^{13}C-labeled populations



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Conclusions

Combination of microcalorimetry + RNA-SIP allows detailed assessment of anaerobic degradation

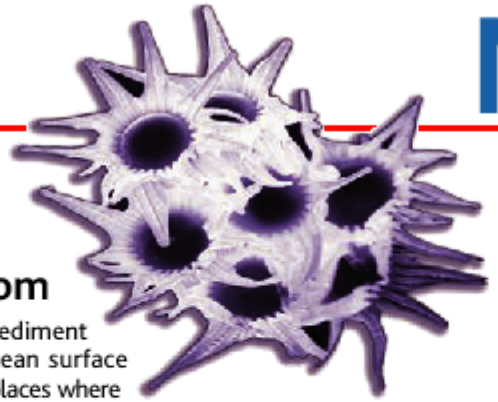
Primary fermentations (+ sulfate reduction) cause maximum activity

***Psychrilyobacter atlanticus* first stimulated fermenter (known to form H_2 + acetate)**

Secondary fermentations take over after sulfate depletion

Completely oxidising SRB didn‘t have a chance due to rapid sulfate depletion

NetWatch



IMAGES

Microbes in Bloom

A briny desert lake and sediment 5000 meters below the ocean surface are just two of the unlikely places where microbes prosper. The Microbiological Garden, tended by Heribert Cypionka of the University of Oldenburg in Germany, shows off the bugs dwelling in these exotic environments and in habitats closer to home. The site features more than 20 photo essays on microbial topics. You can tag along on bug-hunting expeditions, learn how to isolate luminescent bacteria from herring, and observe the bugs that inhabit the scum on the surface of a stagnant pool. Some microbes make the gallery because of their beauty, such as these yeast spores (*Emericella stellamaris*; above) that resemble flowers.

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