Demonstration of a facilitation between microalgae to face ammonia toxicity in a High Rate Algal Pond

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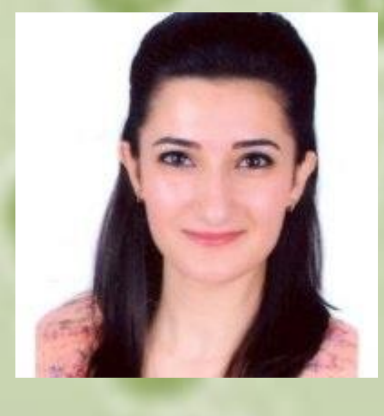

Demonstration of facilitation between microalgae to face ammonia toxicity in a High Rate Algal Pond

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INTRODUCTION

Wastewater treatment using extensive microalgae cultivation in High Rate Algal Ponds (HRAPs) has gained increasing interest for bioremediation and potential biomass valorization. The presence of growth inhibitors in such systems may damage the algae culture and thus influence the stability and economic viability, which are of crucial importance during the wastewater treatment process.

Several studies highlighted the importance of biotic factors, particularly microalgal diversity, in maintaining stable biomass productivity in HRAPs (Cho et al. 2017, Gales et al. 2019). However, in most studies, the successional trends of microalgal species growing in HRAPs have been interpreted as responses to predation and/or seasonal factors, while much fewer studies have discussed the role of positive interactions.

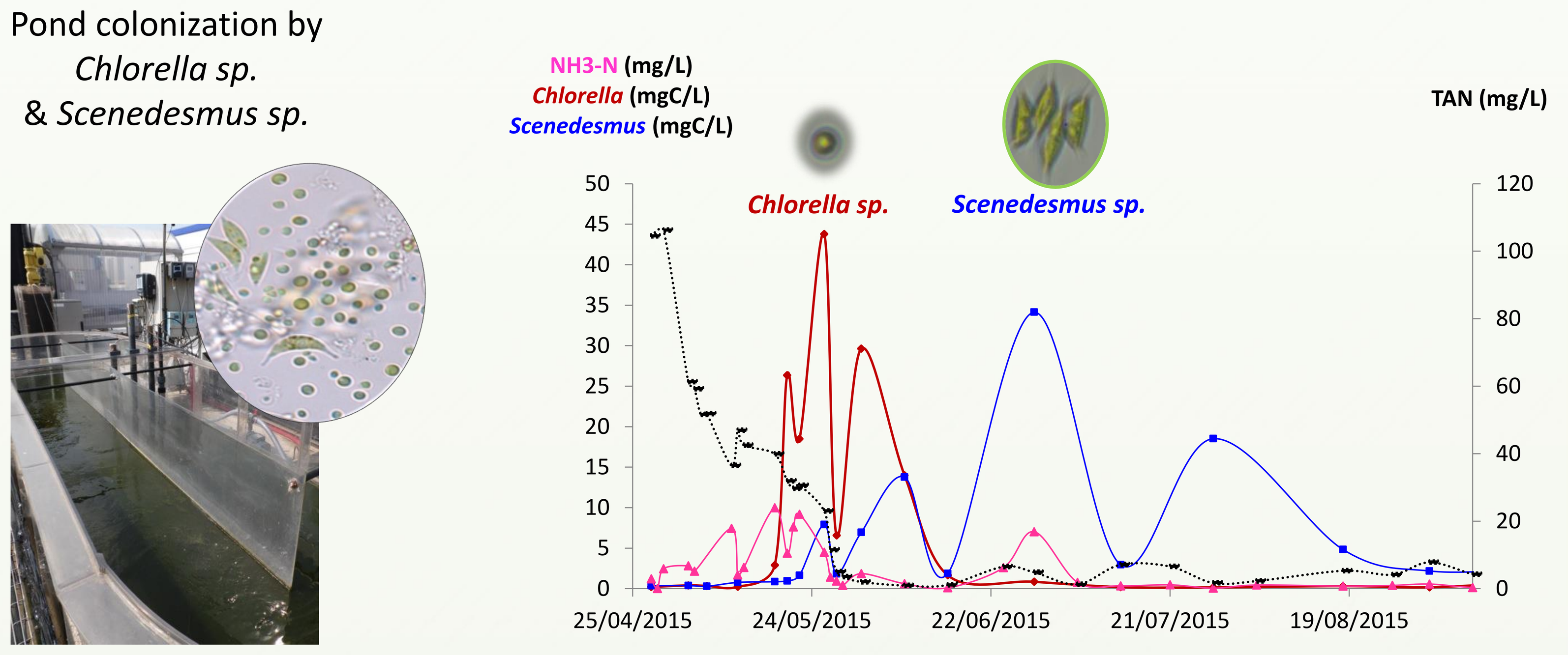
In this study, we validate using both experimental and modeling grounds on single strains the hypothesis of positive interaction between the common freshwater microalgal species (*Chlorella sp.* and *Scenedesmus sp.*) isolated from HRAP under external stress (free ammonia (NH_3) toxicity) during the colonization phase of HRAP.

SUCCESSION OF TWO DOMINANT SPECIES IN HRAP

Is the **succession** of two algal species **due to ecological facilitation?**

Has Chlorella reduced Scenedesmus inhibition by ammonia toxicity?

Pond colonization by *Chlorella sp.* & *Scenedesmus sp.*



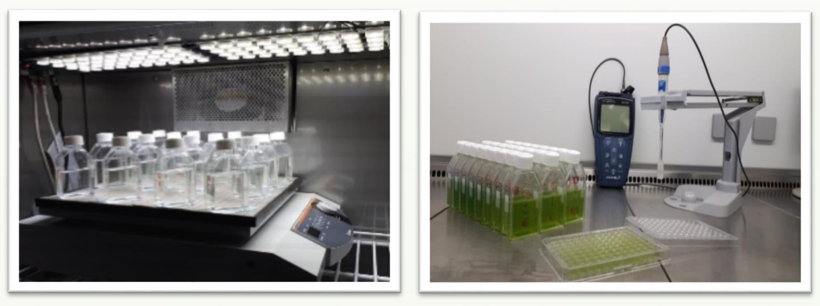
Existing data from Pilot scale HRAP in Northern France (Gales et al. 2019)

Species were identified from microscopy and DNA sequencing while their biomass are given by cell count from flow cytometry converted to mg C/L. NH_3 is determined as a function of Total Ammonia Nitrogen TAN, pH and temperature T (Emerson et al. 1975)

METHODS

1/ Experimental approach

- ✓ **Strains isolation from pilot scale HRAP**
- ✓ **Growth monitoring under different conditions of**
  - **T**otal **A**mmonia **N**itrogen (1,2 – 188 mg/L)
  - **pH** (6 - 9)



Batch cultures monitoring

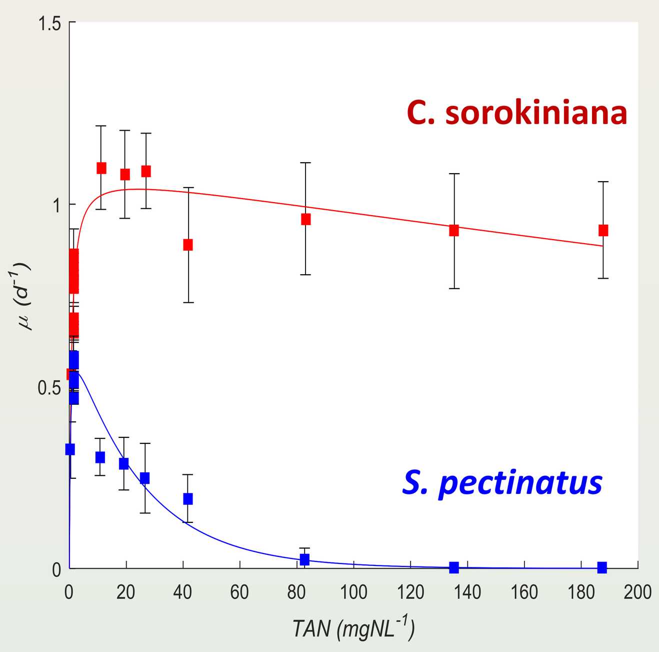
- Physico-chemical analyses: TAN & pH
- cell mass measurements: fluorescence & particulate organic carbon
- DNA isolation, PCR and sequencing (Sanger method)

2/ Modeling approach

- ✓ **Calibration of the growth functions parameters on experimental data in monoculture**
- ✓ **Mathematical simulation of both species in assembly**
- ✓ **Model validation on HRAP data**

RESULTS

1/ Growth kinetics of *C. sorokiniana* and *S. pectinatus* as functions of the limiting resource (TAN) & the toxic nitrogen form (NH₃)



Proposed growth function (*) $\mu(TAN, NH_3) = \mu_{max} \frac{TAN}{TAN+k} e^{-\frac{NH_3}{ki}}$

μ_{max}: the maximum growth rate (d^{-1}),

k: the affinity to the substrate (mgL^{-1}),

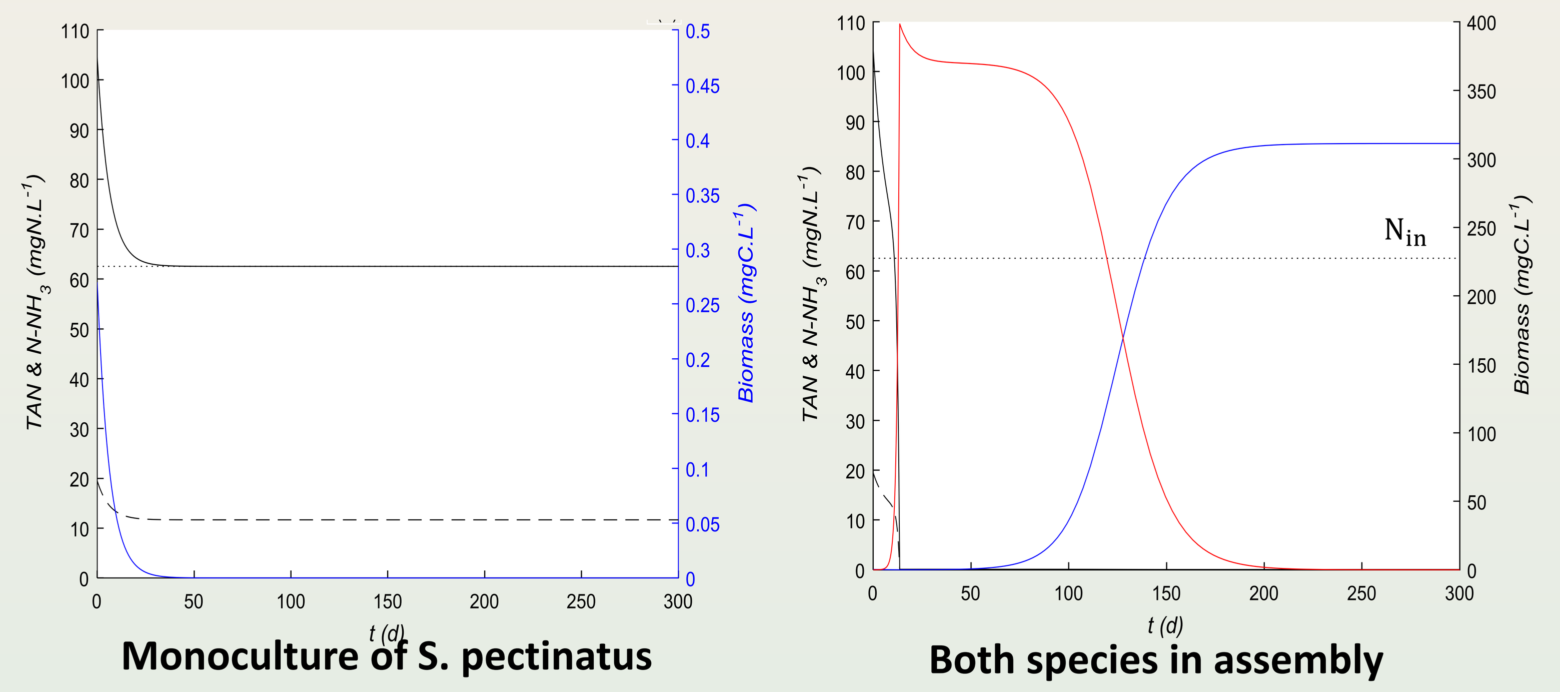
k_i: the inhibition constant of free ammonia nitrogen (mgL^{-1}).

Parameters	*C. sorokiniana*		*S. pectinatus*
μ_{max} (d^{-1})	1.04	>	0.59
k (mgN/L)	0.59	>	0.15
ki (mgNH$_3$-N /L)	130.2	>	2.313

C. Sorokiniana: fast, tolerant to high NH_3: **Pioneer species**

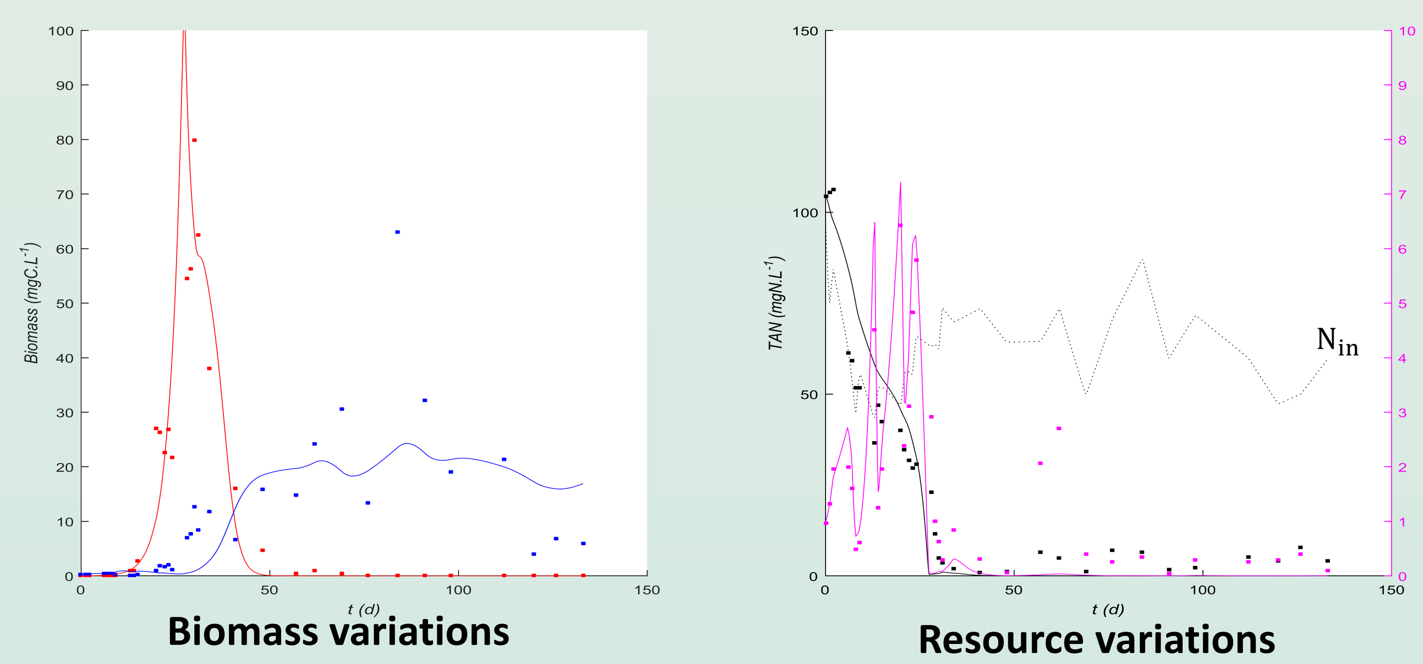
S. pectinatus : low, sensitive to high NH_3 with strong affinity: **Specialist species**

2/ Prediction of species dynamics under a constant dilution rate (*D=0.16* d^{-1}) and a high TAN concentration (N_{in}=*62.5* mgL^{-1})



Monoculture of S. pectinatus	**Both species in assembly**
S. pectinatus is washed out from the pond in the absence of *C. Sorokiniana*	Pond colonization by *S. pectinatus* thanks to the initial presence of *C. sorokiniana*
Non-resilient system	**Facilitation followed by competition**

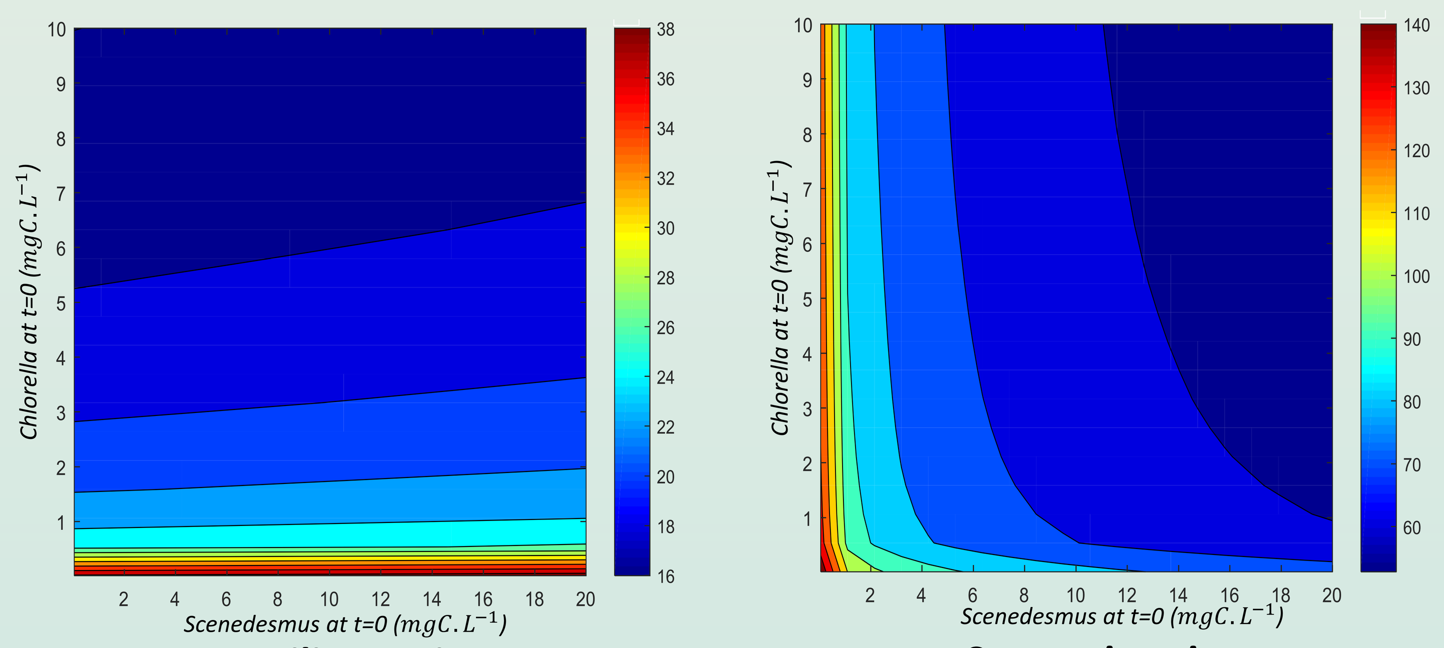
3/ Validation of facilitation in HRAP under fluctuating conditions of N_{in}, pH & T and a constant dilution rate (D=0.16 d^{-1})



Biomass variations **Resource variations**

- Comparison of model simulation to HRAP data using:
- calibrated growth functions from (*),
- mortality terms to take into account the grazing effect on each algal species in the HRAP,
- smaller yield parameters to take into account the presence of denitrifying bacteria in the HRAP.

4/ Resilience and succession times as proxies of the facilitation efficiency



Resilience time **Succession time**

- To ensure a rapid reduction of NH_3 toxicity and rapid development of *Scenedesmus* (< 16 days)

→ Bioaugmentation by increasing the initial concentration of *Chlorella up* to 5 mgC/L.

- To ensure a rapid succession occur (< 60 days)

→ Bioaugmentation by increasing the initial concentration of *Scenedesmus* up to 12 mgC/L.

CONCLUSIONS

- **The first colonization of HRAP by microalgae under a severe chemical condition derives from the rapid growth of pioneer species, such as *C. sorokiniana*, which facilitated the subsequent colonization of low growth specialists such as *S. pectinatus*.**
- **The microbial successions are not only regulated by climatic conditions or predation, but also by interactions between species based on the ability to modify their growth conditions.**
- **The control of algal production in HRAP under chemical stress may be possible by modifying the initial populations' densities in the pond.**

References :

- Cho, D.-H. et al. Microalgal diversity fosters stable biomass productivity in open ponds treating wastewater. Sci. Rep. 7, 1979 (2017).
- Gales et al. Importance of ecological interactions during wastewater treatment using High Rate Algal Ponds under different temperate climates. Algal Res. 40, 101508 (2019).
- Emerson, K., Russo, R. C., Lund, R. E. & Thurston, R. V. Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Board Can. 32, 2379–2383 (1975).