Do all biogeochemical cycles work at elevated temperatures that exist at deep-sea hydrothermal vents ?

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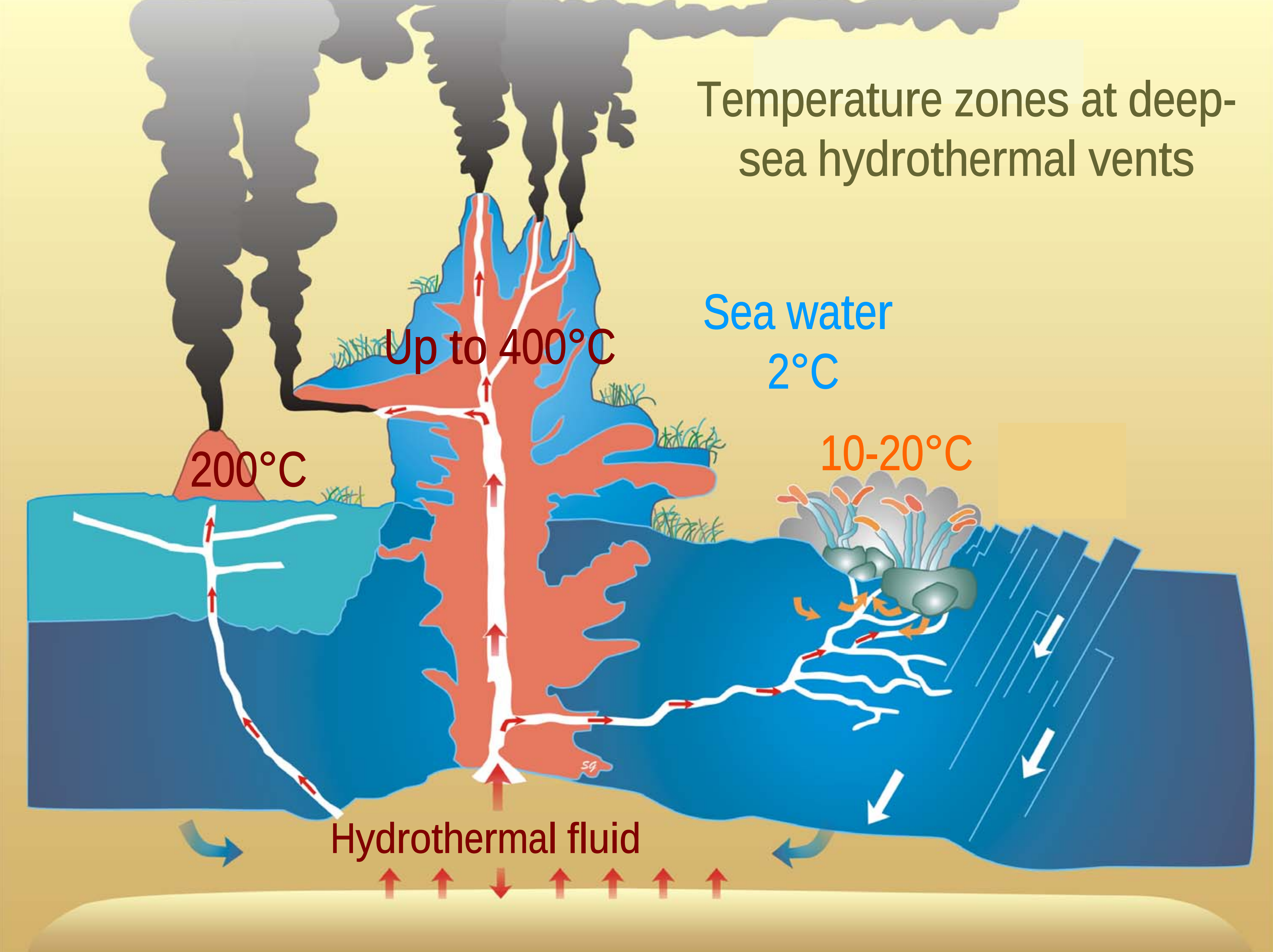
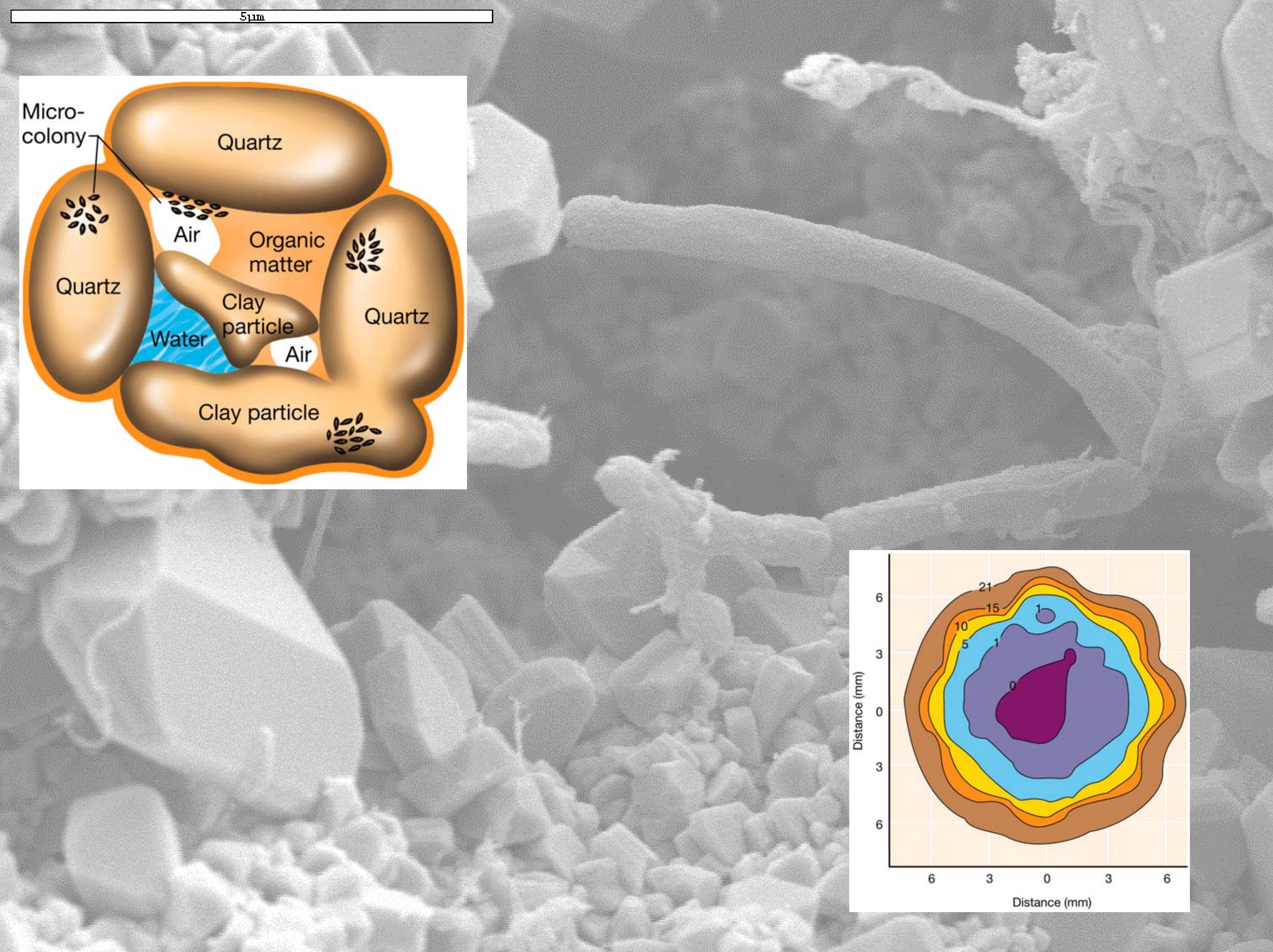
Outline

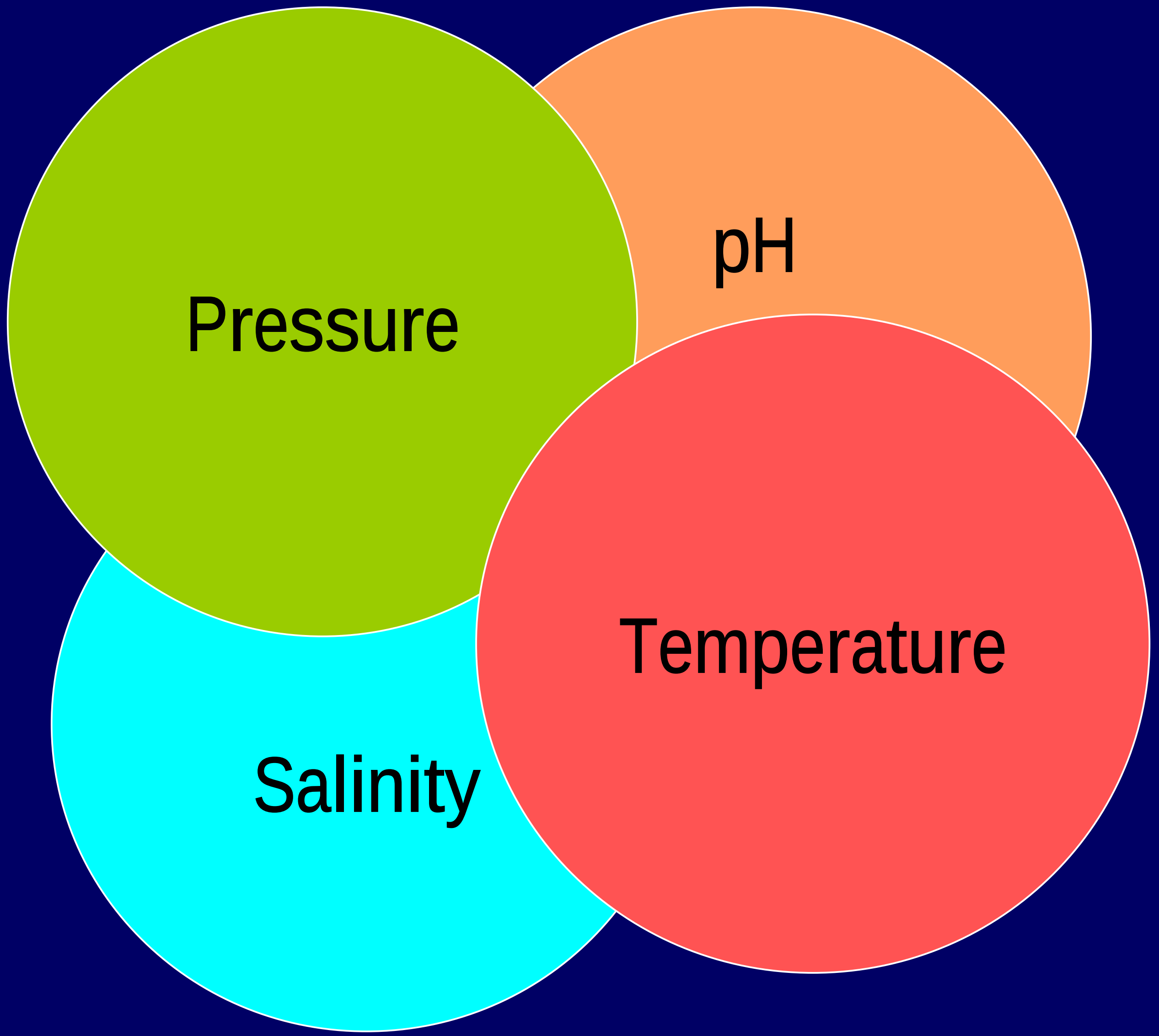
- Background
  - Microbial physiology
  - Microbial metabolism
  - Methods in microbial ecology
- Biogeochemical cycles at high temperature
  - Carbon
  - Nitrogen
  - Sulfur
  - Iron
- Novel approaches
- Conclusions


Temperature zones at deep-sea hydrothermal vents
Sea water 2°C
Up to 400°C
200°C
10-20°C
Hydrothermal fluid
SG

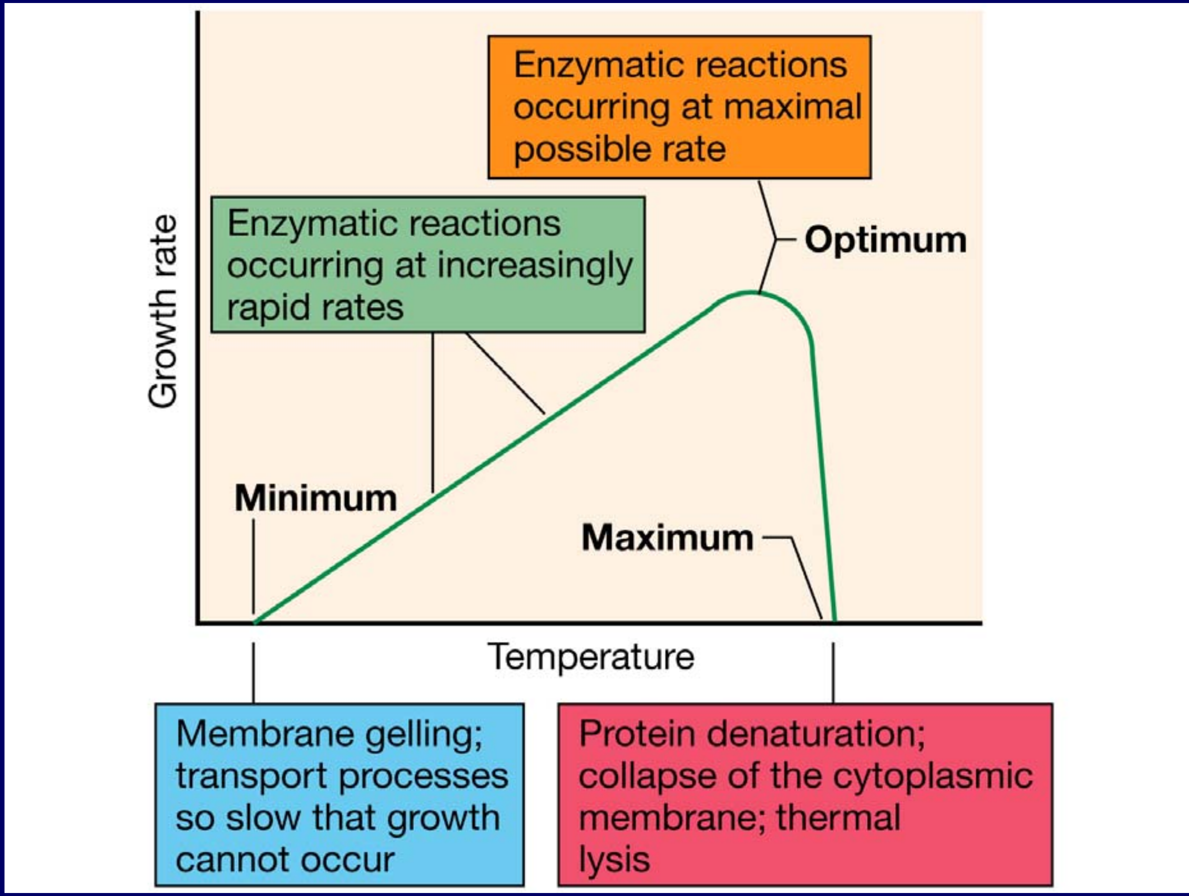

1µm


5μm
Micro-
colony
Quartz
Air
Organic
matter
Quartz
Clay
particle
Water
Air
Quartz
Clay particle
21
15
10
5
1
1
0
Distance (mm)
6
3
0
3
6
Distance (mm)
6
3
0
3
6

Environmental parameters affecting microbial life



Responses of microorganisms to temperature



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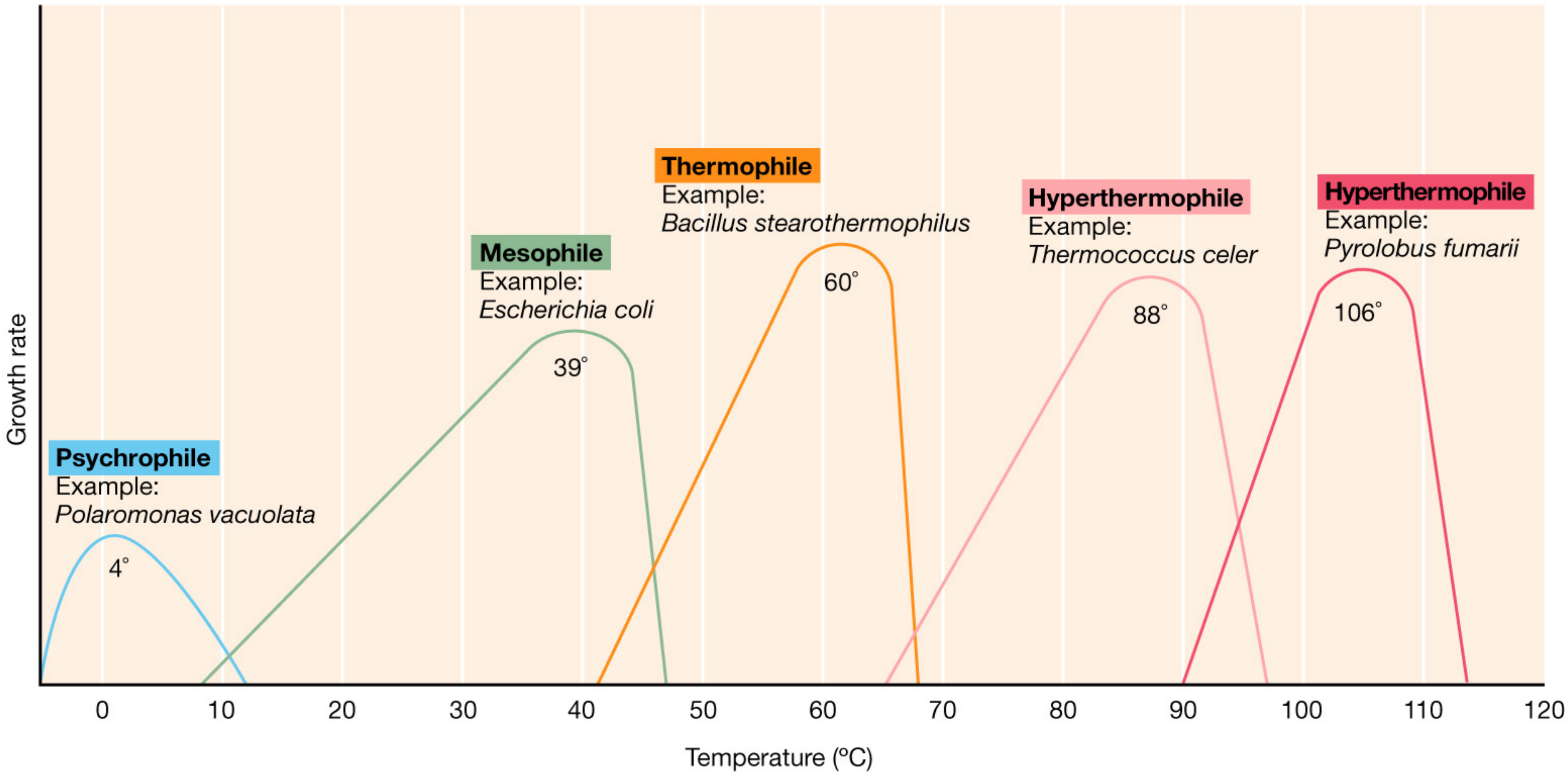

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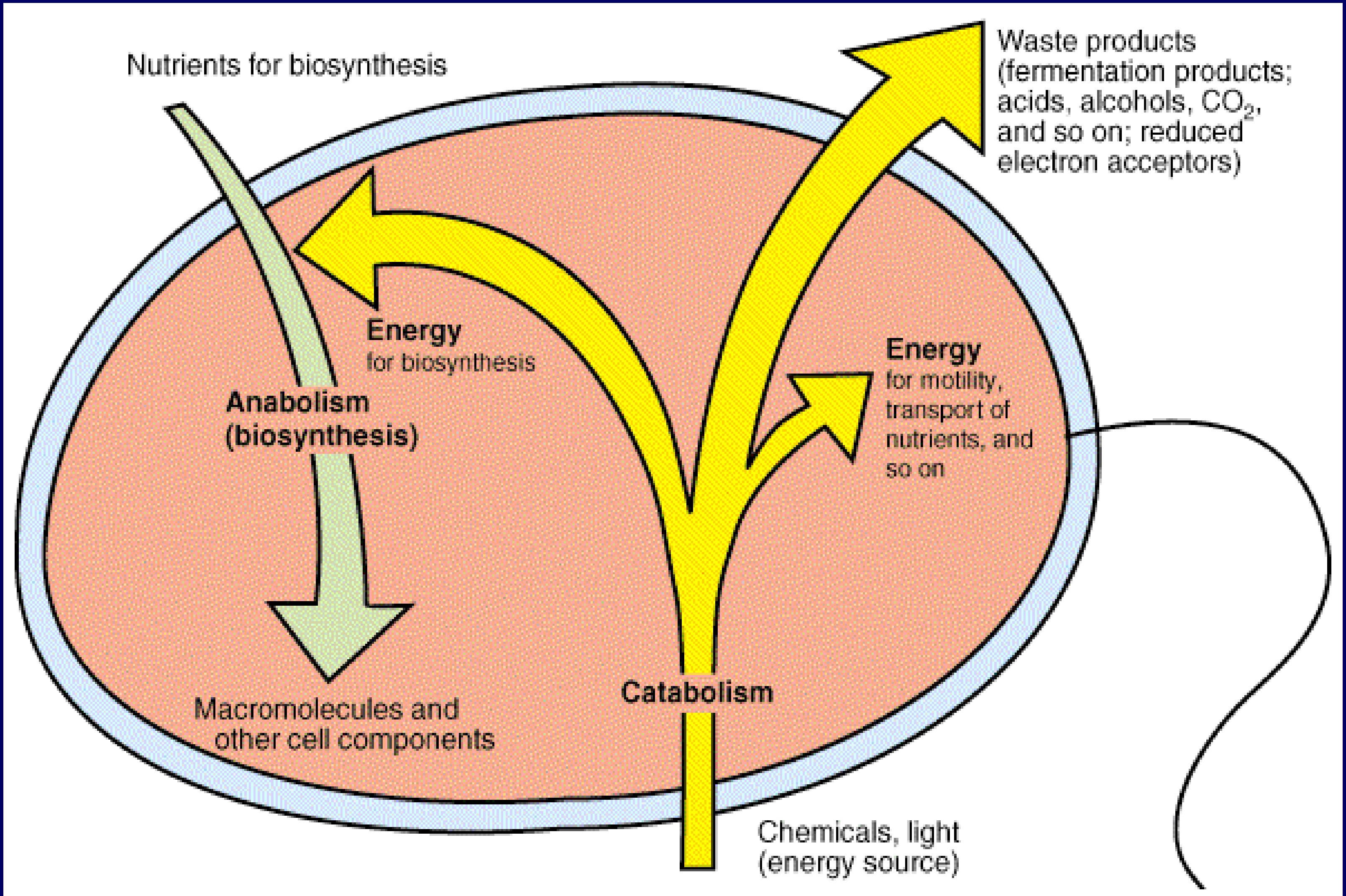
Microbial cell metabolism



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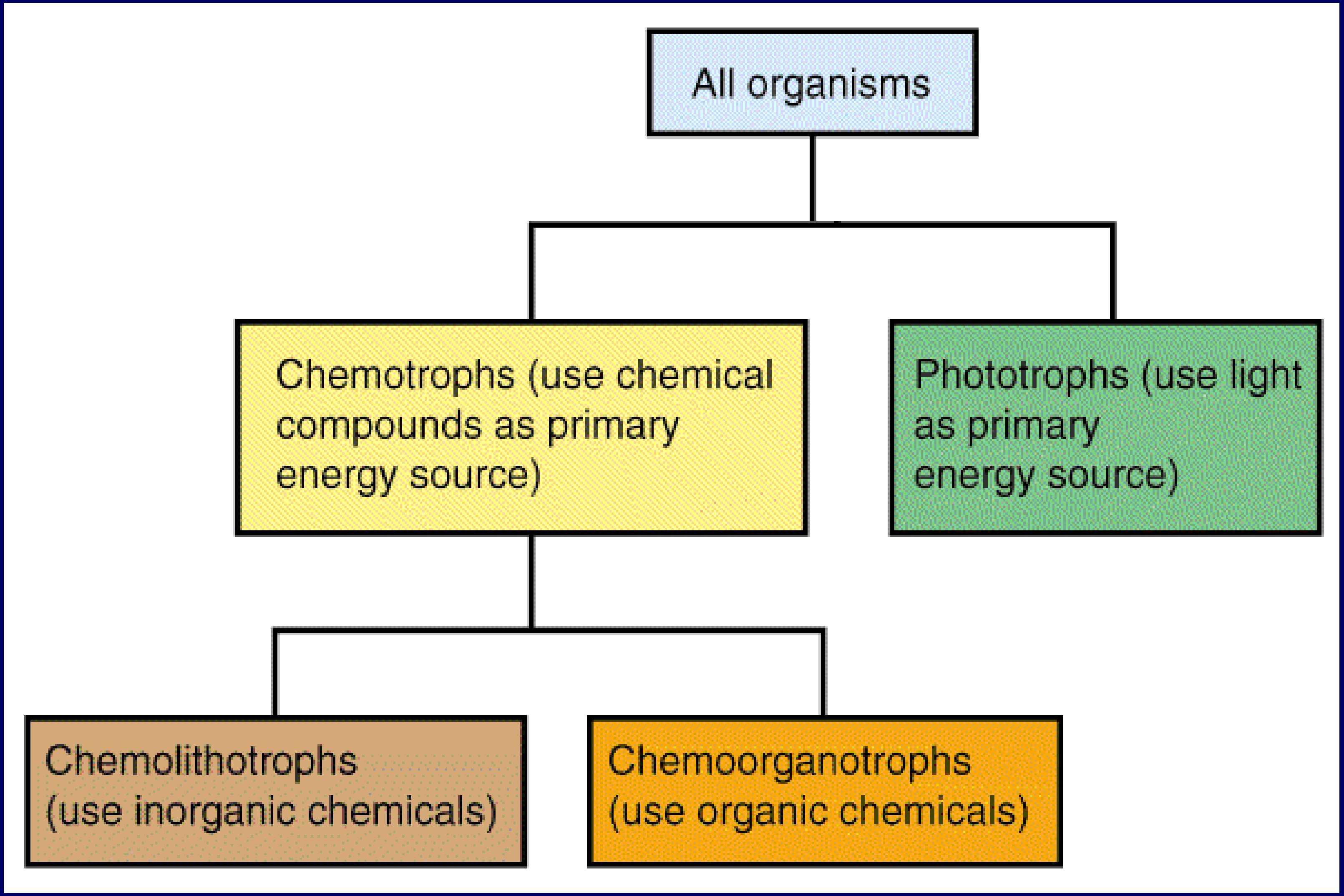
Energy sources



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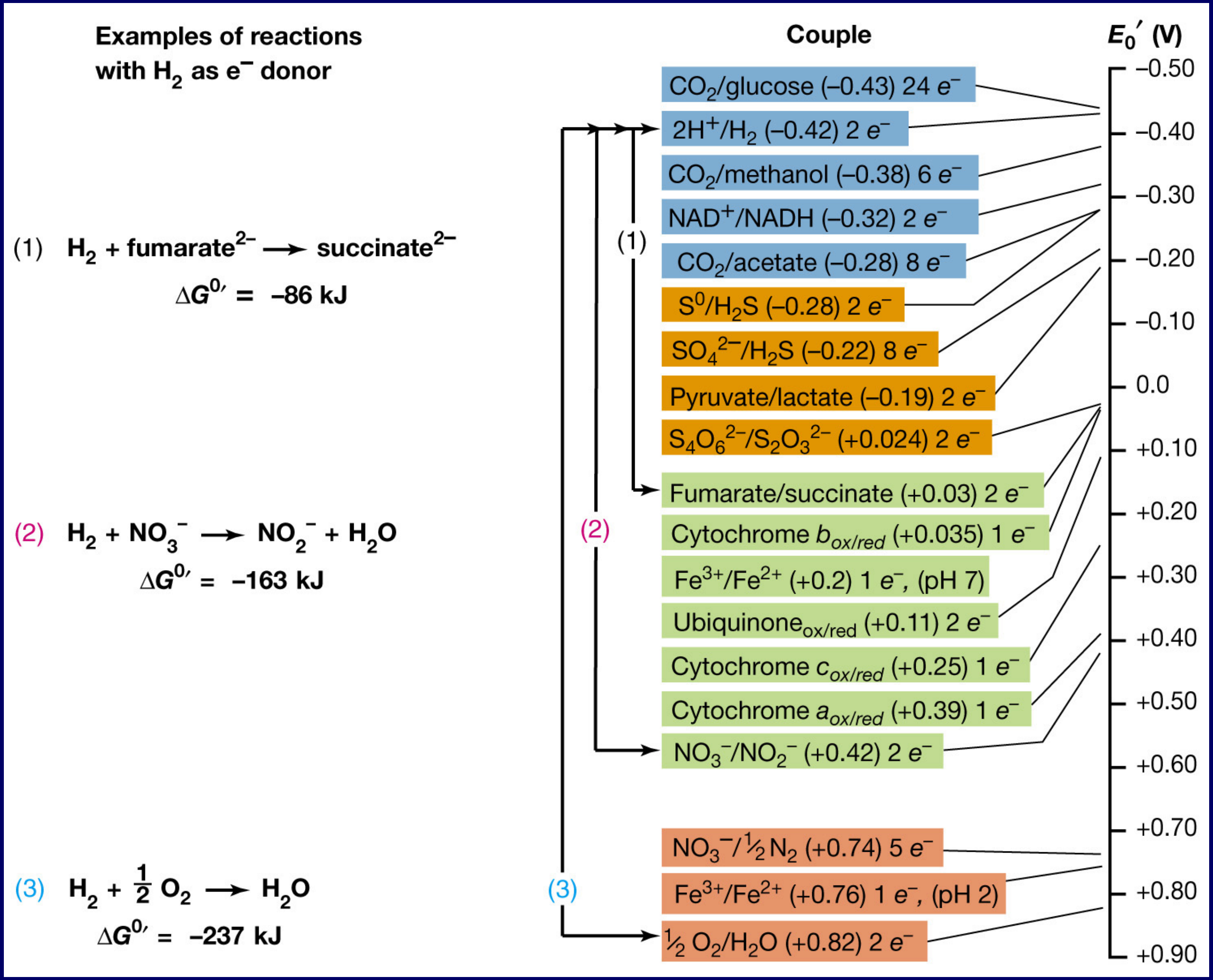
Redox couples: electron tower



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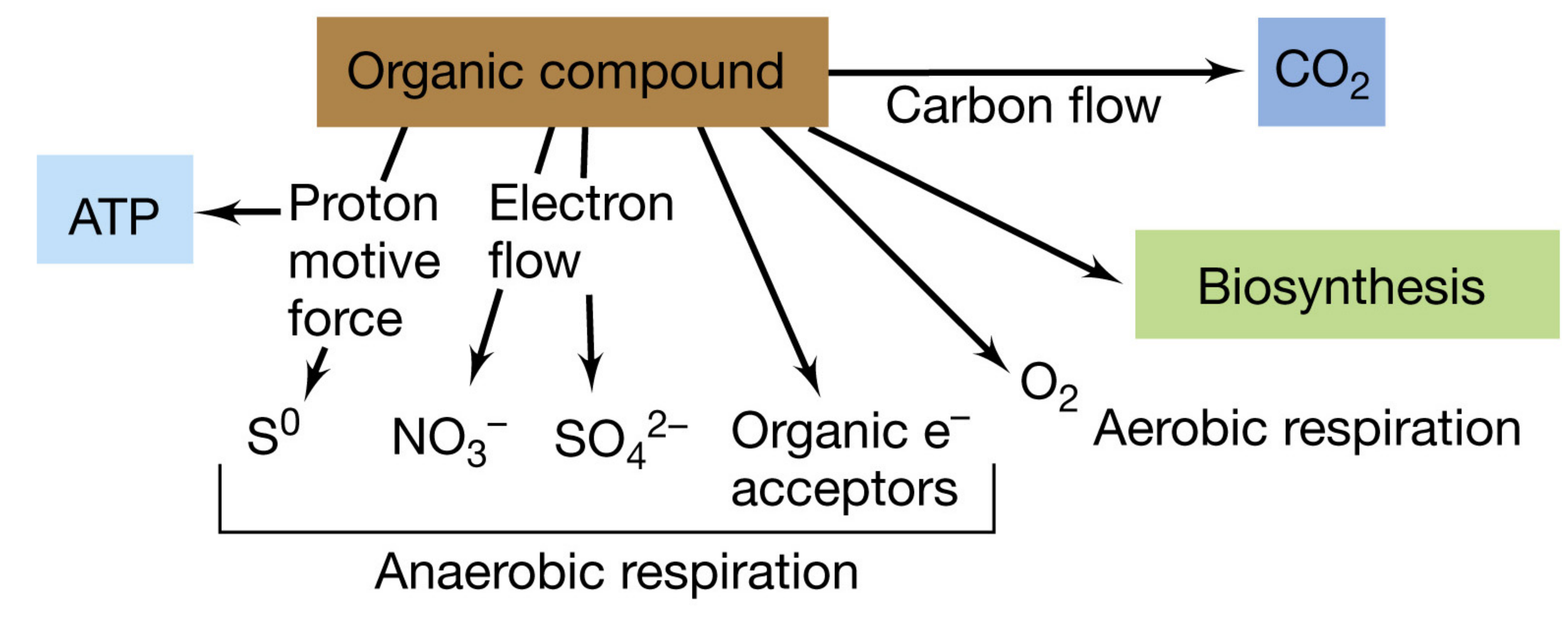
Chemoorganotrophic metabolisms



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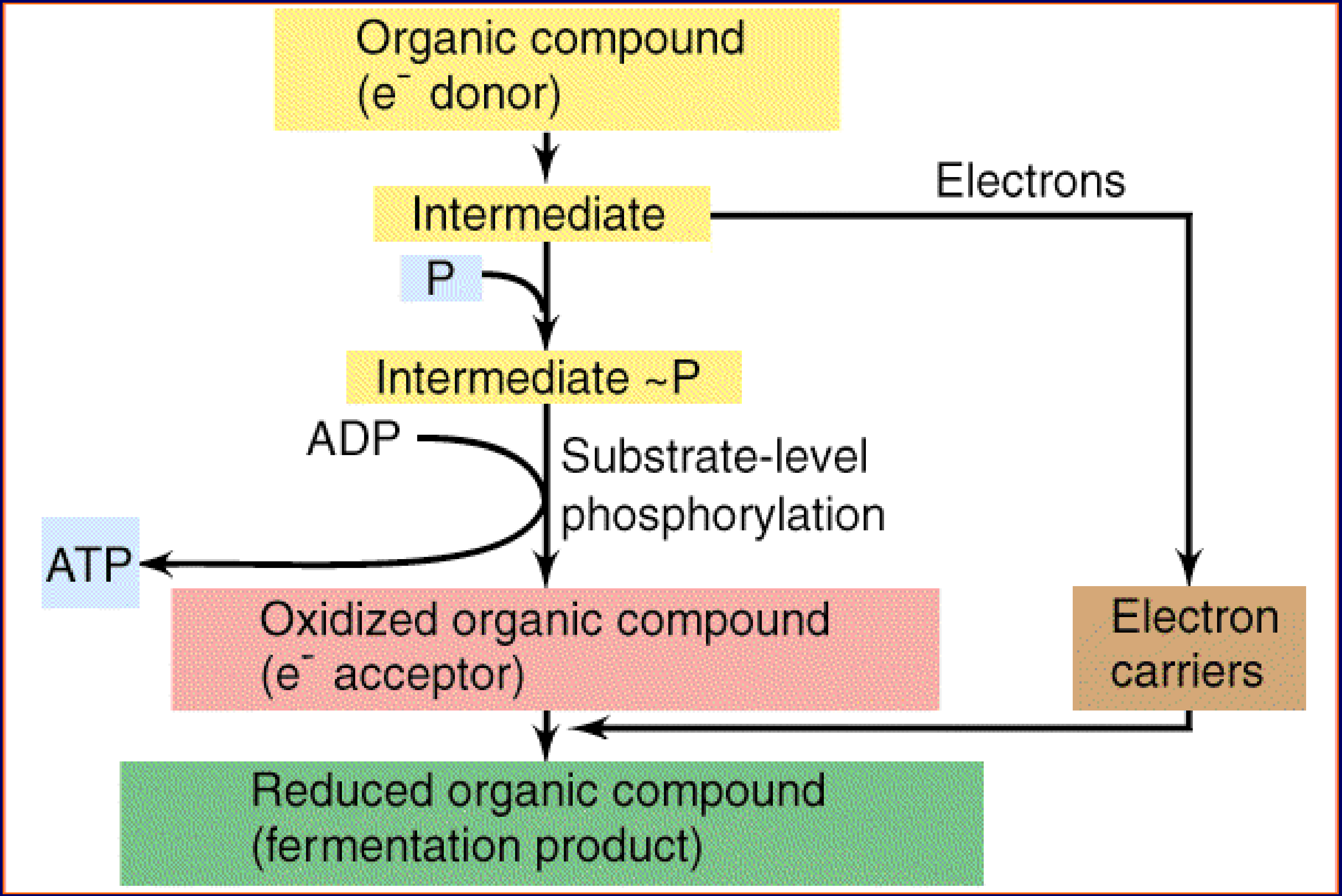
Fermentation



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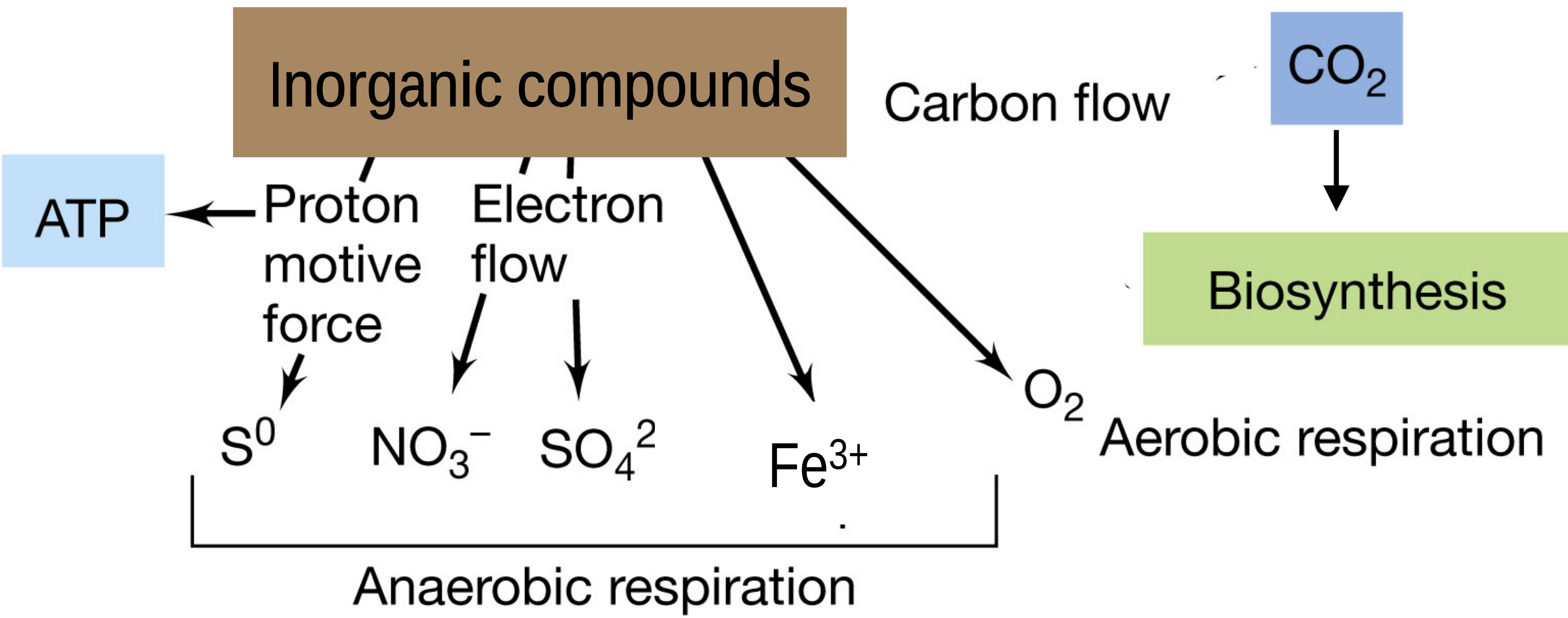
Organic carbon sources/electron donnors

- Hexoses, pentoses
- Polysaccharides
- Proteins
- Amino acids
- Organic acids
- Lipids
- Hydrocarbons

Chemolithotrophic metabolisms



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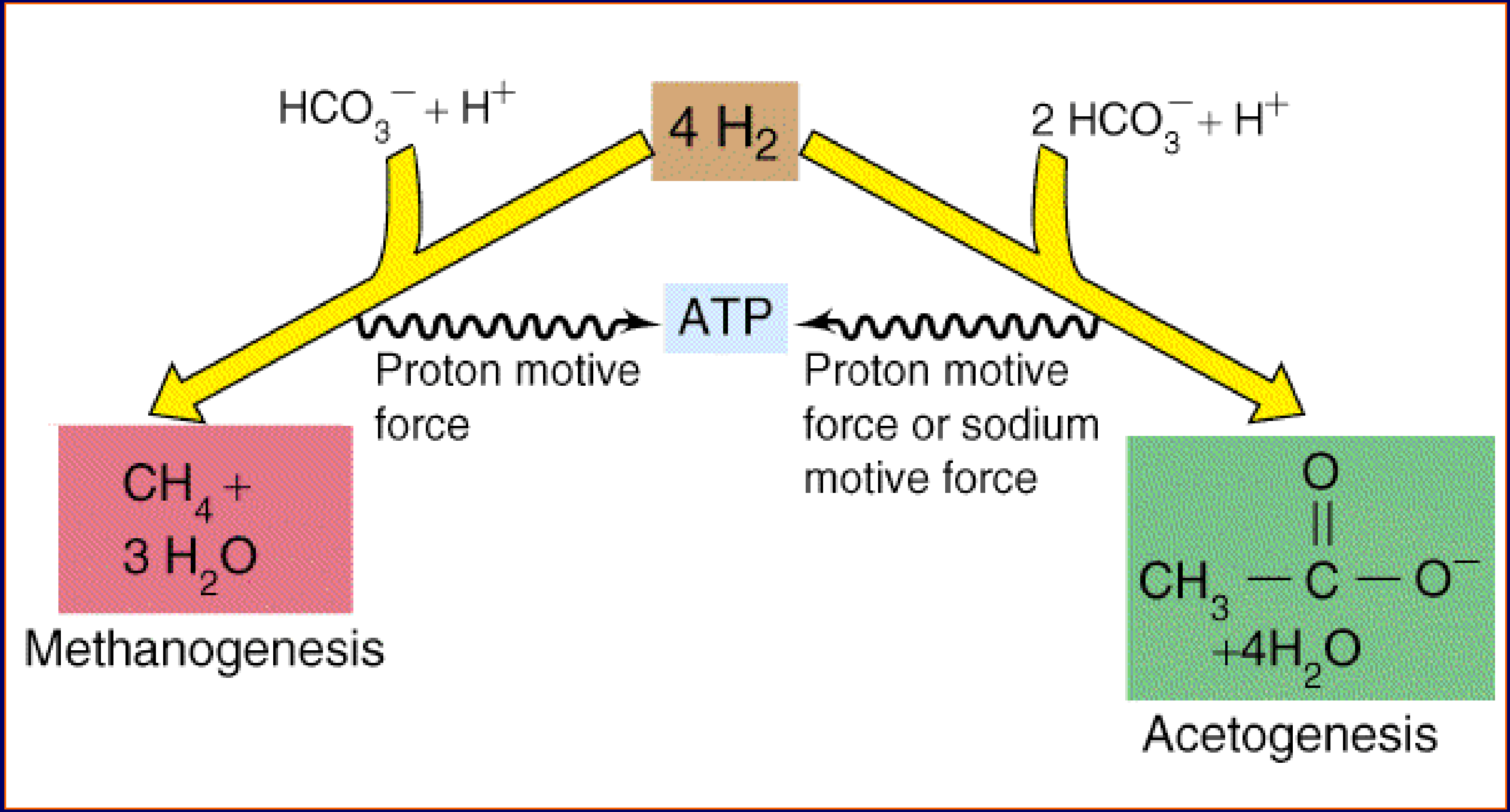
CO_2: electron acceptor



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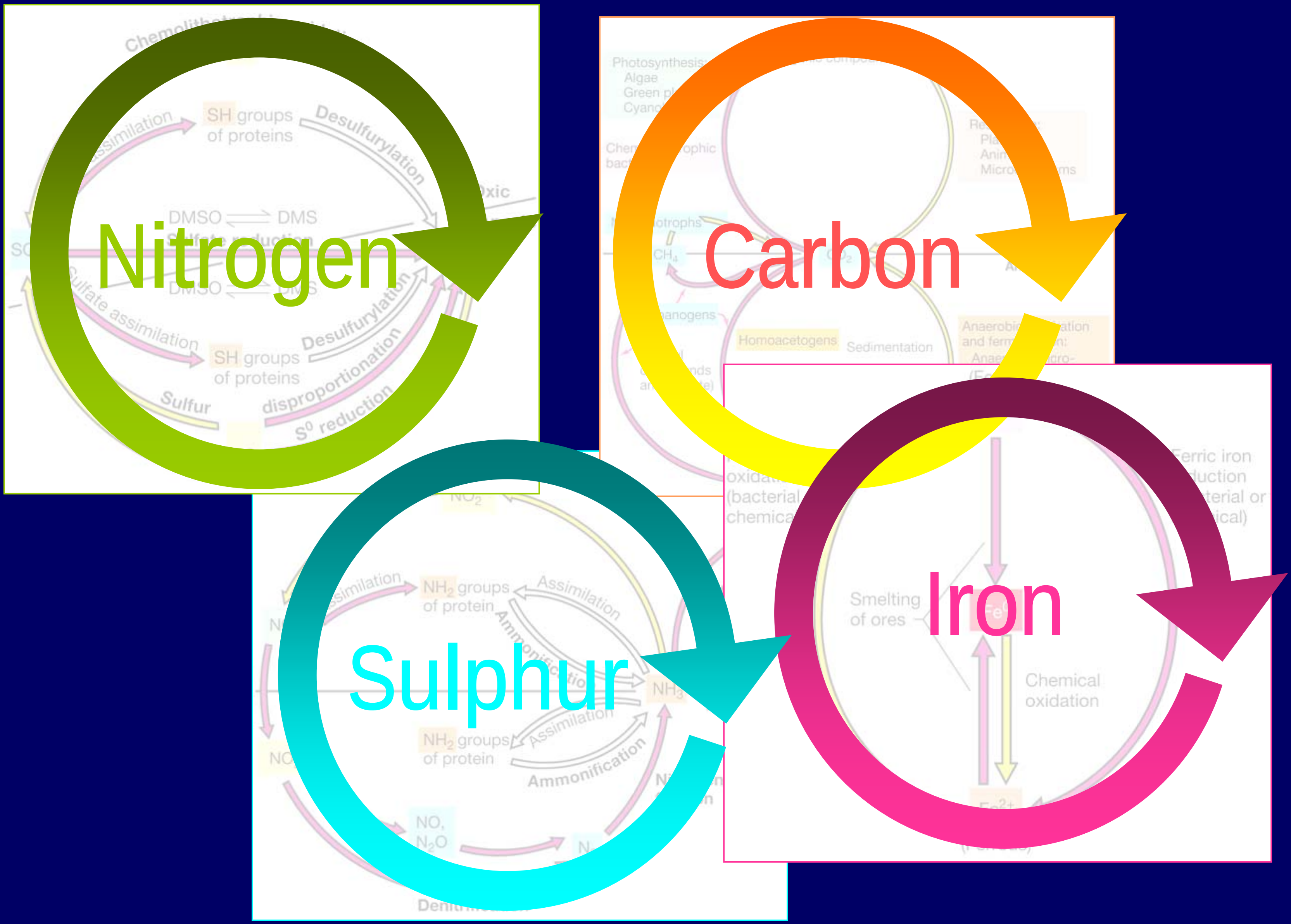
Other electron acceptors

- Chlorate (ClO_3^-) => Chlorine
- Mn^{4+} => Mn^{2+}
- Fe^{3+} => Fe^{2+}
- Selenate => Selenite
- Arsenate => Arsenite
- DMSO => DMS
- Fumarate => Succinate

Biogeochemical cycles



Microbial ecology of hydrothermal vent chimneys

Chimney sampling

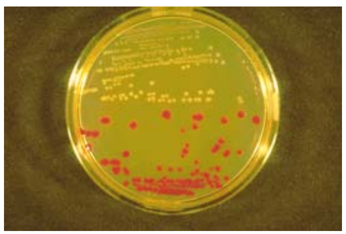
Sample preservation

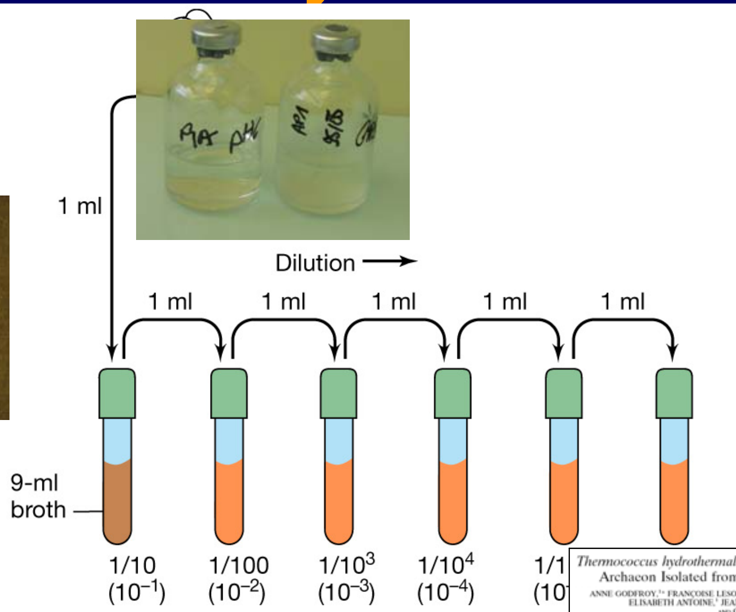
Activity

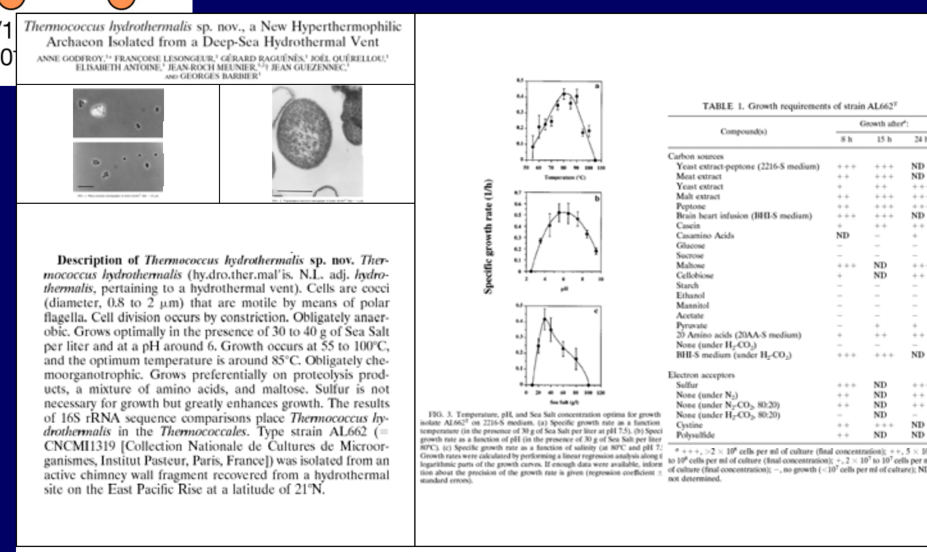
Molecular diversity studies

Cultures

Microbial diversity in hydrothermal vent chimneys : cultural approaches







Thermococcus hydrothermalis sp. nov., a New Hyperthermophilic Archaeon Isolated from a Deep-Sea Hydrothermal Vent

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Description of *Thermococcus hydrothermalis* sp. nov. *Thermococcus hydrothermalis* (hy.dro.ther.mal'is. N.L. adj. *hydrothermalis*, pertaining to a hydrothermal vent). Cells are cocci (diameter, 0.8 to 2 μm) that are motile by means of polar flagella. Cell division occurs by constriction. Obligately anaerobic. Grows optimally in the presence of 30 to 40 g of Sea Salt per liter and at a pH around 6. Growth occurs at 55 to 100°C, and the optimum temperature is around 85°C. Obligately chemoorganotrophic. Grows preferentially on proteolysis products, a mixture of amino acids, and maltose. Sulfur is not necessary for growth but greatly enhances growth. The results of 16S rRNA sequence comparisons place *Thermococcus hydrothermalis* in the *Thermococcales*. Type strain AL662 (= CNCMI1319 [Collection Nationale de Cultures de Microorganismes, Institut Pasteur, Paris, France]) was isolated from an active chimney wall fragment recovered from a hydrothermal site on the East Pacific Rise at a latitude of 21°N.

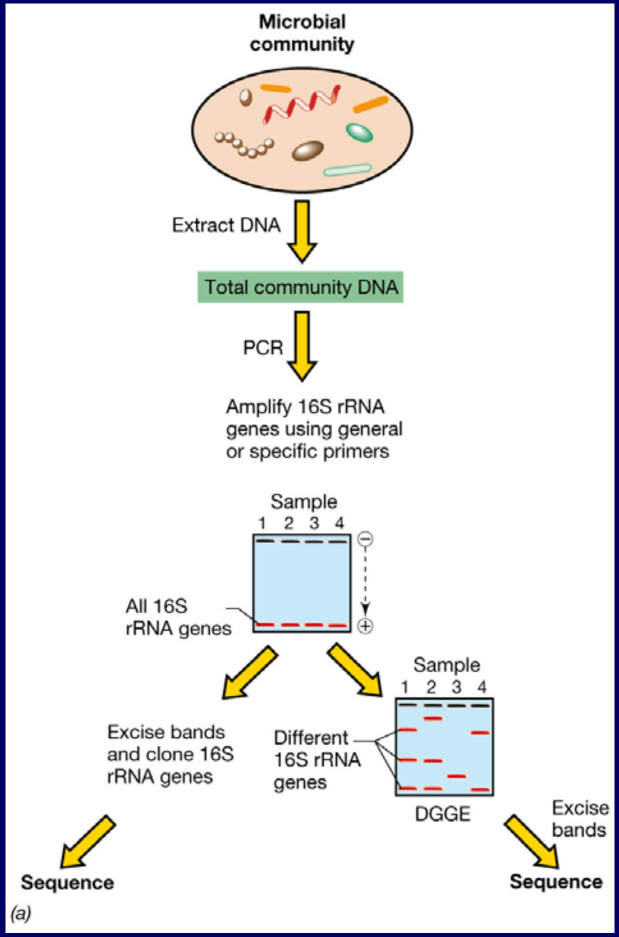
FIG. 3. Temperature, pH, and Sea Salt concentration optima for growth isolate $AL662^T$ on 2216-S medium. (a) Specific growth rate as a function temperature (in the presence of 30 g of Sea Salt per liter at pH 7.5). (b) Speci growth rate as a function of pH (in the presence of 30 g of Sea Salt per liter 80°C). (c) Specific growth rate as a function of salinity (at 80°C and pH 7. Growth rates were calculated by performing a linear regression analysis along t logarithmic parts of the growth curves. If enough data were available, inform tion about the precision of the growth rate is given (regression coefficient ± standard errors).

TABLE 1. Growth requirements of strain $AL662^T$

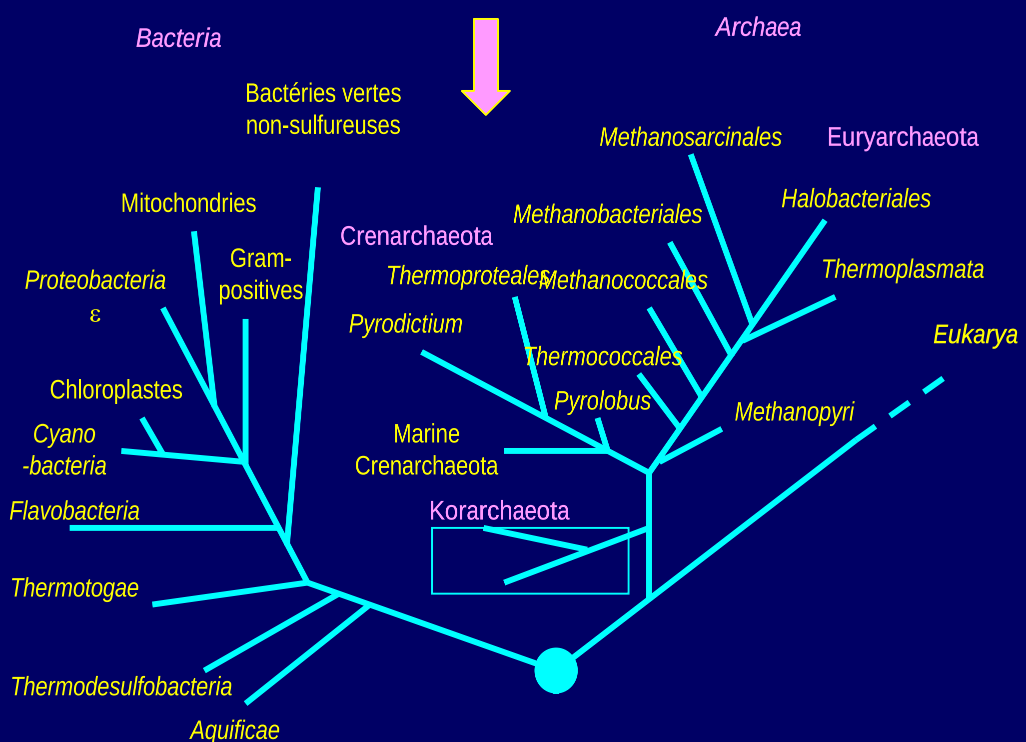
Compound(s)	Growth after[a]:		
	8 h	15 h	24 h
Carbon sources			
Yeast extract-peptone (2216-S medium)	+++	+++	ND
Meat extract	++	+++	ND
Yeast extract	+	++	+++
Malt extract	++	+++	+++
Peptone	++	+++	+++
Brain heart infusion (BHI-S medium)	+++	+++	ND
Casein	+	++	++
Casamino Acids	ND	−	+
Glucose	−	−	−
Sucrose	−	−	−
Maltose	+++	ND	+++
Cellobiose	+	ND	++
Starch	−	−	−
Ethanol	−	−	−
Mannitol	−	−	−
Acetate	−	−	−
Pyruvate	−	+	+
20 Amino acids (20AA-S medium)	+	++	++
None (under H_2-CO_2)	−	−	−
BHI-S medium (under H_2-CO_2)	+++	+++	ND
Electron acceptors			
Sulfur	+++	ND	+++
None (under N_2)	++	ND	++
None (under N_2-CO_2, 80:20)	++	ND	++
None (under H_2-CO_2, 80:20)	−	ND	−
Cystine	++	+++	ND
Polysulfide	++	ND	ND

[a] +++, >2 × 10^8 cells per ml of culture (final concentration); ++, 5 × 10^7 to 10^8 cells per ml of culture (final concentration); +, 2 × 10^7 to 10^7 cells per ml of culture (final concentration); −, no growth (<10^7 cells per ml of culture); ND, not determined.

Microbial diversity in hydrothermal vent chimneys : Molecular approaches

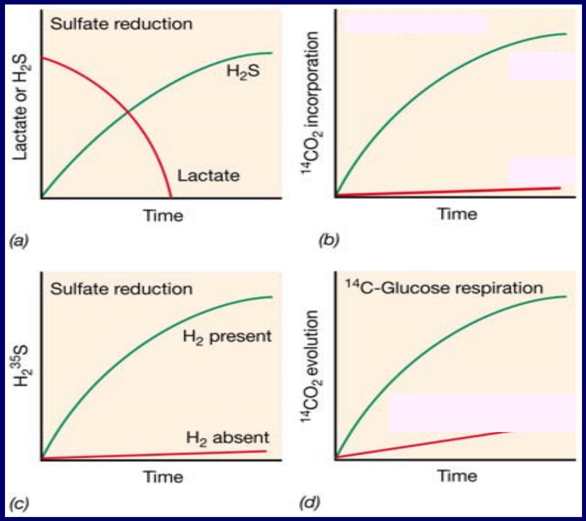


16S rRNA genes
or
functional genes



Microbial diversity in hydrothermal vent chimneys : metabolic activities

Samples



Incubation with labeled substrates
(stable or radioactive isotopes or fluorescent molecules)

Hydrolytic activity
Sulphate reduction
etc…

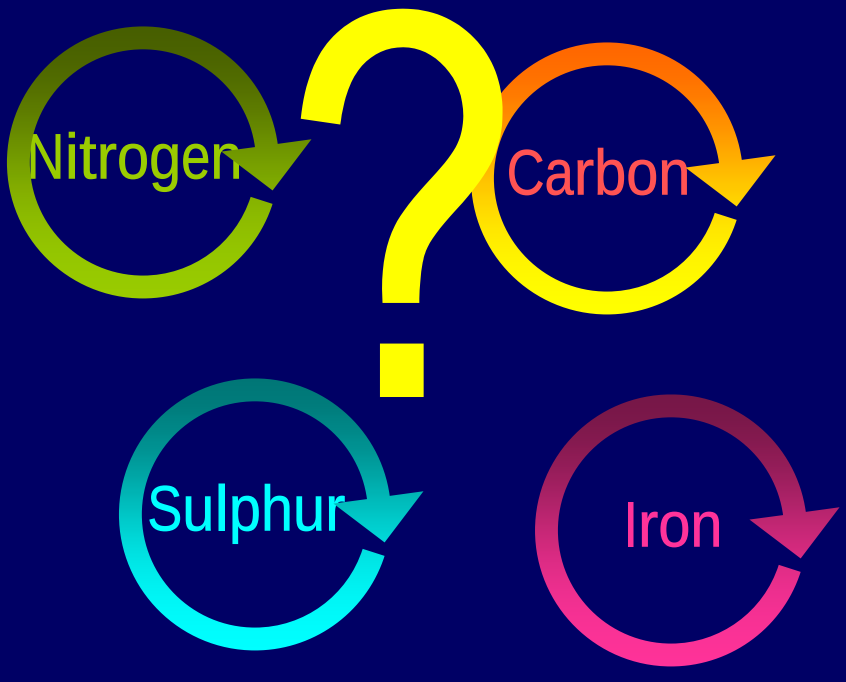
Conclusions (1)

For a given strain, cultural approaches give informations about carbon sources, electron donnors and acceptors, and suitable environmental conditions for this strain.

Molecular approaches give informations about phylogeny (sometimes linked to metabolism) and/or functions (functional genes).
Environmental conditions for a given clone are uncertain.

Measurements of metabolic activities confirm this activity exists for the conditions of the assay.

What do we know about biogeochemical cycles at high temperature in deep sea-hydrothermal vent chimneys

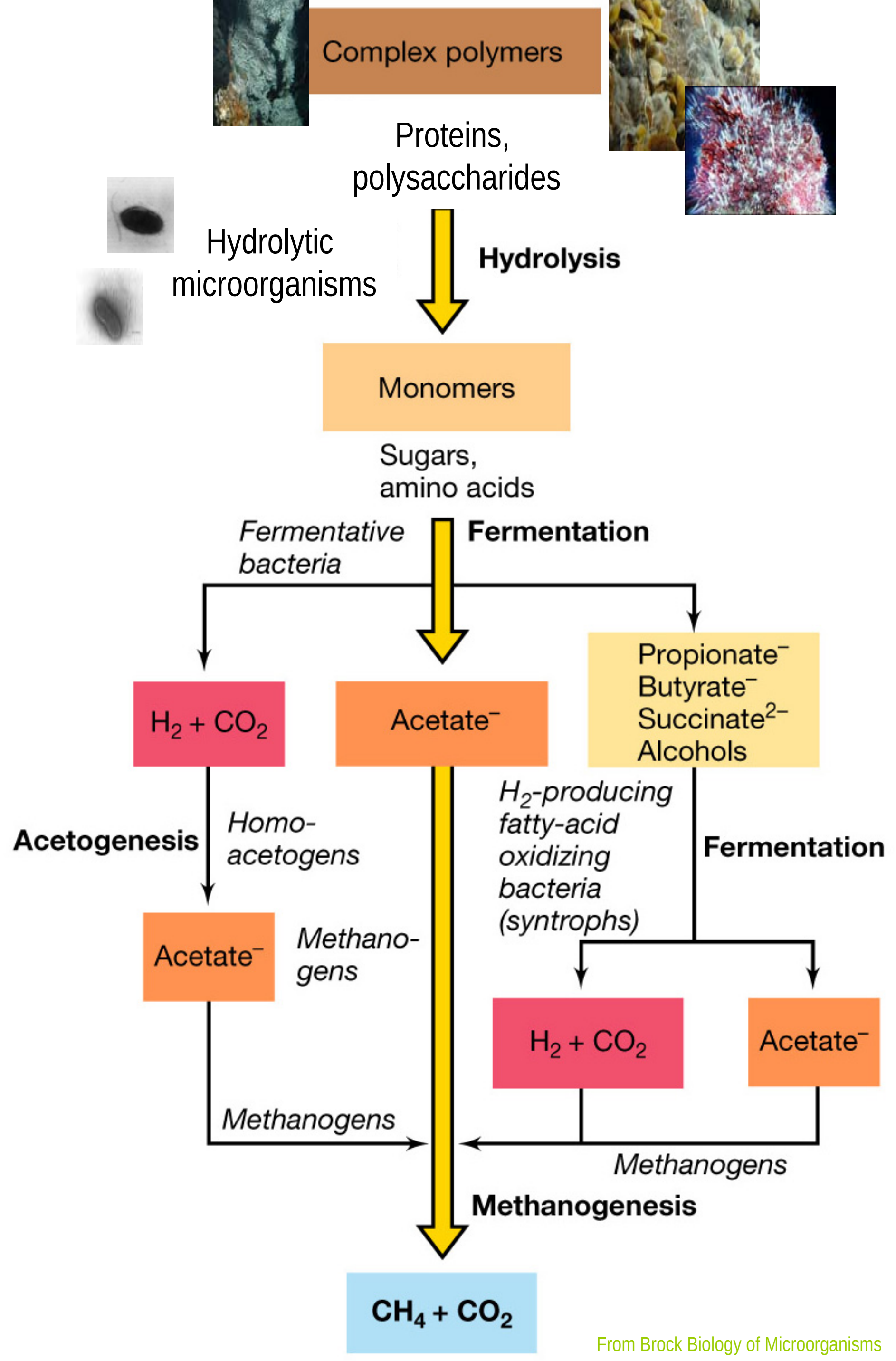


Please, note that

- Data used for this presentation were collected from published papers and pooled.
- Locations of vent sites were not taken into account.
- We apologize for possibly missing data.
- Please let us know…

Carbon cycle

Organic matter degradation/
Organic matter synthesis



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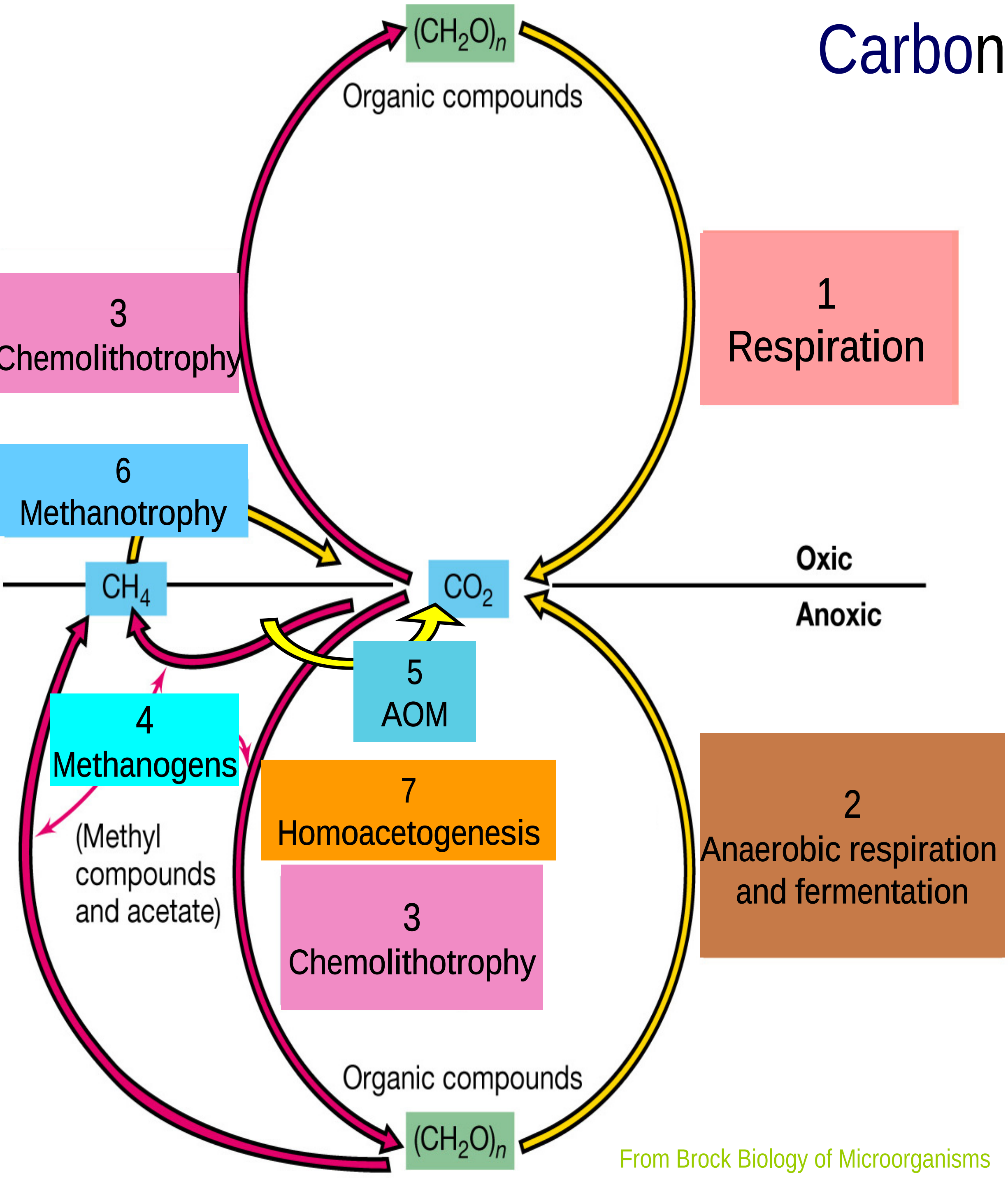
Carbon cycle



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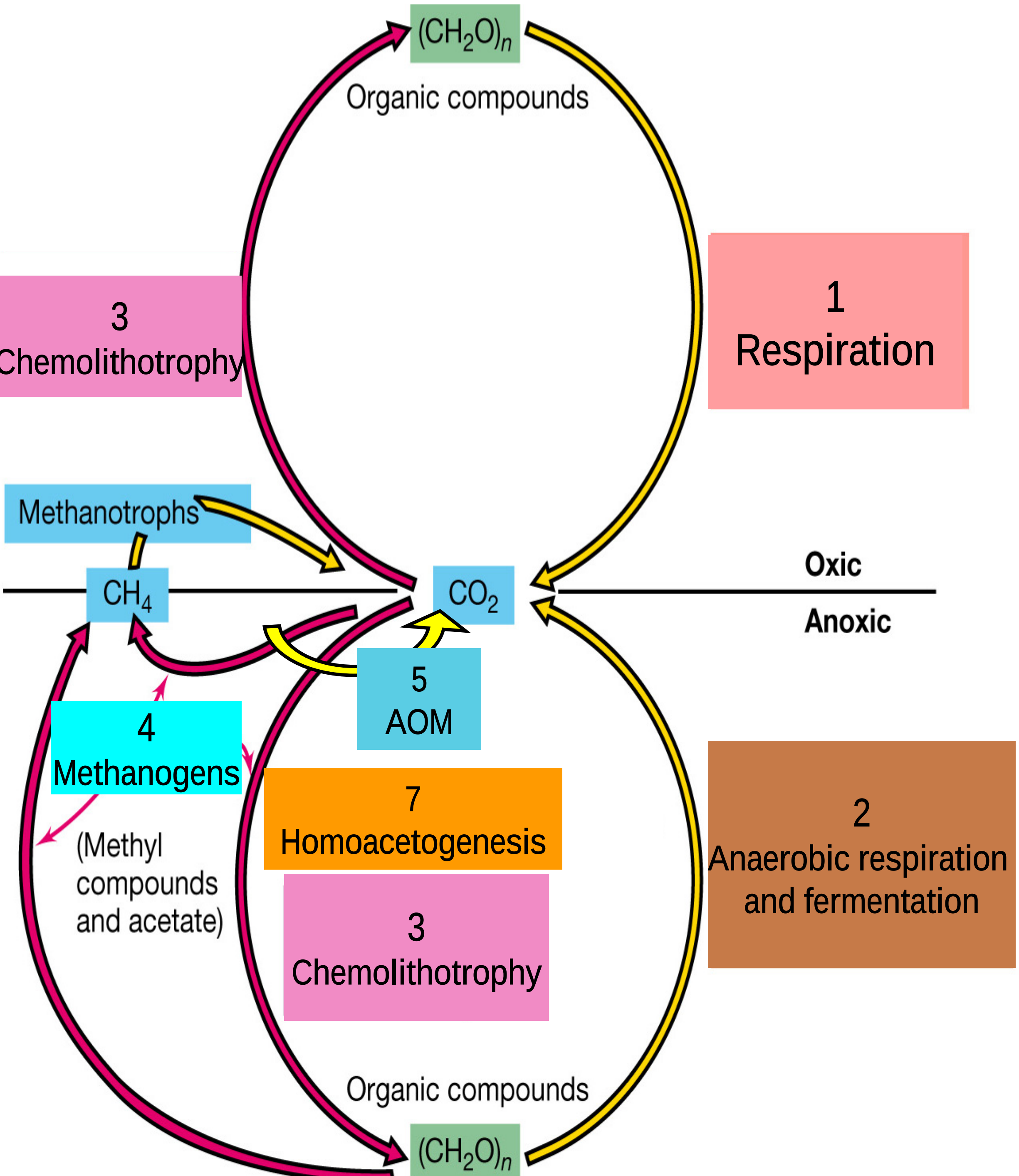
1
Aerobes and
Microaerophiles
Oceanithermus
Vulcanithermus
Aeropyrum camini
Thermus sp.
T° op max 85°C

2
Caminicella
Vulcanibacillus
Caloranaerobacter
Thermosipho
Marinotoga
Tepidibacter
Deferribacter desulfuri
Sulfurospirillum
Desulfurococcus sp
Staphylothermus
Thermococcus
Pyrococcus
Palaeococcus
Pyrodictium abyssi
Aciduliprofundum
T°op max 95°C

3
Archaeoglobus
Persephonella
Desulfurobacterium
Balnearium
Thermovibrio
Deferribacter
Caminibacter
Nautilia
Thermodesulfobacterium
Thermodesulfatator
Sulfurimonas
Hydrogenimonas
Lebetimonas
Ignicoccus
Pyrolobus
T°op max 106°C

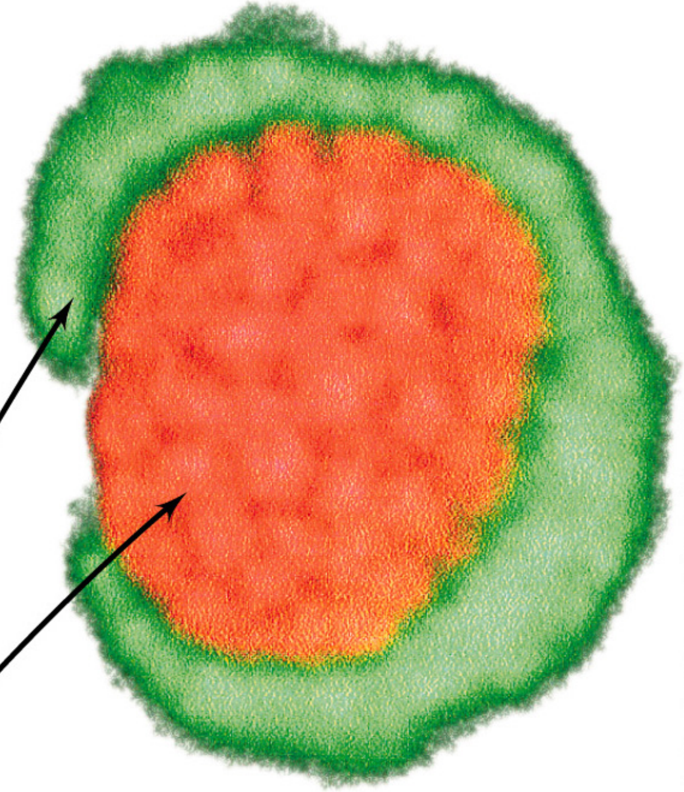


4
Methanoarchaea
(H_2)
Methanocaldococcus
Methanotorris
Methanopyrus
T° op max 98°C

5
AOM
Anaerobic oxidation of
methane
Molecular and activity
Evidence

6
Methanotrophy
CH_4 oxidation
NO

7
Homoacetogenesis
NO



(a)

Reaction	Organism	$\Delta G^{0\prime}$(kJ)
$CH_4 + 2\,H_2O \longrightarrow CO_2 + 4\,H_2$	Methanogen	+131
$SO_4^{2-} + 4\,H_2 + H^+ \longrightarrow HS^- + 4\,H_2O$	Sulfate-reducer	–156
Sum: $SO_4^{2-} + CH_4 \longrightarrow HCO_3^- + HS^- + H_2O$	Syntrophic reaction	–25

(b)

From Brock Biology of Microorganisms

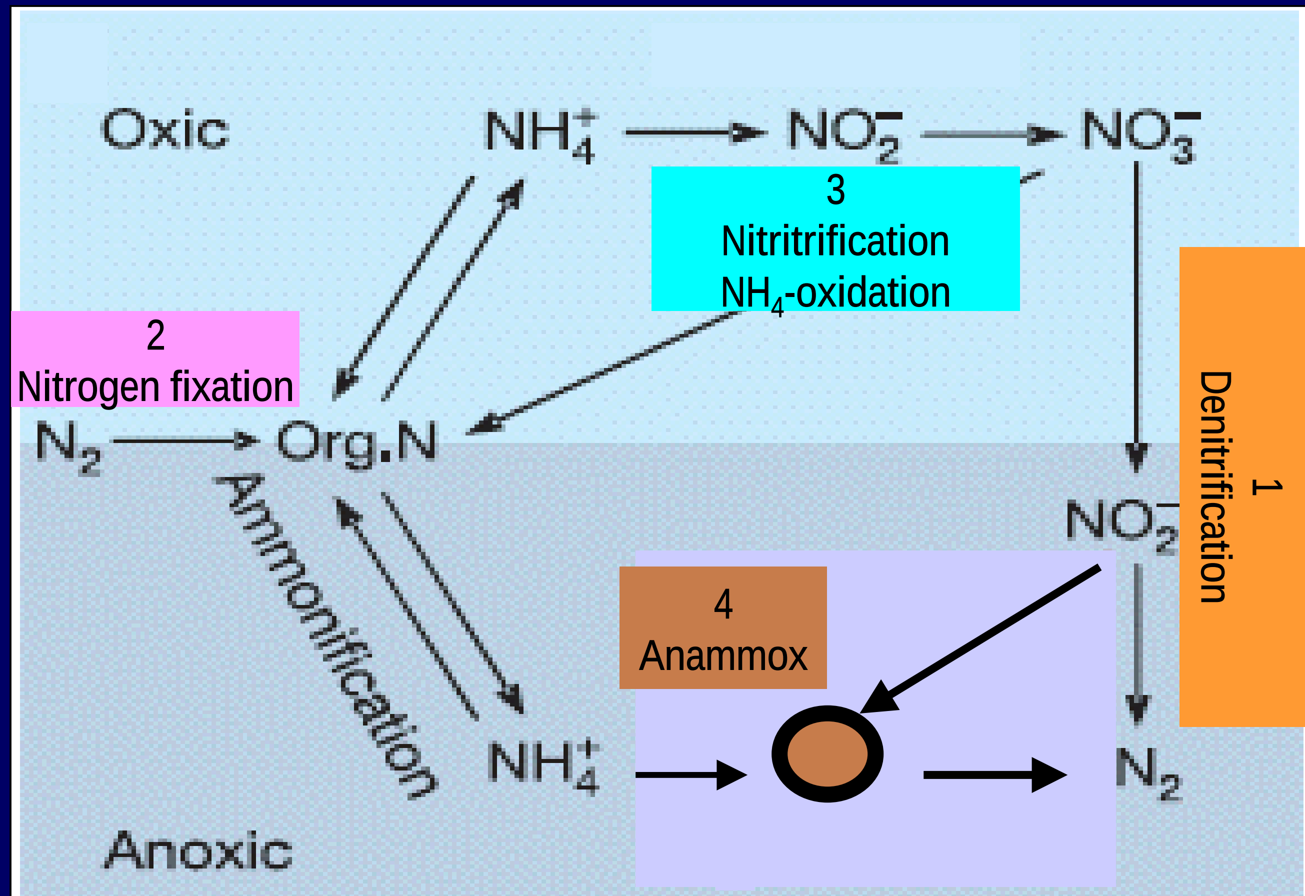
Nitrogen Cycle

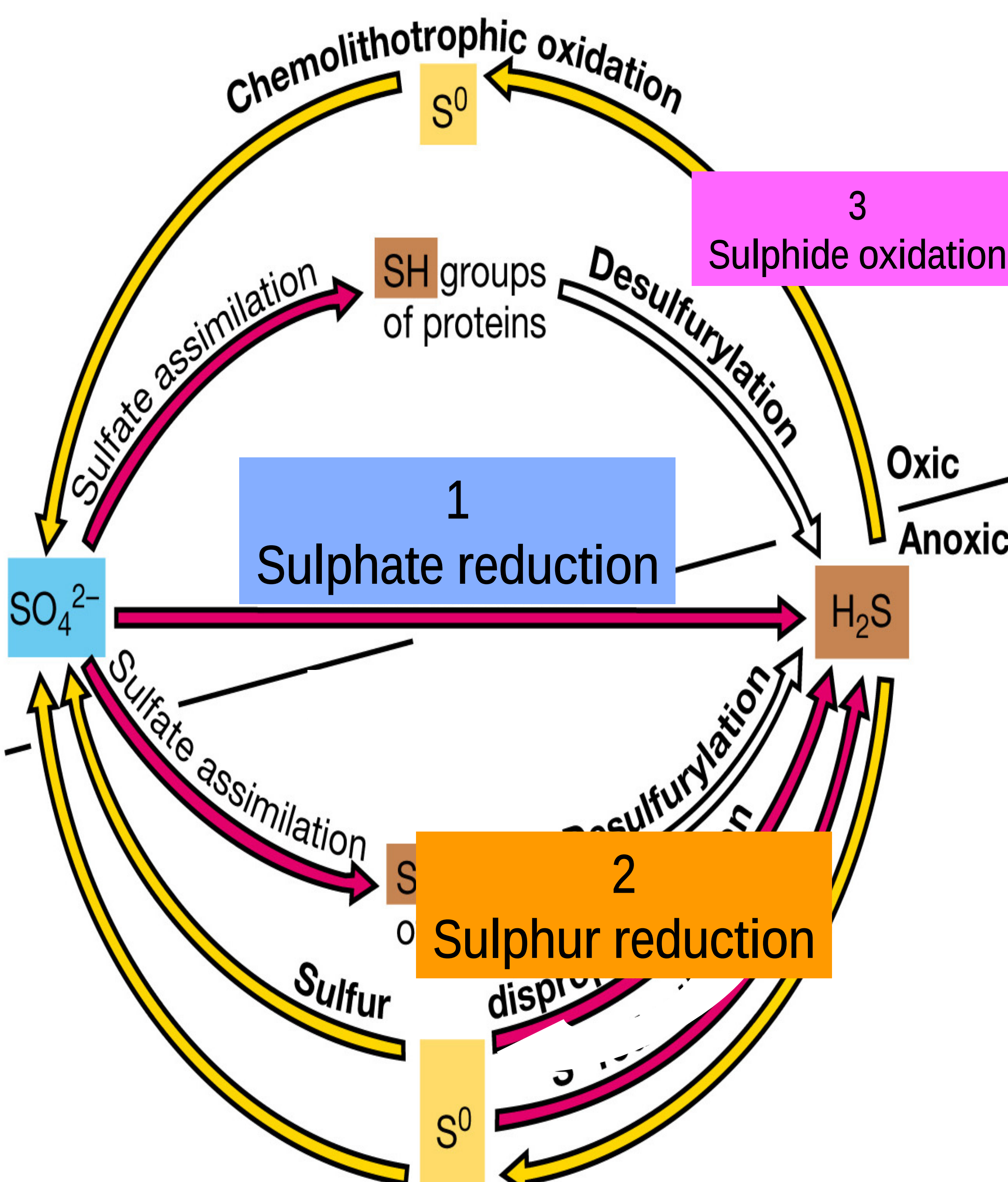
1
Denitrification
NO_3^- reducers
Persephonella
D. crinifex
Deferribacter abyssi
Caminibacter
Sulfurimonas
Pyrolobus
Caldithrix
Geothermobacter
T°op max 106°C

3
Aerobic
NH_4-oxidation

NO
4
Anammox

2
Nitrogen fixation
nifH genes detection
and
M.Jannaschii
str FS406-22
T° op max 90°C





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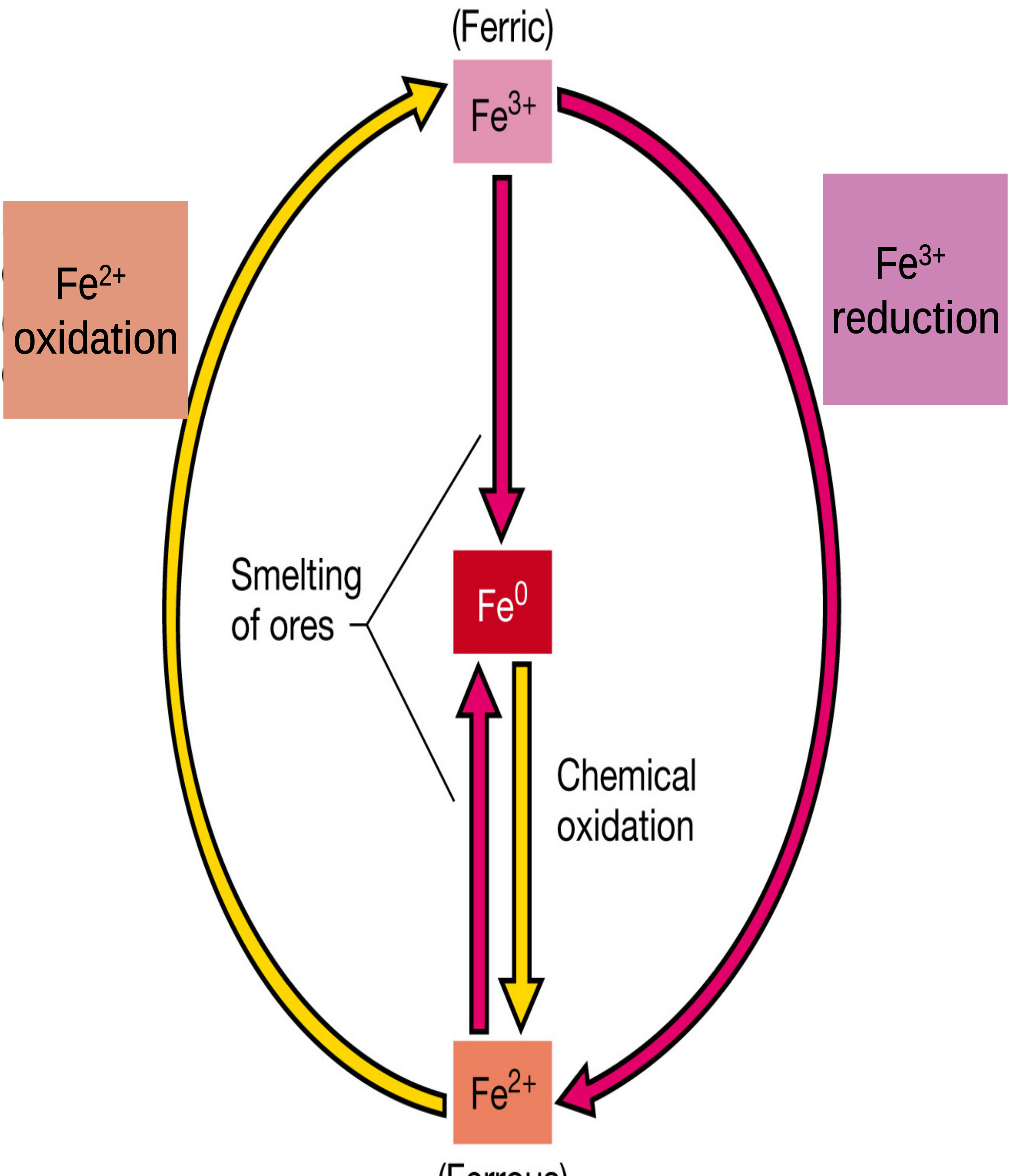
2
Sulphur reduction
Marinitoga
Thermosipho
Persephonella
Desulfurobacterium
Tepidibacter
Sufurospirillum
Desulfurococcus
Staphylothermus
Pyrodictium abyssi
Thermococcus
Pyrococcus
Palaeococcus
Balnearium
Thermovibrio
Deferribacter
Caminibacter
Nautilia
Sulfurimonas
Hydrogenimonas
Lebetimonas
Ignicoccus
T°max 97°C

3
Aerobic (microaerophilic)
S° & thiosulphate oxidation
Persephonella
T° op max 70°C

1
Sulphate reduction
Thermodesulfobacterium
Thermodesulfatator
Archaeoglobus
T° op max 82°C

Iron cycle



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Fe^{2+}
Oxidation
NO

Fe^{3+}
reduction

Geoglobus
Geothermobacter
Strain 121
T° max
up to 121°C!!!

Conclusions (2)

From available microbiology data,
at elevated temperatures existing at deep-sea
in hydrothermal vent chimneys,
C, N, S and Fe cycles do not work completely.
Particularly,
methane, ammonium, and ferrous iron are not oxidized

WANTED

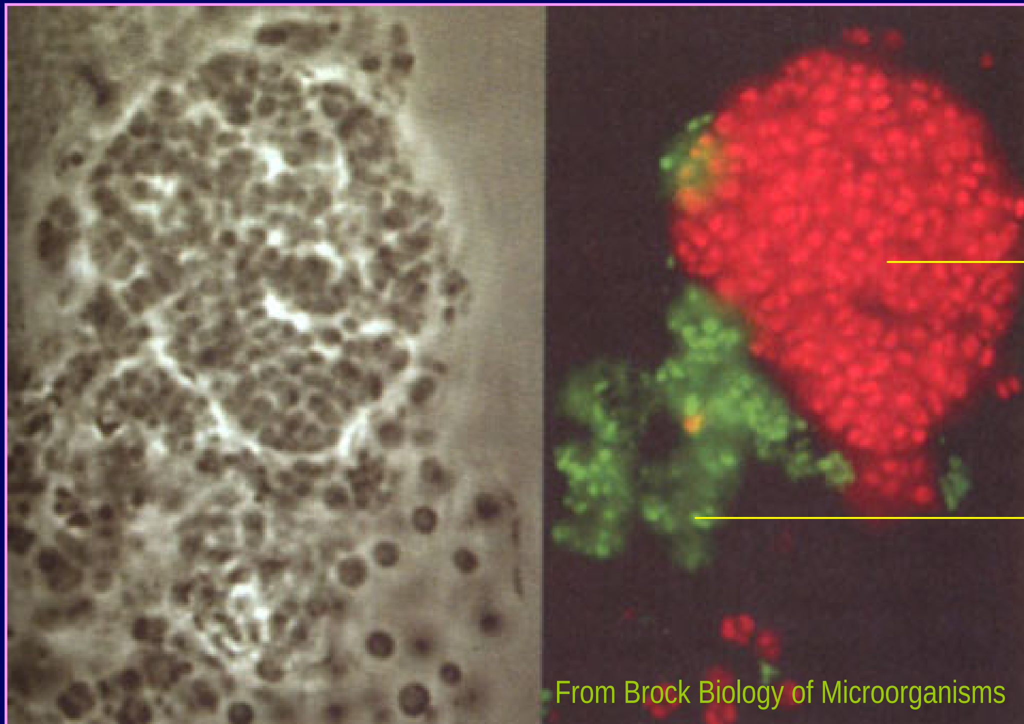
- Methanotrophs (aerobic oxidation of methane)
- Homoacetogens
- Ammonium-oxidizers (both aerobic an anaerobic)
- Sulphide-oxidizers
- Fe^{2+} -oxidizers

How to catch them !
(if they exist…)

Polyphasic approaches consisting of:

- *In situ* and "on board" activity measurements
- Phylogenetic and Functional gene analysis
- Innovative cultural approaches
  - -Gradient culture (Winogradsky columns)
  - -Microbial community cultivation in bioreactors
  - -*In situ* enrichment culture
  - -High throughput cultivation techniques

FISH (Fluorescence *in situ* Hybridization



Ammonium-oxidizers

Nitrification

Nitrite-oxidizers

Developping co-culture techniques

Microbial community cultivation in bioreactor

Hydrothermal chimney sample

Culture conditions

Temperature

pH

Gas sparging

(electron donnors and acceptors)

Dilution rate

(substrates concentration)

Medium composition

Carbon sources

(nature and concentration)

Electron donnors

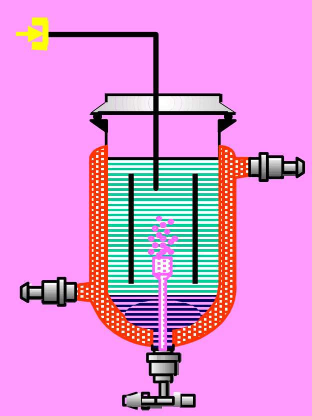
and acceptors

Inoculation

Enrichment cultures

Batch

Continuous culture



Microscopic observation
Phase contrast
epifluorescence

Molecular analysis :

16SrRNA genes diversity

- DGGE/SSCP
- Cloning/sequencing
- Phylogenetic analysis
- *In situ* hybridization
- Quantitative PCR

HPLC analysis of medium
GC analysis of gas exhaust

Sub-cultures and strains isolation

Postec *et al.*, *Extremophiles* **in press** (2007).
Postec *et al.*, *Current Microbiology* **50**, 138 (2005).

- Efficiency of such system to recover a largest diversity of cultivated thermophilic and hyperthermophilic microorganisms from a deep-sea chimney, compared to traditional cultures in vial
  - New species
  - Cultivate the uncultivable

- Efficiency to cultivate microbial communities
  - Population dynamics studies
  - Interactions between microbial populations
  - Influence of various parameters on cultivated microbial communities

Conclusions (final)

- At elevated temperatures (at deep-sea vents) C,N,S Fe cycles do not work completely
- Novel approaches (including those suggested here) should contribute to fill the gaps
- In case of failure, it could be concluded that the high temperature ecosystems must relie on lower temperature ecosystems for recycling some compounds
- But, let’s work first in a multidisciplinary approach to get more data!!