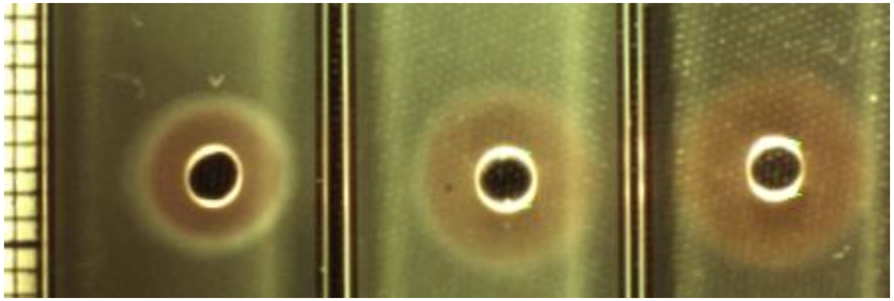
Tempting poision:

Sulfate-reducing bacteria in oxic-anoxic gradients



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www.icbm.de/pmbio

Sulfate-reducing bacteria (SRB)

SRB couple oxidation of electron donors to the reduction of sulfate (or some other electron acceptors)

The main electron donors are fermentation products (H_2, acetate, ethanol, lactate, propionate, butyrate...)

Depending on the species, organic substrates are oxidized incompletely to acetate (fast growing species) or completely to CO_2 (slow growing species)

SRB are known to be anaerobes since 1895 (Beijerinck)

Note: The name sulfate reducer might be restrictive for our thinking. SRB can do more than only reduce sulfate.

Desulfovibrio desulfuricans CSN

our '*E. coli* '

D. desulfuricans **CSN is a typical** ***Desulfovibrio*** **carrying out incomplete oxidation of** H_2**, lactate, ethanol and a few more organic compounds**

However, ***D. desulfuricans*** **CSN has extremely versatile sulfur metabolism and electron transport capacities - like many** ***Desulfovibrio*** **and several other species**

Note: Many SRB do not prefer sulfate reduction if alternate electron acceptors are present.

Reactions of the sulfur cycle catalyzed by SRB

Complete reduction of sulfur compounds

- Reduction of sulfate, thiosulfate, sulfite or elemental sulfur to sulfide

Incomplete reduction of sulfur compounds

- Formation of thionates und electron donor limitation

Disproportionation of sulfur compounds

- Formation of sulfide plus sulfate from intermediate sulfur compounds

Oxidation of sulfur compounds

- Formation of sulfate from sulfide or intermediate sulfur compounds using oxygen or nitrate as electron acceptor

Note: SRB are the only organisms known to carry out an inorganic fermentation and all reactions of the sulfur cycle.

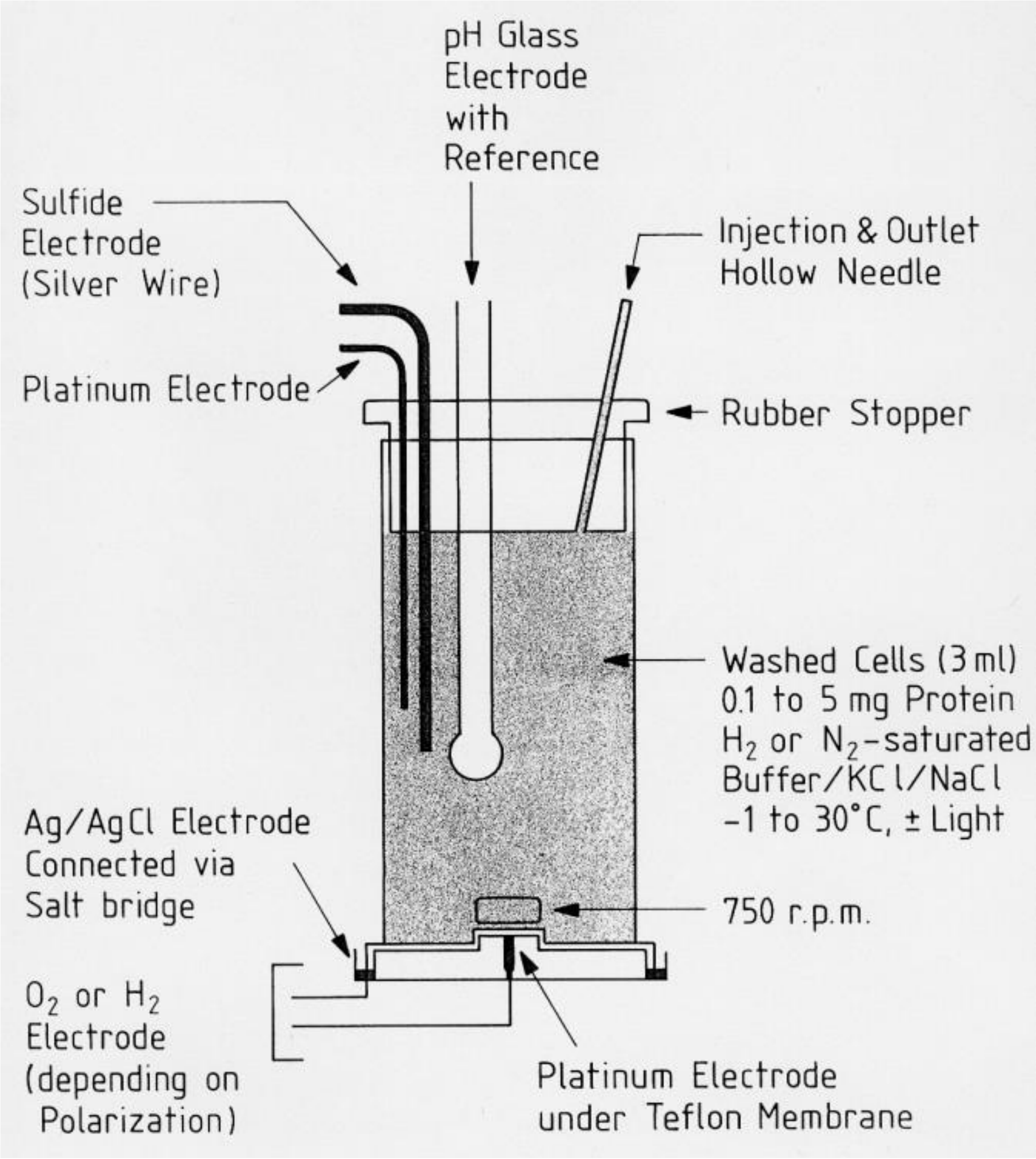
Reactions of the sulfur cycle catalyzed by SRB

Reaction	$\Delta G_0'$ (kJ/mol)
Complete reduction of sulfur compounds	
$SO_4^{2-} + 4\ H_2 + 1.5\ H^+ \rightarrow 0.5\ HS^- + 0.5\ H_2S + 4\ H_2O$	-155
$0.5\ SO_3^{2-} + 0.5\ HSO_3^- + 3\ H_2 + H^+ \rightarrow 0.5\ HS^- + 0.5\ H_2S + 3\ H_2O$	-175
$S_2O_3^{2-} + 4\ H_2 + H^+ \rightarrow HS^- + H_2S + 3\ H_2O$	-179
$S + H_2 \rightarrow 0.5\ HS^- + 0.5\ H_2S + 0.5\ H^+$	-30
Incomplete reduction of sulfate	
$SO_4^{2-} + 2\ H_2 + H^+ \rightarrow 0.5\ S_2O_3^{2-} + 2.5\ H_2O$	-65
Disproportionation of sulfur compounds	
$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + 0.5\ HS^- + 0.5\ H_2S + 0.5\ H^+$	-25
$2\ SO_3^{2-} + 2\ HSO_3^- \rightarrow 3\ SO_4^{2-} + 0.5\ HS^- + 0.5\ H_2S + 0.5\ H^+$	-236
$4\ S + 4\ H_2O \rightarrow SO_4^{2-} + 1.5\ HS^- + 1.5\ H_2S + 3.5\ H^+$	+33[1)]
Oxidation of sulfur compounds	
$0.5\ HS^- + 0.5\ H_2S + 2\ O_2 \rightarrow SO_4^{2-} + 1.5\ H^+$	-794
$0.5\ HS^- + 0.5\ H_2S + NO_3^- + 0.5\ H+ + H_2O \rightarrow SO_4^{2-} + NH_4^+$	-445
$S_2O_3^{2-} + 2\ H_2O + 2\ O_2 \rightarrow 2\ SO_4^{2-} + 2\ H^+$	-818
$0.5\ SO_3^{2-} + 0.5\ HSO_3^- + 0.5\ O_2 \rightarrow SO_4^{2-} + 0.5\ H^+$	-257[2)]

[1)] Promoted by precipitation of H_2S

[2)] Incomplete reduction may result in the formation of H_2O_2

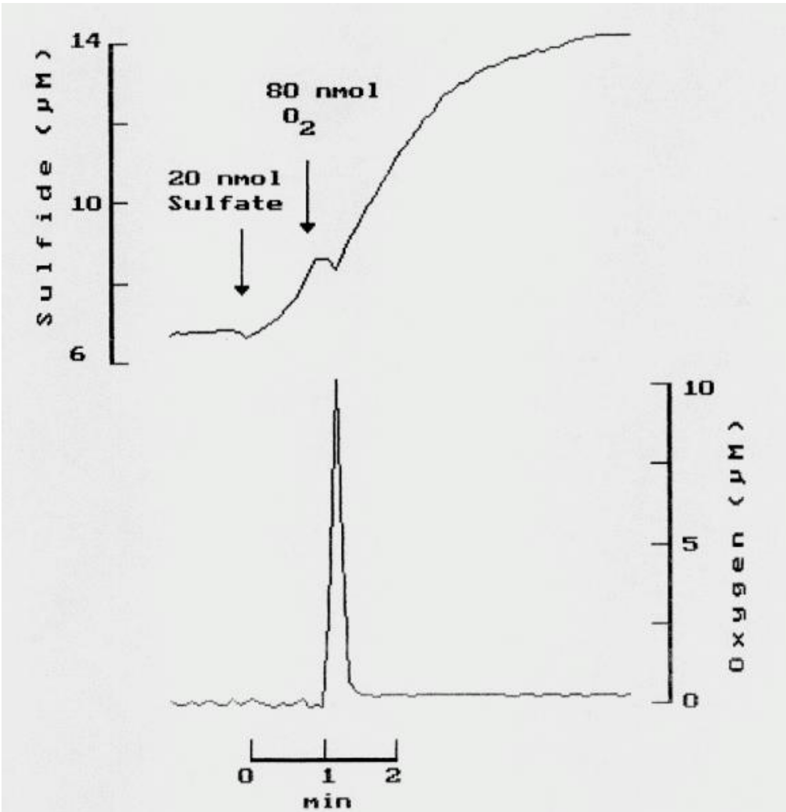
Note: Better download from our homepage than copy out now...



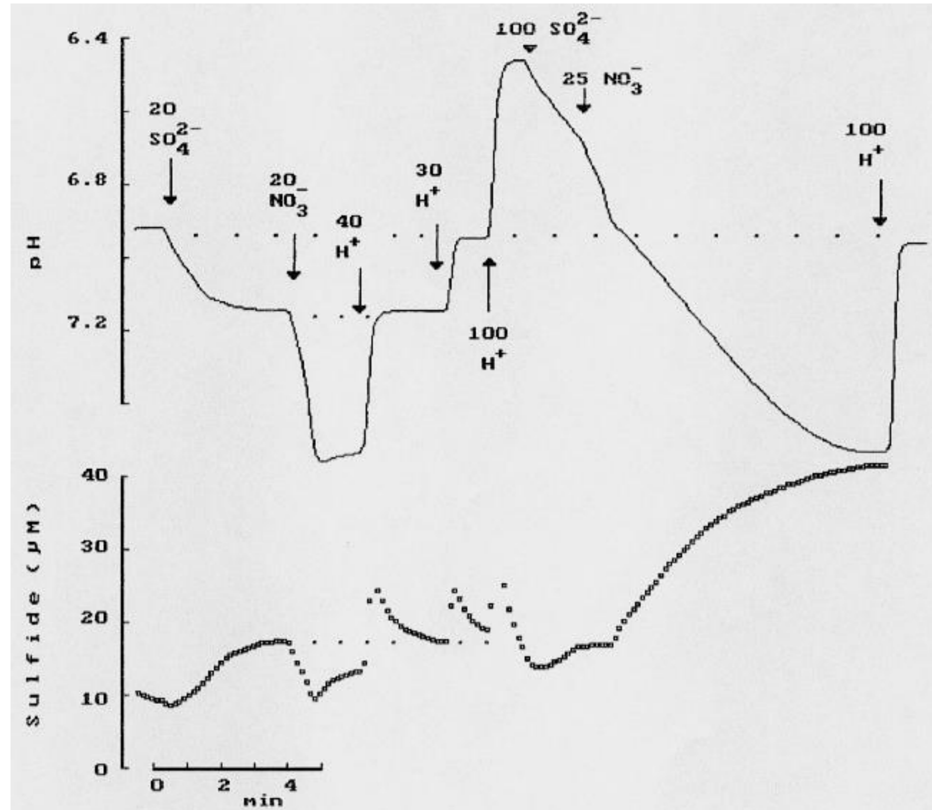
Multi-electrode device for study of SRB metabolism

Cypionka H.
Methods in Enzymol 243:3-14

The preferred electron acceptor



Oxygen preferred vs. sulfate



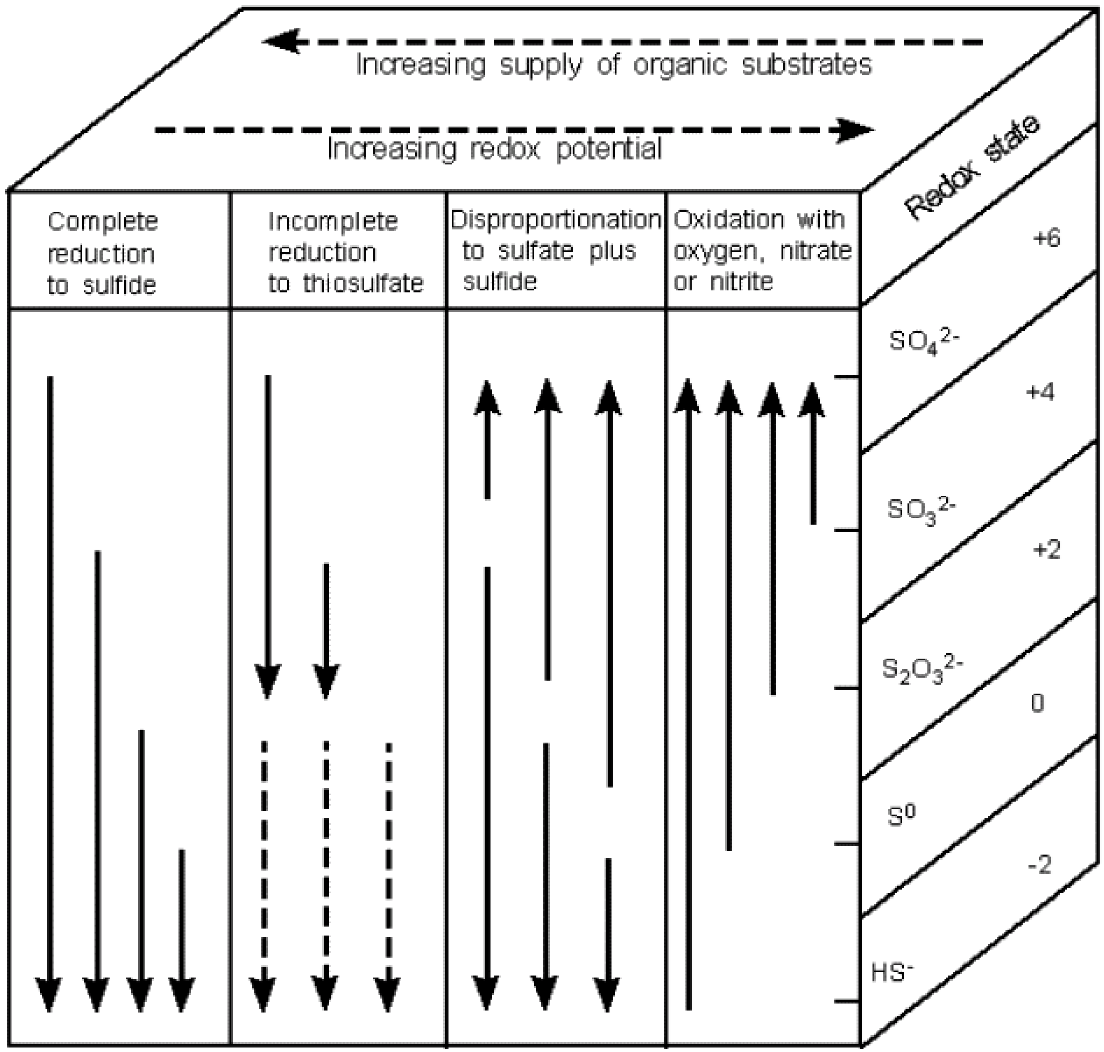
Nitrate preferred vs. sulfate

$SO_4^{2-} + 4\ H_2 + 1.5\ H^+ \rightarrow 0.5\ HS^- + 0.5\ H_2S + 4\ H_2O$

$NO_3^- + 4\ H_2 + 2\ H^+ \rightarrow NH_4^+ + 3\ H_2O$

Krekeler D., Cypionka H.
FEMS Microbiol Ecol 17:271-278

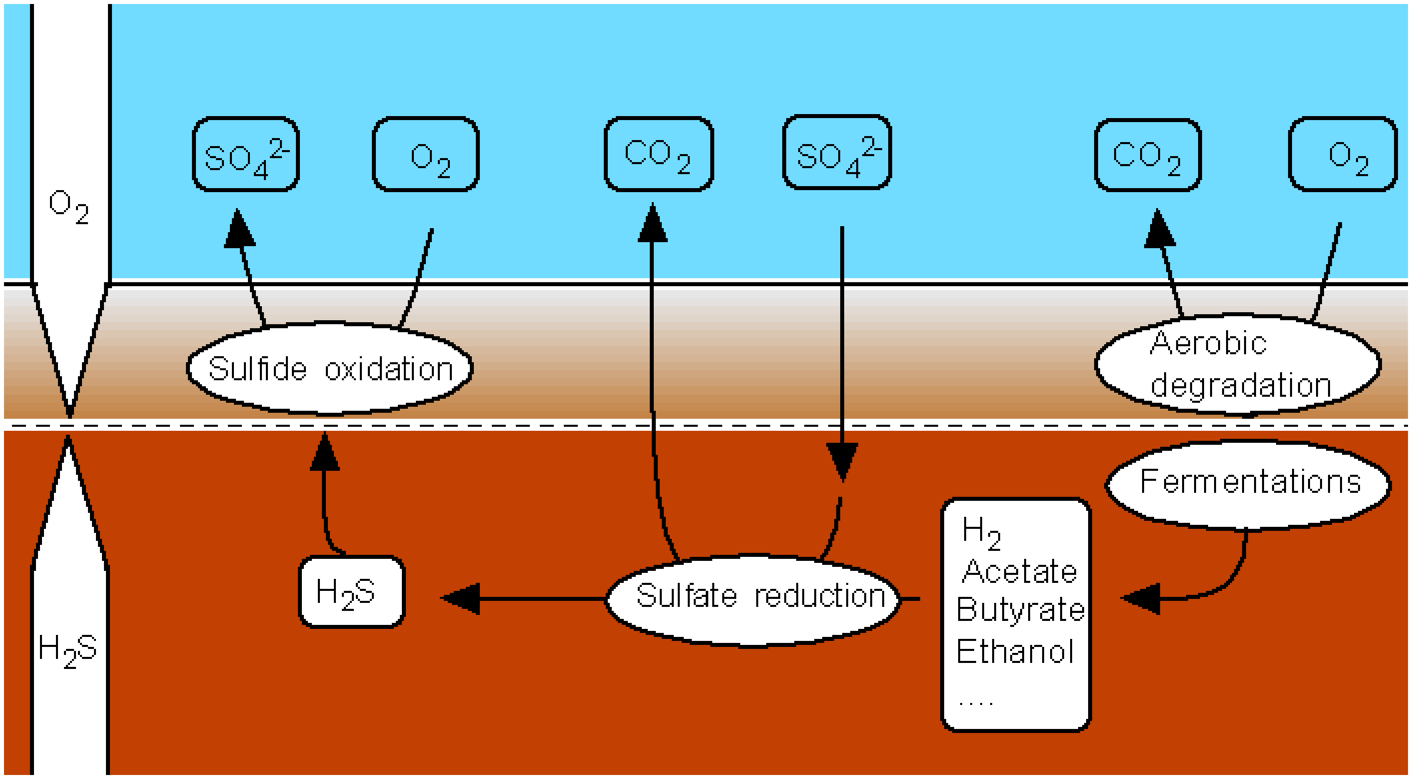
Sulfur transformations by SRB in dependence of the environmental conditions



Anything goes...

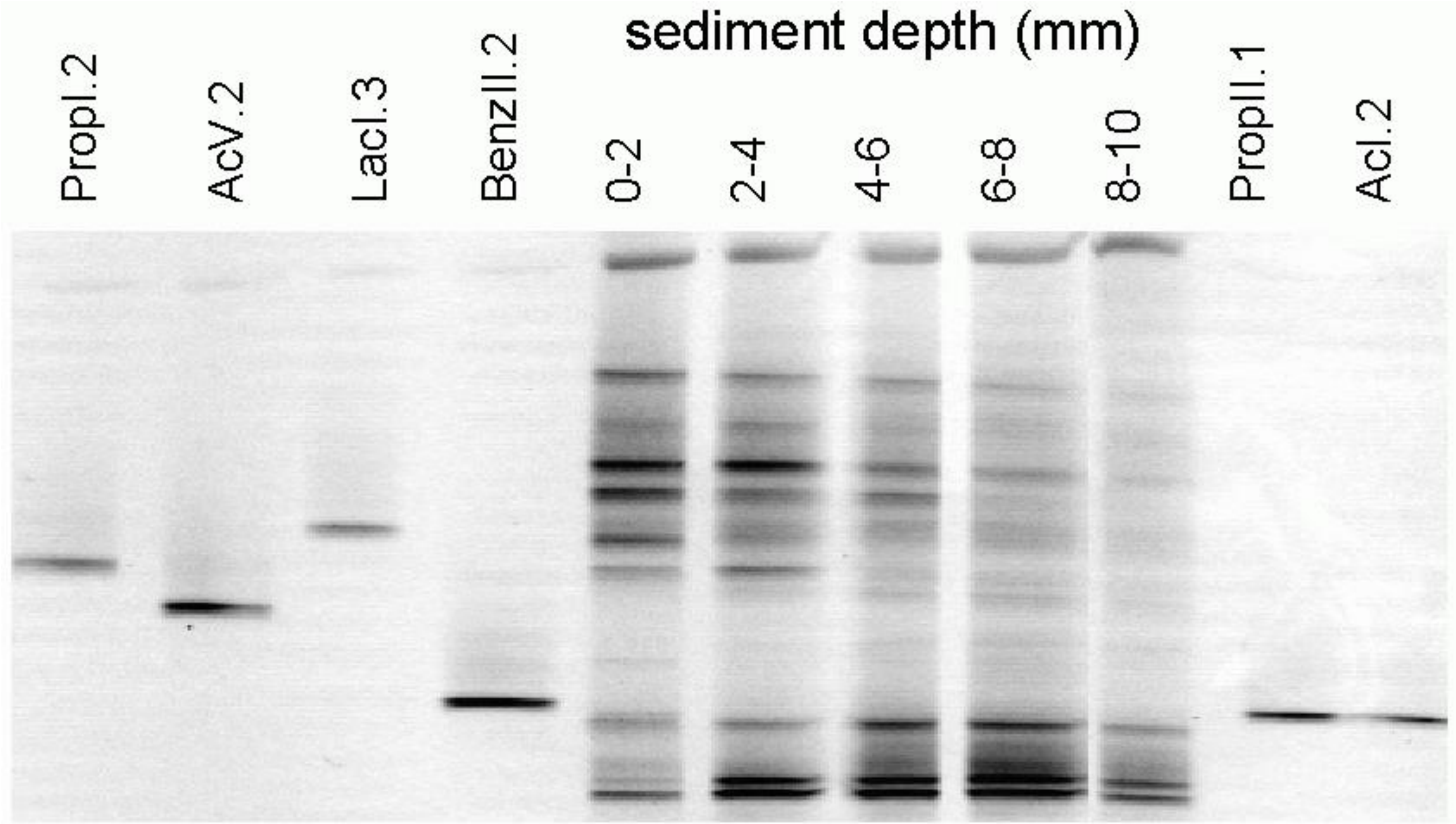
Cypionka, H. in: Microbial Mats, Stal LJ Caumette P (Eds.), p 367-376, NATO ASI Series, Vol G35, Springer, Berlin

Processes at the oxic-anoxic boundary



Note: **Fresh organic substrates enter from the oxic zone.**

Community structure

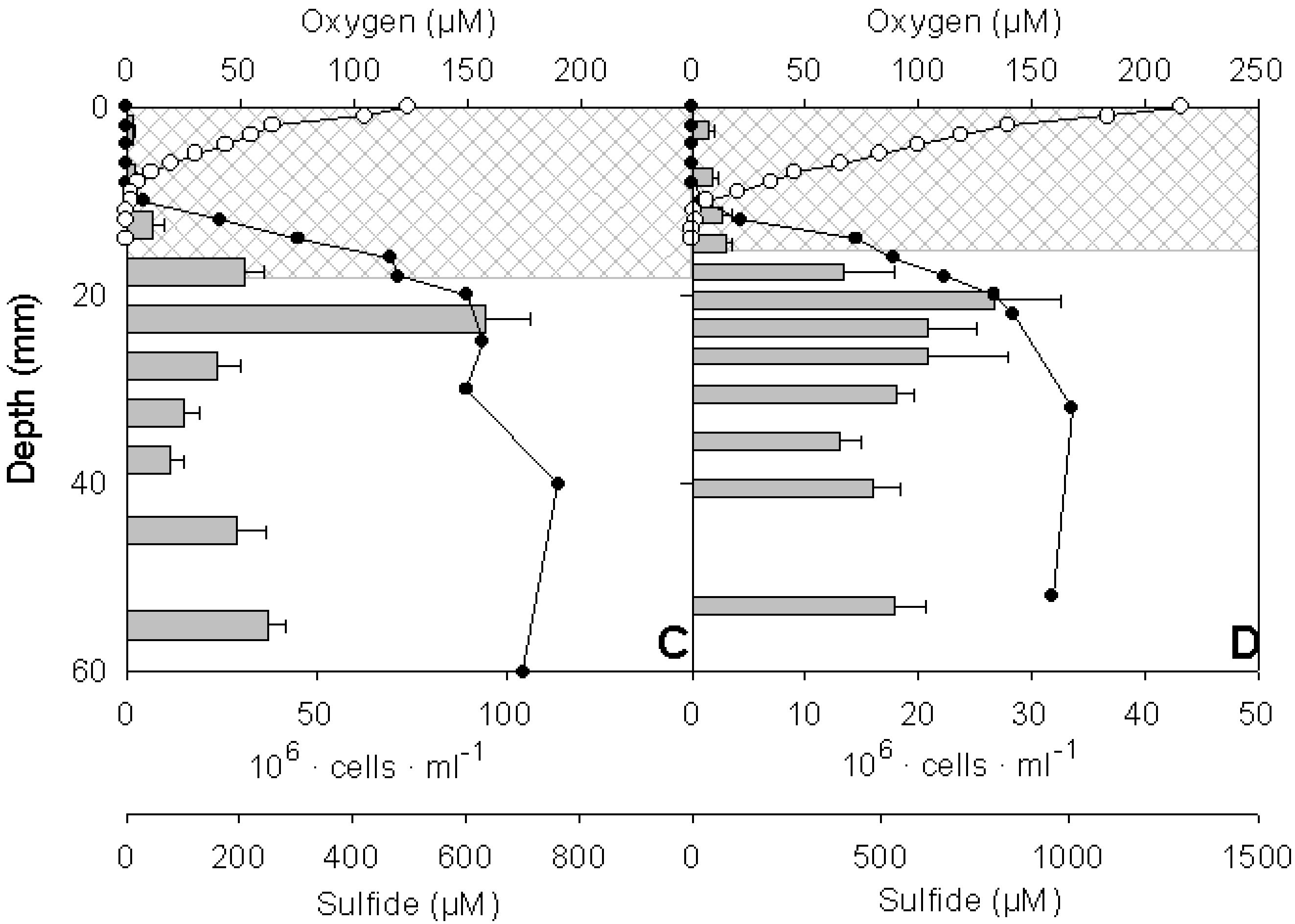


DGGE gels of pure SRB cultures and sediment (0 -10 mm) of a sandy tidal flat at Schiermonnikoog

Wieringa E. et al.
Environ Microbiol 2:417-427

Note: SRB communities in the oxic-anoxic interface are highly structured.

Growth at the oxic-anoxic interface



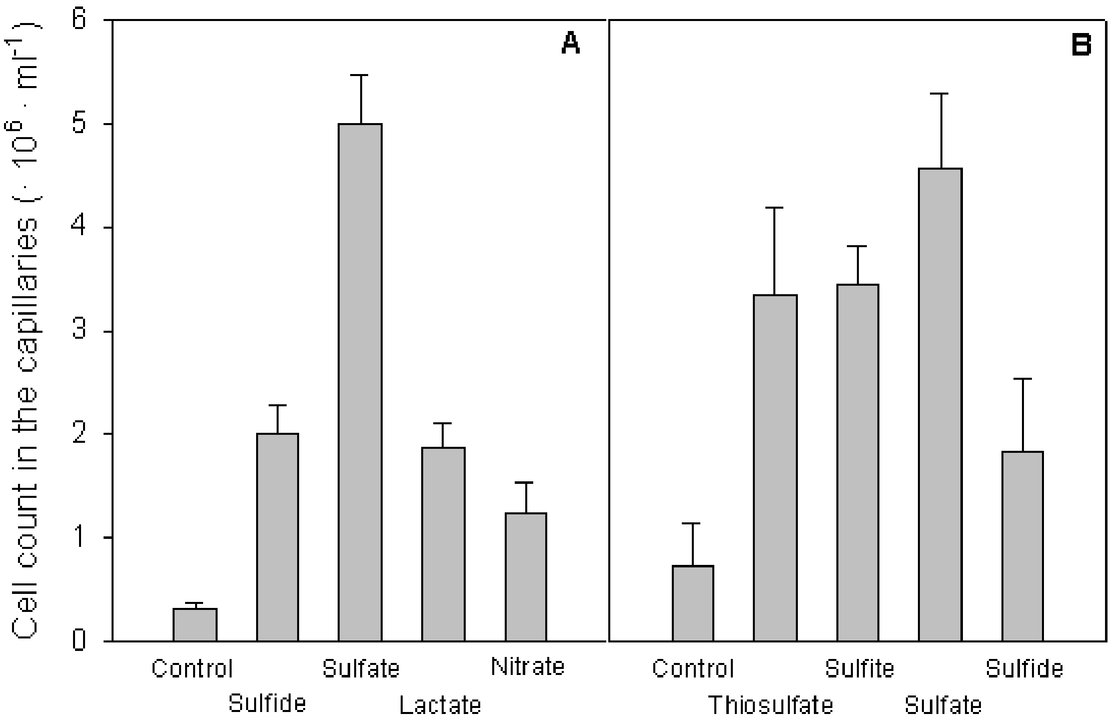
Note: **Maximum cell numbers often directly below the interface (natural communities and pure cultures)**

Growth of sulfate-reducing bacteria in artificial oxygen-sulfide countergradients C: *Desulfovibrio oxyclinae* N13 with sediment particles; D: *Desulfovibrio desulfuricans* CSN;• : oxygen; • : sulfide; bars: cell counts with standard error; hatched area: pink color of resazurin

Andrea Sass

Henrik Sass

Chemotaxis



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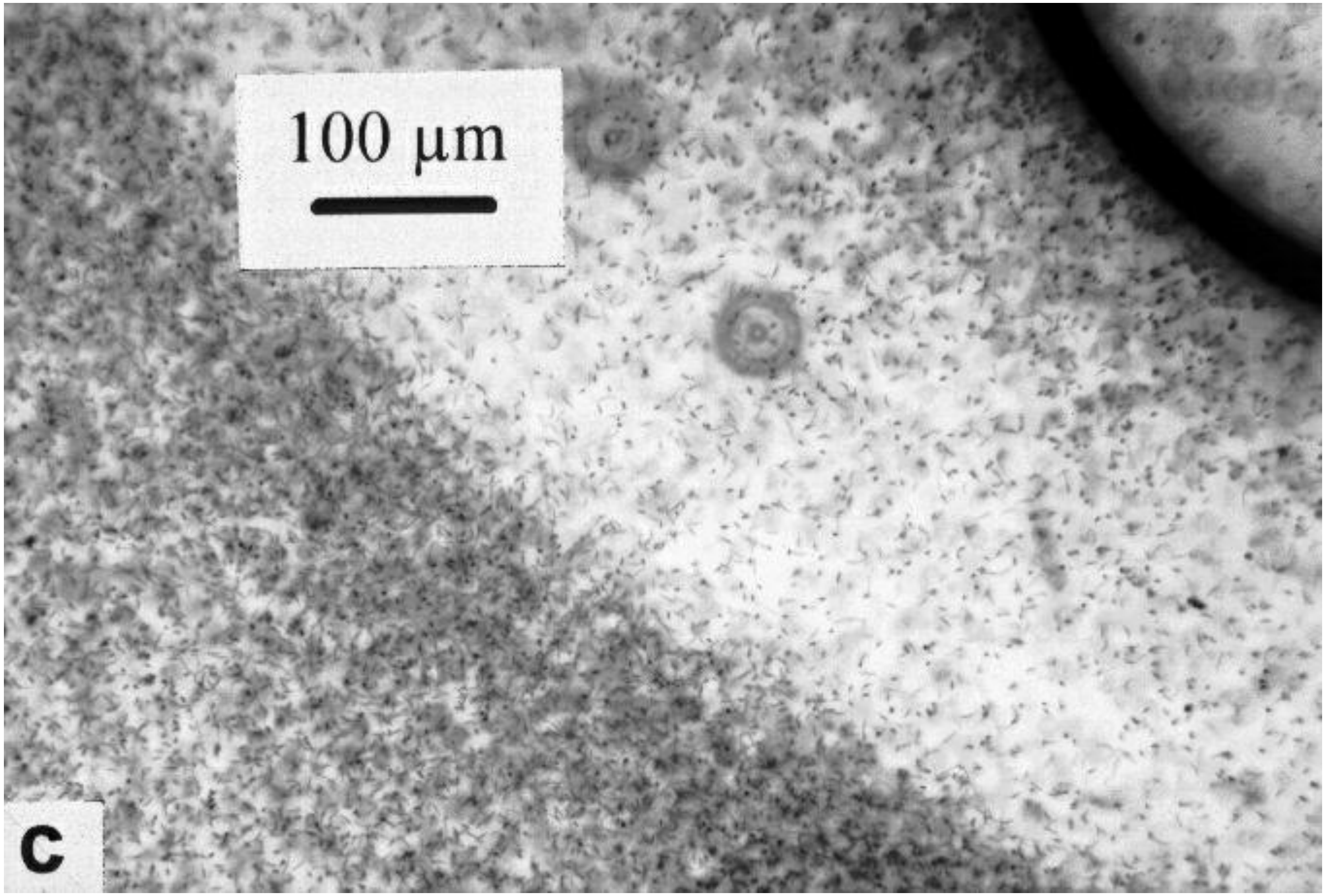
Andrea Sass

Chemotaxis of *Desulfovibrio desulfuricans* CSN
A: Comparison of different electron acceptors and donors, B: Comparison of different sulfur compounds; the concentration of attractants was 10 mM, incubation time1 h at 30°C; cells were grown with lactate (20 mM) and sodium sulfate (10 mM)

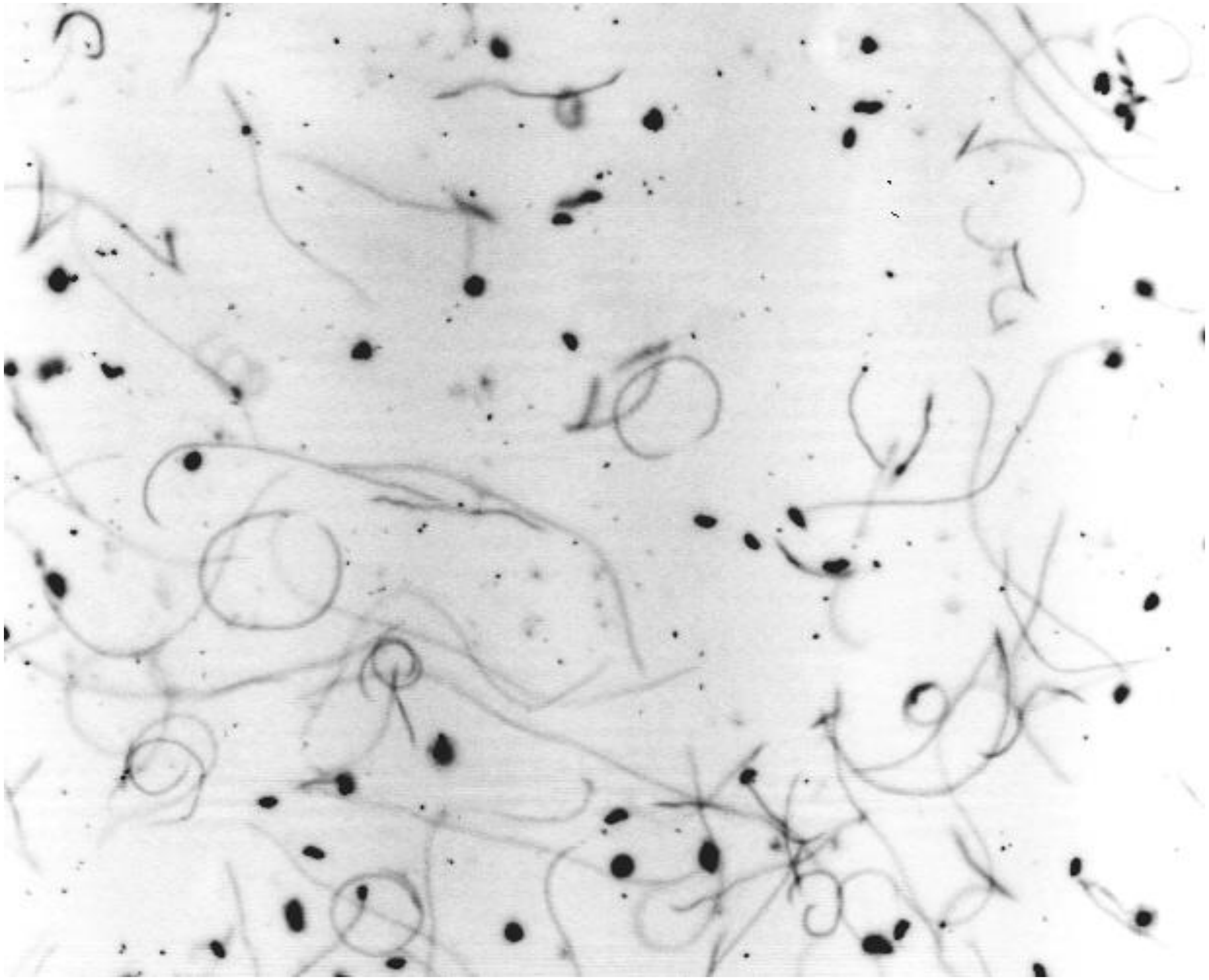
Aerotaxis



Eschemann A.et al. Environ Microbiol 1:489-494

Note: **Band formation does not simply result from aerophobic behaviour.**

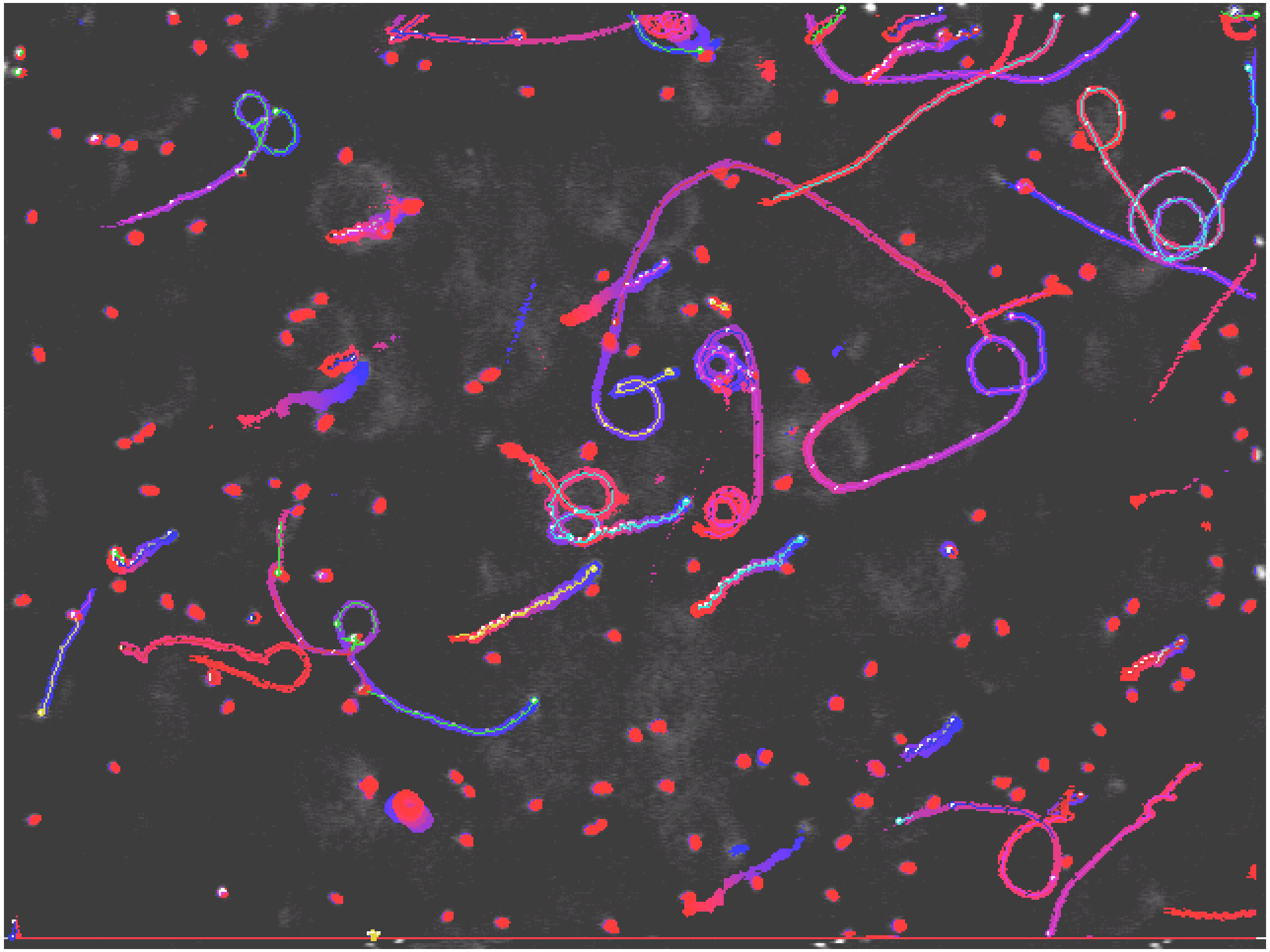
Tracks of SRB



Inversed dark-field photograph, exposure 1 sec

Note reversals and slow (thick) backward-tracks

Tracks of SRB (5 sec, overlay)



Note vectorized tracks

- Time code: from blue to red
- All bacteria turn clockwise (due to influence of the cover slip surface)

Relations of SRB to oxygen

Tolerance

- Survival of 1 day air exposure, sulfide + O_2: increased toxicity

Oxygen respiration

- High rates, energy coupling demonstrated, at least three different oxygen-reducing systems, one periplasmic

Aerobic growth?

- Only in cocultures, in pure culture max. 1 doubling

Various behavioural responses

- Migration, aggregation, band formation

What is a bacterial strategy?

Migration of SRB in response to oxygen

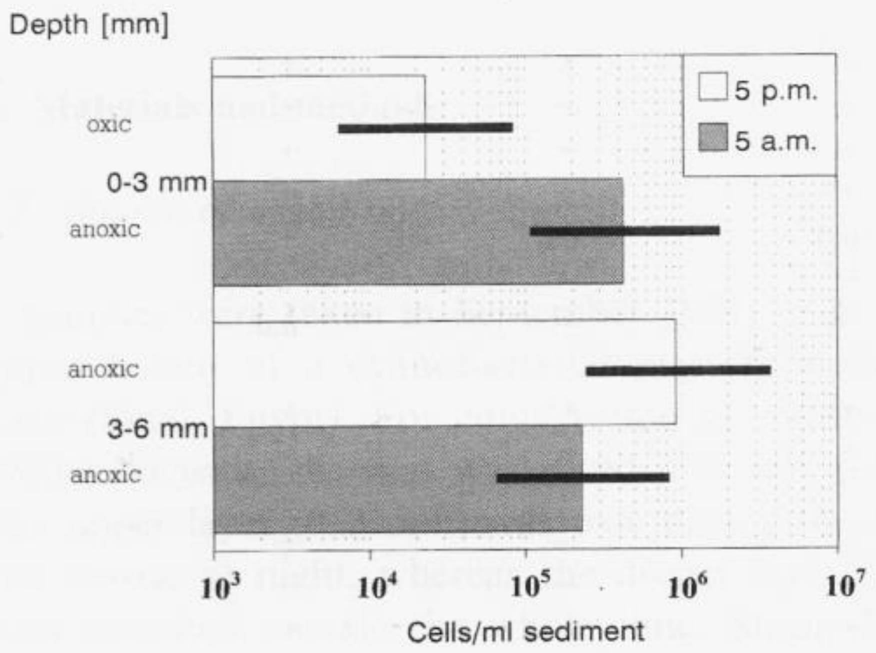


Fig. 1. Vertical distribution of sulfate-reducing bacteria during the day and at night in the microbial mat of Solar Lake (Sinai). The mat was oxic to 3 mm depth during the day, whereas the whole mat turned anoxic during night. The upper layer (0–6 mm) was sampled at two different times (5.00 and 17.00 h) and at two depths (0–3 mm and 3–6 mm). These slices were used as an inoculum for MPN incubations. Bars show 95% confidence intervals.

Note: SRB avoid high oxygen concentrations

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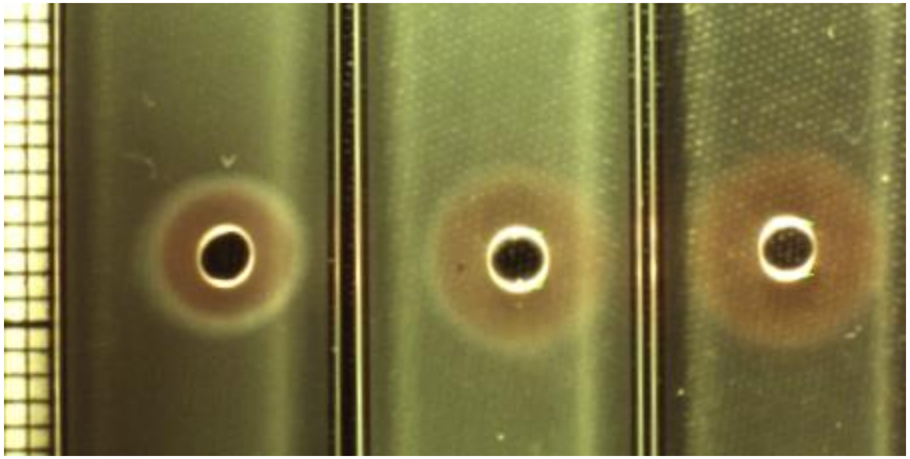
FEMS Microbiol
Ecol 25:89-96

Aggregate formation

- Immotile cells
- In phototrophic bacteria dependent on the sulfide concentration
- Increased survival rates

Note: Aggregation looks as a helpless stopgap, but may be a highly specific behaviour.

Band formation of SRB



OD 10 OD 5 OD 3

Pink resazurin indicates the presence of oxygen

Bands around oxygen bubbles of *Desulfovibrio oxyclinae* in microslide capillaries filled with lactate-containing buffer

Band formation dependent on electron donor

- Lactate, H_2

Band location dependent on the cell density

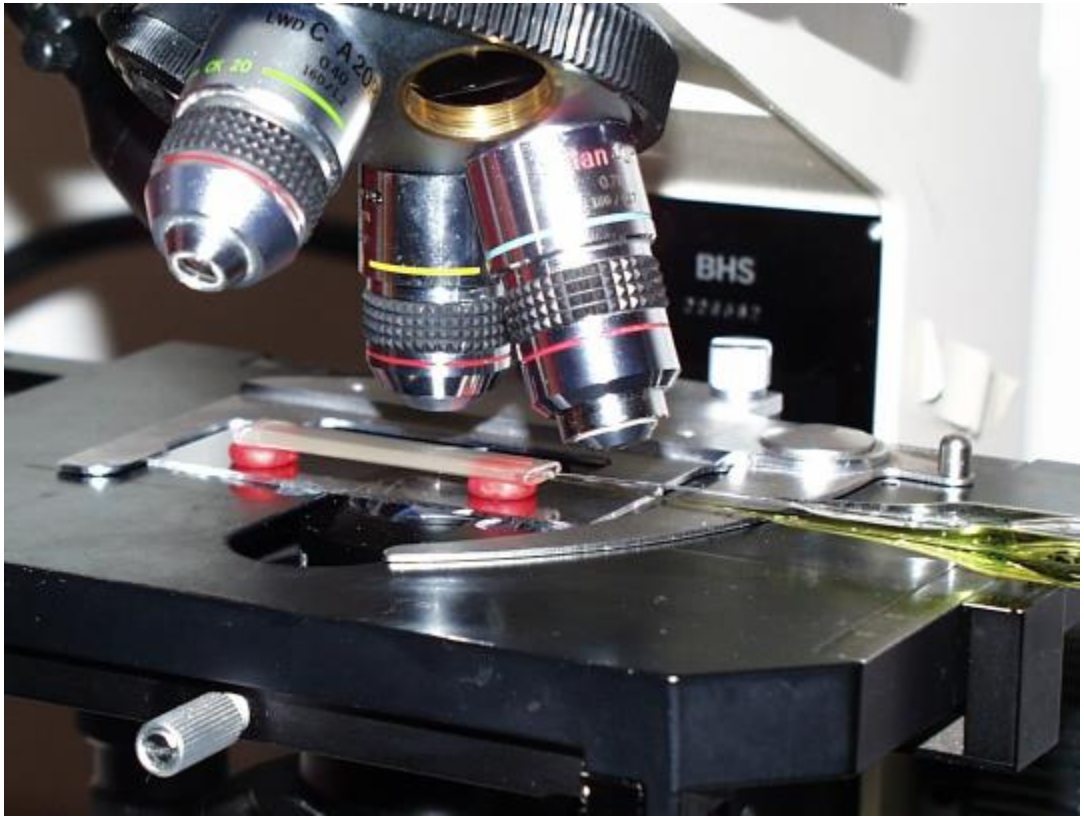
- At high density bands form closer to oxygen bubble

Bacteria control oxygen diffusion

- Overnight anoxic conditions are reestablished

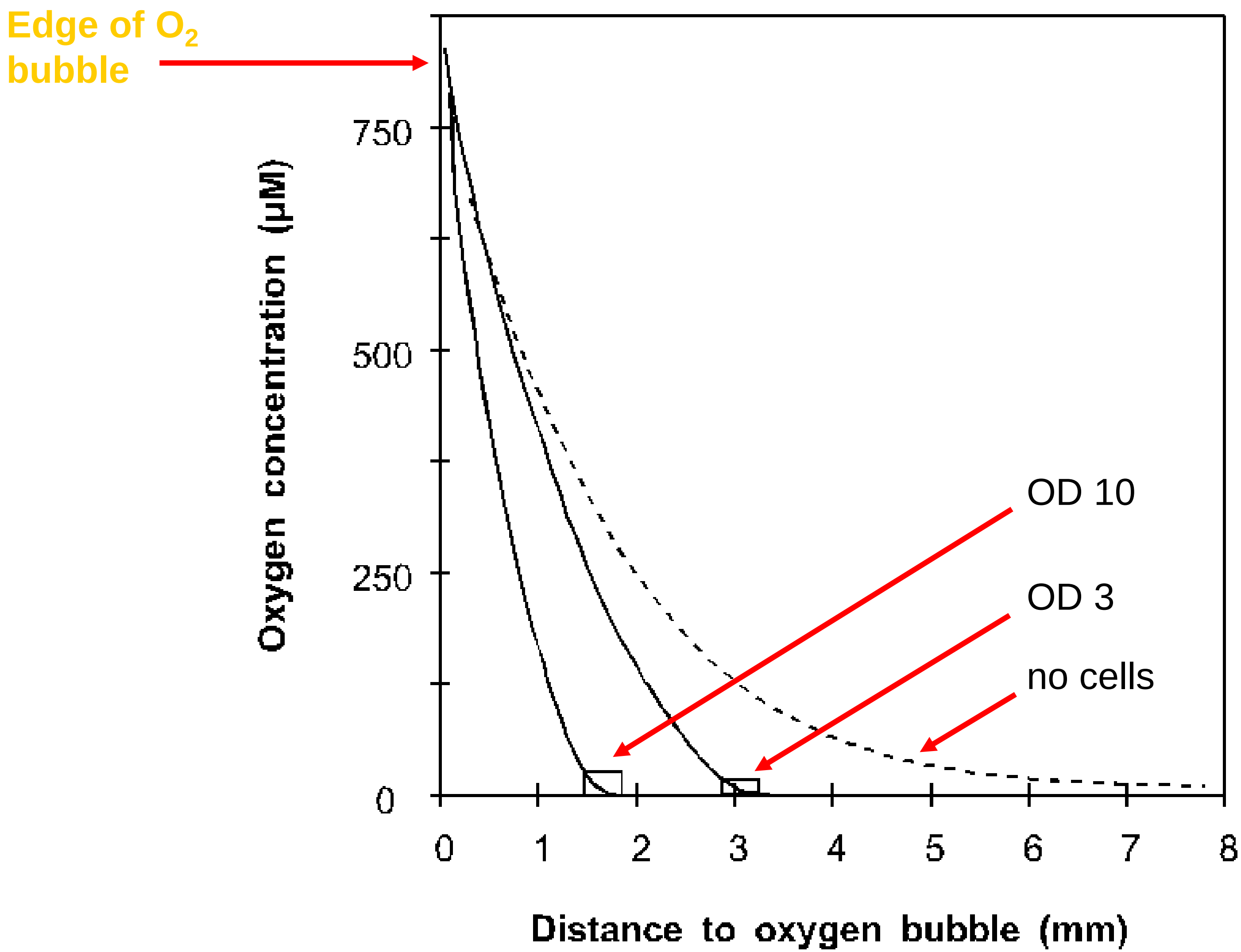
Note: In order to consume oxygen, the bacteria must stay in the oxic zone.

Study of band formation by means of oxygen- and sulfide microelectrodes



Andrea Eschemann, Andrea Sass, Michael Kühl

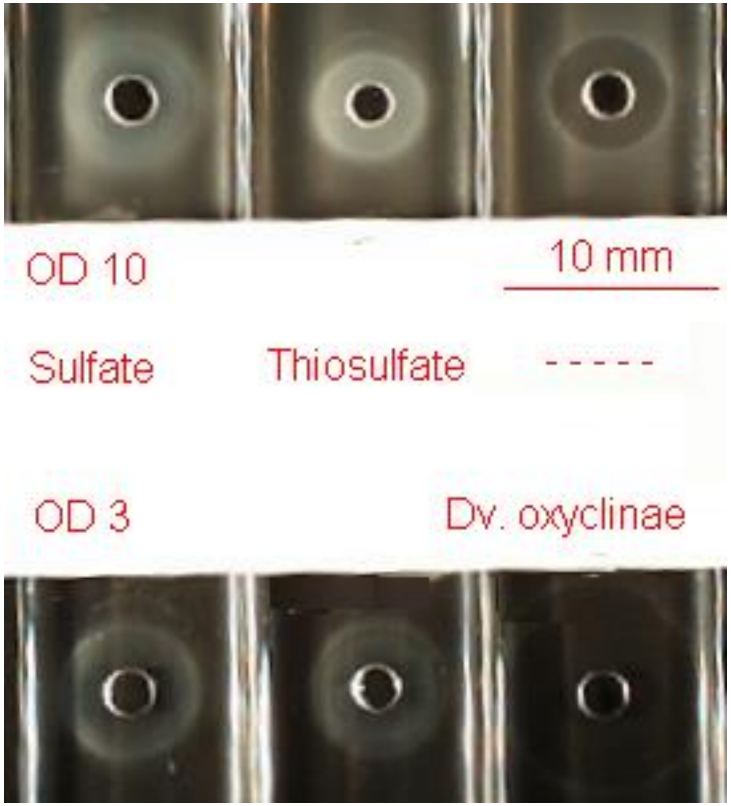
Oxic zones in the presence and absence of SRB



Eschemann et al.

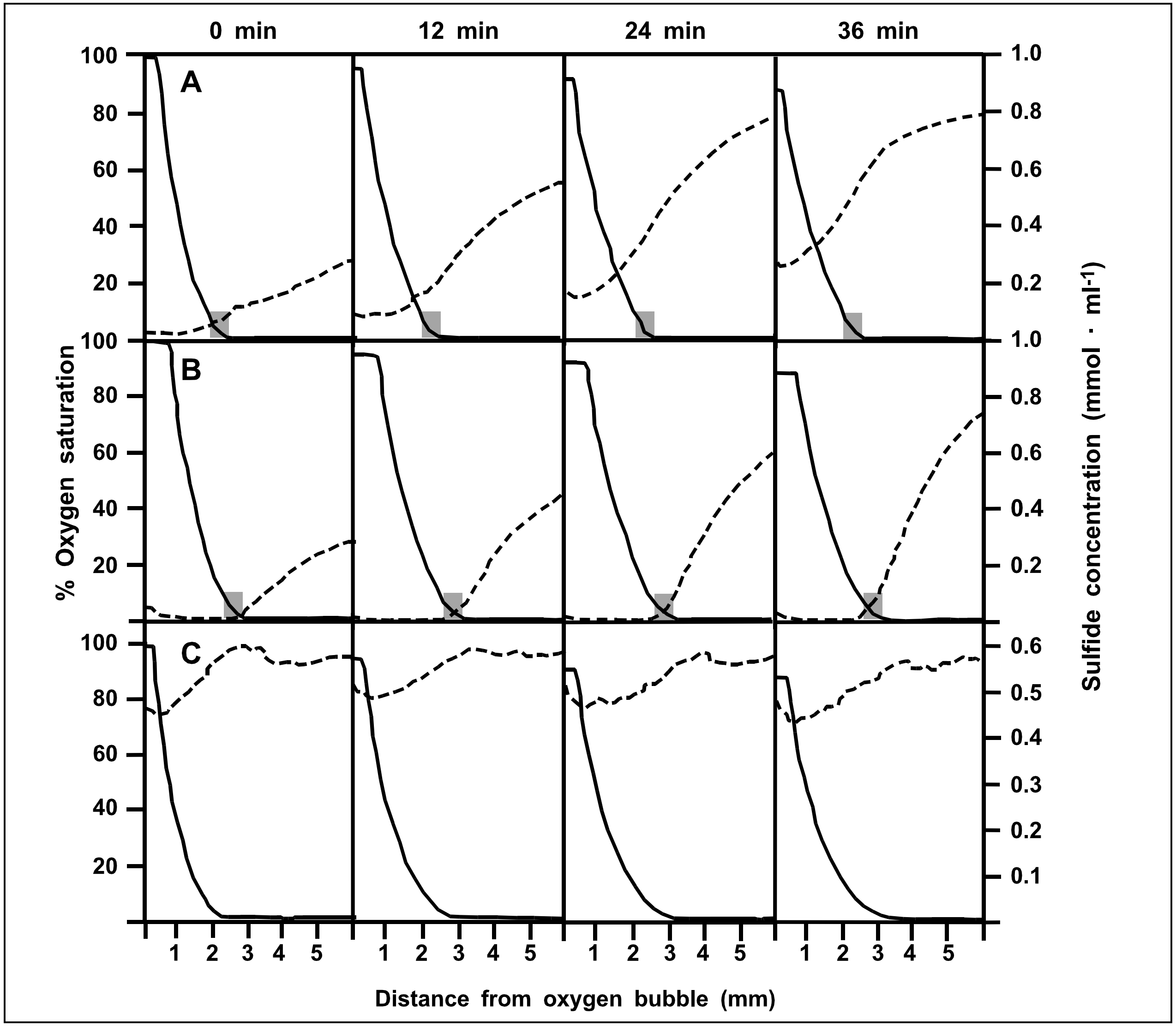
Environ Microbiol 1:489-494

Band formation in the presence and absence of sulfur compounds



Just imagine you had some coffee with glucose, fructose or without sugar...

Band formation in the presence of sulfur compounds



Profiles of oxygen and sulfide around an oxygen bubble in suspensions of sulfate-reducing bacteria A: *Desulfovibrio desulfuricans* CSN (5 mM lactate, 5 mM sulfate), B: *Desulfovibrio oxyclinae* N13 (10 mM lactate, 5 mM thiosulfate), C: Control experiment (2 mM sulfide); gray boxes indicate the location of bacterial bands

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Andrea Sass
Michael Kühl

Firefighter strategy

- At least three different oxygen-reducing mechanisms in SRB
- High rates point on defense mechanism
- Band formation dependent on an electron donor (lactate)
- Ring diameter dependent on cell density
- Removal of oxygen by respiration overnight
- Negative response to pure oxygen
- Accumulation within the oxic zone (forced by aerobic respiration)
- Some of the cells come from the anoxic zone outside (trapped!)

H. Cypionka, Annu Rev Microbiol 54:827-848

Define a strategy as a set of reactions directed to achieve a final aim without higher intelligence being necessary...

Ecological conclusions

- **Names can restrict thinking**
- **Physiological diversity can be displayed by a single strain**
- **Living organisms in our hands remain essential**

Do Microbiology

plus

Molecular Biology....

Thank

you