Algal biomass: Quality and safety aspects

Final IDEA+ conference, 28th of September 2023

Dr Maria Hayes, Teagasc Food Research Centre,
Ashtown, Dublin 15, Ireland.

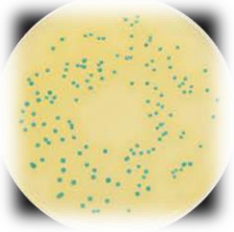
Use of algae in Foods & Feeds – Microbial criteria

- Food producers must comply with the microbiological criteria, as defined in Regulation (EC) No 2073/2005, or on targets in primary production sectors, as defined in Regulation (EC) No 2160/2003.
- Microbiological criteria includes both Food Safety Criteria (FSC) and Process Hygiene Criteria (PHC) as defined in the EC legislation.
- FSC are applicable for food products placed on the market during their shelf-life, while PHC are applicable typically for food during processing or at the end of a production line before putting a product on the market.
- If a FSC is not met, then the food has to be removed from the market or reprocessed (if not at retail).
- Regulation 2073/20054 lays down microbiological criteria for various combinations of food commodities and microorganisms, their toxins or metabolites. It requires food business operators to take measures, as part of their procedures based on GHP and HACCP principles, to ensure compliance with the relevant microbiological criteria. Food business operators should test against these criteria, as appropriate, when validating and verifying the correct functioning of these procedures.

EFSA journal volume 15 Issue 11 2017,
https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2017.e15111

Microbial food contamination

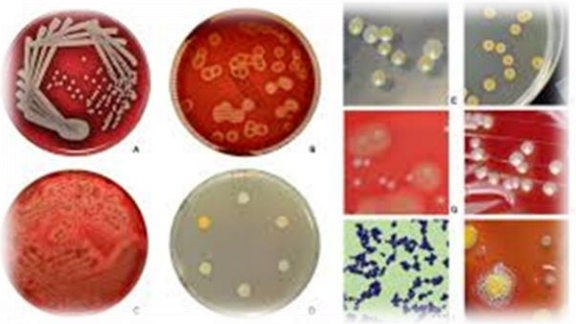
Bacillus cereus



Listeria sp.

Salmonella sp.

Escherichia coli



Staphylococcus aureus

Rice, pasta, baked goods and chilled foods

Milk, cheese, ice cream

Chicken, eggs, vegetables

Meat and Vegetables

Fruits and Vegetables

Microbial safety of biomass

Microalga	Sidestream used for growth	Aerobic plate count [1]	Coagulase postive *Staphylococcus aureus* [2]	Coliforms [3]	*E. coli* [4]	*L. innocua* [5]	*B. cereus* [6]	*Salmonella* sp. [7]
Nannochloropsis sp.	MAF-permeate from open pond culture growing on demineralization process water. No filtration of permeate after storage.	2200 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	Not detected in 25 g	<20 CFU/g	Not detected in 25 g
Defatted Nanncohloropsis	MAF-permeate from open pond culture growing on demineralization process water	1800 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	Not detected in 25 g	<10 CFU/g	Not detected in 25 g
Chlorella sp.	CO_2 from biogas incineration	>3000000 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	Not detected in 25 g	<10 CFU/g	Not detected in 25 g
Mix of algae	Demineralization process water	>3000 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	Not detected in 25 g	<10 CFU/g	Not detected in 25 g
Chloromonas sp.	MAF-permeate from open pond culture growing on demineralization process water	830 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	Not detected in 25 g	<10 CFU/g	Not detected in 25 g

[1] Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 °C by the pour plate technique; [2] Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 1: Method using Baird-Parker agar medium, [3] ISO 4832:2006
Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique, [6] ISO 7932:2004
Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of presumptive Bacillus cereus — Colony-count technique at 30 degrees C,, [7] ISO 6579-1:2017
Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp.

- **Several microalgal cultures were found to contain excessive numbers of aerobic bacteria but with the exception of *Nannochloropsis* sp. none contained in excess of 10 CFU/g of the spore former *Bacillus cereus.* No samples contained excess iodine and heavy metal analysis is on-going.**

What does this mean?

- Products intended for consumption in their raw form should contain less than 100 CFU/g.
- Most cases of food-borne outbreaks caused by the *B. cereus* group have been associated with concentrations above 10^5 CFU/g [1].
- *Listeria* sp. is associated with RTE foods – 1 CFU/g can cause Listeriosis. *L. monocytogenes* -Indications are that the infectious dose is low, possibly less than 1,000 cells[2].
- *E.coli* – VTEC – as low as 10 cells can cause infection[3].
- St*aphylococcus aureus* (SE), an enteroxigenic strain, needs to grow to levels of 10^5 to 10^6 cells per gram or ml to produce 1 µg of SE toxin[4].
- *Salmonella* sp. – low numbers can cause infection – generally heat treatment can kill *Salmonella* sp.

- Depends on application – not safe for infant formula for example as can't have more than 1000 microbial cells present in infant formula.

Not safe microbiologically

Heavy metals in foods

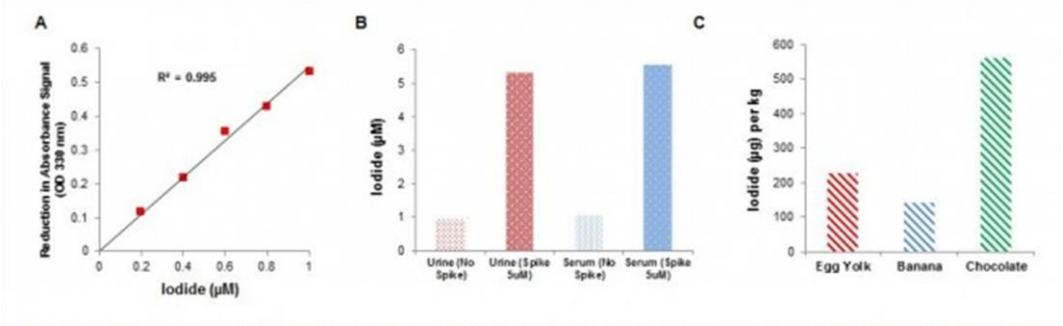
- Commission Regulation (EU) 2023/915 replaces Commission Regulation (EC) 1881/2006
- Sets maximum levels for lead, cadmium, mercury, and arsenic in a wide range of foods.
- Analysis ICP-MS.

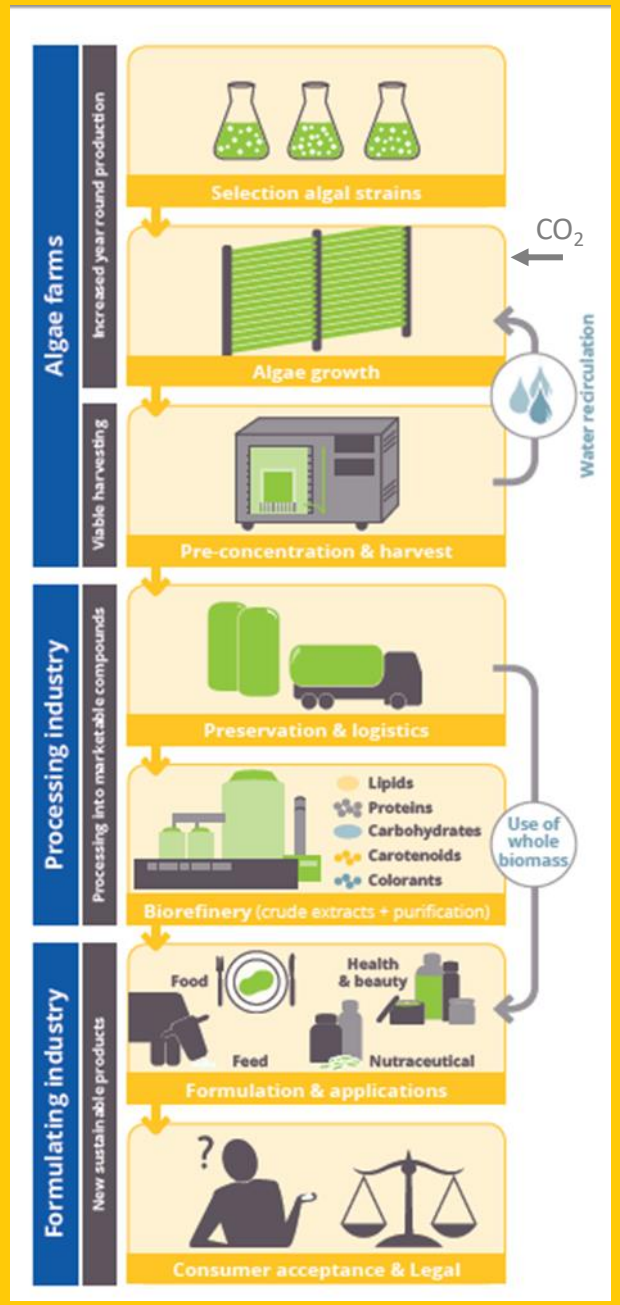
HEAVY METAL (mg/Kg)		FOOD TYPE				
	INFANT FORMULA	*FRUIT/VEGETABLES*	*MEAT*	*FUNGI*	*OILS/FATS*	SUPPLEMENT
ARSENIC	0.01	0.01 - 0.5	0.01-0.5	0.01-0.5	0.01-0.5	0.1
LEAD	0.01	0.1 - 0.8	0.1	0.1-0.8	0.1	3
CADMIUM	0.005	0.02 - 0.5	0.05-0.15	0.02-0.5	0.05-0.15	3
MERCURY	0.01	0.01	0.3-1	0.01	0.1	0.1

For mercury, currently under Regulation (EC) No 396/2005 of the European Parliament and of the Council (2) a maximum residue level (MRL) for algae and prokaryotic organisms is established at the default level of 0,01 mg/kg.

Iodine in algae for food

- Commission Regulation (EC) No 1881/2006.
- EFSA set a tolerable upper intake level (UL) of 600 µg of iodine per day for adults (EFSA, 2006) and 200 µg of iodine per day for children.
- Dried algae can be accumulate iodine.
- *Chlorella* sp. can accumulate iodine to a content of 1,300 mg kg^{-1} and the iodine content in cyanobacteria *Spirulina platensis* can be as high as 2,000 mg kg^{-1}.





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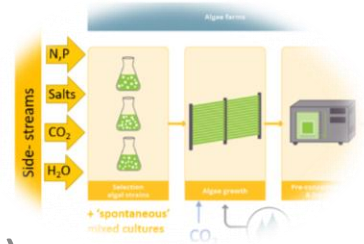


Caoimhe Gargan, Min Su, Karen Hussey (Teagasc)

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains in North-West Europe).

Website: www.nweurope.eu/idea

Full partners:

Session 4 overview

- Algae biomass quality and safety aspects - Maria Hayes (TEAGASC, Ireland)
- **First screening of application potential of algae biomass - Joran Verspreet (VITO, Belgium)**
- Potential of algae biomass for food/feed applications - Maria Hayes (TEAGASC, Ireland)
- Potential of algae biomass extracts for agro-applications – Yana De Ruyter/ (PCfruit, Belgium)
- Techno-economic & sustainability considerations - Mohammed El Ibrahimi/ (VITO, Belgium)
- Discussion

First screening of the application potential of algae biomass

Final IDEA+ event, September 2023

Joran Verspreet

Possible application areas for algae grown on side streams

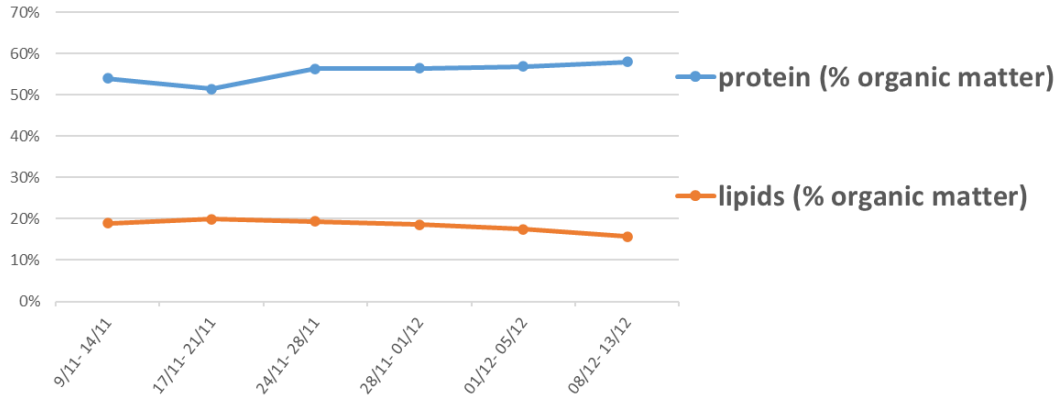
Possible application areas for algae grown on side streams

Important aspects

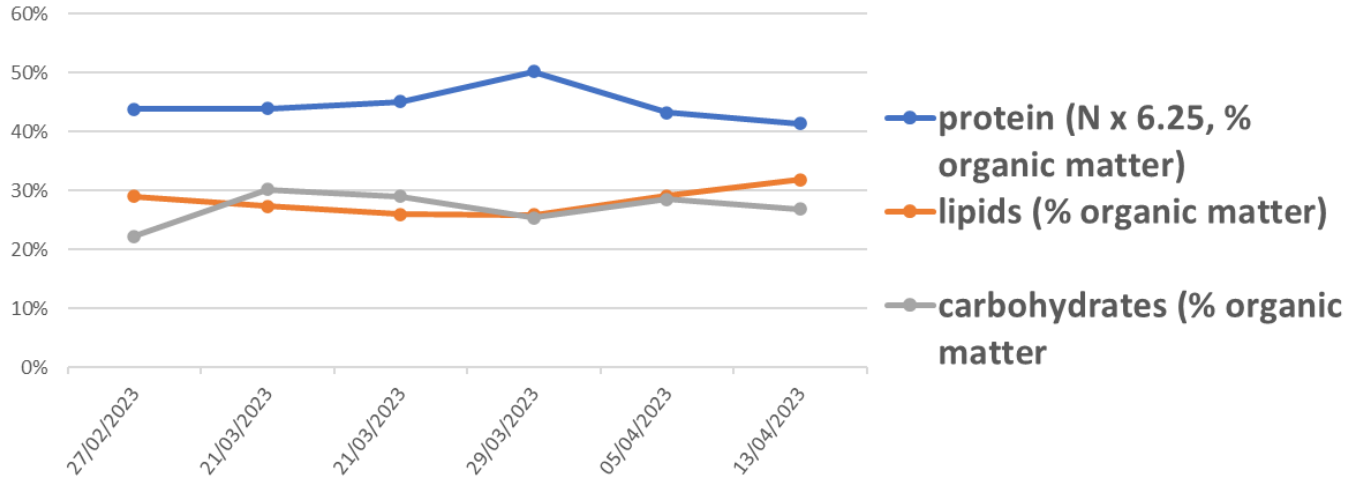
- Required amounts
- Safety & legal requirements
- Product value
- Quality
- Composition stability

Composition stability

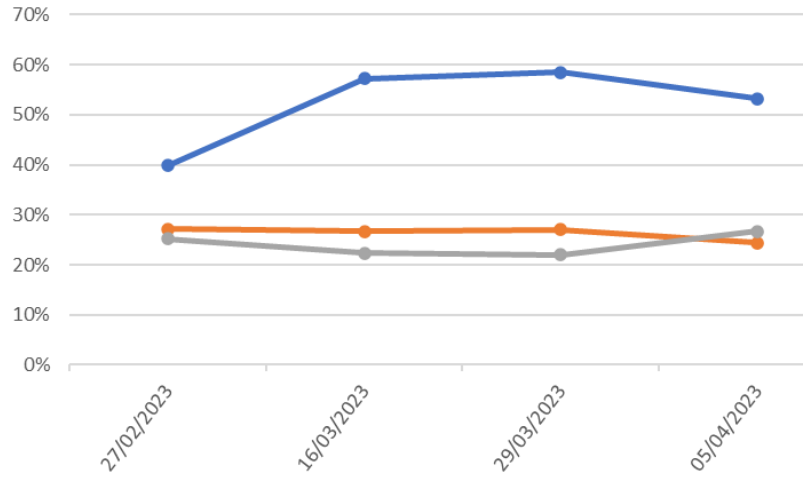
Chlorella grown on CO_2 from biogas combustion



Chloromonas with medium based on permeate obtained by filtration open pond culture

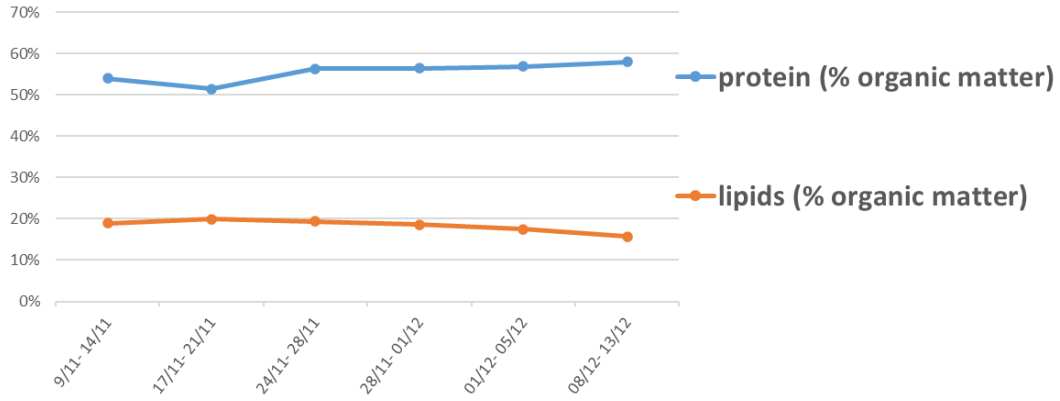


Chloromonas with regular medium

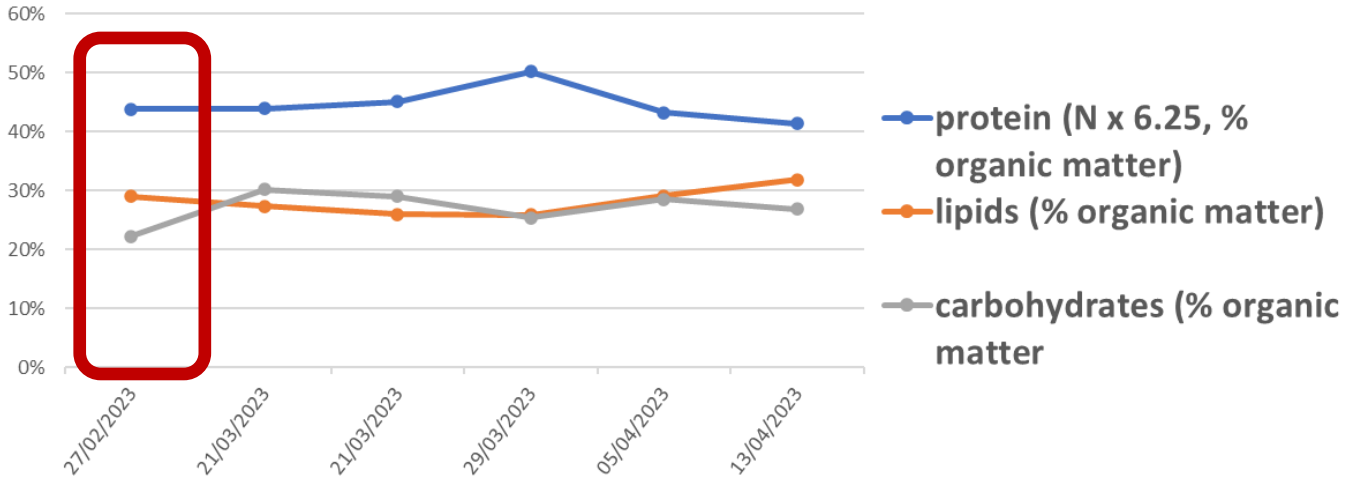


Composition stability

Chlorella grown on CO_2 from biogas combustion

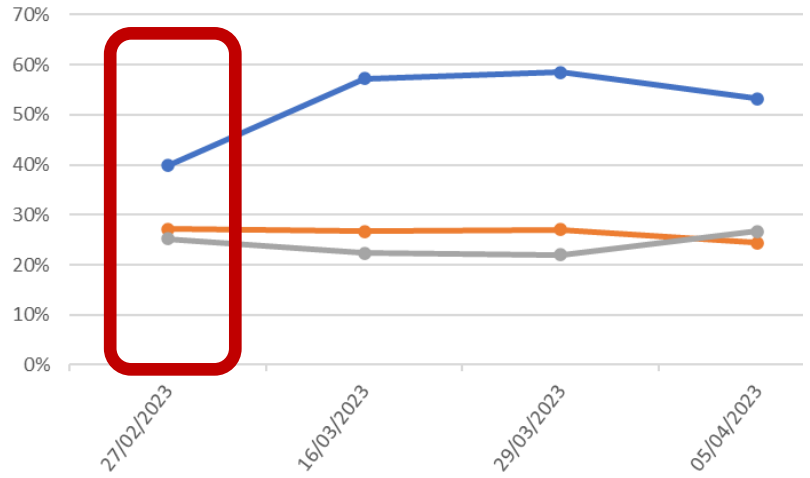


Chloromonas with medium based on permeate obtained by filtration open pond culture



Start of cultivation & changing weather conditions

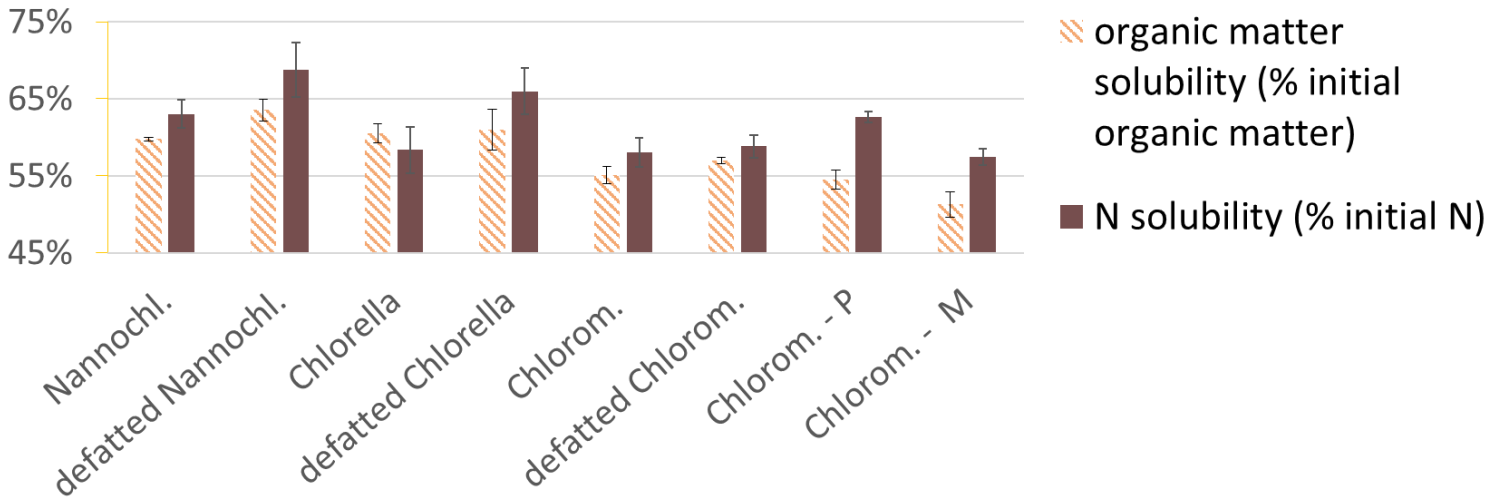
Chloromonas with regular medium

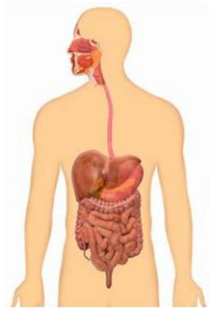


Start of cultivation & changing weather conditions

Food

- Digestibility should be considered for food applications





Oral phase simulation

Gastric phase simulation

Small intestinal phase simulation

- Poster #20

EVALUATION OF THE ANTIMICROBIAL ACTIVITY AND DIGESTIBILITY OF ALGAE CULTIVATED ON SIDESTREAMS

Verspreet J., Simons Q., Van den Bosch B., Soetemans L., and L. Bastiaens

VITO, Conversion and Separation Technologies department, Boeretang 200, 2400 Mol, Belgium, leen.bastiaens@vito.be

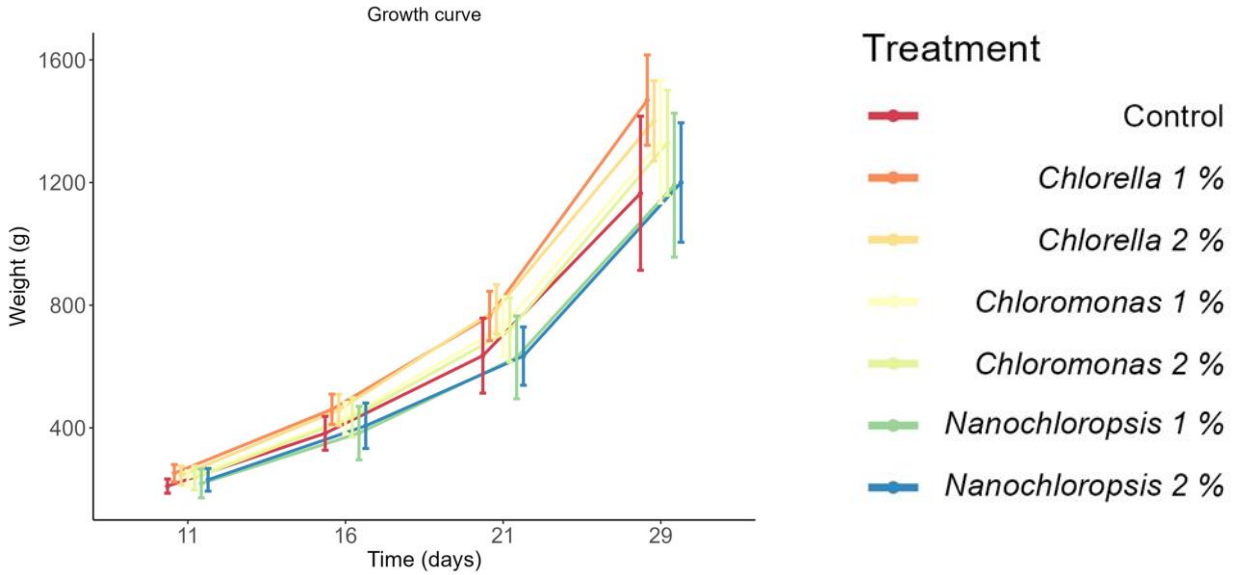
Feed

Including defatted algae in broiler diets (1-2%)

- impacts intestinal morphology
  - Length of intestinal tract tended to increase in algae-supplemented broilers
  - Villi width, and crypt width and depth seemed to increase for most algae-supplemented broilers

- *In vitro* trials suggested that *Lactobacillus amylovorus* can use digested *Chloromonas* and *Chlorella* fractions as a growth substrate

- May also affect broiler performance



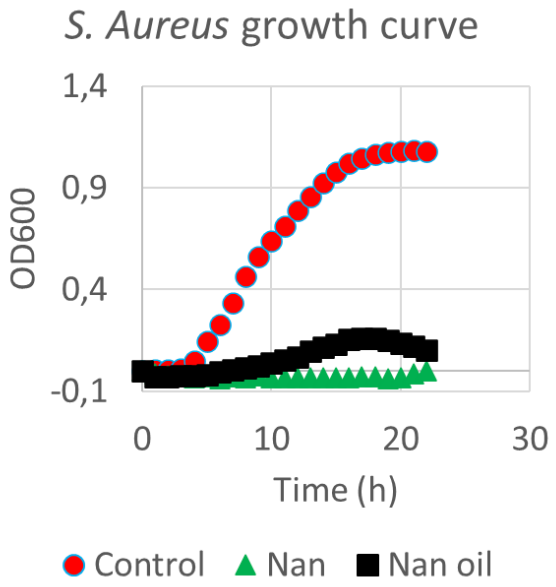
- Poster #21

INCLUDING DEFATTED ALGAE BIOMASS IN BROILER DIETS: IMPACT ON INTESTINAL MORPHOLOGY

Van Nerom S.[1], Lourenço M.[1], Robbens J.[1], Verspreet J.[2], and Bastiaens L.[2]

Antimicrobial activity

- Valuable activity for food, feed, cosmetic and agro applications
- Certain ethanol extracts of algae grown on side streams were shown to have antimicrobial activities
  - At 1 g DM/L for *E.coli and S. aureus*
  - At 5-10 -20 g DM/ L for *B. cinerea*



Tomato leaves infected with *Botrytis cinerea*

- Poster #20

EVALUATION OF THE ANTIMICROBIAL ACTIVITY AND DIGESTIBILITY OF ALGAE CULTIVATED ON SIDESTREAMS

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Potential of algal biomass for food and feed applications

Final IDEA+ conference, 28th of September 2023

Dr Maria Hayes, Teagasc Food Research Centre,
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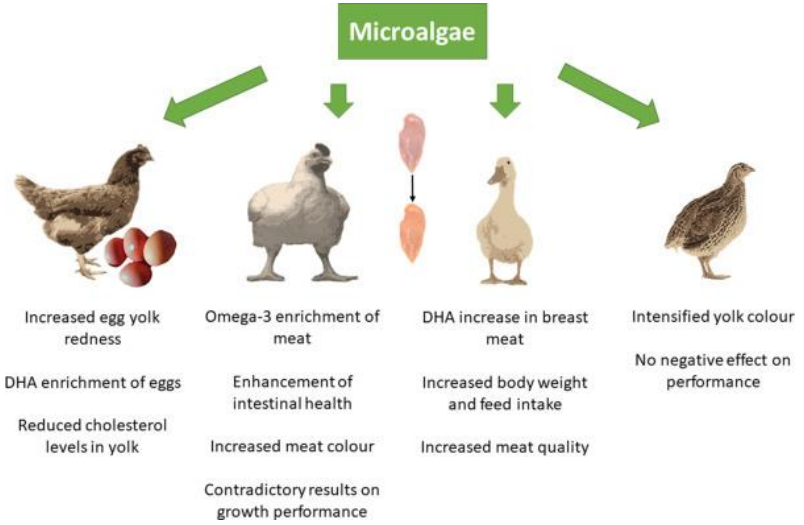
Microalgae for feed

- Traditionally, microalgae have been used as a sustainable resource for domestic livestock, poultry and aquaculture production due to their diverse nutritional profiles, i.e., carbohydrates, essential fatty acids and amino acids, carotenoids, and vitamins.
- Microalgae can result in (1) cholesterol-lowering effect in animals, (2) improved immune response (3)enhanced milk quality and production yield in cows.



https://www.sciencedirect.com/science/article/abs/pii/B9780128212189000098

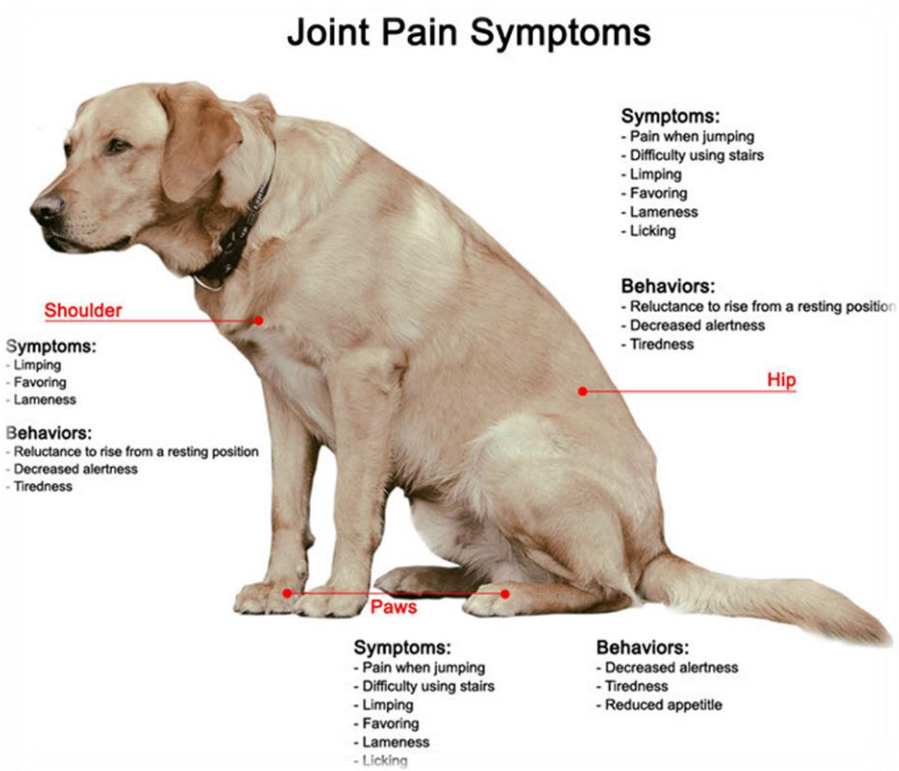
eagasc
Agriculture and Food Development Authority

Health benefits of algal extracts for animals

- Cyclooxygenase (COX) catalyses the conversion of arachidonic acid into prostaglandins (PGs), which play a significant role in health and disease in the gastrointestinal tract (GI) and in the renal, skeletal, and ocular systems.

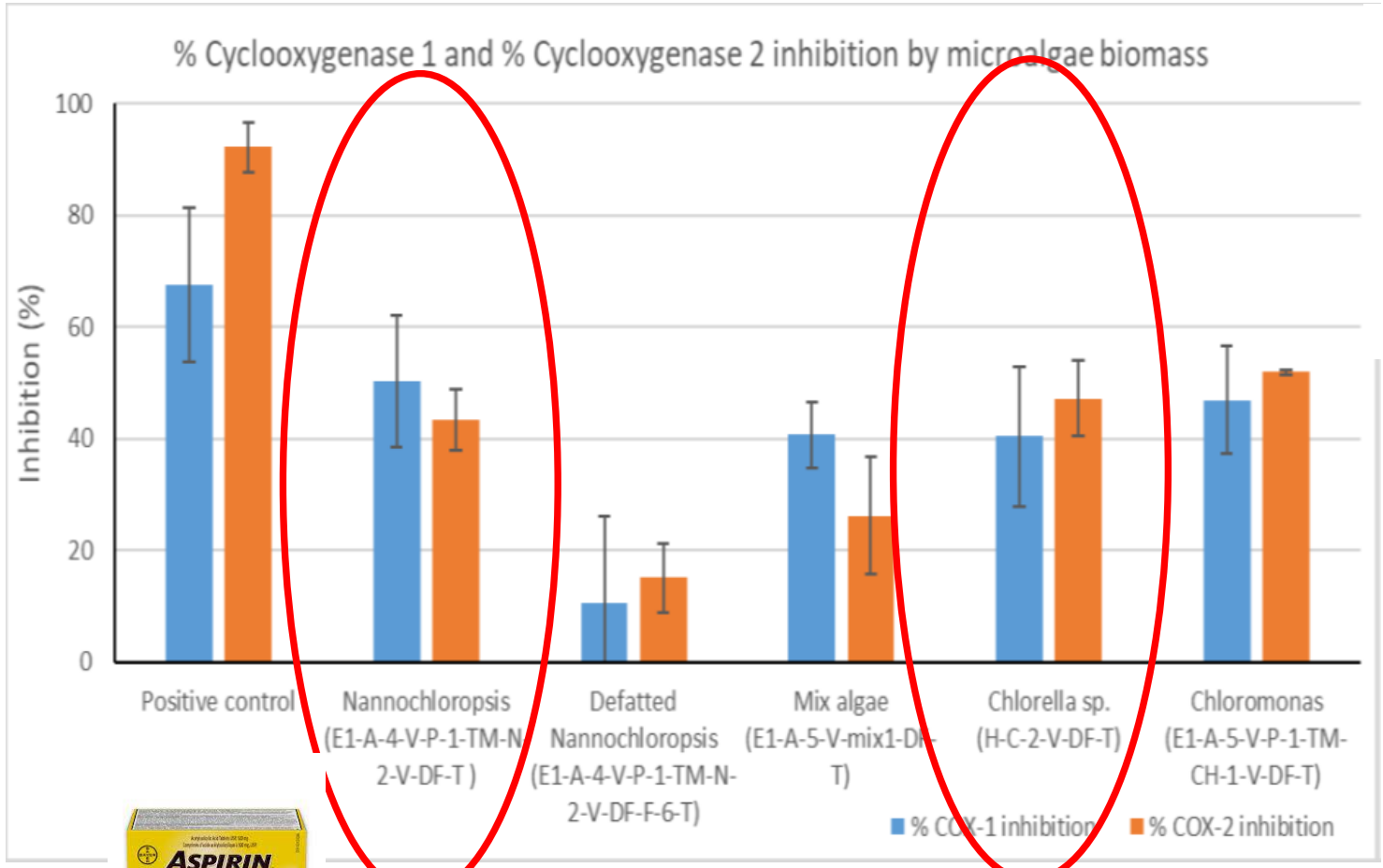
- Inhibition of COX-1 often elicits GI toxicity in animals and humans.

- COX-2 inhibitors have benefits including healing of ligament or tendon tears in the skeletal system in animal models.



Reference: Radi, Z. A. Toxicologic Pathology, 37: 34-46, 2009

COX inhibition by whole microalgae



Microalgae	Protein (%w/w)	Fat (%w/w)	Moisture (%w/w)	Ash (%w/w)
Nannochloropsis (E1-A-4-V-P-1-TM-N-2-V-DF-T)	44.93 ± 0.23	2.23 ± 0.45	4.05 ± 0.06	6.69 ± 0.36
Defatted *Nannochloropsis* (E1-A-4-V-P-1-TM-N-2-V-DF-F-6-T)	52.44 ± 0.41	0.21 ± 0.04	5.81 ± 0.09	7.71 ± 0.01
Chloromonas (E1-A-5-V-P-1-TM-CH-1-V-DF-T)	39.00 ± 0.23	10.85 ± 2.72	5.45 ± 0.75	5.73 ± 0.46
Chlorella sp. (H-C-2-V-DF-T)	48.25 ± 0.16	0.90 ± 0.40	5.06 ± 0.15	10.31 ± 0.09
Mix algae (E1-A-5-V-mix1-DF-T)	23.08 ± 0.07	0.27 ± 0.12	4.08 ± 0.11	40.35 ± 0.11

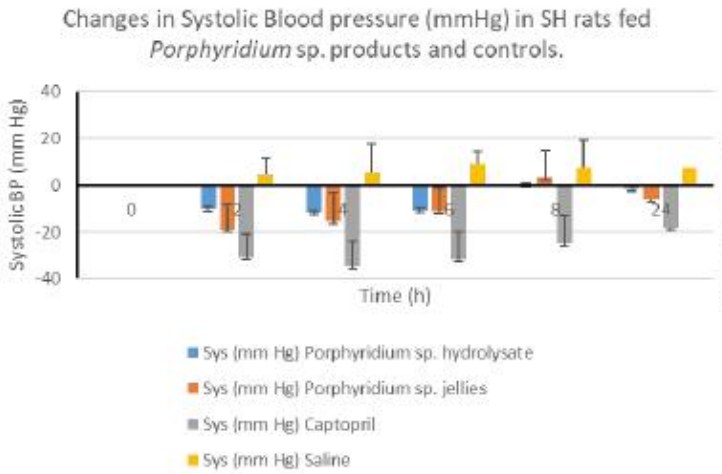
AOAC methods used to determine protein, fat, moisture and Ash content

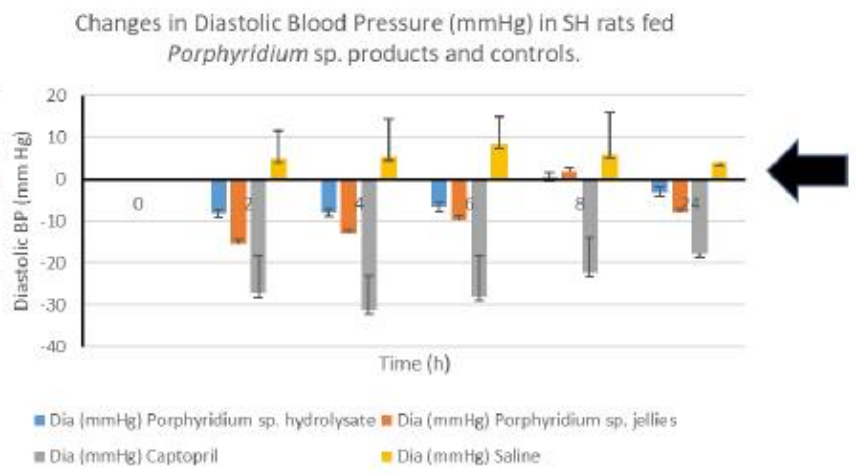
Heart health benefits of *Porphyridium* protein extract and product development

Interreg North-West Europe IDEA
European Regional Development Fund

Development of an ACE-1 inhibitory hydrolysate containing treat and assessment of antihypertensive activity *in vivo*

Gelatine recipe *Porphyridium sp.*
- 570g ddH_2O
- 12 g Gelatine
- 5g Sucrose
- 1g ascorbic acid
- 1g flavouring lemon essence
- 1g food red colouring
- 6g (1%) *Porphyridium sp.* permeate 3kDa





Environmental benefits of algae

- 1.14 million dairy cows
- 940,000 beef cattle
- 4 million sheep

- Beef cattle ~230 g CH_4/ per day.
- Dairy cow grazing on pasture emits about 320-330 g CH_4/per day.
- Sheep ~ 22-23 g CH_4/per day.

- In 2022 Irish dairy farms produced over 8.7 billion litres of liquid milk. The milk was then processed into over 1.7 million tonnes of high quality butter, cheese, infant food, powders and functional ingredients and exported to over 130 global markets.
- Irish meat sector - 120,000 individual farmers and generating total sales of > €4.5bn, with 2020 exports of approximately €3bn. 5th largest exporter in the world.

Algal ingredients to mitigate GHGS

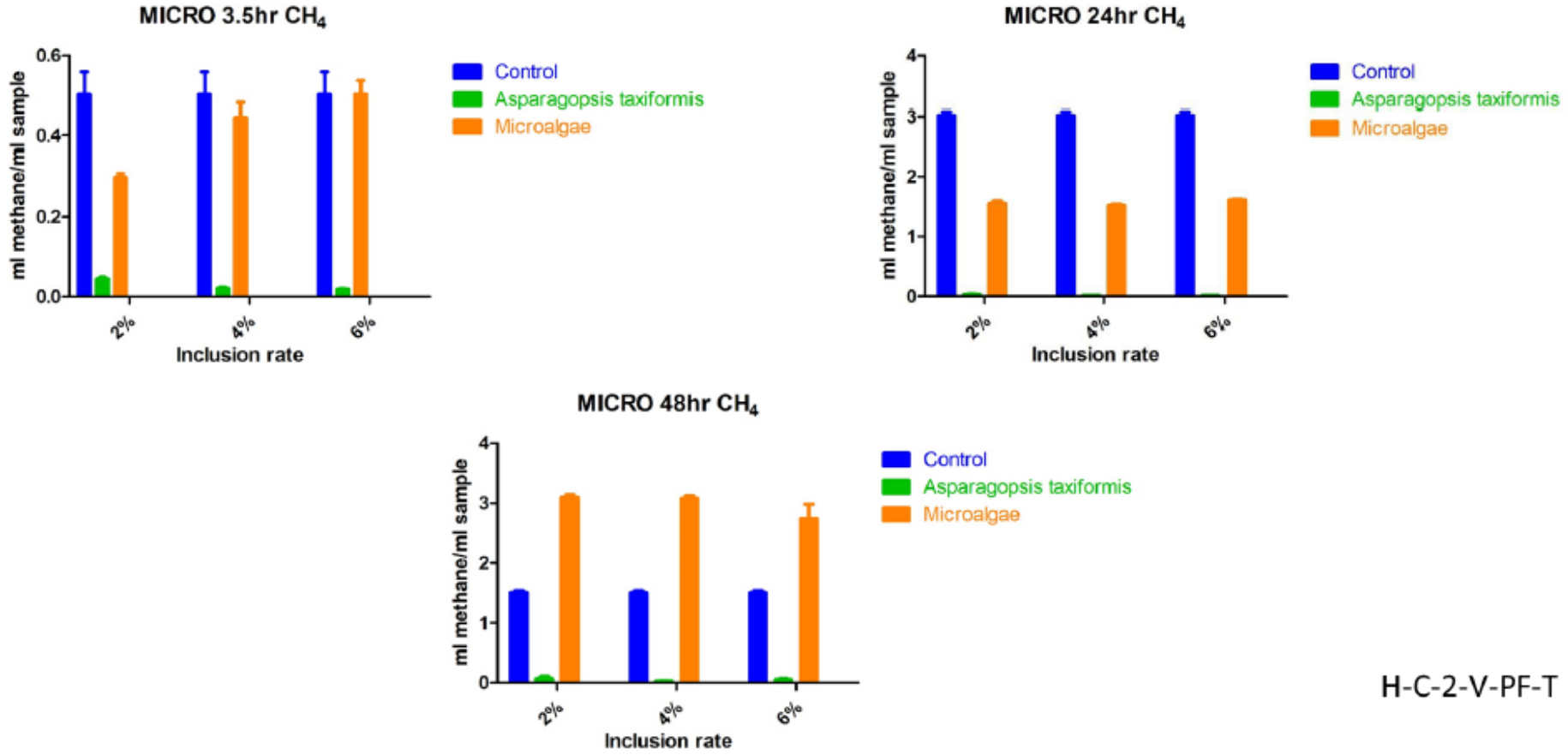
- Methane gas is formed by anaerobic archaea coupled with bacteria, protozoa, and fungi in the rumen ecosystem.

- Antioxidants
- Antimicrobial action (inhibition of Co-Enzyme M)
- Prebiotic potential
- Polysaccharides

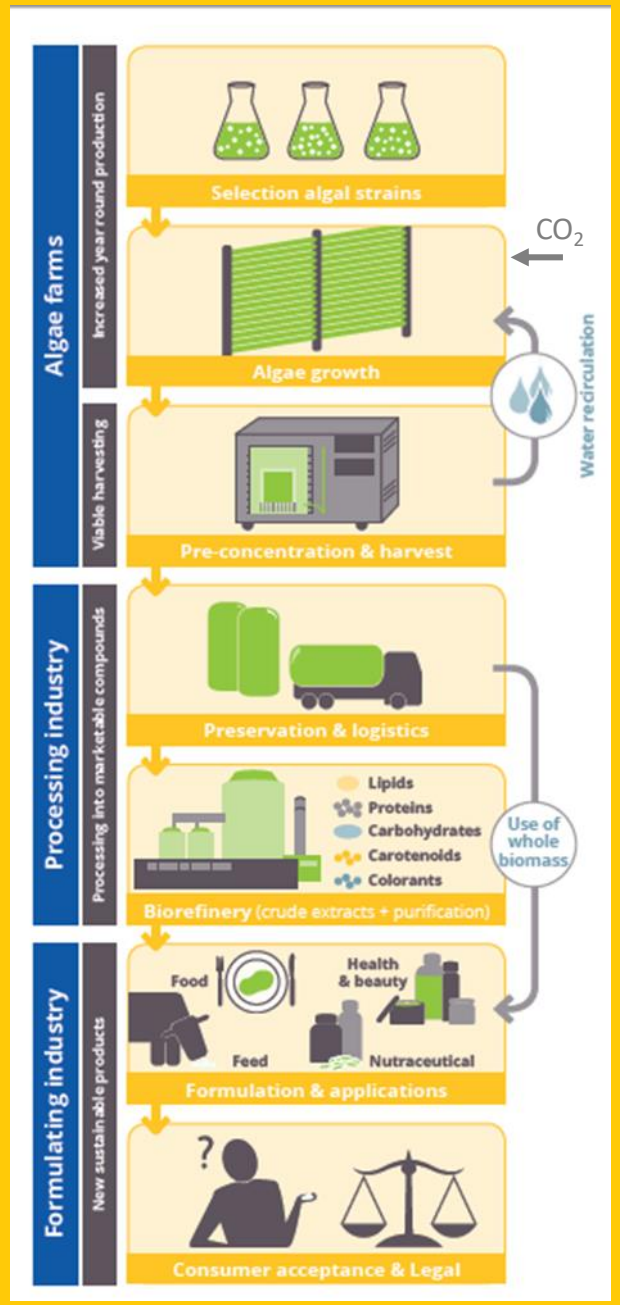
Volta Greentech, Protos and Coop Sweden -pilot project on Gotland island produce methane-reduced meat – 80% CH_4 reductions

Algal ingredients to mitigate GHGS

Potential to mitigate Methane & Ammonia with *Chlorella* sp.



H-C-2-V-PF-T



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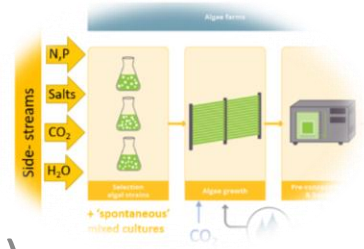

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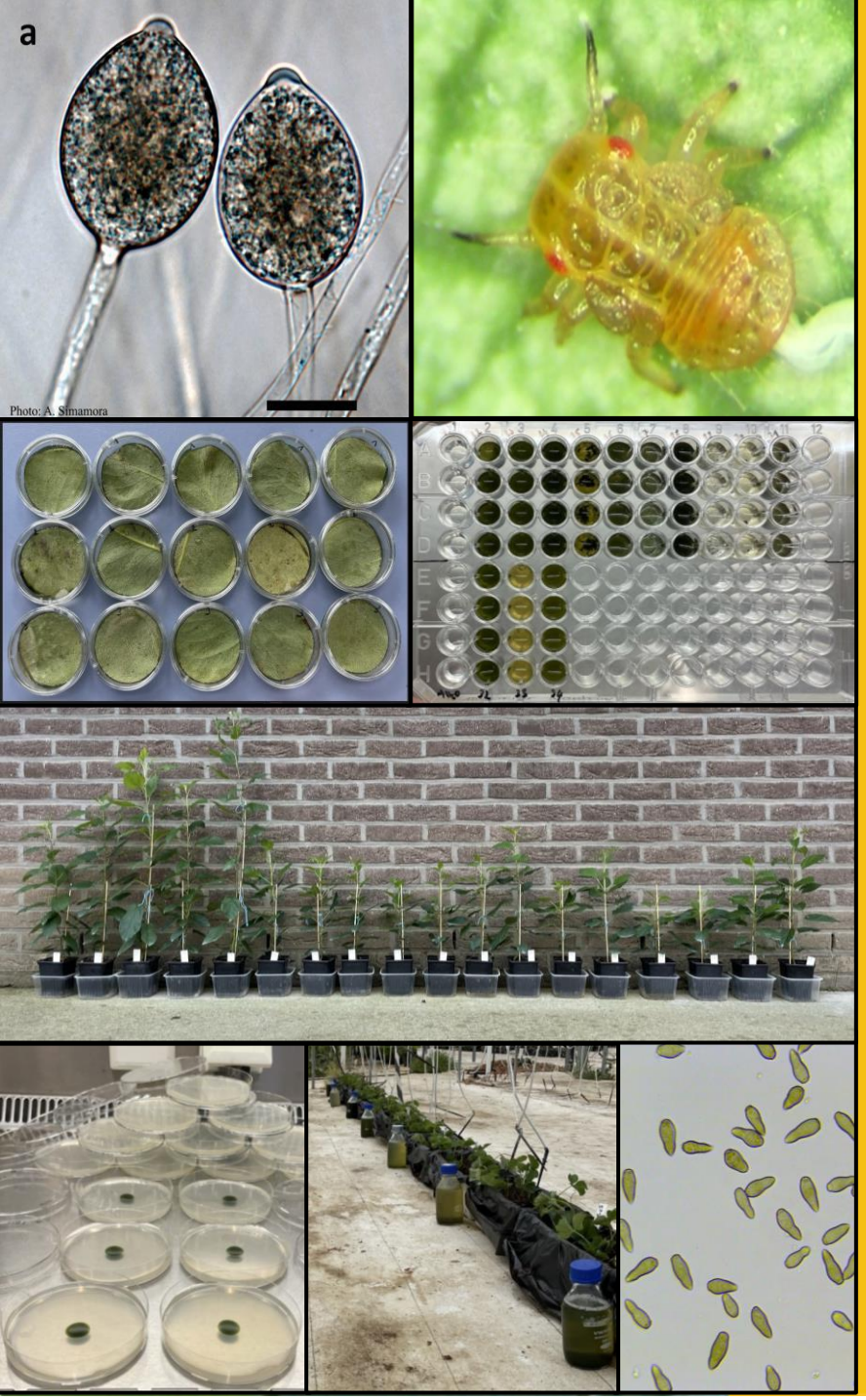
Full partners:



The effect of microalgae extracts on biotic and abiotic stress in fruit crops

Final IDEA+ event, September 2023

Yana De Ruyter

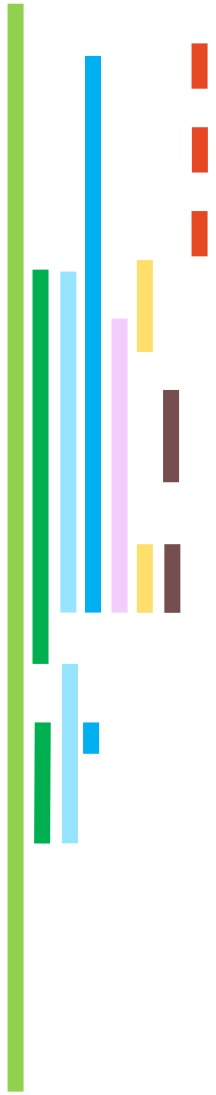
Interreg North-West Europe IDEA

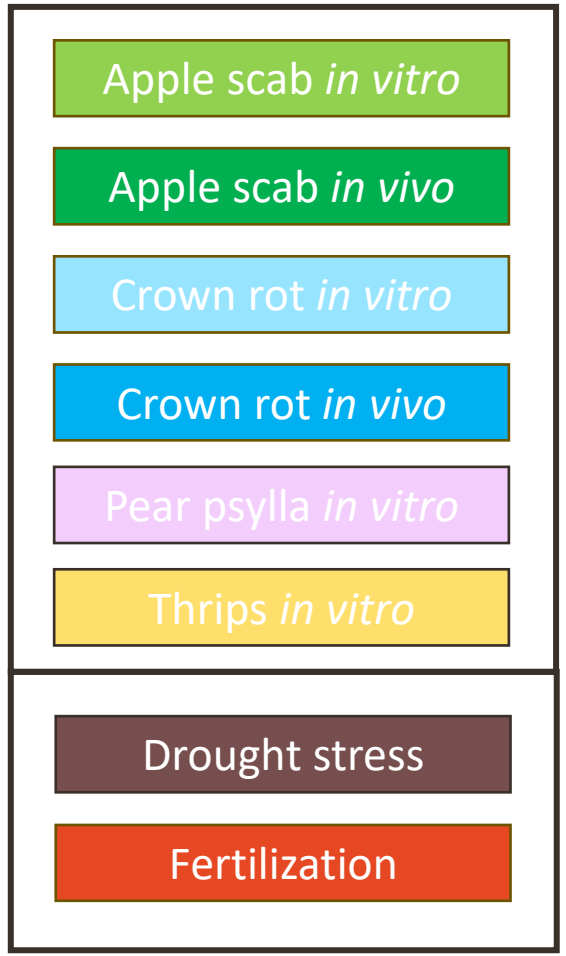
pcfruit

PROEFCENTRUM FRUITTEELT VZW

IDEA + extracts vs stress factors

Bead milled	Location 1 - algae from algae pond -> MAF-> desalted
	NAN Growth on permeate of process water 1
	NAN Growth on process water 1
	Algae from pond location 1 --> MAF pilot --> centr --> BM
	Algae from pond location 1 2023 -> Mixed biomass C desalted
	SCE grown on digestate 30/03-20/04/2023
Water extract	Algae from pond
	NAN Grown on permeate of process water 1
	NAN Grown on process water 1
	Algae from pond location 1 (test 2)
	CHM grown on permeate of process water
	Algae from pond location 1 - 2023 - Mixed biomass C desalted
	Algae from pond location 1 - 2023 - no pH adaptation Mixed biomass C (pH 6,2-6,5) desalted
Ethanol extract	Algae from pond
	NAN Grown on permeate of process water 1
	NAN grown on permeate of process water
	Residue of water extract from CHM grown on permeate of process water
Residue after water extract	Algae from pond
	NAN grown on permeate of process water 1
	NAN grown on process water 1
Residue after ethanol extract	Algae from pond
	NAN grown on permeate of process water 2





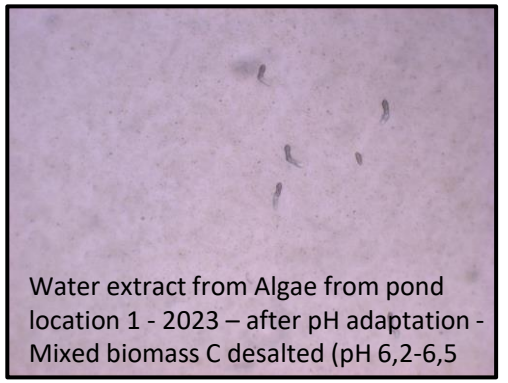
Biotic stress

Abiotic stress

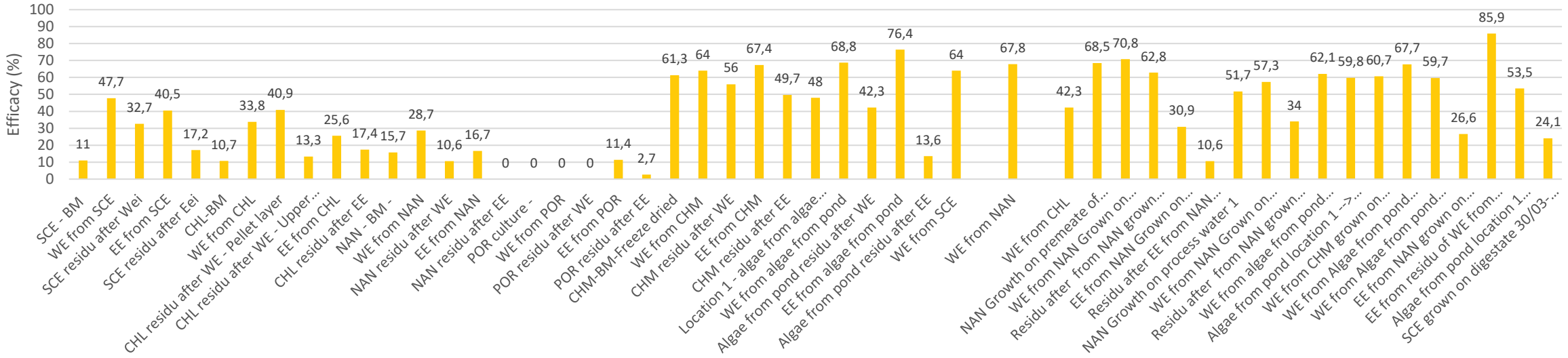
Abbreviations
NAN = *Nannochloropsis*
CHM = *Chloromonas*
SCE = *Scenedesmus*

Biotic stress: diseases - fungi

- Apple scab (*Venturia inaequalis*)
  - *In vitro:* germination trial



Effect of beadmilled microalgae and microalgae extracts on germination of ascospores from *V. inaequalis*

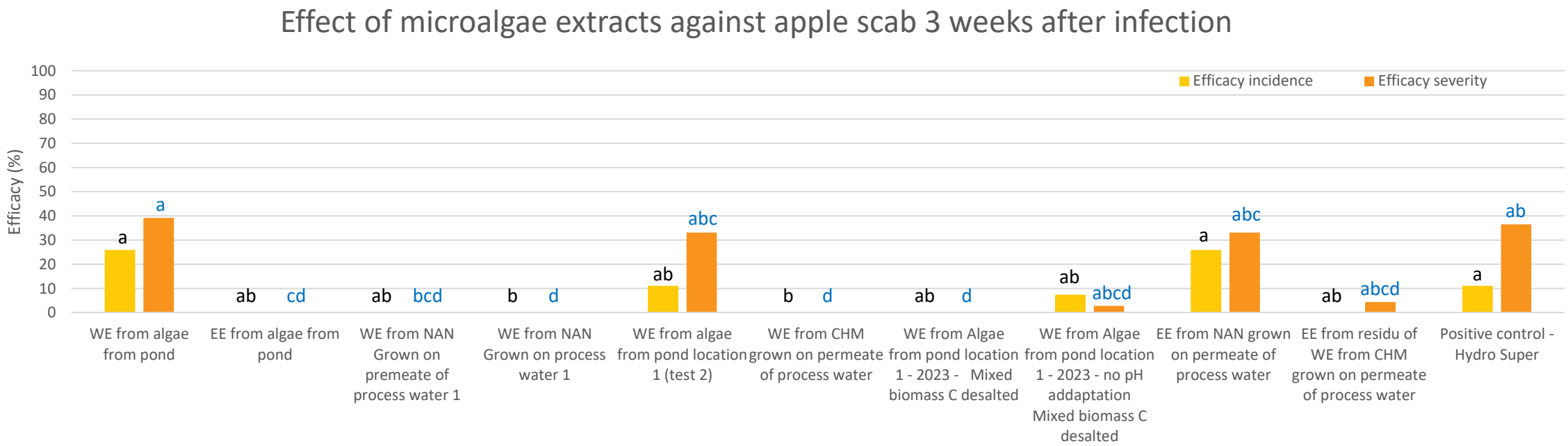


Biotic stress: diseases - fungi

- Apple scab (*Venturia inaequalis*)
  - *In vivo:* infection trial

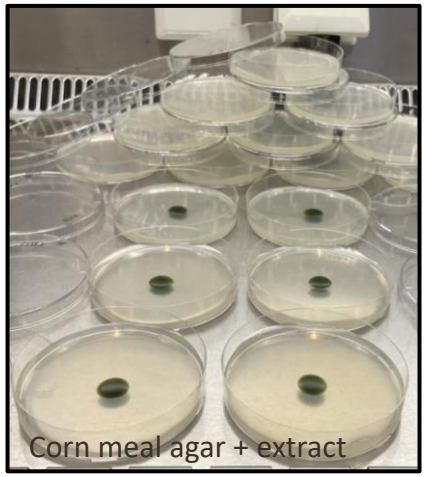

Climate chamber


Apple scab lesions



Biotic stress: disease fungi

- Crown rot (*Phytophthora cactorum*)
  - *In vitro:* mycelium growth trial
    - no promising results


Corn meal agar + extract


Mycelium growth

  - *In vivo:* infection trial
    - Data still pending

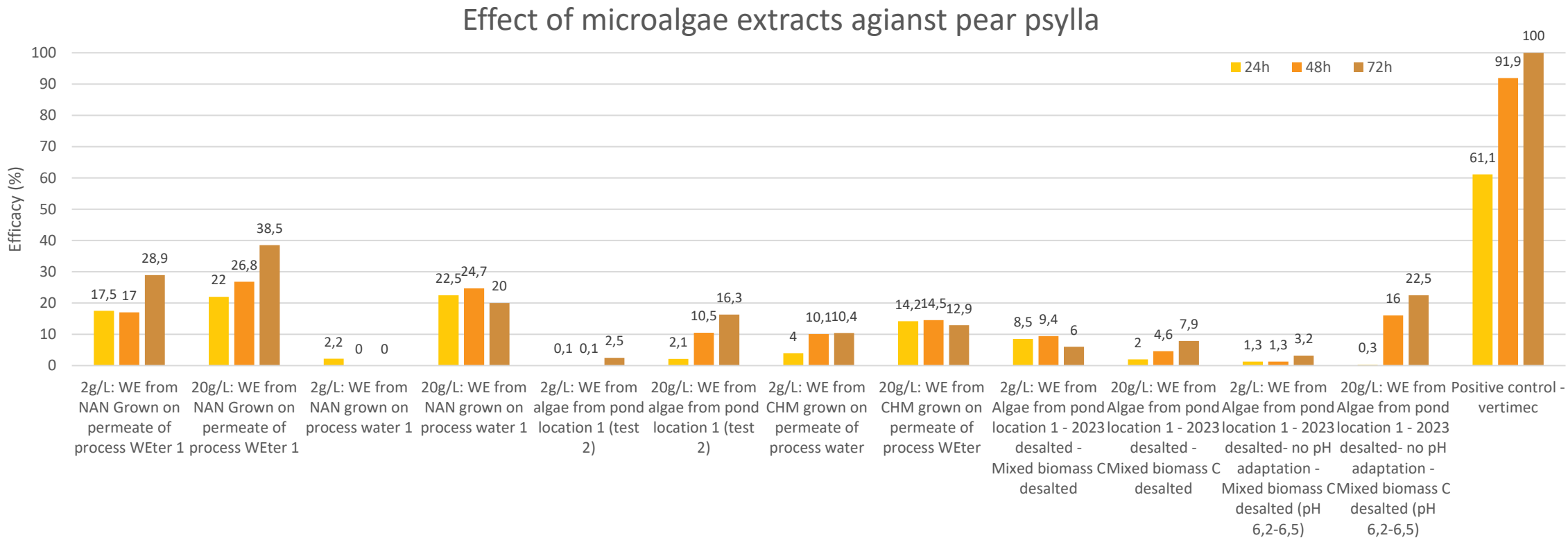

Infected plants, with their designated test product

Biotic stress: pests

- Pear psylla (*Cacopsylla pyri*)
  - *In vitro:* susceptibility trial


Potter spray tower

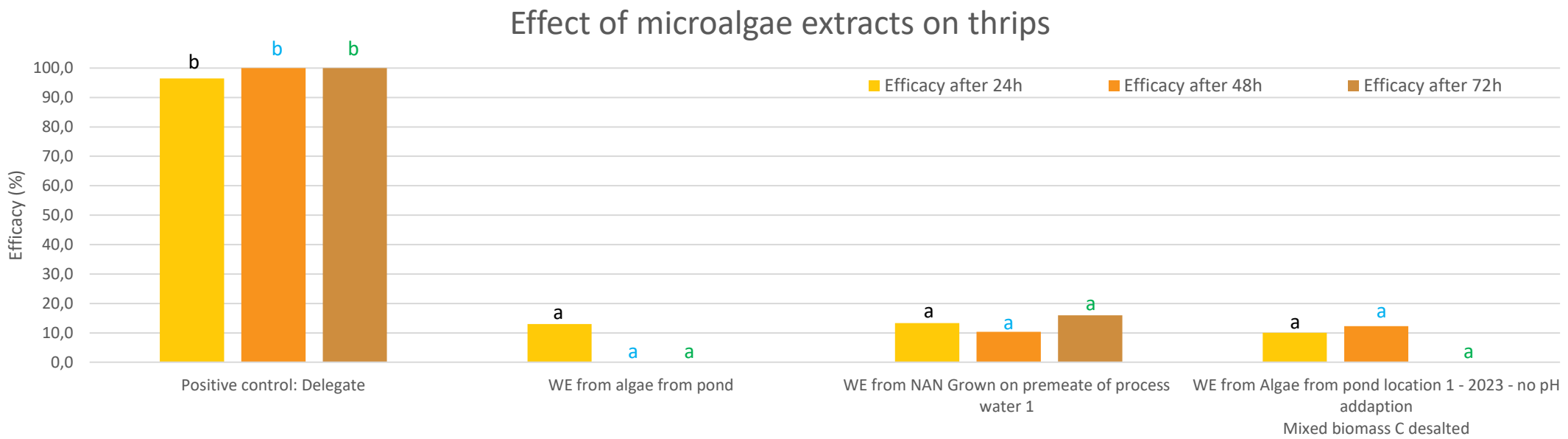

Petri dish with sprayed pear psylla



Biotic stress: pests

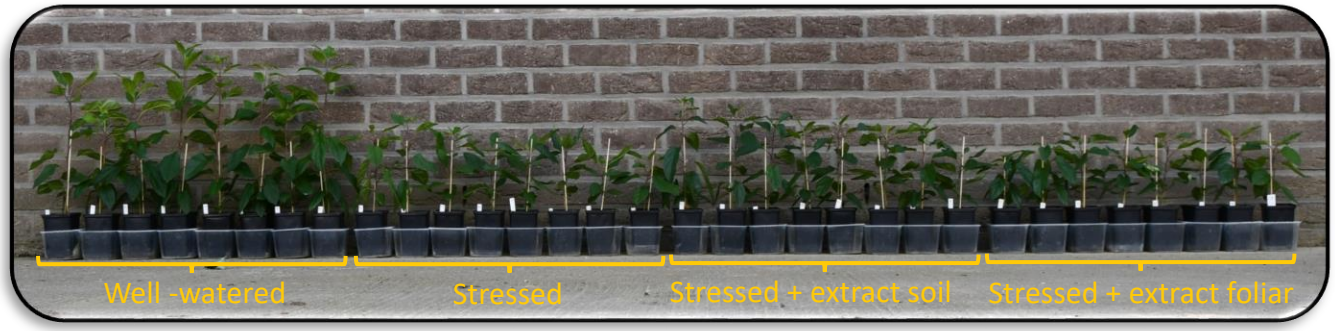
- Thrips
  - *In vitro*: susceptibility trial

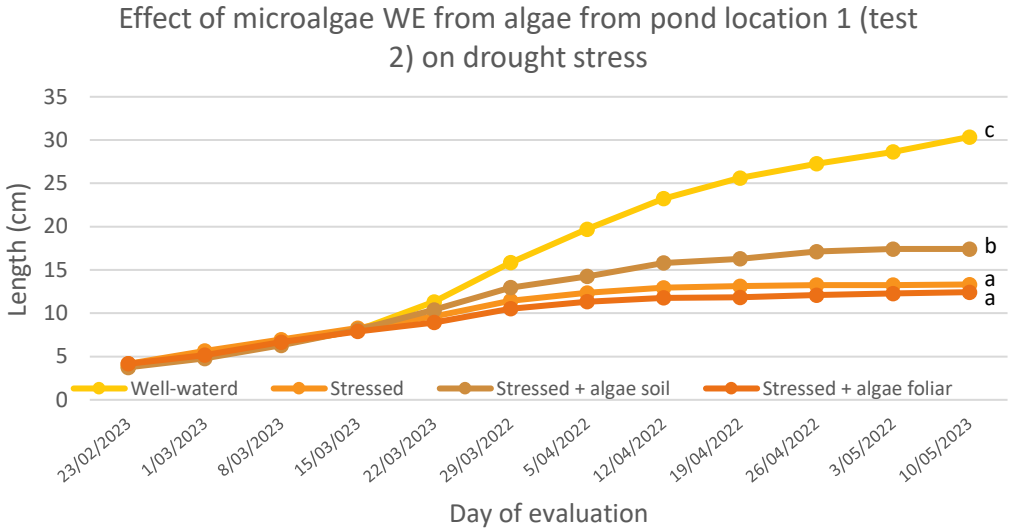
WE from NAN grown on permeate of process water 1



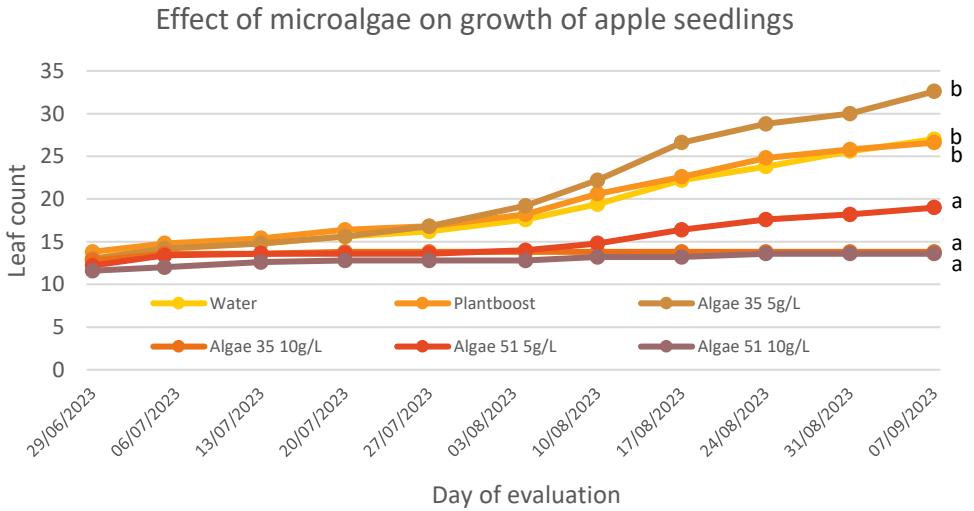
Abiotic stress

- Drought stress

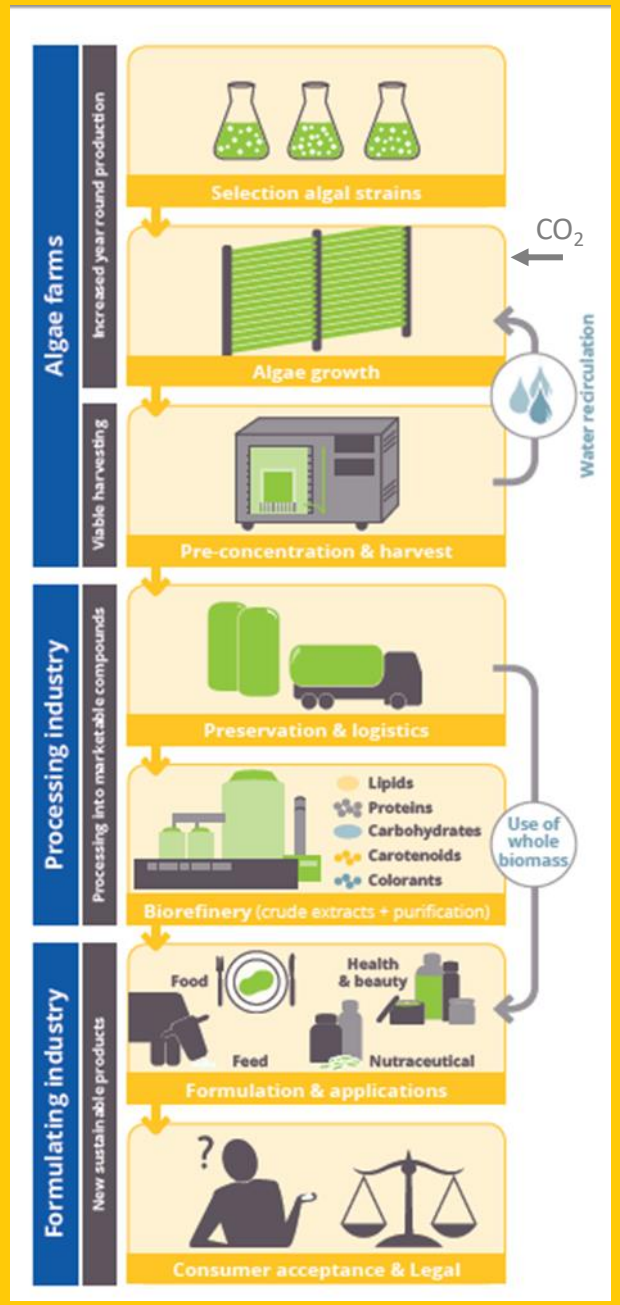




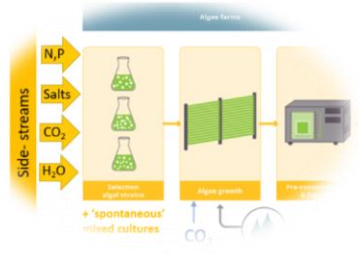
- Fertilization



35: NAN grown on permeate of process water 1
51: SCE grown on permeate 30/03-20/04/2023



Acknowledgements



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Techno-Economic and Sustainability Assessment of Side Streams Utilization in Microalgae Cultivation

Final IDEA+ event, 28 September 2023

Mohammed El Ibrahimi

Outline

Introduction

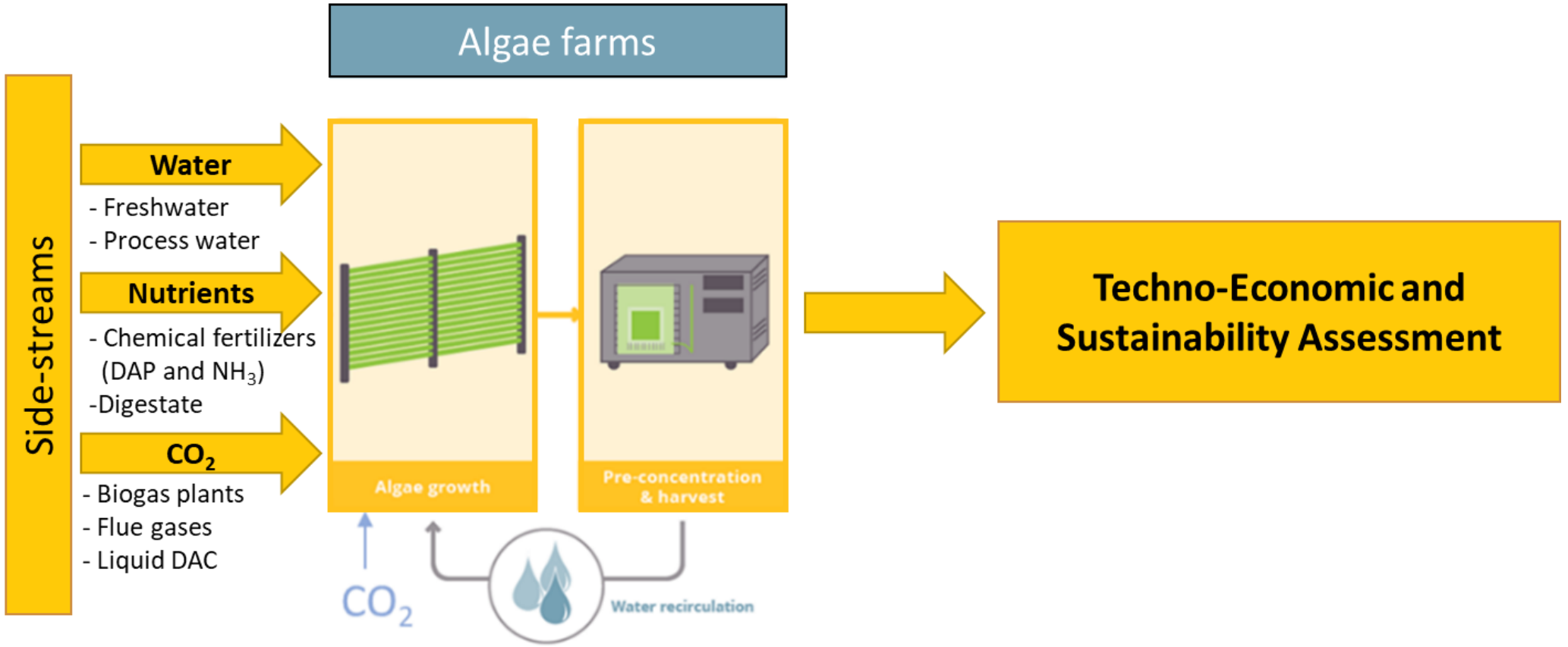
Case 1: Implications of using low carbon-containing process water

Case 2: Implications of using digestate as a nutrient source

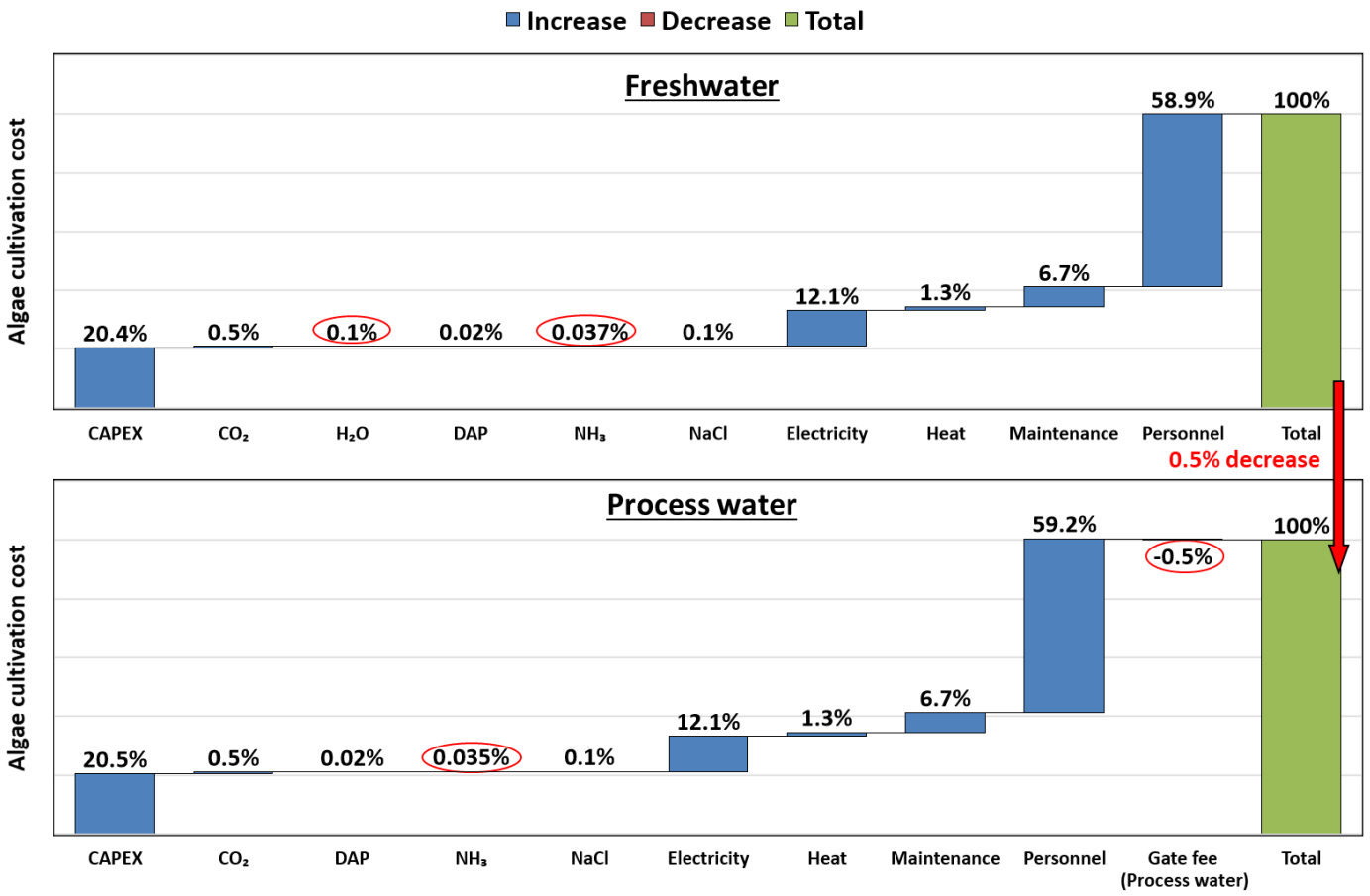
Case 3: Implications of using different CO_2 sources

Recommendations to cultivators

Introduction



Case 1: Implications of using low carbon-containing process water



Economic impacts

- Generation of revenue through gate fee imposition.
- Reduction in operating expenditures due to lowering freshwater and ammonia purchase.

Environmental benefits

- Relieving the strain on freshwater resources, which is particularly important in regions facing water scarcity.
- Contributing to the preservation of natural ecosystems and biodiversity.

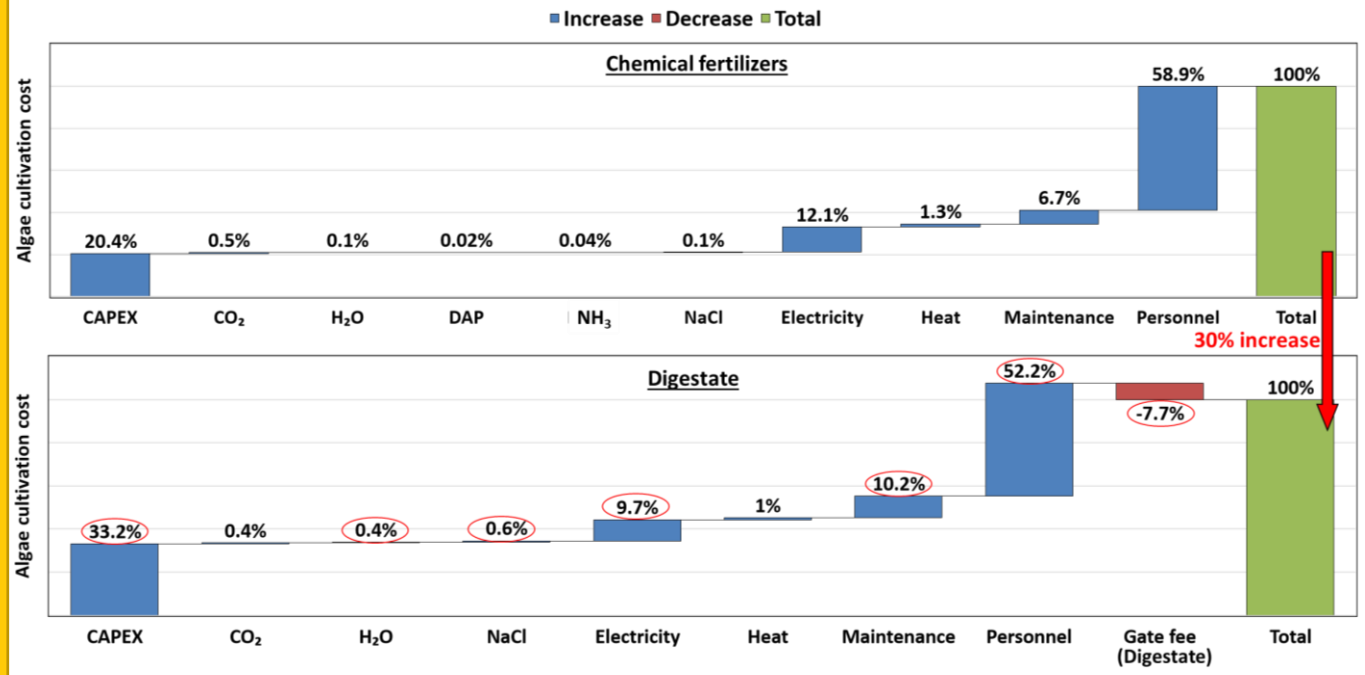
Impact limitations

- The magnitude of these benefits is not expected to be significant (in the investigated cultivation system) due to:
  - The quantity of the used process water is low since the MAF system recycles most of the water.
  - The expenses associated with water and nutrients purchase have a low impact on the overall cultivation cost.

These economic and environmental gains should not be neglected as they may become more considerable under different operational conditions and larger cultivation scales.

Case 2: Implications of using digestate as a nutrient source

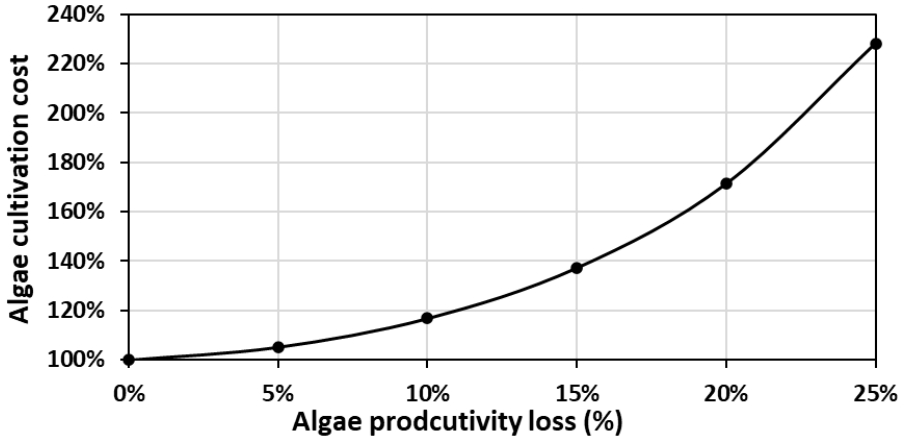
Economic implications



- The pre-treatment equipment substantially increase the capital and operating expenses of the algae cultivation system.
- Electricity and water purchase further contribute to increasing operational costs.
- Using digestate can provide minor cost savings associated with the lower needs for purchasing chemical fertilizers.
- Revenues can be generated through imposing a gate fee, collected in exchange of admitting the digestate.

Algae cultivation using digestate remains less economically attractive than chemical fertilizers.

- Hypothesis: Using digestate may trigger lower algae productivity rates, thus significantly increasing cultivation costs.
- Maintaining optimal nutrient balances and turbidity levels can be essential for ensuring favorable growth conditions and adequate economic performance.



Case 2: Implications of using digestate as a nutrient source

Environmental and health impacts

Water Usage: Significant water quantities are required for digestate dilution, which can further exacerbate water scarcity problems in regions with limited water resources.

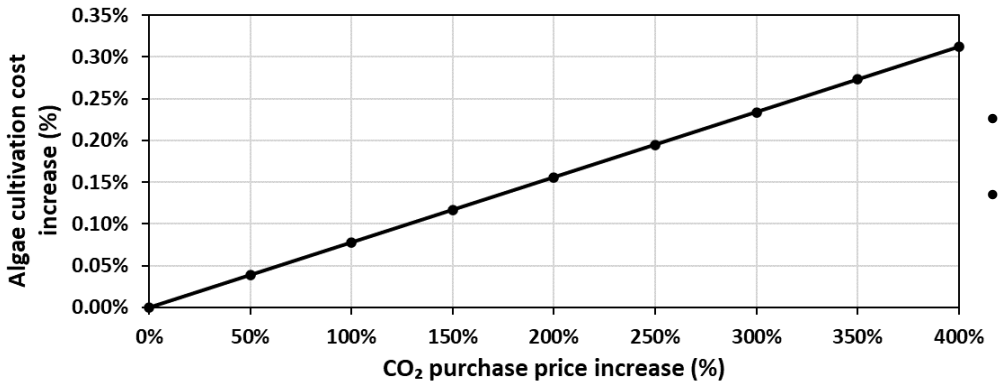
Methane Emissions: Digestate can still emit methane during its storage. Storing the amount of digestate considered in the present assessment could result in significant GHG emissions to the atmosphere.

Energy Usage: Pre-treating digestate demands considerable energy input, which contributes to further increasing the emissions of the cultivation system.

Health repercussions: Cultivating microalgae using digestate can introduce safety concerns, primarily related to potential contaminant transfer. Depending on its source, digestate can contain heavy metals and organic pollutants, which may affect the safety of resulting products.

Case 3: Implications of using different CO_2 sources

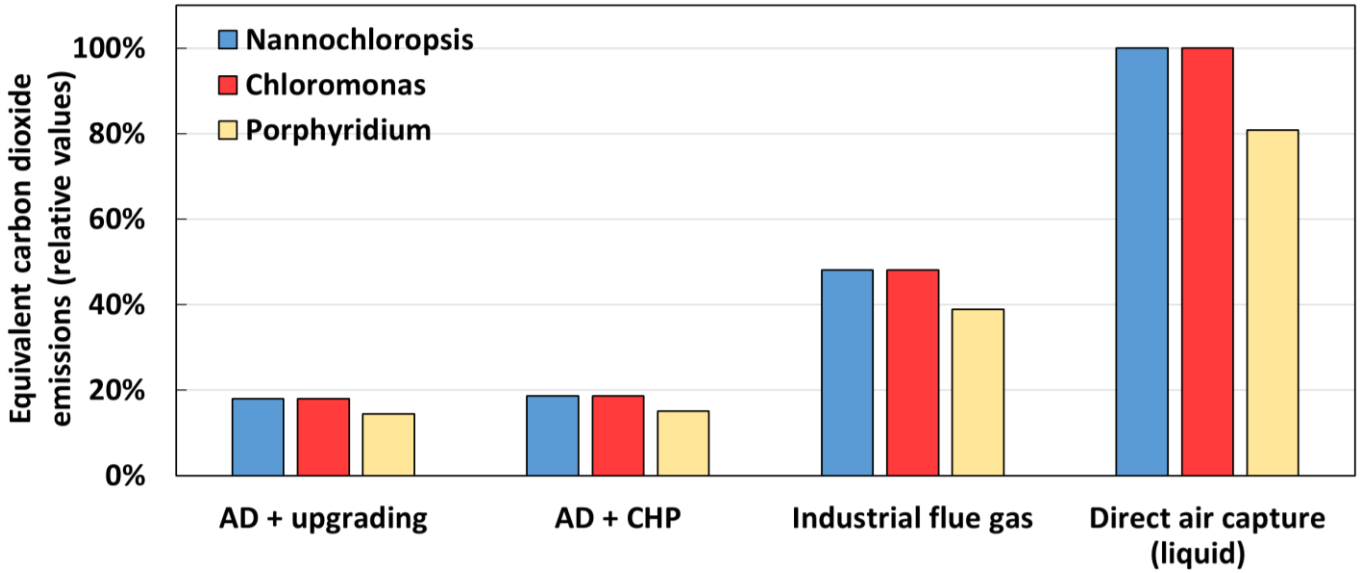
Economic impacts



- The CO_2 price has a small impact on the microalgae cultivation cost.
- These results are due to the small contribution of the CO_2 purchase costs to the total cultivation costs.

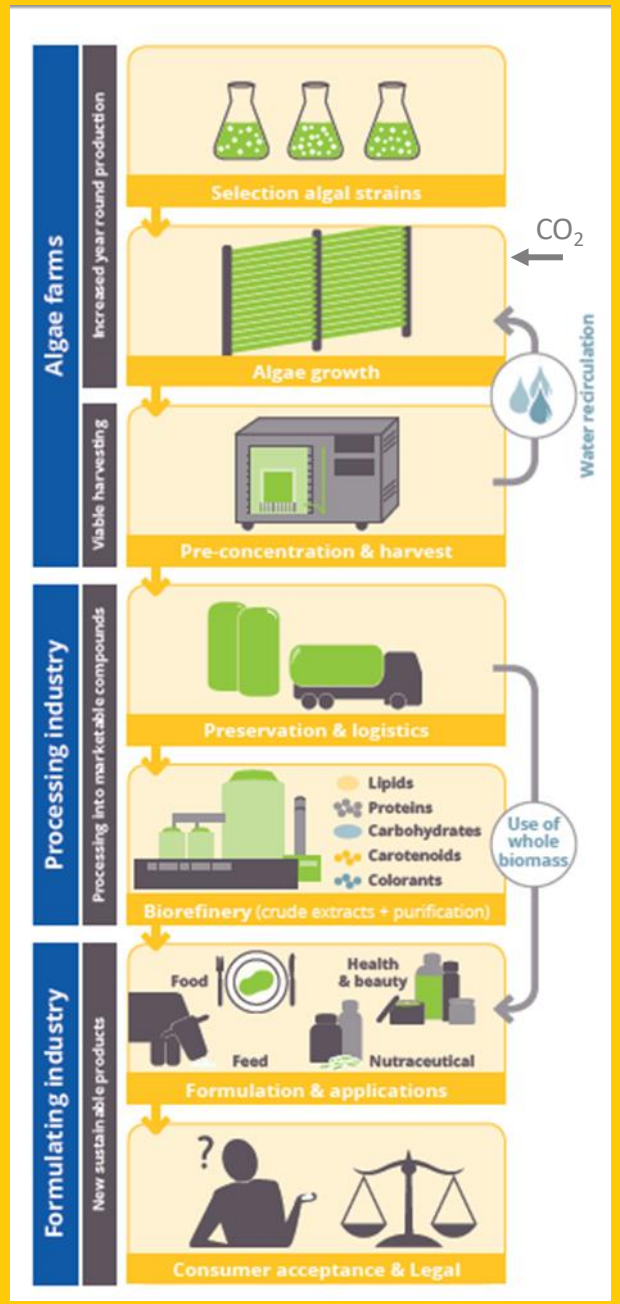
Environmental implications

- Growing algae on CO_2 from biogas plants has the lowest carbon footprint among the considered CO_2 sources.
- CO_2 sourced from liquid DAC plants results in the highest CO_2-e emissions.
- This is a result of the low concentration of CO_2 in the air, which drives up the need for electricity and chemicals use.



Recommendations to cultivators

- **Limitations of using process water**: Cultivating algae using process water is not expected to result in considerable economic and environmental gains. These benefits can, however, become more prominent under different conditions (e.g., larger cultivation scales, no water recycling).
- **Economic considerations associated with digestate use**: Pre-treating digestate has significant impacts on the economic performance of the algae cultivation system.
- **Digestate GHG emissions reduction**: Emissions can be mitigated by sourcing digestate from nearby AD plants with immediate supply and covered storage systems. The carbon footprint can further be reduced by using renewable energy for digestate pre-treatment.
- **Algae-based product safety**: Quality control procedures need to be implemented to ensure the safety of the microalgae-based products, especially when digestate is involved. Regular testing of digestate, microalgae biomass, and final products can help identify and manage potential contaminants.
- **CO_2 sourcing optimization**: Prioritize CO_2 from biogas plants to lower the carbon footprint of the algae cultivation system. Nearby biogas plants must be preferred to reduce transport-related emissions and improve environmental sustainability.



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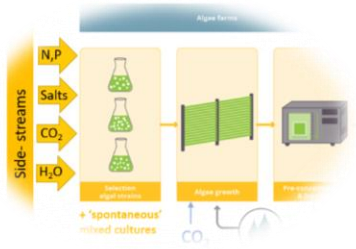

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