Ammonia-based method to control the predator *Poterioochromonas malhamensis* on *Chlorella vulgaris* massive cultures

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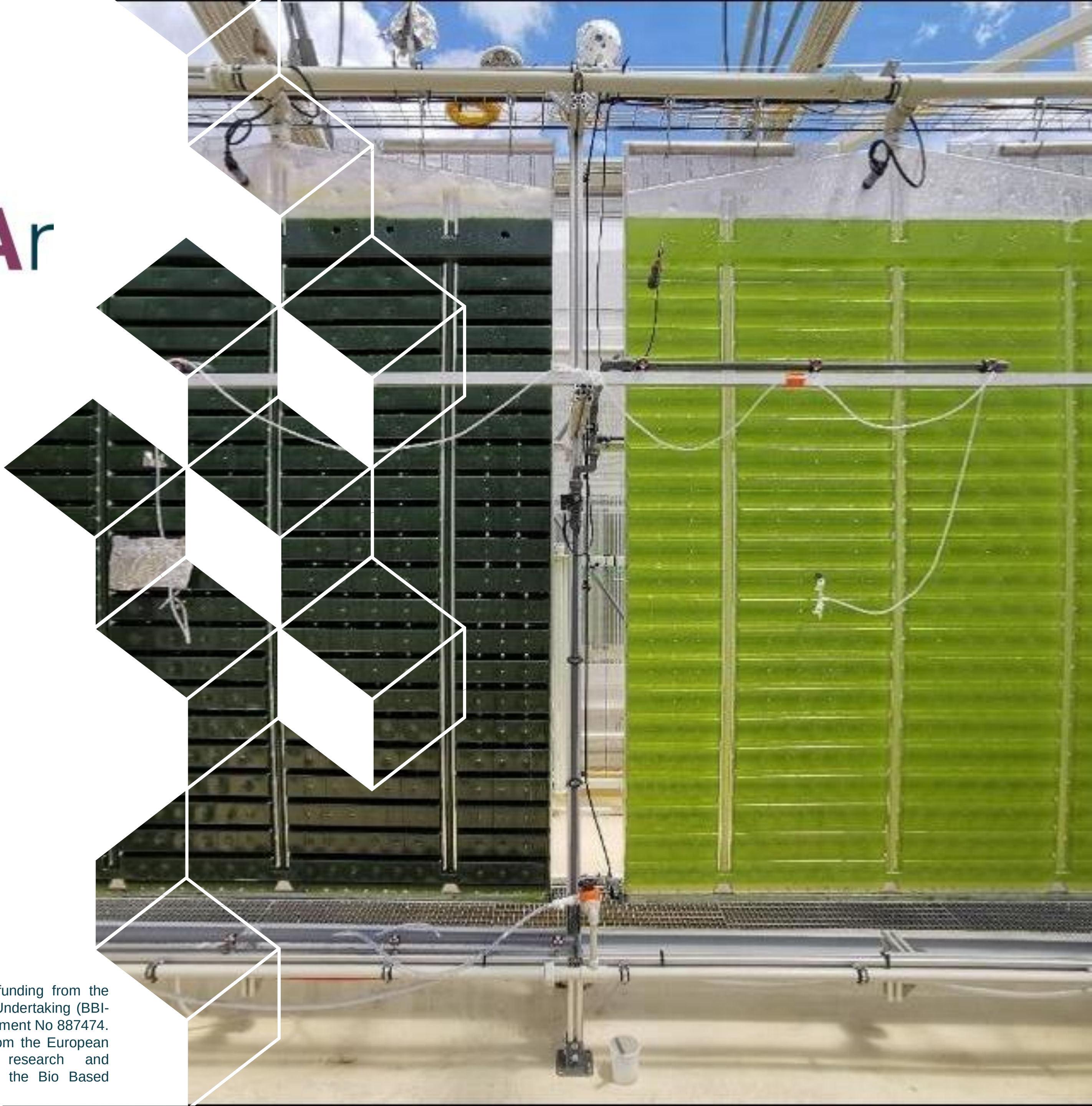


The nenuPHAr value chain and final products

Microalgae production in photobioreactor

Starch recovery

Bacteria production in fermenter

PHA recovery

Formulation and compounding

Processing into products

Recycling

Flexible transparent film for packaging

Thermoformed food plastic tray for cheese

Roll on bottle

3D printing filament

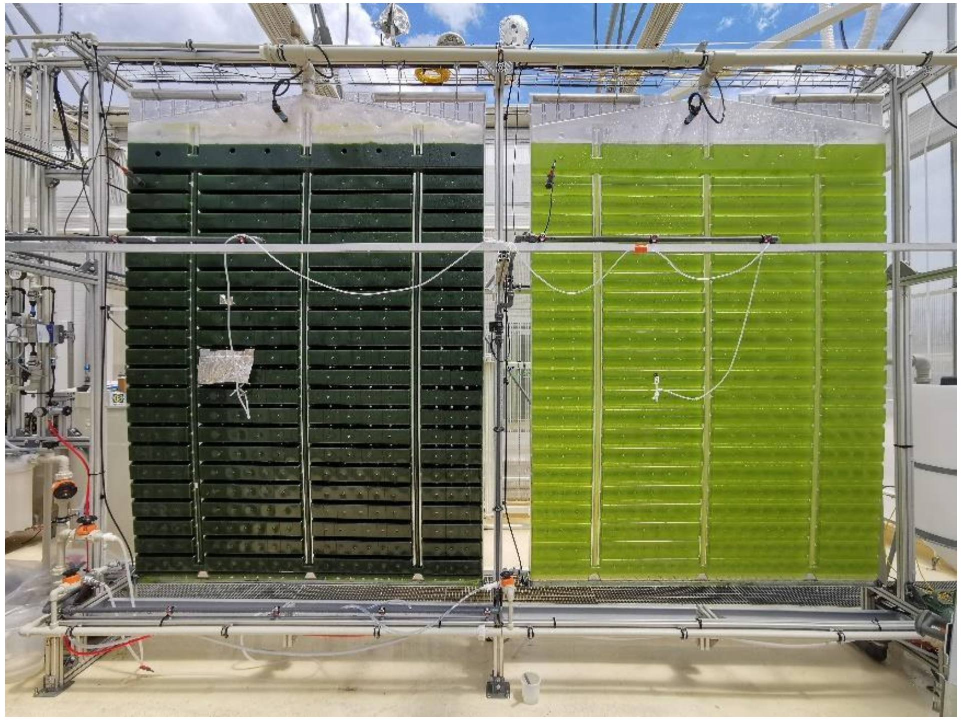
Stand up pouch

Plastic cup

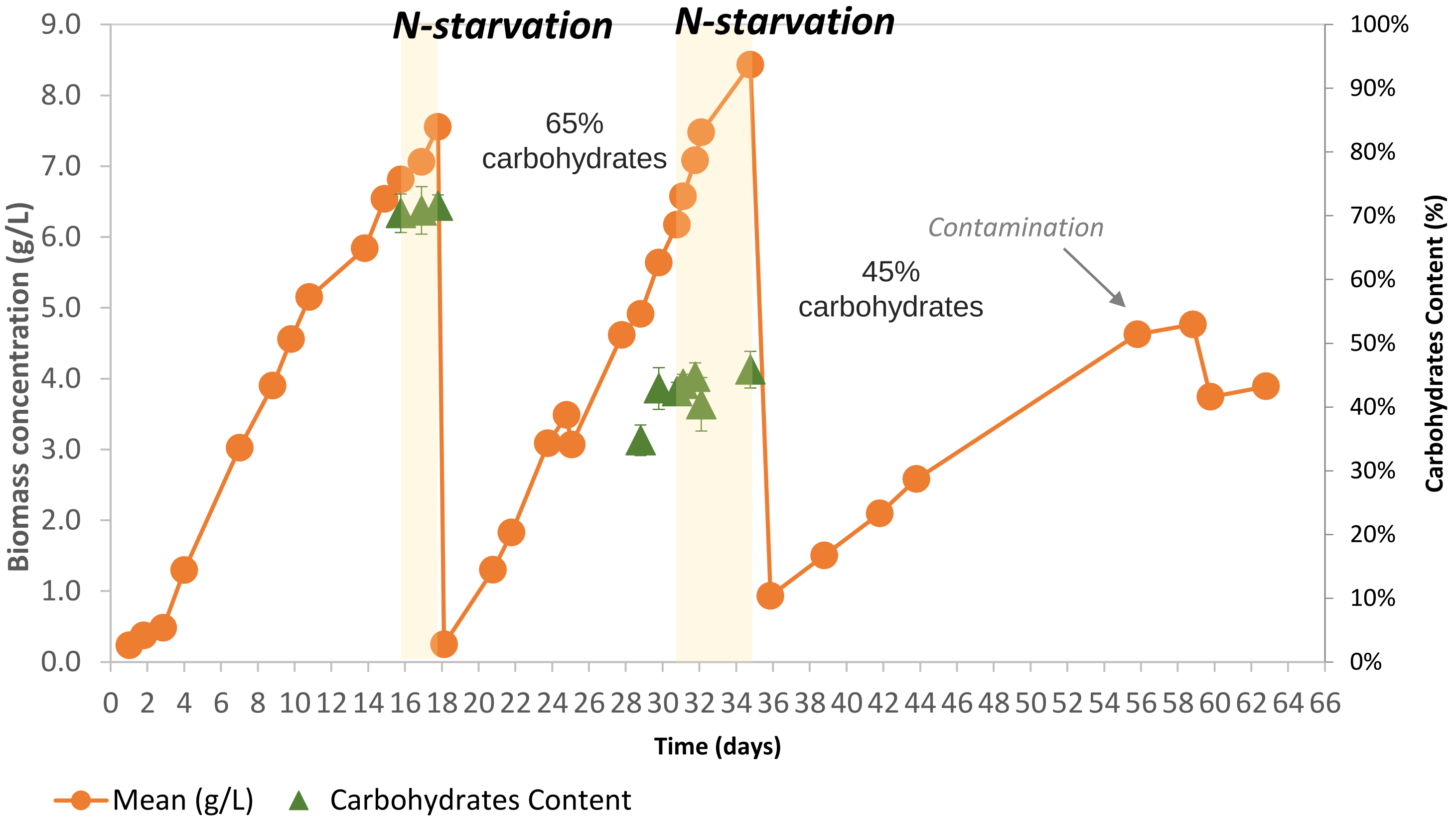
Medical yarn

Agrotextile landscape fabric

Starch production from *C. vulgaris* (CCALA 942)



180L Flat panel airlift photobioreactor



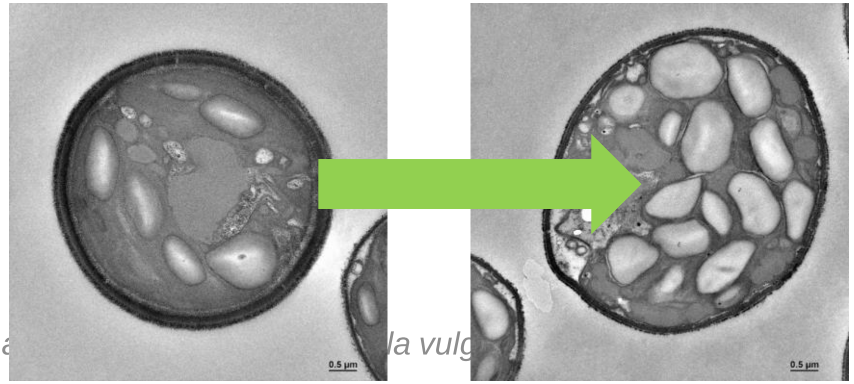
N-starvation

N-starvation

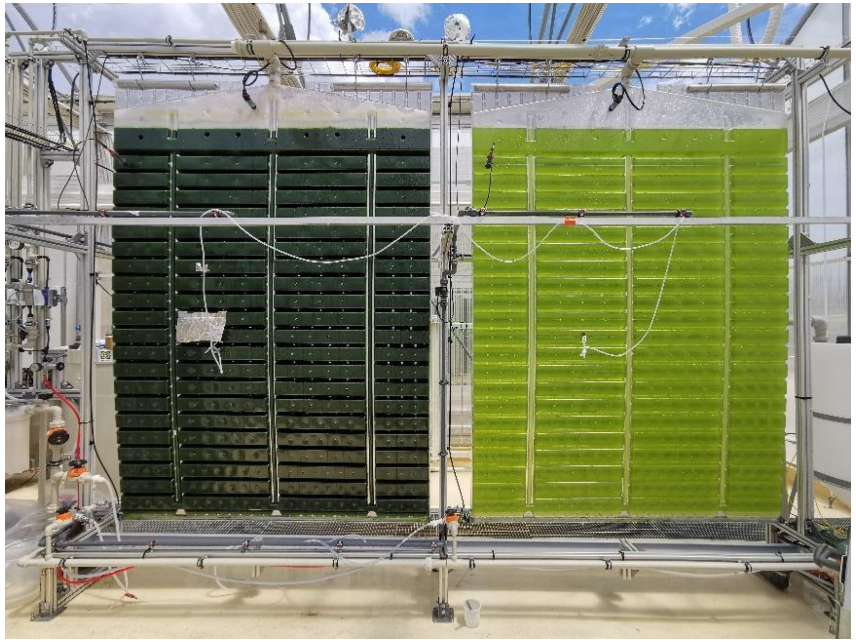
65% carbohydrates

45% carbohydrates

Contamination

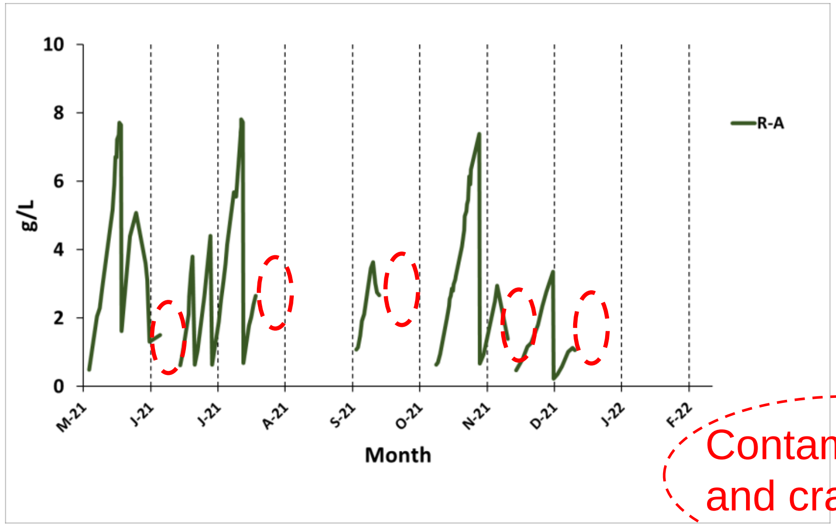


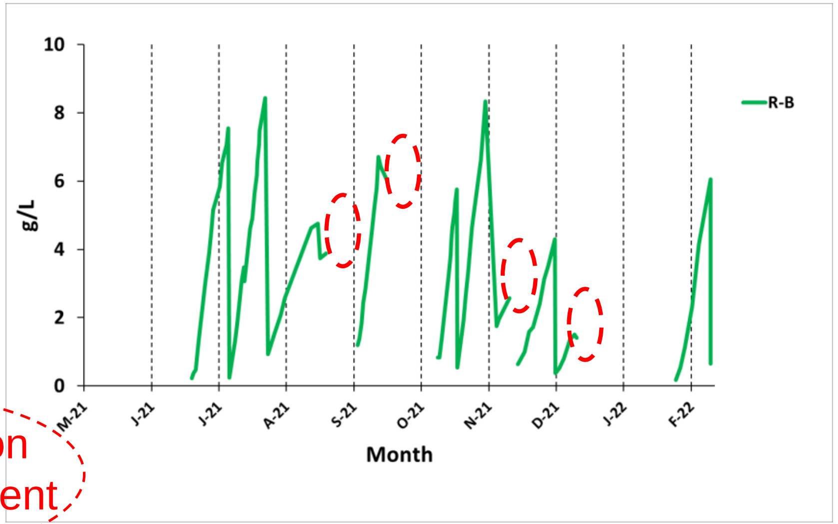
Contamination events



- Frequent culture **crash**
- High **impact** on biomass **production**
- **Early harvest** without starch accumulation
- Time-consuming **cleaning** and **sterilization**
- Extra **man work**

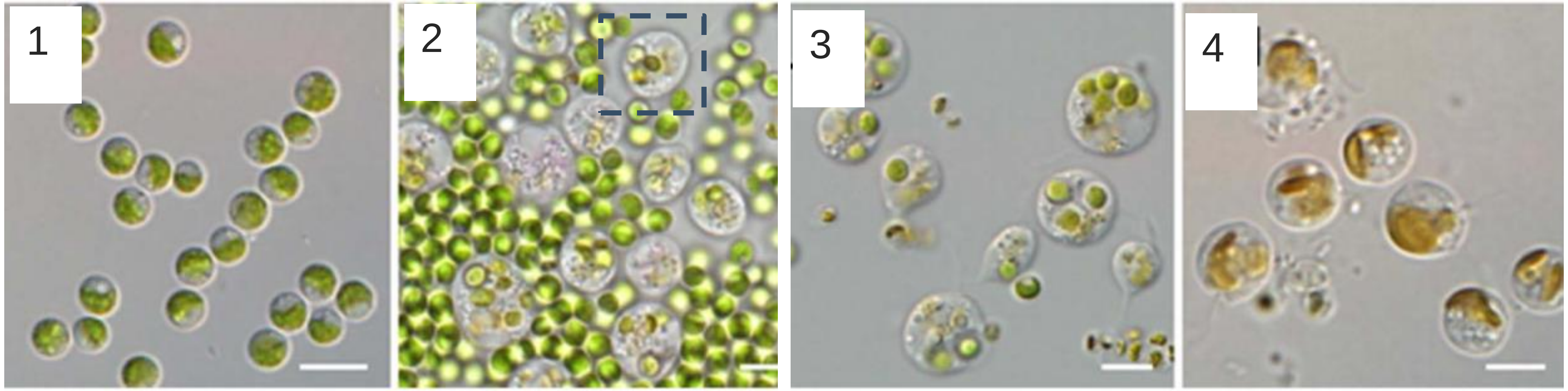
Closed reactor:
No contaminations

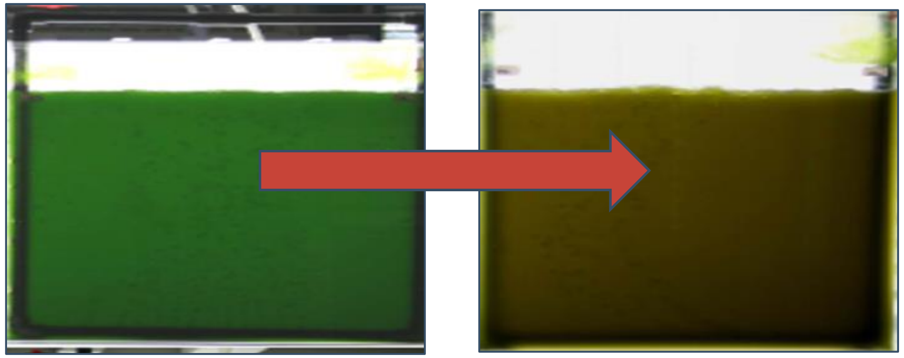




Contamination and crash event

Predator: *Poterioochromonas malhamensis*





Chlorella culture turns from **green** → deep **brown** within a few days

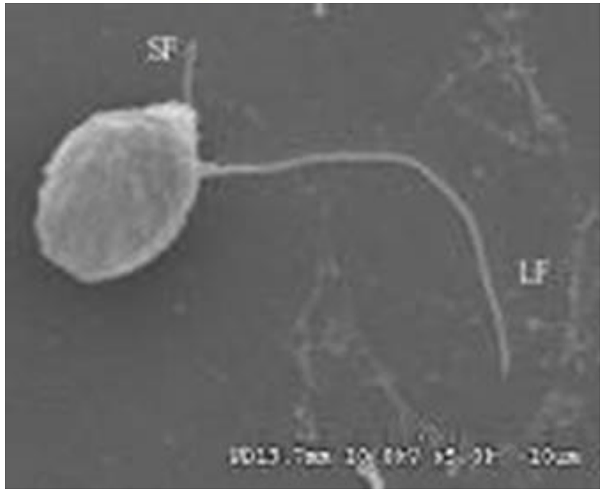
(Ma et al., 2018)

Predator: *Poterioochromonas malhamensis*

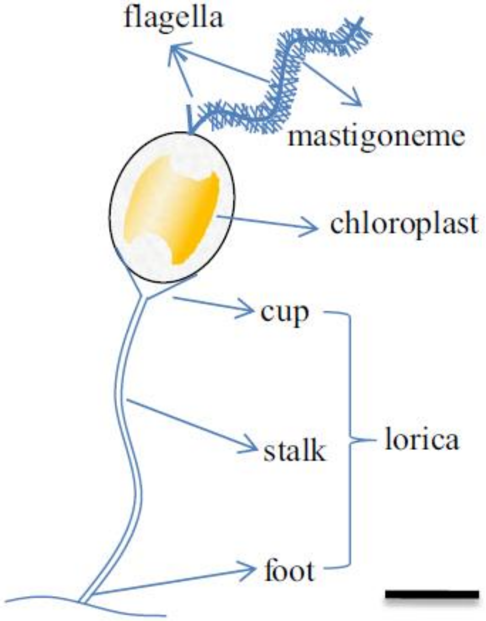
Golden-brown algae, mostly freshwater
Flagellated microalgae
- two heterodynamic flagella
- Graze on a diverse range of prey

Sphere or ellipse: 5-10 µm in diameter
Maximum diameter to reach at preying is 25 µm
No rigid cell wall

Fig. 1 Diagram of key cell morphological characteristics of *Poterioochromonas*. Scale bar = 5 μm



(Guo and Song, 2010)



(Ma et al., 2023)

Mode of nutrition

Autotrophic	Chemoheterotrophic	Phagotrophic
Growth rate extremely low (lower photosynthetic ability, lack of CO2 concentrating mechanisms)	Higher growth rate and higher cell size when cultivated with dissolved organic matter (glucose)	Predominant nutritional mode
Higher protein and fucoxanthin content	Higher sugar content	

Growth rate of *P. malhamensis* ↑
- Carbohydrate content ↑
- C:N ratio ↑ and

(Wei et al., 2020)

Contamination control techniques

Chemical treatment	**Lethality Factor**	**Disadvantages**
NH_4HCO_3 (He et al., 2021) 100 L raceway ponds	Concentration of NH_3	Not applicable for systems anticipating lipids or carbohydrates accumulation upon nitrogen starvation
Sodium Dodecyl Benzene Sulfonate (SDBS) (Wen et al., 2021) 40 000 L raceway pond	Toxicological effects of surfactants	Foaming effect in aerated reactors

Physical treatment	**Letality Factor**	**Disadvantages**
Ultrasonication (Wang et al., 2018) 60 L raceway ponds	Mechanical Pressure	Higher facility cost

Altered cultivation conditions	**Letality Factor**	**Disadvantages**
CO_2-mediated low culture pH (Ma et al., 2017) 100L raceway ponds	High-pressure inactivation Low culture pH reduced the cytoplasmic pH	Higher operational cost

$$NH_4^+ \leftrightharpoons NH_3 + H^+$$

NH_3 equilibrium controlled by pH and T

$$NH_3 - N\left(\frac{mg}{L}\right) = AN\left(\frac{mg}{L}\right) \times [1 + \frac{10^{-pH}}{10^{-(0.1075+\frac{2725}{T})}}]^{-1}$$

Objective

Develop an **effective strategy** to **control** the **contamination** by ***P. malhamensis***, applicable at **mass-scale *Chlorella*** production facilities.

Steps to develop the method

Step 1: Determination of **ammonia** concentrations **tolerated** by ***C. vulgaris***

Step 2: Determination of **mortality** of ***P. malhamensis*** **under** the selected **ammonia** concentration

Step 3: **Validation** of the effectiveness of ammonia-based control method **in a co-culture** of *C. vulgaris* and *P. malhamensis.*

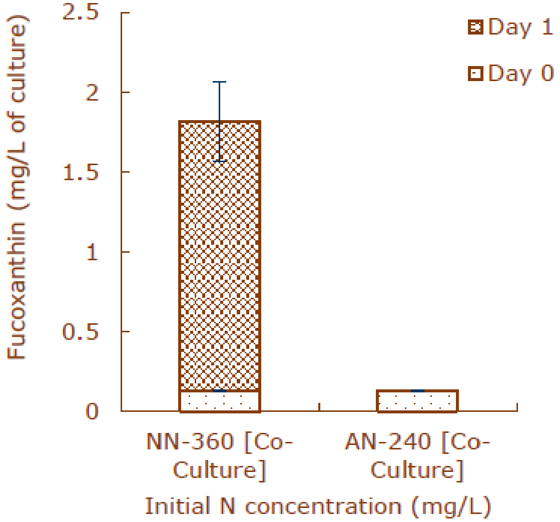
Step 4: **Up-scaling** into semi-industrial pilot units and **validation**

Step 5: **Optimization**

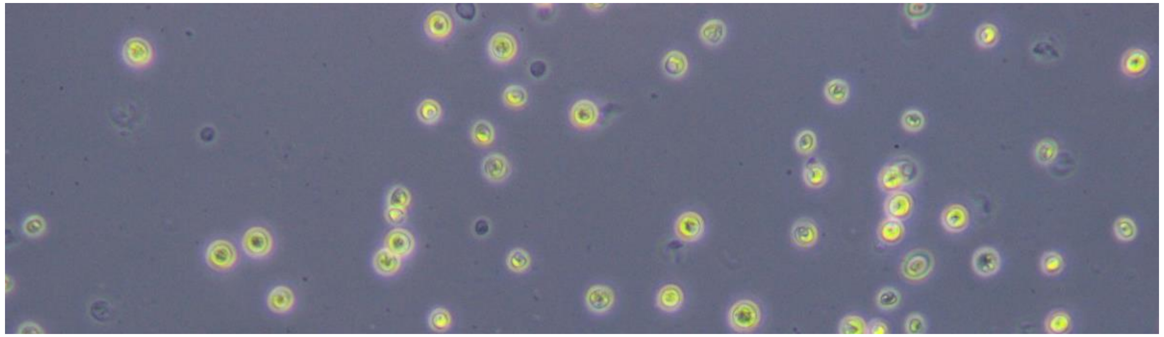
Detection method

- **Same size** as *Chlorella* → Not possible automated cell count
- **Motility** → Visual check on microscope → **Recommended method**
- Possible extraction and **determination** of **fucoxantine** → too late…

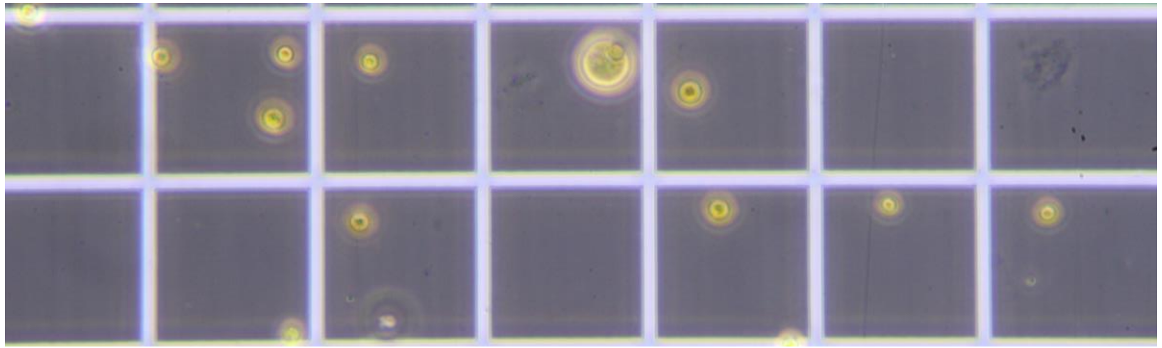
HPLC fucoxantine determination



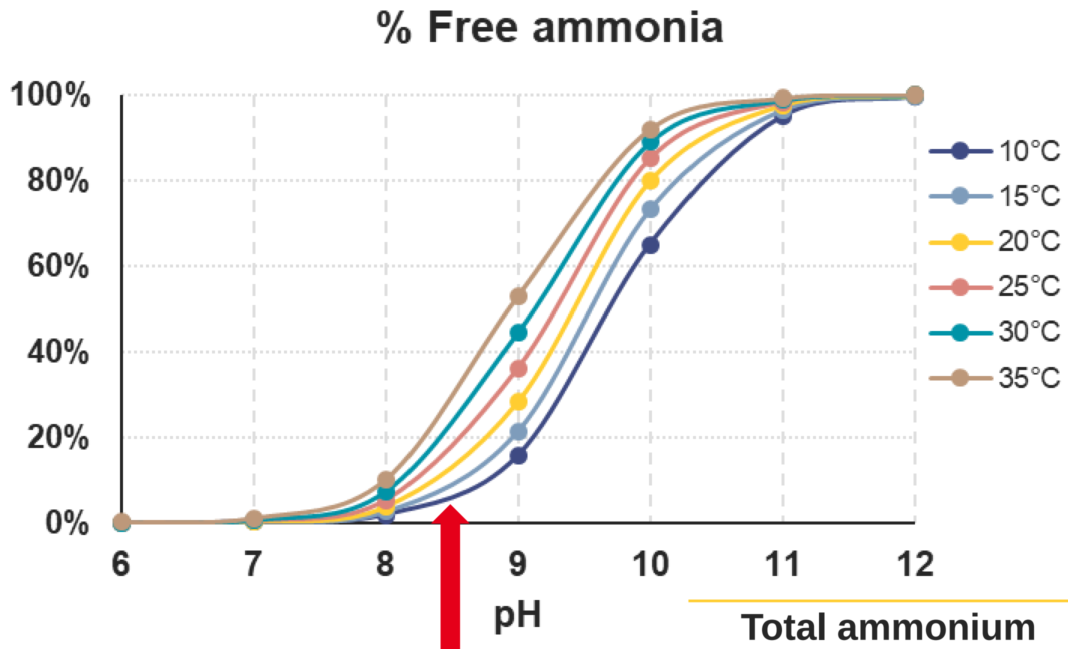
Same size (autotrophy) → **Motility** detection



Different size (phagotrophy) → Size and **Motility** detection



Step 1. Determination of ammonia concentrations tolerated by *C. vulgaris*

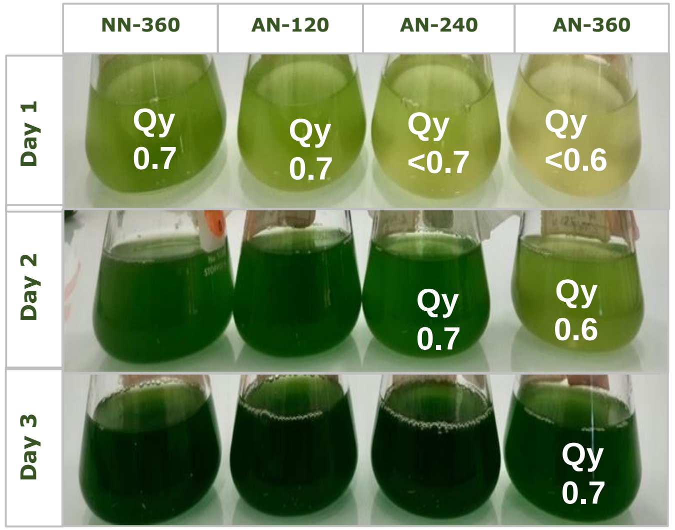


pH 8.5

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

$$FAN = \frac{TAN}{1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T(K)}\right)}}}$$

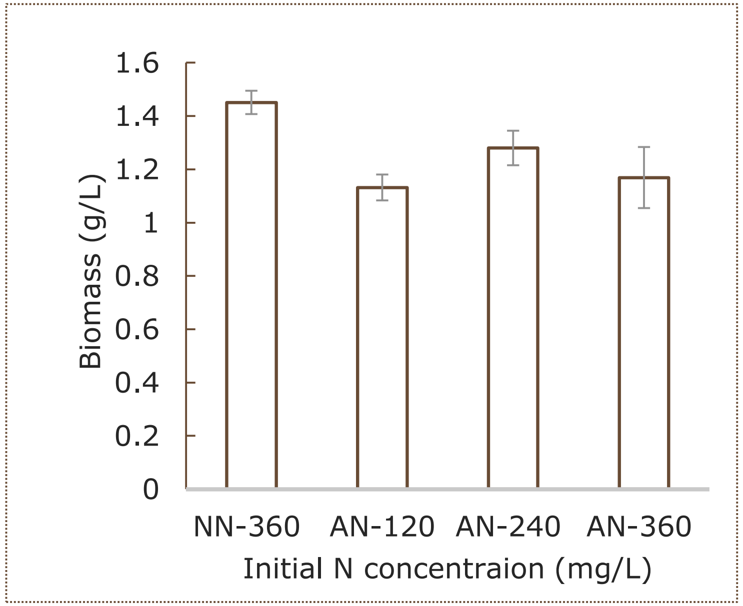
(Xia and Murphy, 2016)



Total ammonium (mg/L)	Free ammonia at pH=8.5 (mg/L)
120	23.03
240	45.68
360	69.17
Control in NO_3 360	0

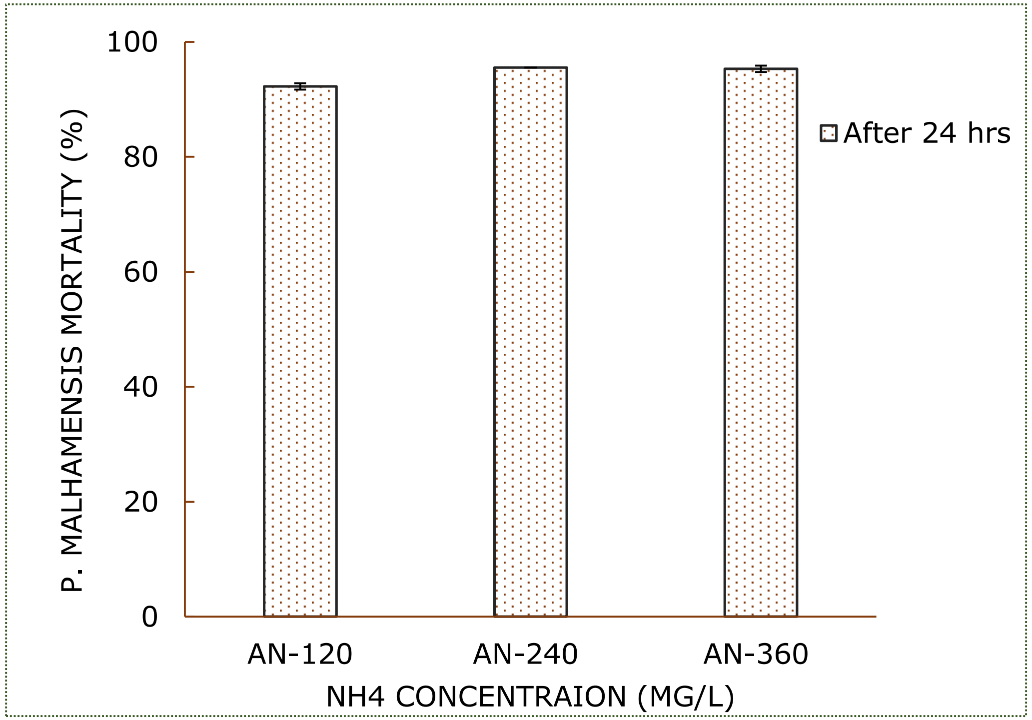
Jiang et al., 2021

OK → 36.8 mg/L NH_3

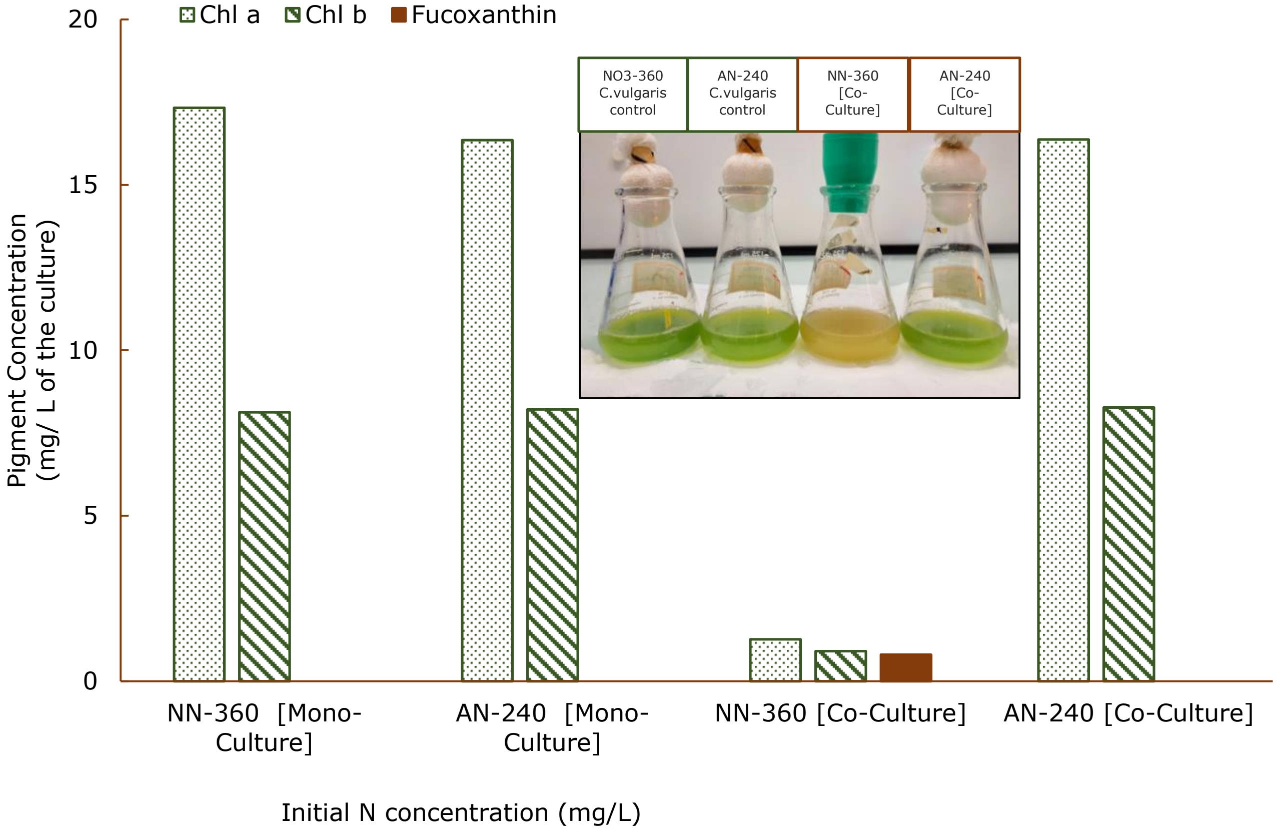


Step 2: Mortality of *P. malhamensis* under ammonia
Step 3: Validation in a co-culture

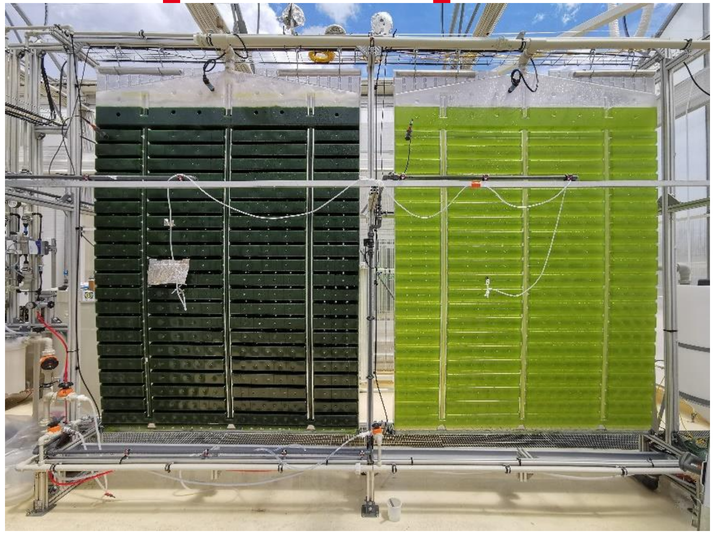
Mortality of *P .malhamensis* in 24h



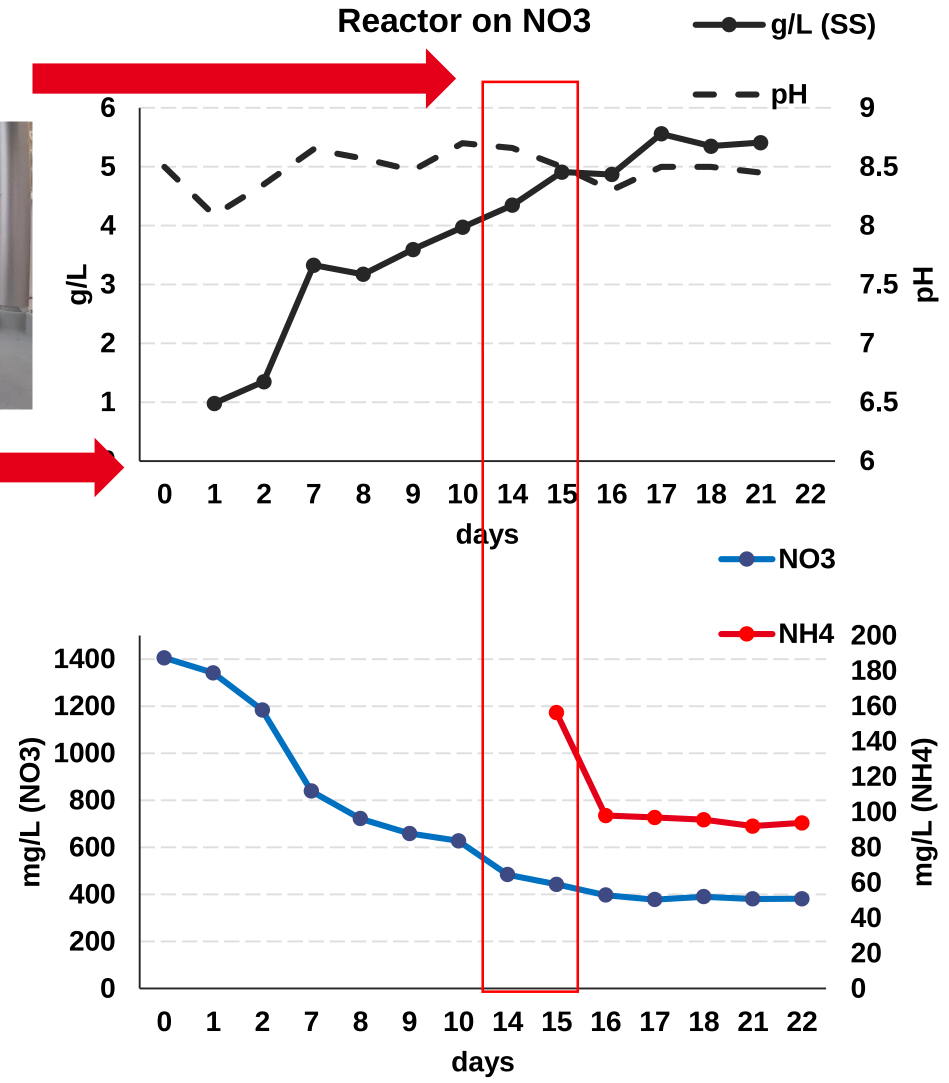
Mortality of *P .malhamensis* and survival of *C.vulgaris* in 24h



Step 4: Up-scaling the method



P. malhamensis attack



Innoculation of *P. malhamensis* and *C.vulgaris*

Reactor in **NO_3** ; **Summer** conditions (25°C - 35°C)

Attack after **15 days** (around 100 mg/L left of N)

Control method:

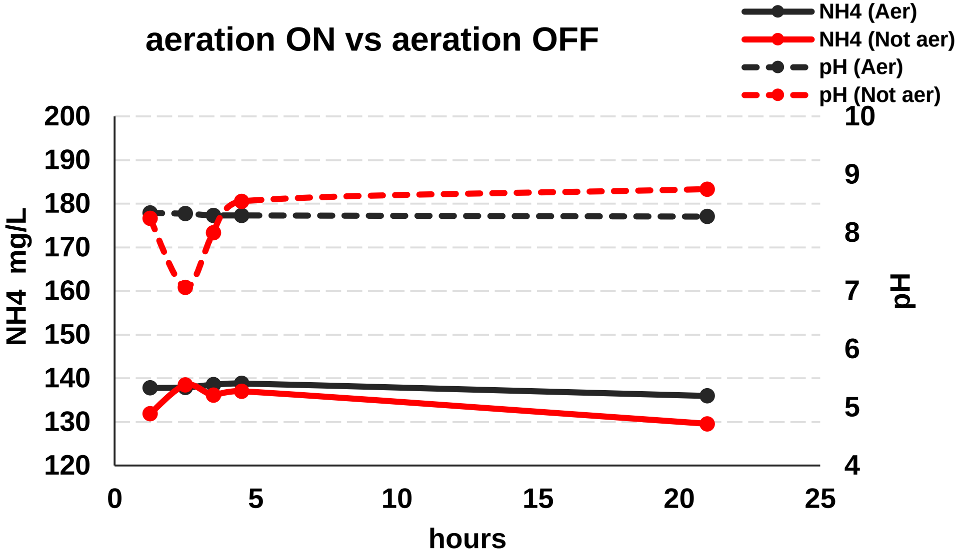
- **Aeration** and CO_2 **stopped** during 24h
- **pH rised** until 8.5
- **160 mg/L** of NH_4 (24,3-42 mgNH_3/L; 25-35°C)

Step 5: Optimization

Stop aeration during 24h:

- Fouling
- Reduction of productivity

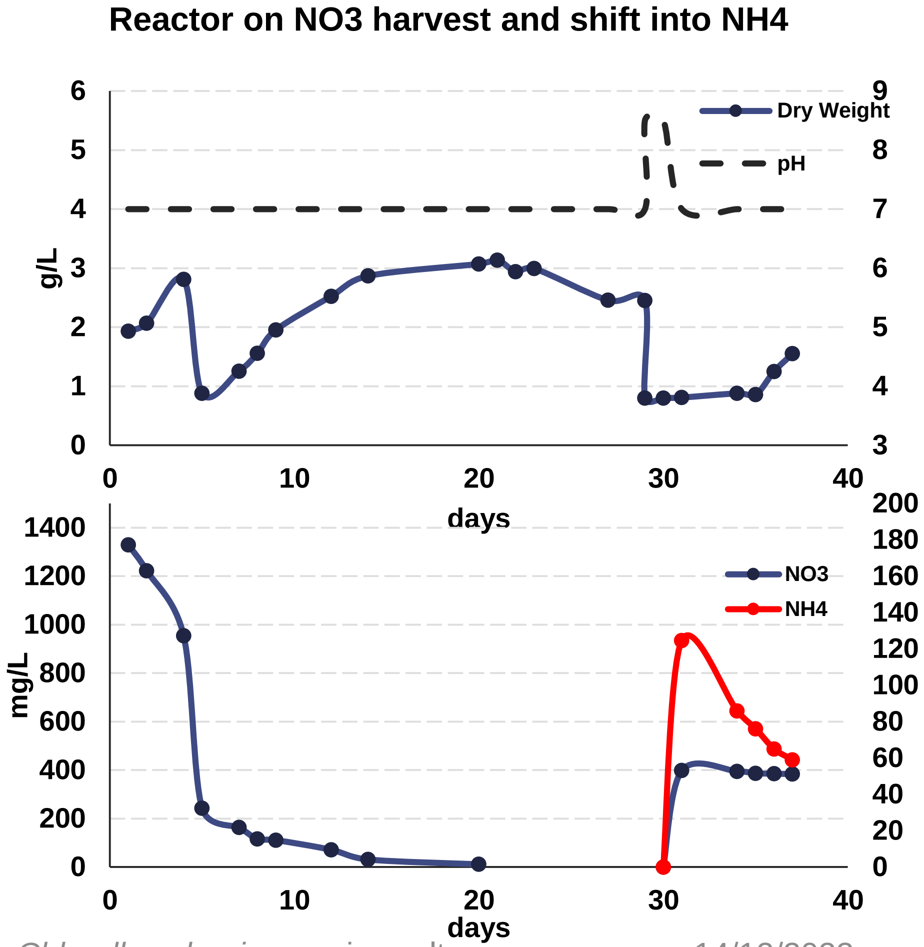
Effect of aeration on NH3 stripping:



Continue **aeration is OK** during treatment

NH_4 stops N starvation: (accumulation phase)

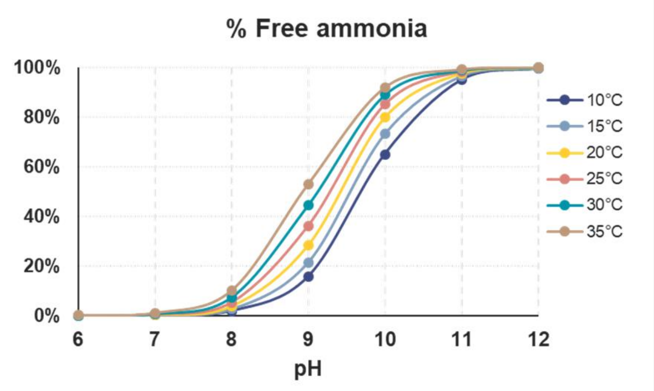
- Harvest after detection of contaminant
- Reinoculation in NO_3 + NH_4



Conclusions

- **Ammonia** is an efficient tool to control *P. malhamensis* in *C. vulgaris* massive cultures: 20-40 mg/L of **NH_3** during 12-24h.

- **NH_3** can be provided by **NH_4^+**:
  1. Controlling the pH around 8.5, affected by: photosynthesis, NH_4 uptake, CO_2 injection
  2. Close look at temperature (summer vs winter conditions)



- **NH_3** stripping is negligible (do not stop aeration)

- Treatment feasible:
  1. **While** culturing
  2. **After** reinoculation with contaminant

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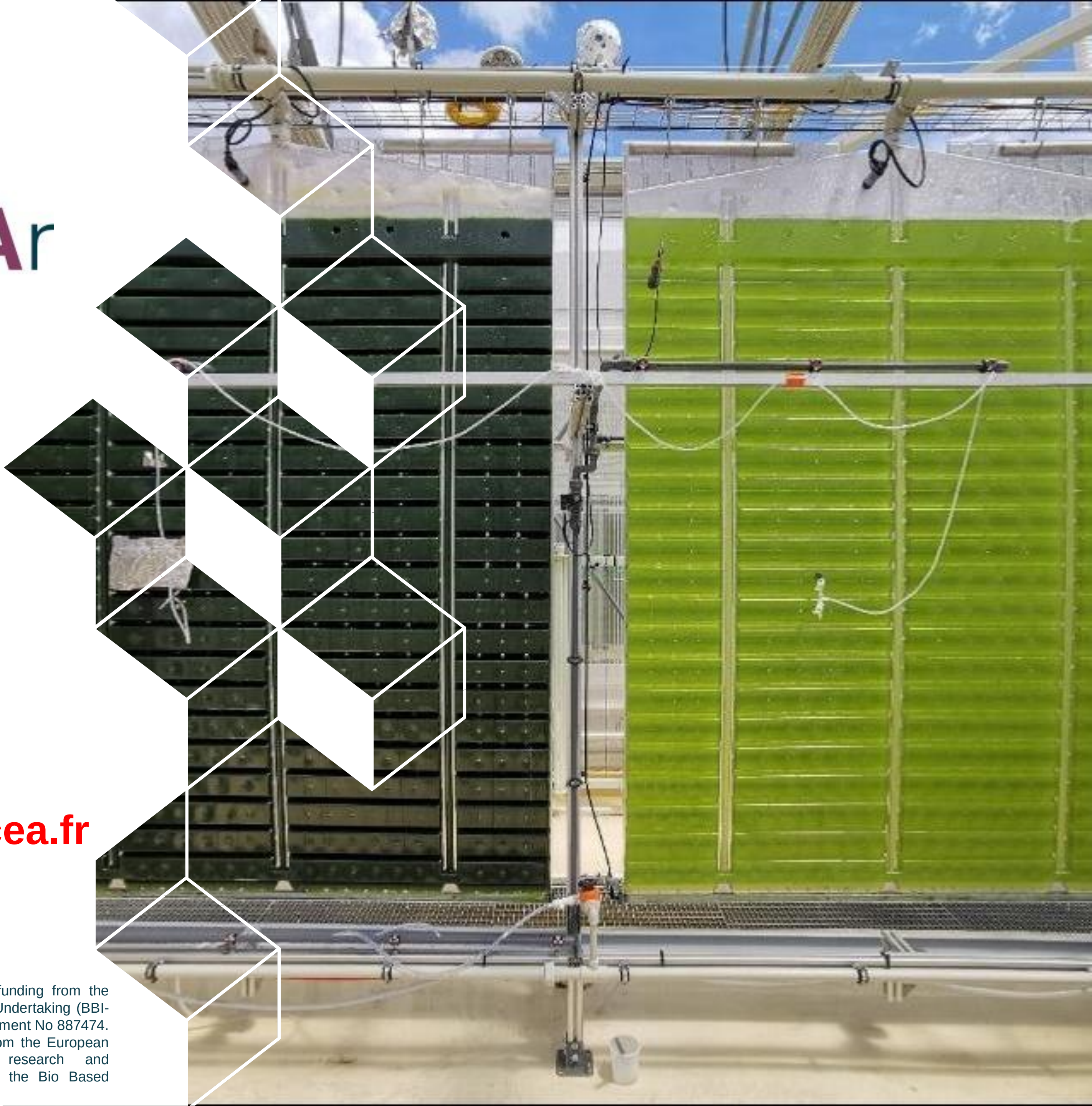

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