

Bioprocesses in the Dark

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Agenda

- What is a «dark» environment?
- Is there cellular growth in the dark?
- What are the benefits?
- Examples for bioprocesses in the dark
- Conclusions

The HES-SO > *Haute Ecole Spécialisée de Suisse Occidentale* <
University of Applied Sciences and Arts Western Switzerland

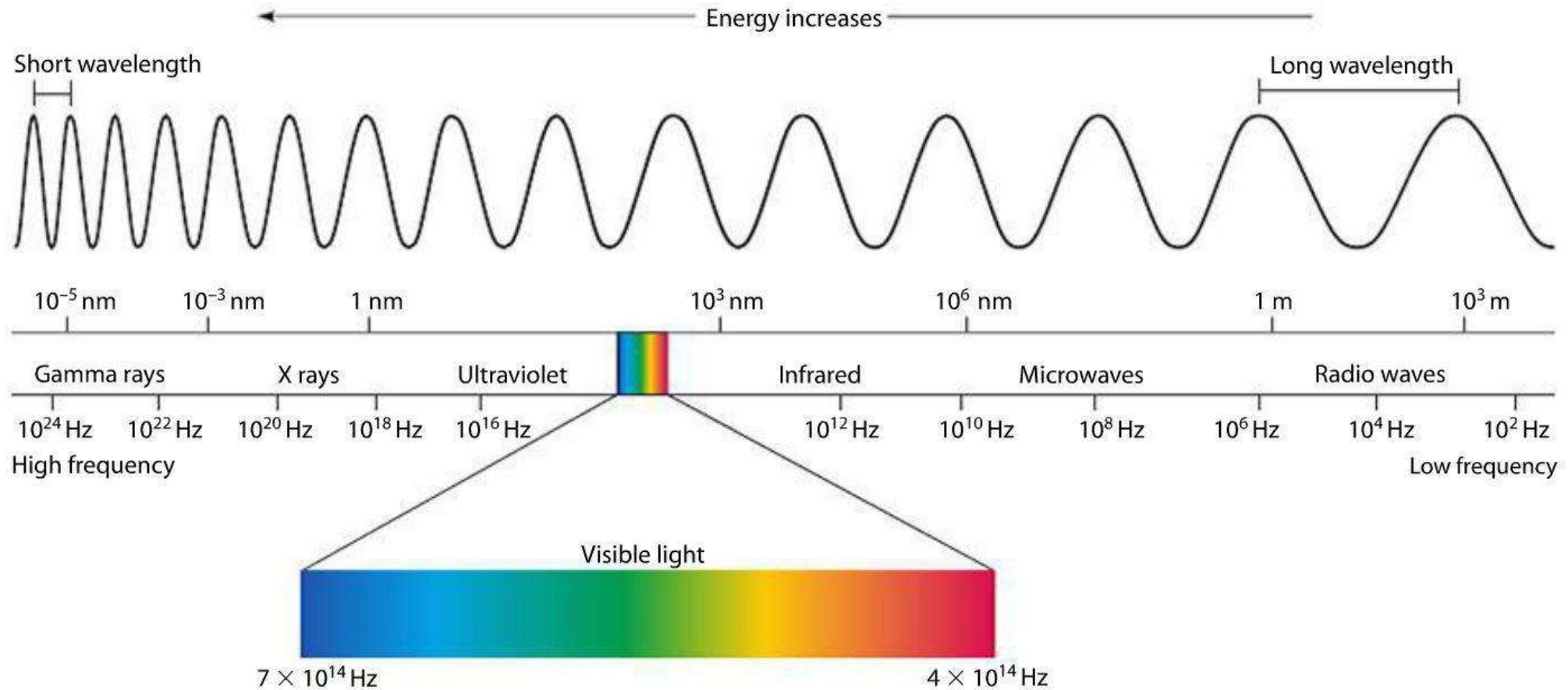


- ~21'000 students
- 28 schools
- 6 faculties
- 46 Bachelor programs
- 20 Master programs

... and Master studies in Applied Biosciences and Food Technology!

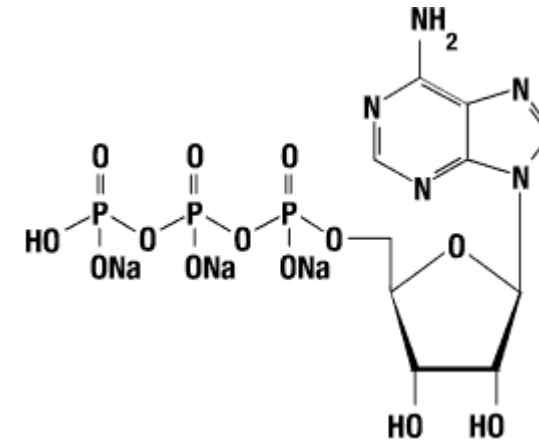
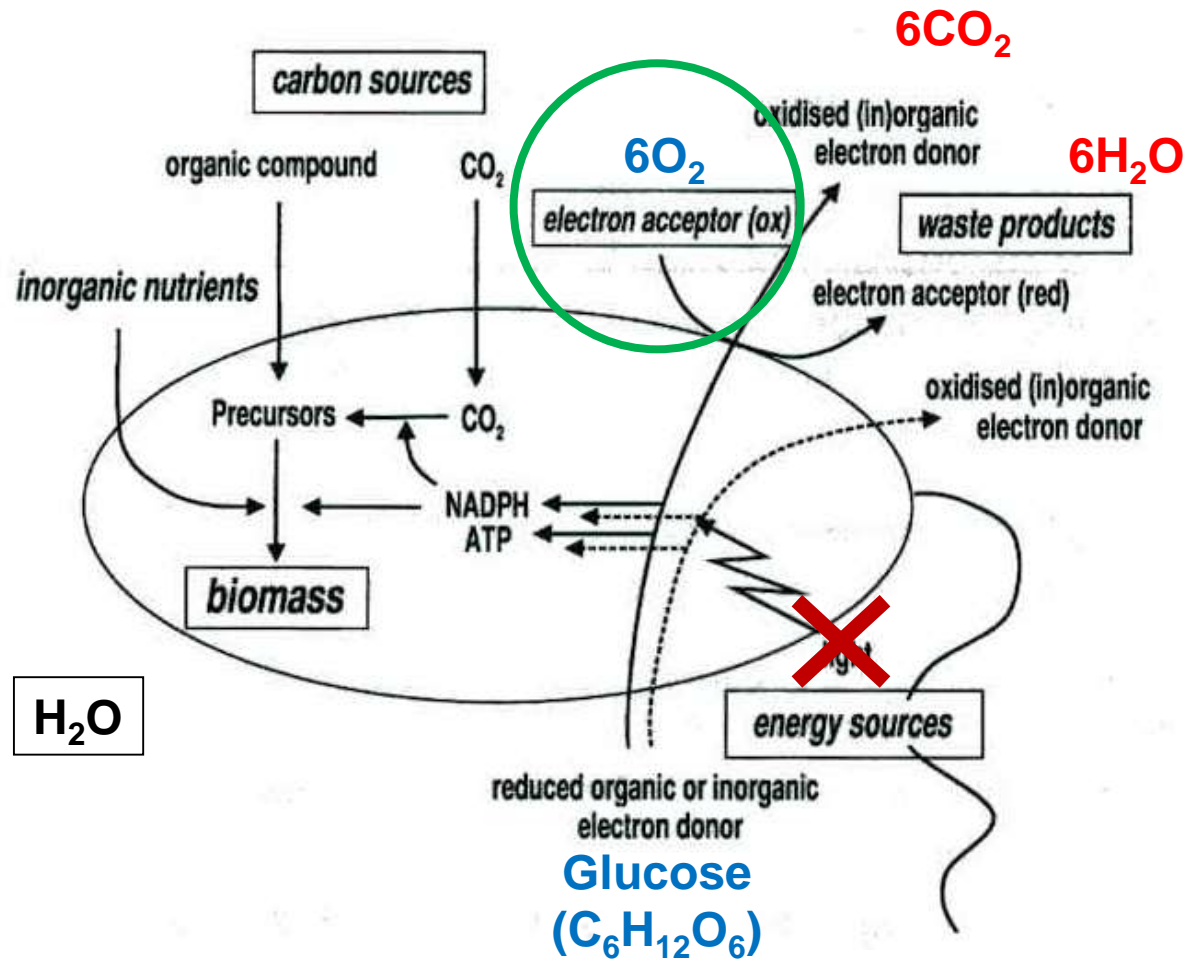
What is a dark environment?

The electromagnetic spectrum:

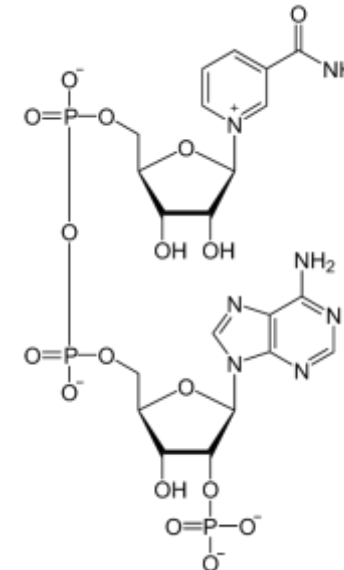


Dark = UV and IR are not visible for the human eye!

Is there cellular growth in the dark?



Adenosin triphosphate (ATP)



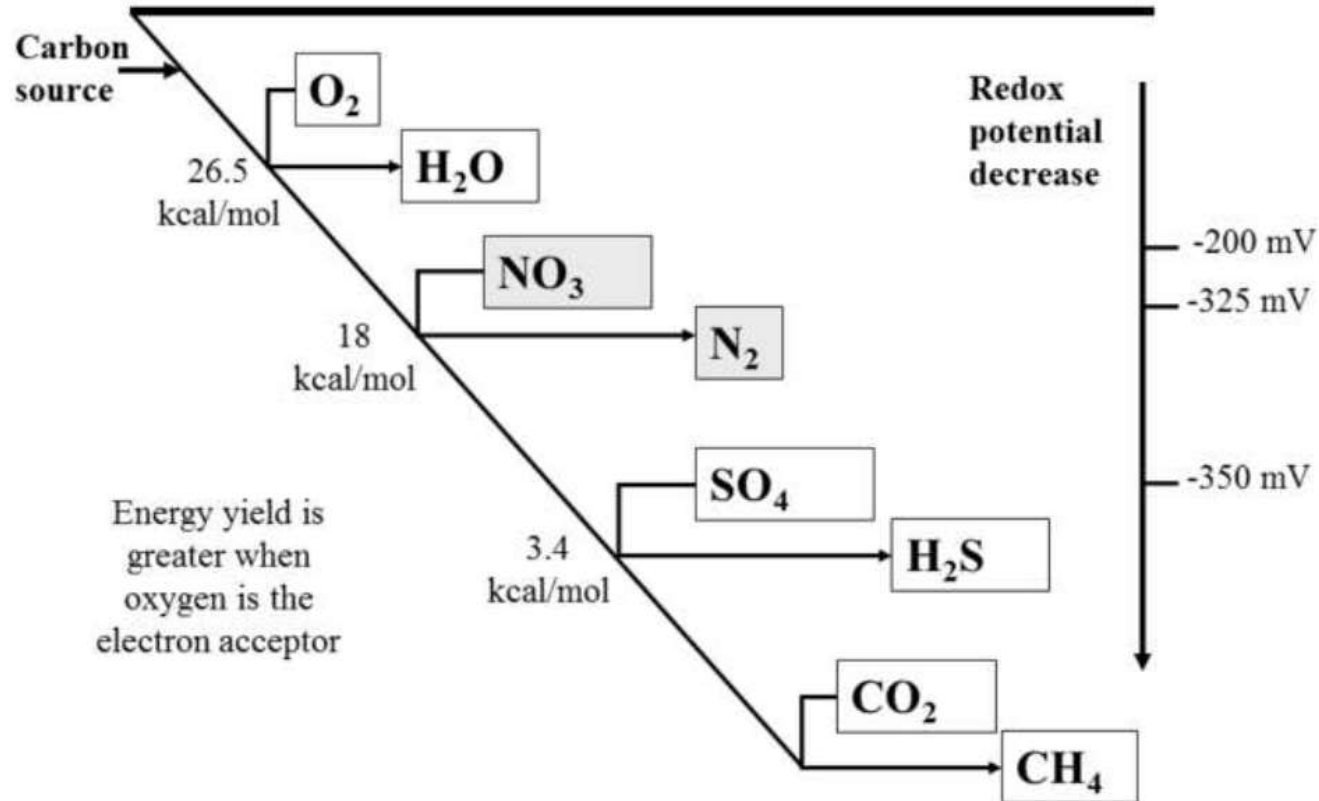
$$1 \text{ NADPH} = 2 \text{ ATP}$$

Simplified sketch of the physiological function of nutrients for the growth of microorganisms.

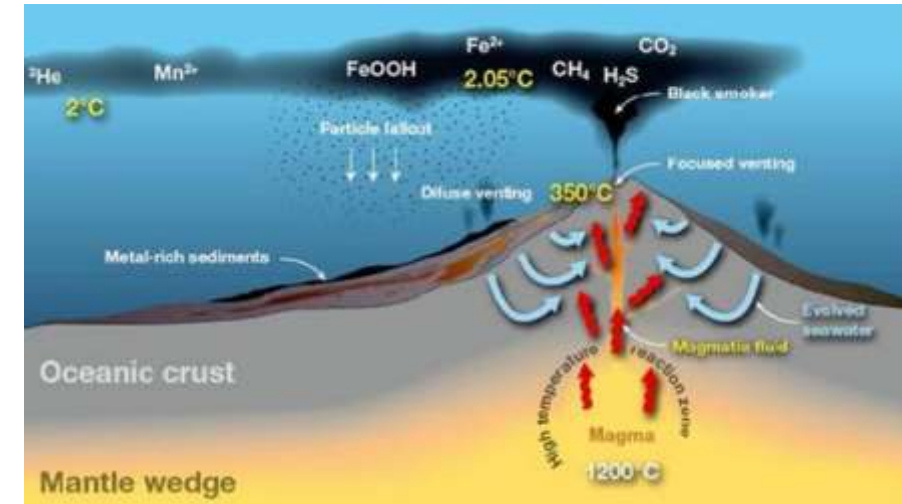
Nicotinamide adenine dinucleotide phosphate

Typical electron acceptors

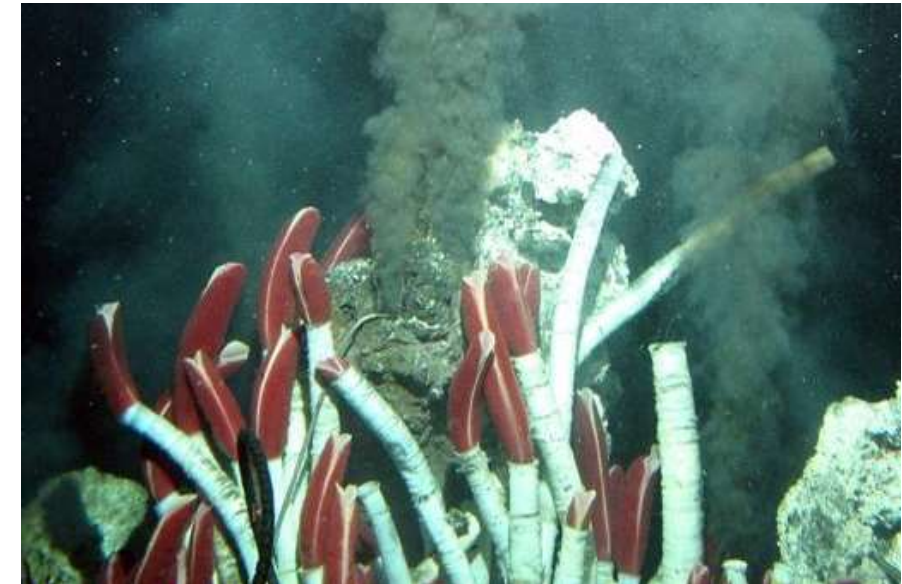
Respiratory processes



Growth is slower without oxygen!



https://www.ncl.ac.uk/media/wwwnclacuk/teacherstoolkit/emilijakopustaite/Teachers%20notes_hydrothermal%20vents%20background%20information.pdf



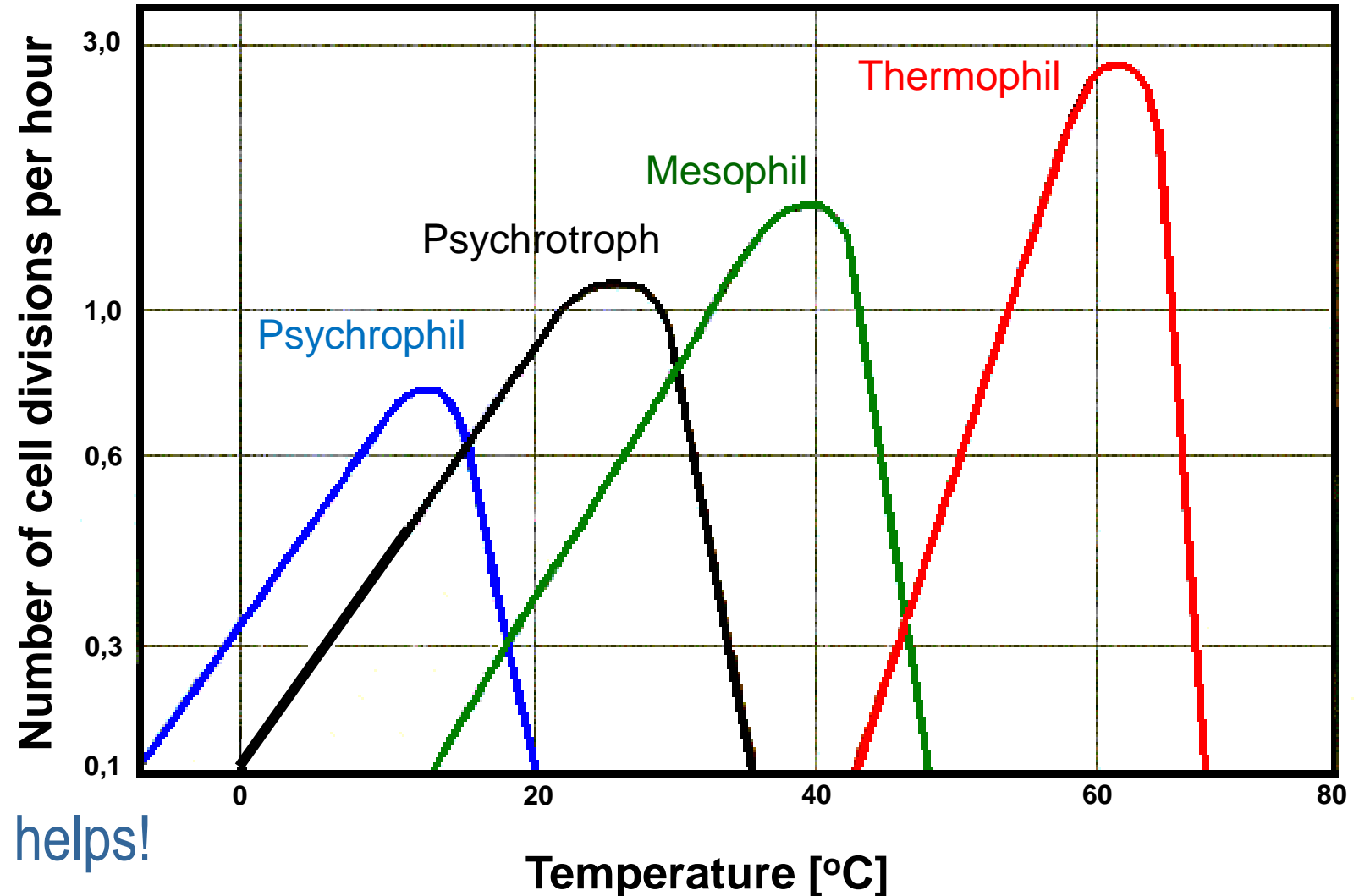
<https://blogs.agu.org/thefield/2019/03/04/the-discovery-of-riftia/>

Growth of microorganisms is influenced by the Arrhenius equation

$$k = Ae^{\frac{E_A}{RT}}$$

Diagram illustrating the Arrhenius equation components:

- k : rate constant
- A : frequency factor or pre-exponential factor
- e : mathematical quantity, e
- E_A : activation energy
- R : the gas constant
- T : kelvin temperature



➡ Optimal temperature helps!

Energy gain and energy needs

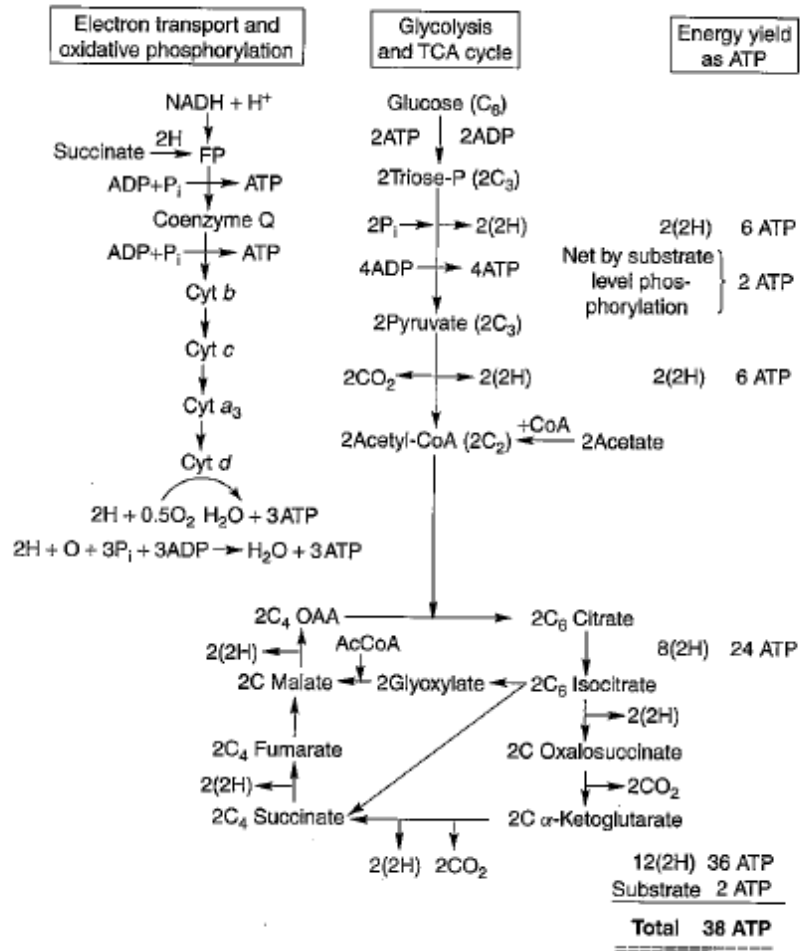


TABLE 5.2 The Biosynthetic Capabilities of a Bacterial Cell^a

Chemical component	Percent of dry weight	Approximate molecular weight	Number of molecules per cell	Number of molecules synthesized per second	Number of molecules required to synthesize per second	Percent of total synthetic energy required
DNA	5	2,000,000,000	1	0.00083	60,000	2.5
RNA	10	1,000,000	15,000	12.5	75,000	3.1
Protein	70	60,000	1,700,000	1,400	2,120,000	88.0
Lipids	10	1,000	15,000,000	12,500	87,500	3.7
Polysaccharides	5	200,000	39,000	32.5	65,000	2.7

^a Reprinted with permission from Lehninger (1971). *Escherichia coli* is about $1 \times 1 \times 3 \mu m$ in size; it has a volume of $2.25 \mu m^3$, a total weight of $10 \times 10^{13} g$, and a dry weight of $2.5 \times 10^{-13} g$. The rates of biosynthesis were averaged over a 20 minute cell division cycle.

Fig. 9-9. Theoretical energy yield as ATP from glycolysis and the TCA cycle. The calculations shown here assume that each pair of hydrogen atoms (2H) released from the substrate yields 3 ATP. The reaction shown as $ADP + P_i \rightarrow ATP$ represents the action of ATP synthase. Two turns of the TCA cycle are required to completely oxidize the 2 acetyl-CoA derived from glucose. Each 2H generated by the system yields 1 molecule of water ($2H + 0.5O_2 \rightarrow H_2O$). Overall reaction: $C_6H_{12}O_6 + 38P_i + 38ADP + 6O_2 \rightarrow 6CO_2 + 6H_2O + 38ATP$. Total ATP from TCA cycle: $12(2H) + 6O_2 + 36P_i \rightarrow 6H_2O + 36ATP$.

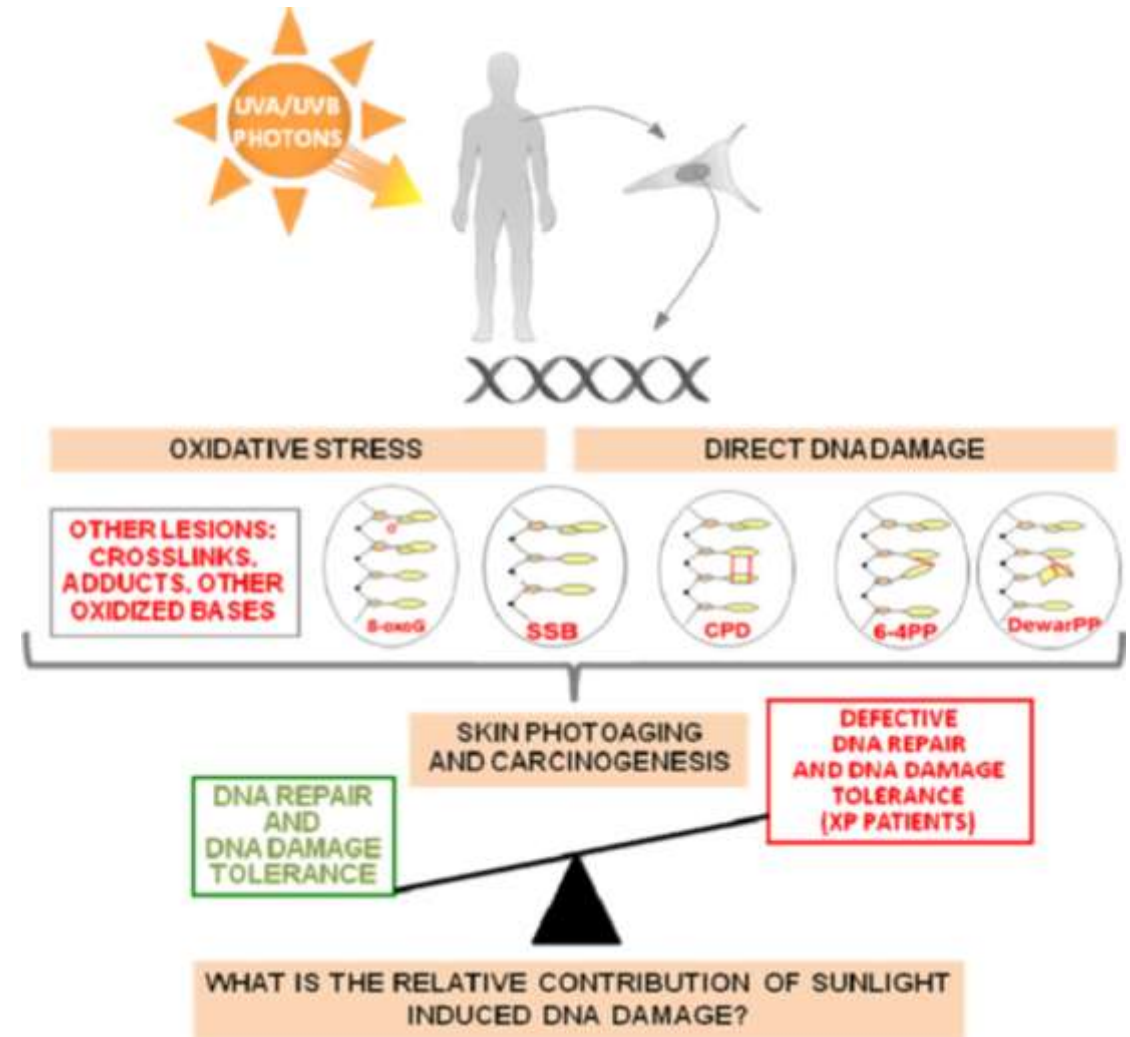
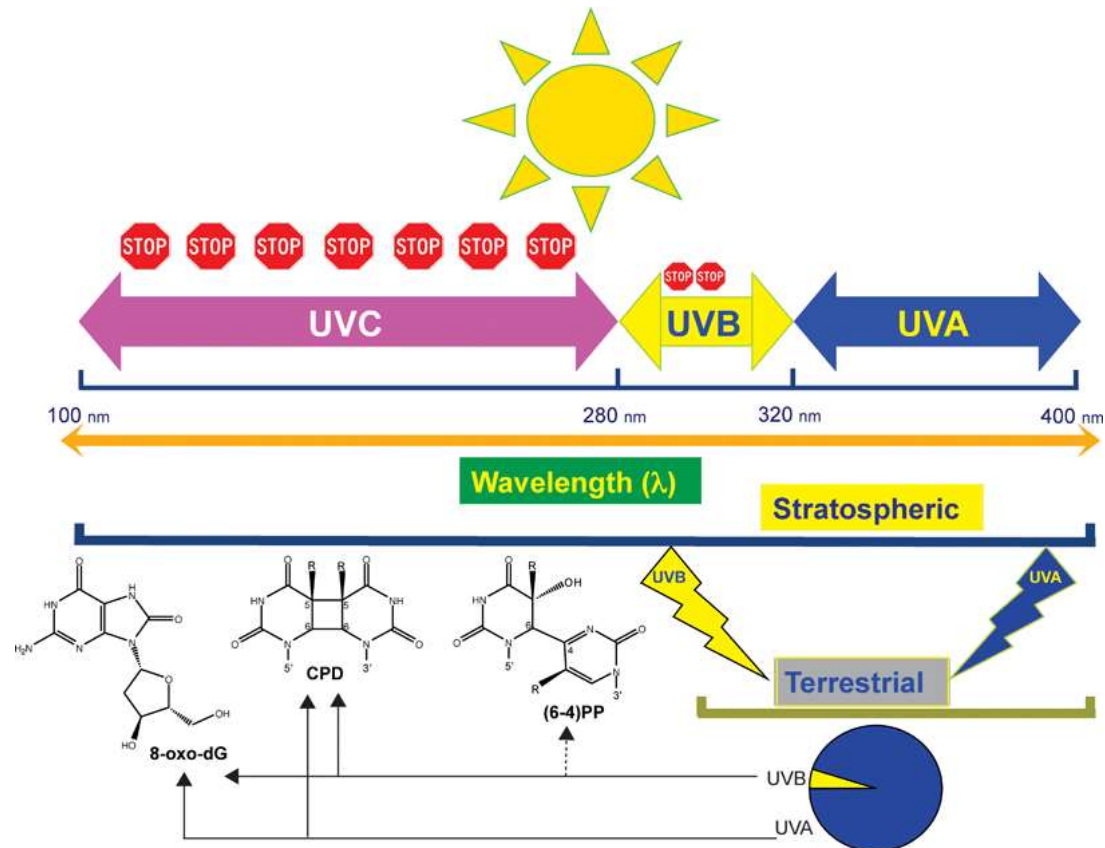


A constant energy (and carbon) flow is needed!

Wonderful, there is growth without sunlight!

Are there even advantages?

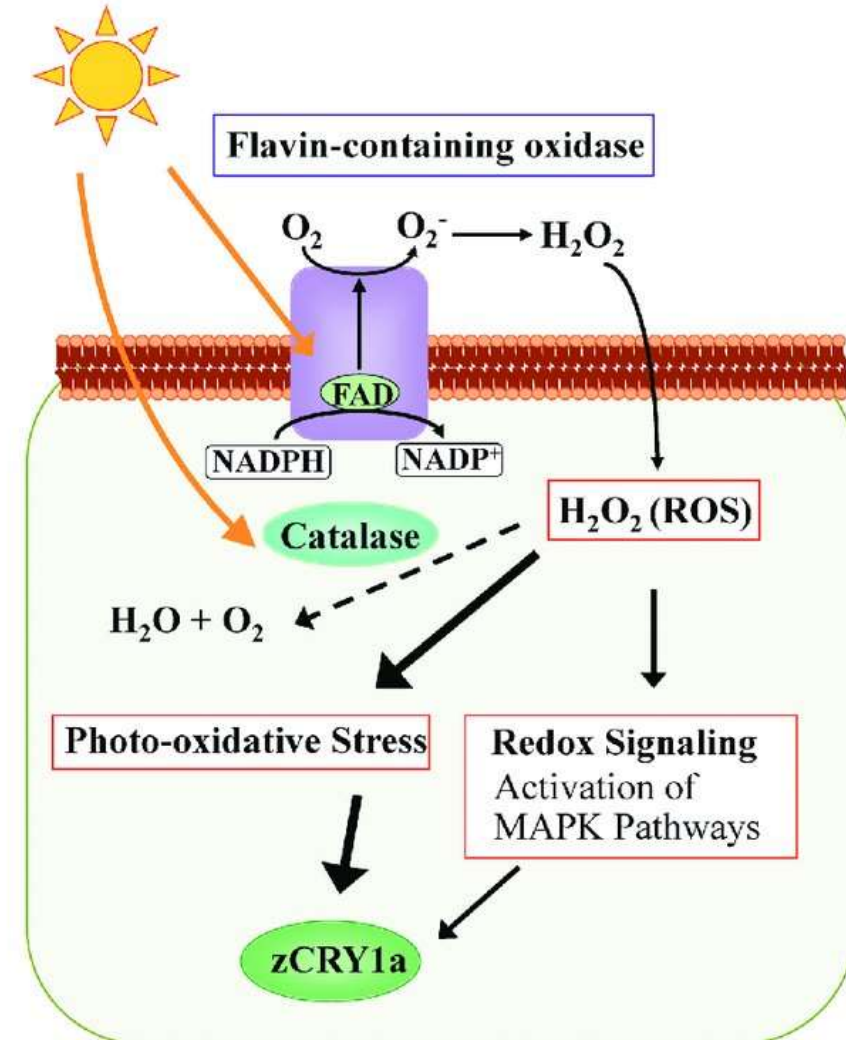
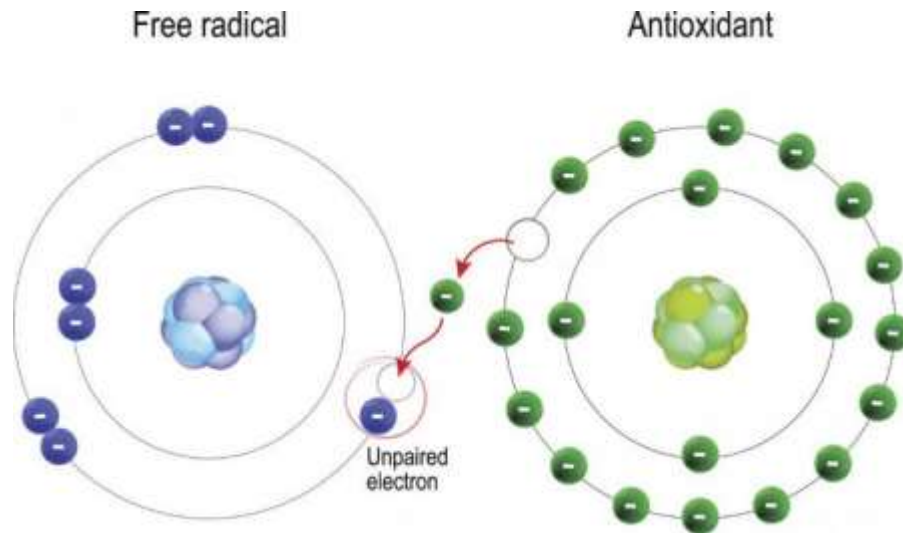
- Less DNA damage!



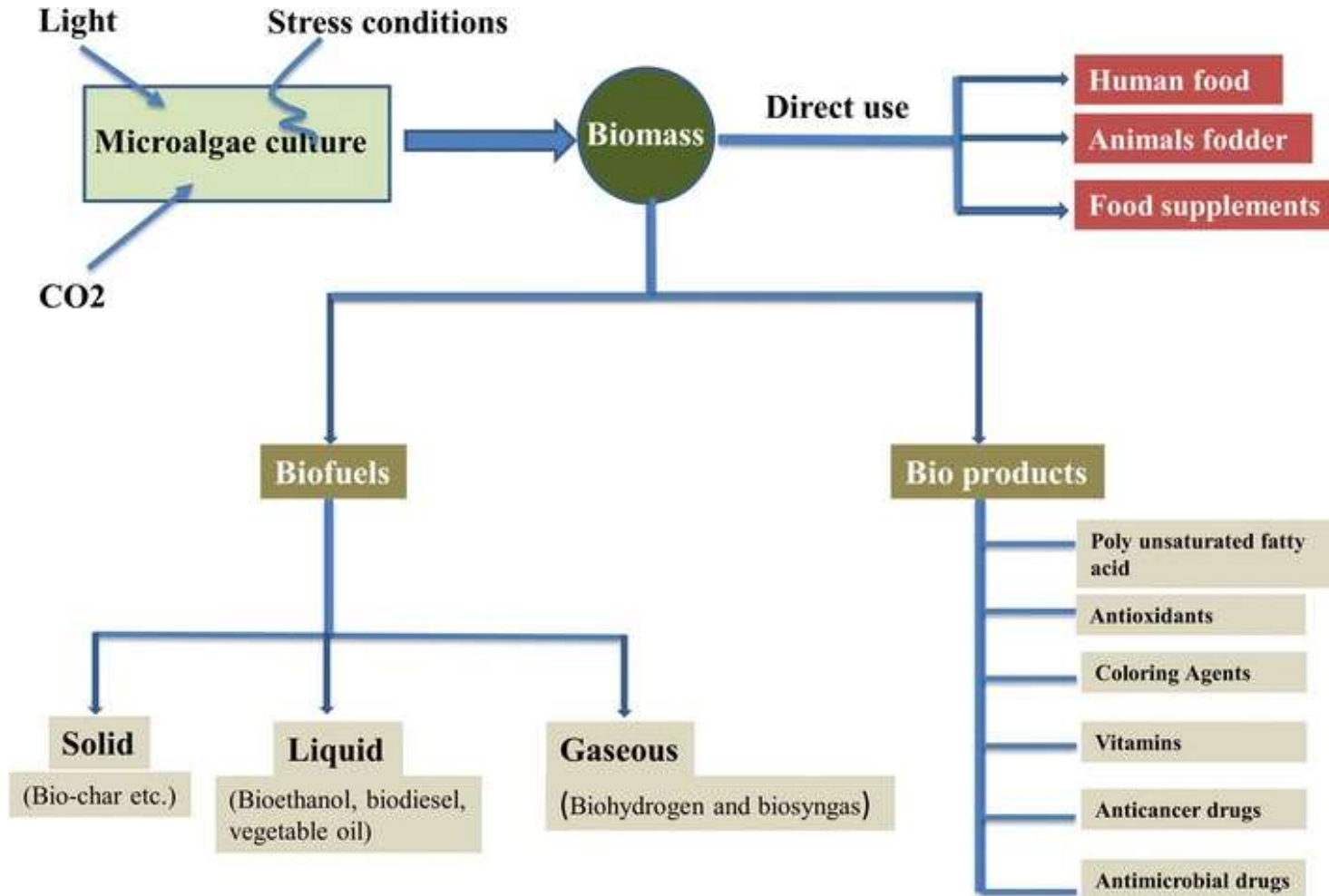
Wonderful, there is growth without sunlight!

Are there even advantages?

- **Less oxidative stress!...**
...but also loss of sense for day time in zebra fishes.



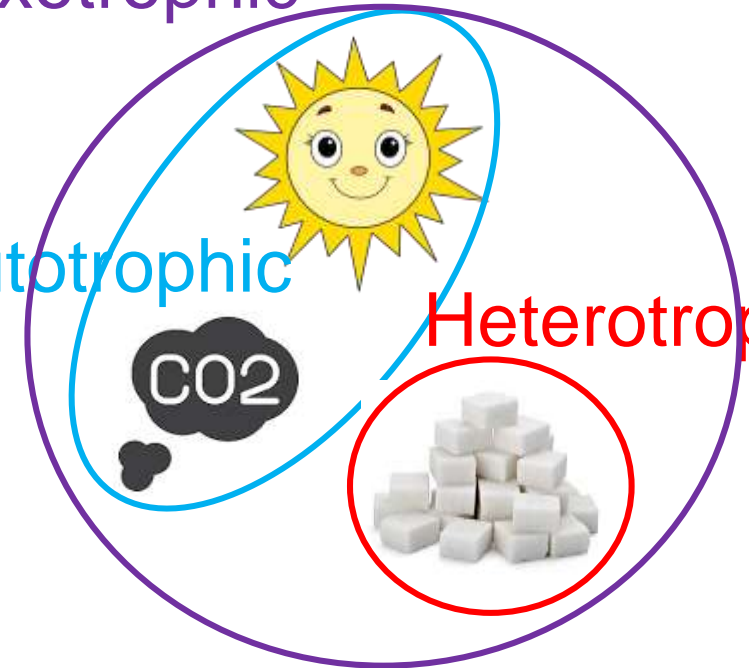
Can we even use phototrophic microorganisms and grow them under dark conditions?



Mixotrophic

Autotrophic

Heterotrophic



Large potential for heterotrophic growth!

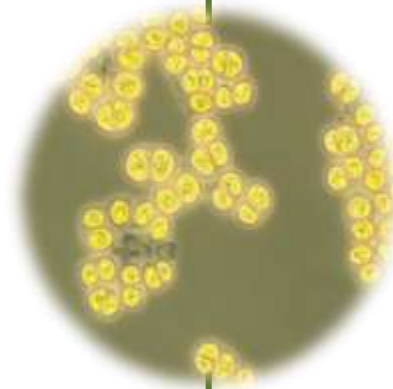
Tested species as a part of an EPFL Master study

Tetraselmis suecica



- Green algae
- Marine
- Flagella

Tetraselmis species



- Green algae
- Marine
- No flagella

Chlorella Vulgaris

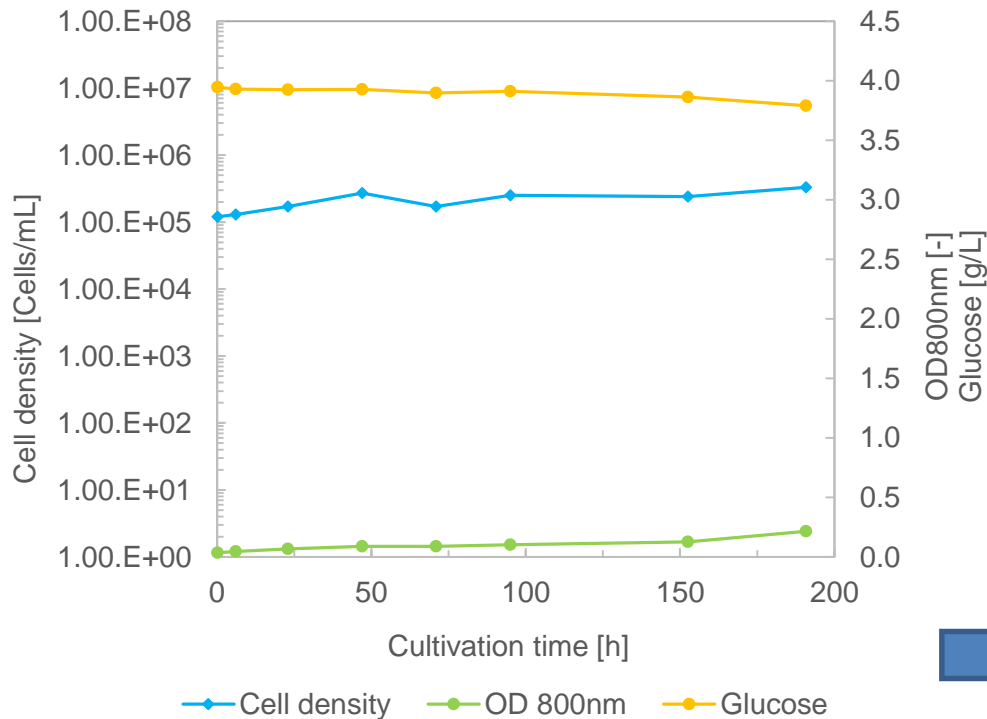


- Green algae
- Unicellular
- Freshwater
- No flagella

Heterotrophic growth – *Tetraselmis species*

Medium: Walne medium + peptone + yeast extract + meat extract

Inoculum: Autotrophic culture



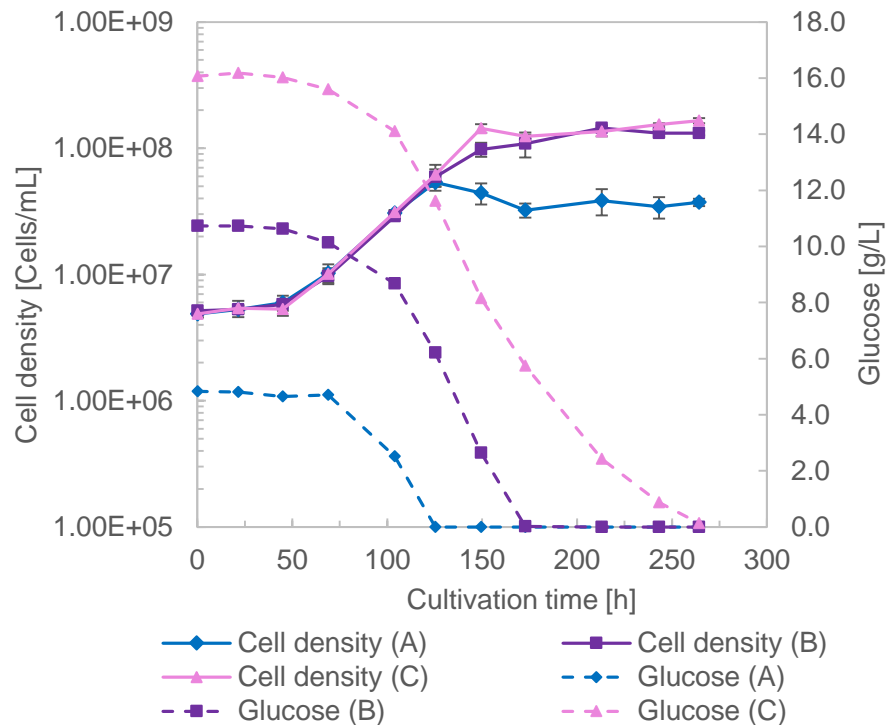
2 L Bioreactor fermentation under 100 % heterotrophic conditions:

- No glucose assimilation
- Practically no growth
- Adhesion on bioreactor wall



Tetraselmis sp. needs long adaptation time!

Heterotrophic growth – *Chlorella vulgaris*



A: 5 g/L glucose, B: 10 g/L glucose,
C: 15 g/L glucose



Chlorella vulgaris grows easily on glucose and was scaled-up to 300 L!

Growth	Glucose concentration		
	(A) 5 g/L	(B) 10 g/L	(C) 15 g/L
$\mu_{\text{Cell count}} [\text{h}^{-1}]$	0.028	0.028	0.033
$P_{sp} \left[\frac{\text{Cells}}{\text{g}_{\text{glucose}} \cdot \text{h}} \right]$	$8.02 \cdot 10^7$	$6.12 \cdot 10^7$	$3.80 \cdot 10^7$

Conclusions & Outlook



- Growth in the dark is biochemically possible without any negative aspects.
- Important: Enough energy and carbon input!
- Phototrophic organisms can be grown heterotrophically.
- Cell physiology not fully understood (e.g. influence of nutrient limitations).

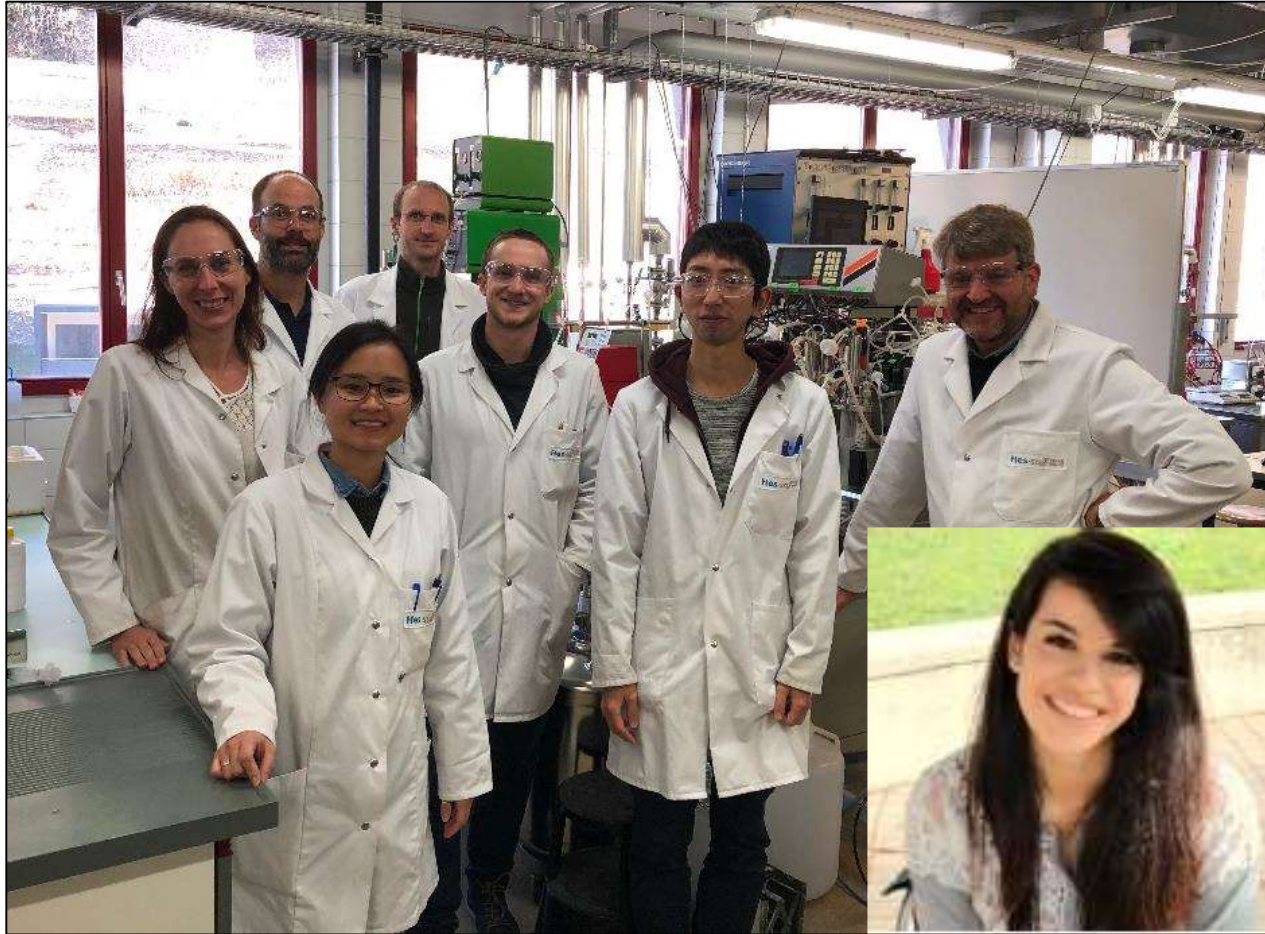
Brandnew: Innosuisse project with Alver



"Alver offers a range of *high protein* vegan foods containing our micro-algae **Golden Chlorella®**"



Many thanks for your attention,
many thanks to the team...



Priscilla Tremblay
Julien Pott
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Shuzo Arai
Nils Hanik
Luc Malbois
Simon Maréschal
Camila Utsunomia
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