

Measurement of intra- and extracellular cytokinin content of algae cultures and application of *Scenedesmus sp.* cultures for plant growth promotion – resulted the new foliar fertilizer of Albitech Ltd.

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Introduction

Albitech Biotechnological Ltd.
Budapest, Berliini Research Center since 2006

- freshwater algae
- research, development microalgae based products
- production of foliar fertilizers

Algafix microbiological biostimulator
Since 2011

Patent application No. HU-P 1300274
Permit no. 04.2/353-4/2014



Algafix
mikrobiológiai lombtrágya

Industrial potencial of microalgae in Albitech

- Why do use microalgae?
 - Low demand for raw materials, simple production
 - Algae suspension could be ready for use

Some microalgae suspension based products on the market



Extracts

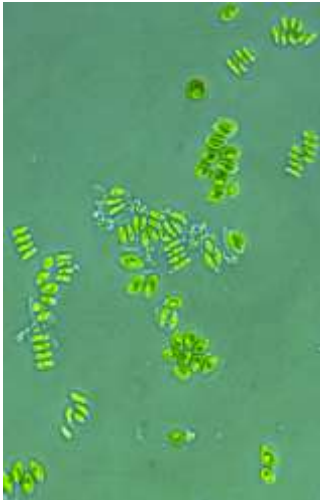


„Green design”



Microalgae as foliar fertilizer

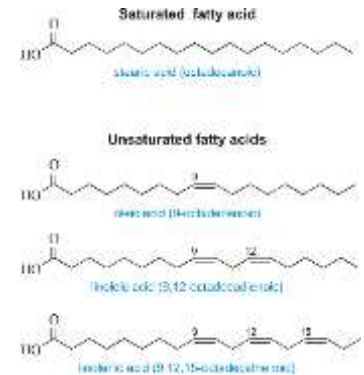
- Foliar fertilization => crop yield enhancement
- Suspension of microalgae –promotion of plant growth and stimulation of germination
- Auxins, gibberellins, cytokinins



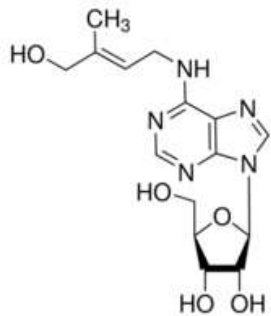
Trifsn and Bularda, 2015

How does it effect?

- Vitamins, amino acids, fatty acids
- Macro and micro elements
- *Plant hormones*

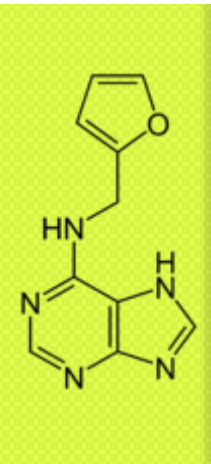


- Trans-zeatin riboside is the most effective natural cytokinin



- *Kinetin*

- Known as artificial cytokinin
- In the nature it could be synthesized among stress conditions as a degradation product from DNA through furfural as an intermediate (oxidative stress)
- Effects: inhibits the production phytoalexins as free radical scavenger, SAR(systemic acquired resistance) induction, it could be a possible anti-stress hormone etc.



Hormone content of algae cultures- examples from literature

- Many algae can produce plant hormones
- Macroalgae
 - Extracts of *Ecklonia maxima* és *Macrocystis pyrifera* contained ca. 0,05 nmol/ml of cytokinins (Stirk et. al. 2004)
 - *Hypnea musciformis* contained 0,454 nmol/g dried weight of cytokinins (Yokoya et al, 2010).
- Microalgae
 - 24 strains were tested by Stirk and Ördög et. al. (2013). The highest detected amount of cytokinins was 21,4 nmol/g lyophilized dry weight (*Stigeoclonium nanum* MACC-790) Lyophilized *Chlorella minutissima* had 7,1 nmol/g cytokinin content.

Strains and samples

- *Scenedesmus sp.* K2012 BEA D01_12 (Sco)
- *Chlorella minutissima* K2012 41 (Chlo)

Proliferation was stimulated by the addition of *Azospirillum brasilense* NCAIM (P) 001411 (ABT) bacterial culture in a low concentration



Liquid culture

Centrifugation

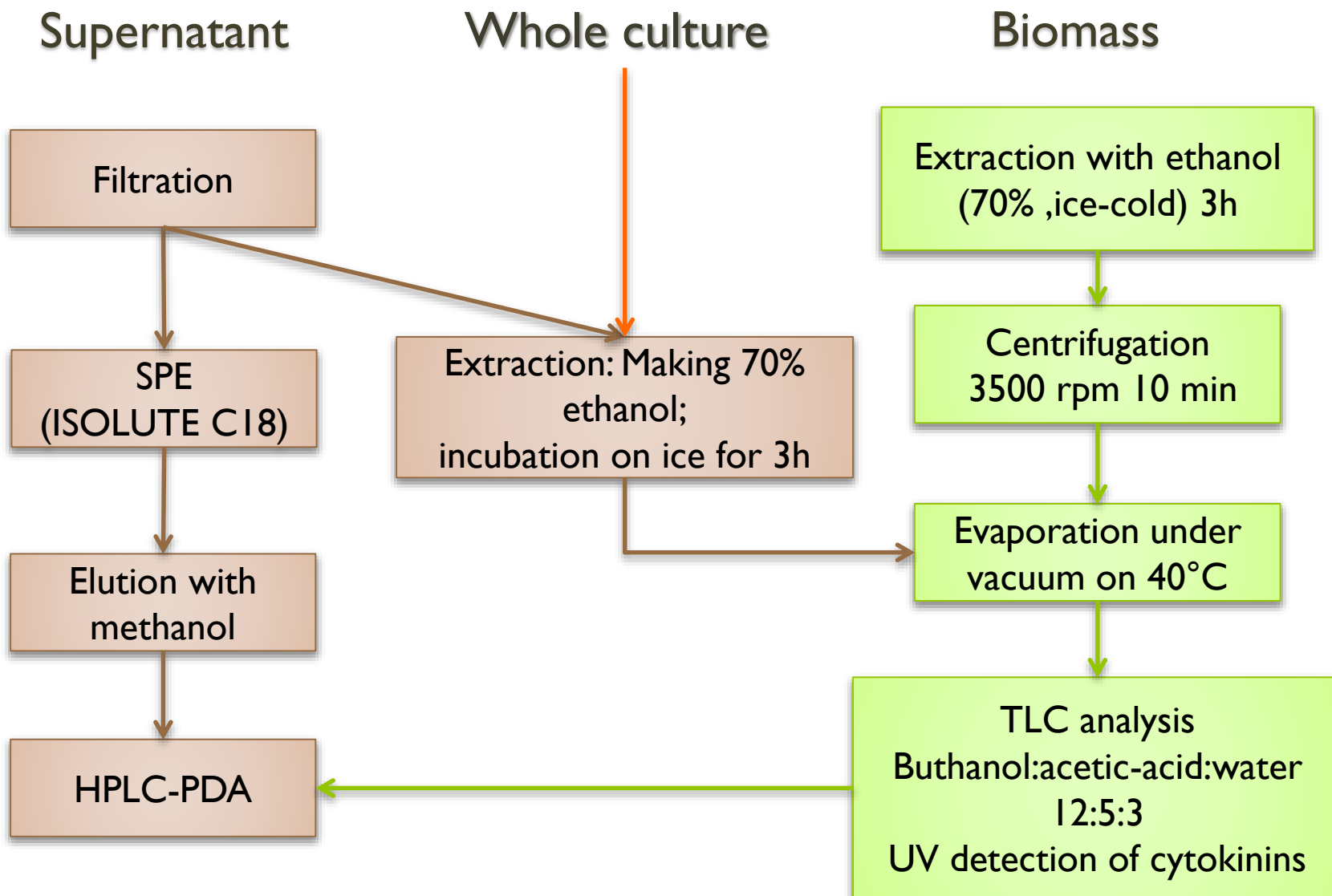
Biomass

Lyophilization



Supernatant

Extraction of cytokinins



Analitical results - TLC and HPLC

Cytokinin production on 20 °C with TLC

Culture	Age of cultures	Alga cell number (db/ml)	Detected cytokinins		
			biomass	supernatant	whole culture
Sco	3 days	7,7*10 ⁵	-	-	ZR
Sco+ABT	3 days	7,9*10 ⁵	-	ZR	ZR
Sco	6 days	7,2*10 ⁶	KIN	ZR	ZR+KIN
Sco+ABT	6 days	8,1*10 ⁶	KIN	KIN	KIN

Sco= *Scenedesmus sp.*; ABT=*Azospirillum brasilense*; KIN=kinetin; ZR=zeatin riboside

Formation of most important cytokinins in algae cultures at room temperature (HPLC-PDA)

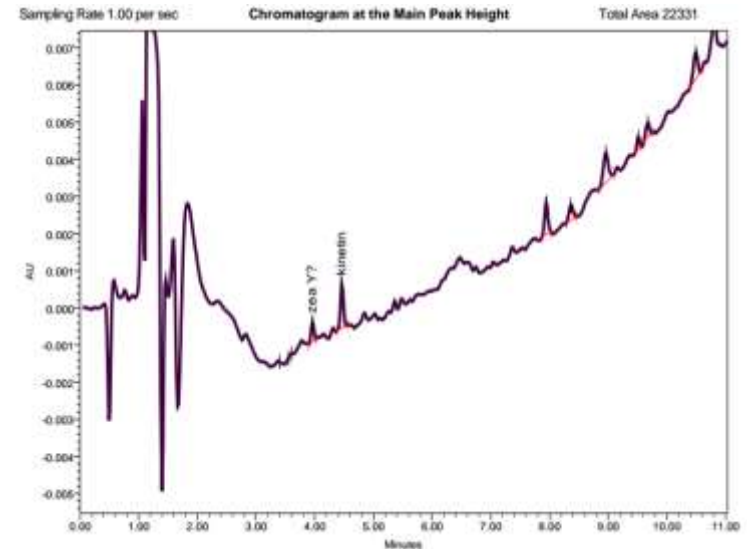
Culture	Age of cultures	Alga cells/ml	Kinetin (nmol/ml)	Trans-zeatin ribozid (nmol/ml)	Zeatin (nmol/ml)
Sco	6 days	8,30E+06	1,79	0,98	0,01
Sco+ABT	6 days	9,00E+06	2,21		
Chlo	6 days	9,70E+06	0,16		
Chlo+ABT	6 days	1,02E+07	0,57		

Chlo= *Chlorella minutissima*

Hormone production experiment

- Kinetin and zeatin derivative contents of the **biomass** of *Scenedesmus sp.* cultures grown on culture media with different nitrogen and phosphorus content and at various temperatures were examined.

	Lower	Medium	Higher
Temperature	16°C	25°C	32°C
KNO ₃	0,4 g/l	1 g/l	1,8 g/l
K ₂ HPO ₄	0,1 g/l	0,2 g/l	0,4 g/l



- Traces of kinetin and a materia shown structural similarity to zeatin.
- Trans-zeatin riboside had no detectable.
- Kinetin was detected, maximum of 1,86 nmol/g lyophilized biomass, which could be achieved at 25 ° C, with increased N and P content. Zeatin was detectable only under such circumstances around the limit of detection.

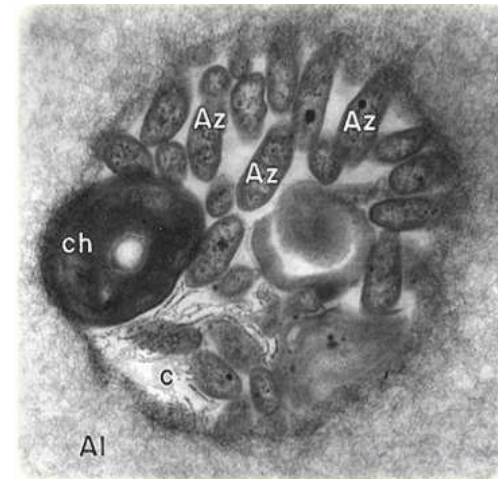
Whole cultures contained as much cytokinin (kinetin) per ml as measured in 1 g lyophilized Sco biomass.

Culturing algae with soil bacteria-cofermentation - literature

- Increased proliferation and uptake of ammonium (also in cell and culture level)
 - *C. sorokiniana* és *B. pumilus*,
 - *C. vulgaris* és *A. brasilense*
- The bacteria select indole-3-acetic acid and other unknown signaling molecules which are supplied to the algae cells.
- Glutamine synthetase and glutamate dehydrogenase activity increased as well as the production of photosynthetic pigments and nitrogen and phosphorus uptake of algal cells
- Wastewater treatment
(de-Bashan and Bashan 2008, 2011)



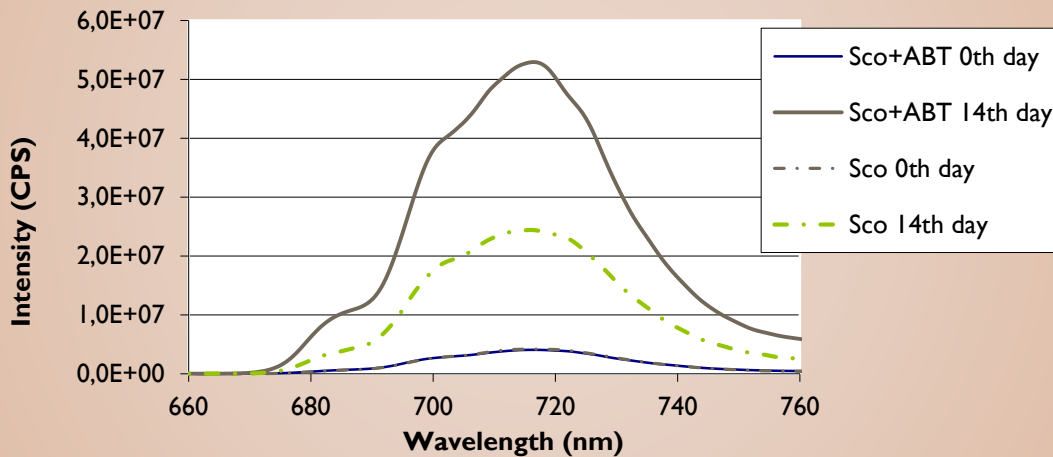
Alginate beads



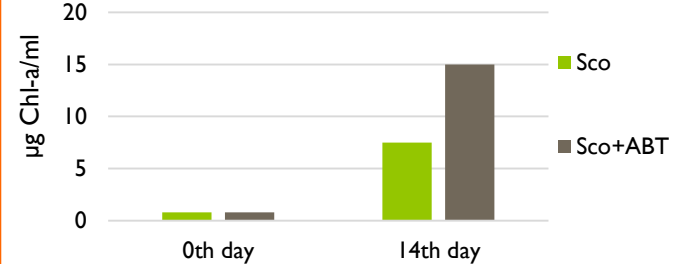
<http://www.bashanfoundation.org/gmaweb/proyectos2/ilin6.html>

Culturing algae with soil bacteria-cofermentation – experiments

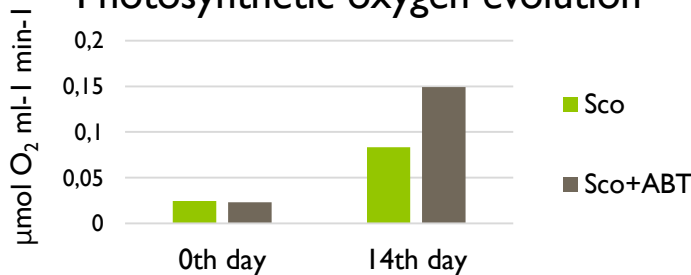
77K fluorescence spectra



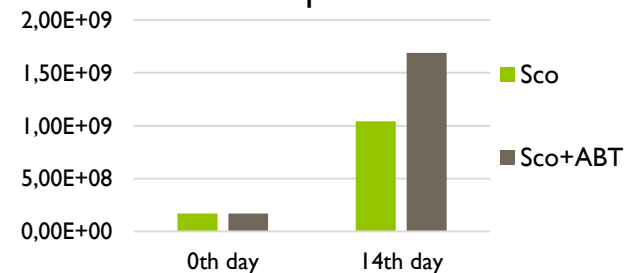
Chlorophyll-a content of Scenedesmus cultures depending on time



Photosynthetic oxygen evolution

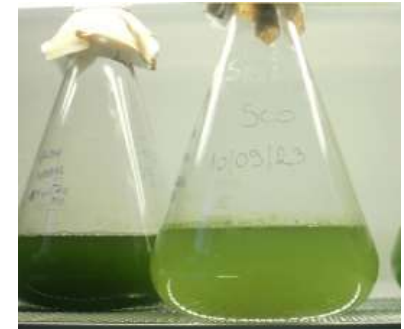
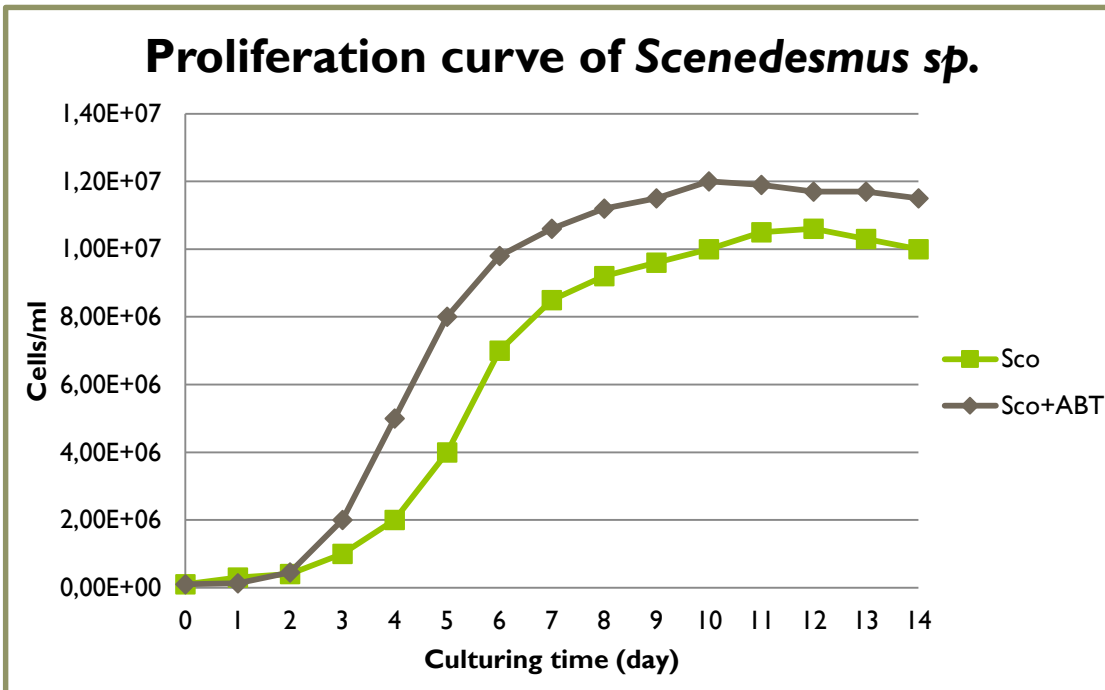


Total integrals of 77K fluorescence spectra



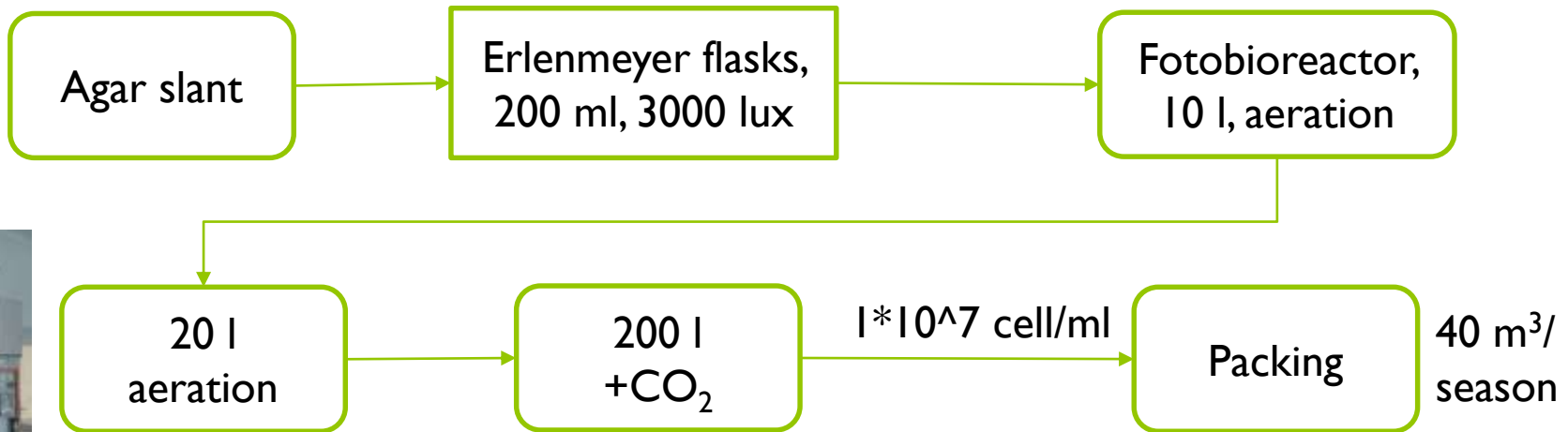
Measurements were made at Eötvös Loránd Science University
 Department of Plant Anatomy and Department of Plant Physiology and Molecular Biology

Culturing algae with soil bacteria-cofermentation – practice



- Industrial possibilities, shorter growing period, more active cells

Production technology



Experiences of the last few years

- Frost damage/infection suffered crops were saved - hormone effects -induction of SAR- stress tolerance
- Greater amount of bees in the treated area



Barely infected
by fungus



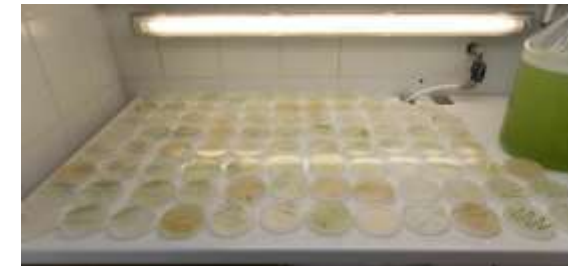
5th day after
algal treatment



12th day after
algal treatment

Making culture collection – for developments of the future

- **What is the goal?- strains for (easy) production**
- **Special algae**
 - halofita microalgae – foliar fertilizer
 - termotolerant – power plant CO₂ recycling
- **Isolation conditions - saline lakes, temporary waters; in summer**
- **Screening**
 - PGPR - green algae - ca. 100 sp.
 - PUFA – diatoms – 10 sp.
- **Maintenance- glass tube with agar slant**



Conclusions I.

- In our collection about 100 green alga and diatoma strains are available.
- The production technology efficiency increased at co-cultivation with *Azospirillum brasilense*.
- Co-cultivation increased the photosynthetic activity of *Scenedesmus sp.* more than 2x in two-week-long fermentation period
- Cytokinins were detected from of *Scenedesmus sp.*, *Scenedesmus sp.* – *A. brasilense* mixed cultures, then *C. minutissima* and *C. minutissima* - *A. brasilense* mixed cultures as well.

Conclusions II.

- Spraying of 5 - 18 l doses on one hectare Sco liquid culture could be as effective as 100 g biomass extracted from 1000-5000l algal culture.
- We could verify the efficiency of Algafix by field and greenhouse studies for both agricultural and horticultural purposes.
- It was effective on crop yield, plant height and head diameter, leaf surface area, root mass and flower number both in monocot and dicot plants.
- We could observe the effect of SAR induction aimed by Algafix.
- Experiences warrant further researches about the effects on stress tolerance and attraction of bees.

Acknowledgments

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Thank you for your attention!

