Algae4IBD
Health from aquatic resources

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MICROALGAE AS A POTENTIAL FUNCTIONAL INGREDIENT FOR AQUAFEEDS: ENHANCING ANTI-INFLAMMATORY ACTIVITIES THROUGH CULTIVATION FACTORS

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Introduction

- Aquaculture is the fastest-growing food production sector that uses aquafeeds formulated with ingredients that fish do not consume in their natural environment.
- Pro-inflammatory ingredients (e.g. soy) in aquafeeds can promote foodborne inflammation which, and can cause inflammatory bowel disease (IBD) in fish (Peng et al 2020).
- Microalgae are promising sources of bioactive compounds with antioxidant, anti-inflammatory and cancer-preventive properties. **The application of microalgae with enhanced bioactivity that allow the prevention and alleviation of IBD adds value to the biomass and can be leveraged through its application in aquafeeds, as a mitigation strategy of the pro-inflammatory ingredients commonly present in fish diets, to improve animals' biological performance**.

GOAL

- **Optimize the cultivation of microalgae species to promote target-bioactive compound induction to mitigate inflammatory bowel disease**
- **Investigation of metabolites induction through nutritional modulation in *Tetraselmis chui* and *Phaeodactylum tricornutum* in different cultivation modes**

Methods

Experimental design

Nutrients (NO_3/PO_4) modulation

T. chui			*P. tricornutum*		
Fed-batch	Batch	Replenishment	Fed-batch	Batch	Replenishment

Batch: Single application of nutrients in the beginning of the cultivation
Fed-Batch: Daily adjustment of the target nutrient to a specific concentration
Replenishment: Batch with nutrient replenishment 24h prior to sampling

Nitrate trial
- Batch: 3 mM NO_3
- Fed-batch: 2 mM NO_3
- Replenishment: 2 mM NO_3

Phosphate trial
- Batch: 0.15 mM PO_4
- Fed-batch: 0.25 mM PO_4
- Replenishment: 0.25 mM PO_4

Microalgae cultivation and analysis

Microalgae culture analysis	
Nitrate concentration	Ultraviolet spectrophotometry (Armstrong, 1963)
Phosphate concentration	Spectrophotometry - Spectroquant®, Supelco® (Merk)
Microalgae dry weight (DW) in culture	Spectrophotometry with a calibration curve
Culture stress evaluation (Maximum Quantum Yield of PSII; Fm/Fv)	Chlorophyll *a* fluorescence - AquaPen 110-C (Photon systems instruments)

Microalgae cultivation conditions	
Temperature	22 °C for *T. chui*, 17 °C for *P. tricornutum*
Irradiance (L:D)	75-0 µmol.m^{-2}.s^{-1}, 14 Light : 10 Dark
System	100 mL flasks in incubated shaker (*n*=3), working volume 50 mL

Results

Nitrate modulation during cultivation

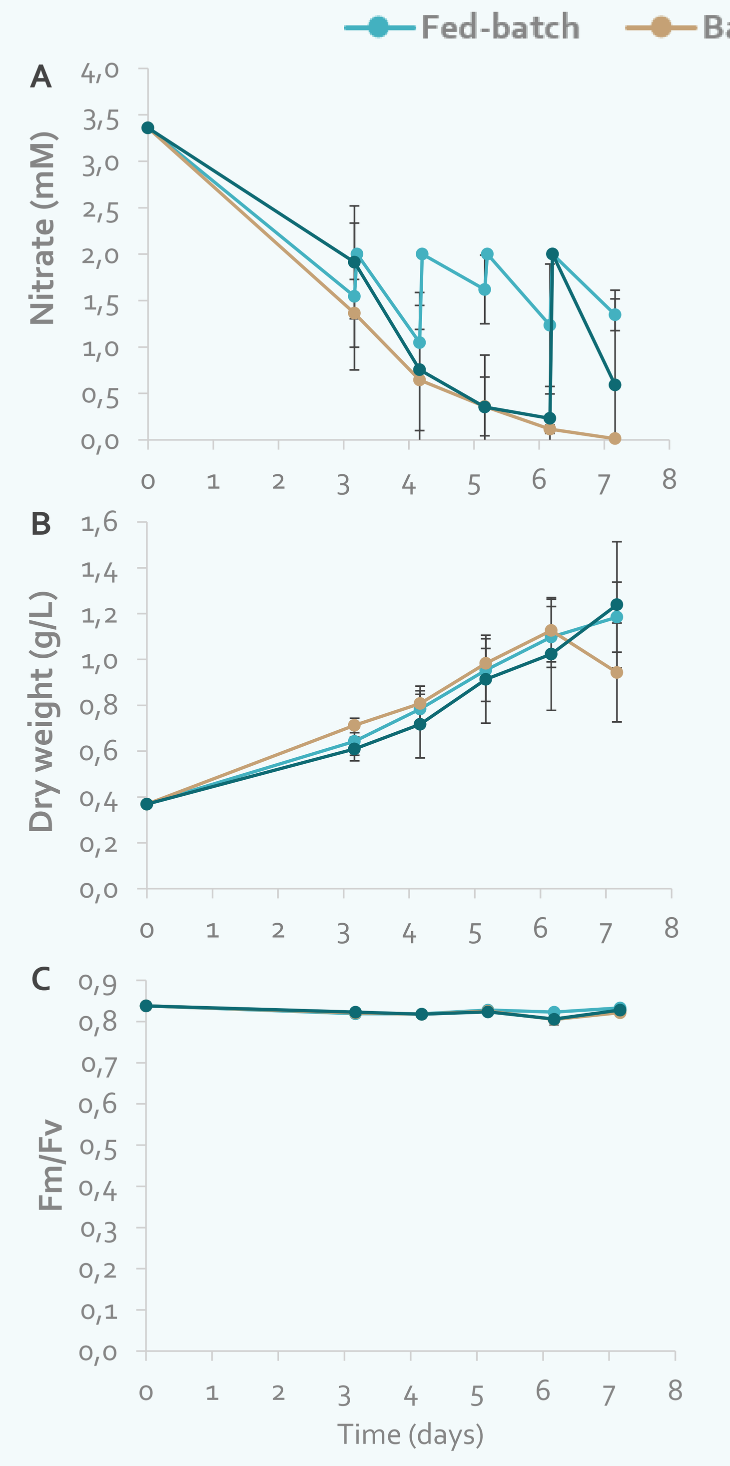

Fig. 1: *T. chui* metabolite induction by nitrate modulation throughout time: A) Nitrate concentration, B) Microalgae dry weight, and C) Maximum quantum yield of PSII (*n*=3)

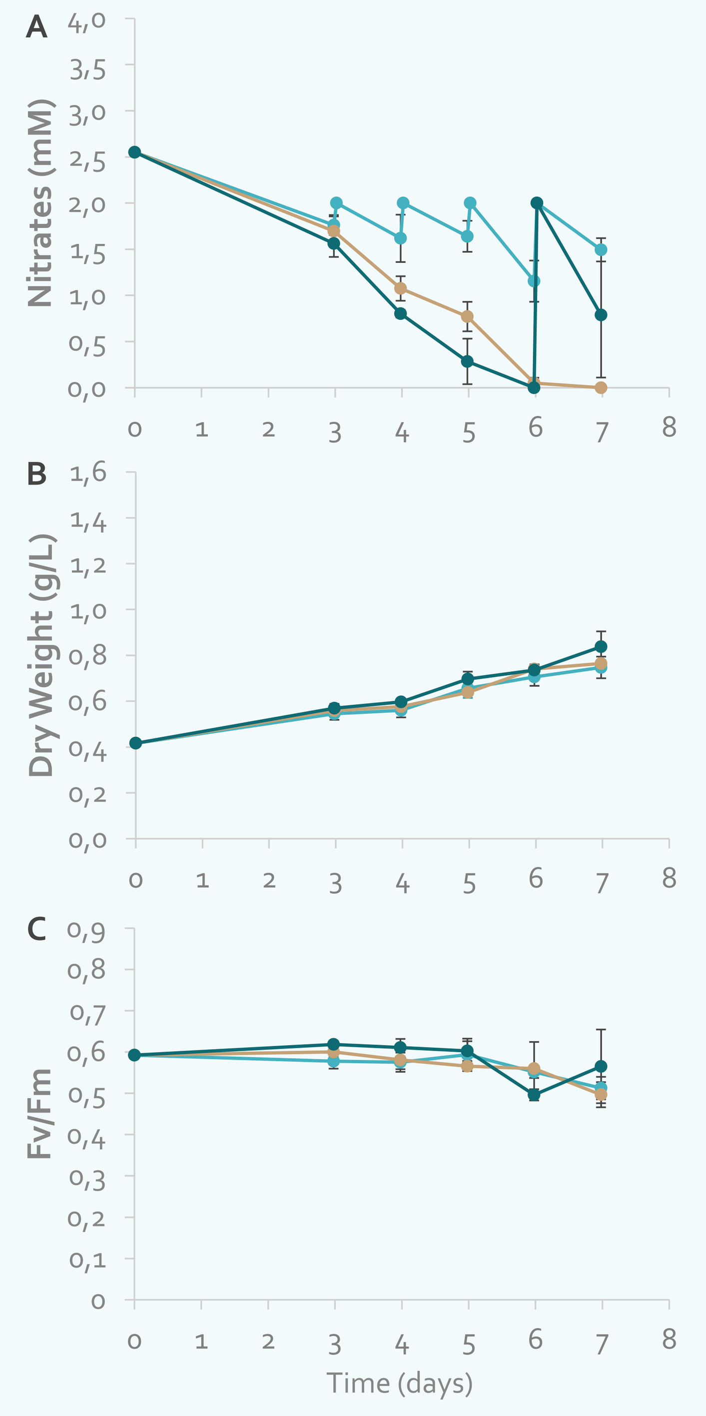

Fig. 2: *P. tricornutum* metabolite induction by nitrate modulation throughout time: A) Nitrate concentration, B) Microalgae dry weight, and C) Maximum quantum yield of PSII (*n*=3)

- Nitrate was fully consumed in 7 days of batch. Although no significant changes in final DW were observed, *T. chui* showed a significant superior specific growth (µ = 0.165 ± 0.006 d^{-1}) compared to *P. tricornutum* (µ = 0.090± 0.004 d^{-1}). After starvation, replenished cultures showed higher nitrate consumption than fed-batch showing that nitrate limitation led to stress in PSII where Fv/Fm values were significantly lower in batch compared to the other conditions.

Phosphate modulation during cultivation

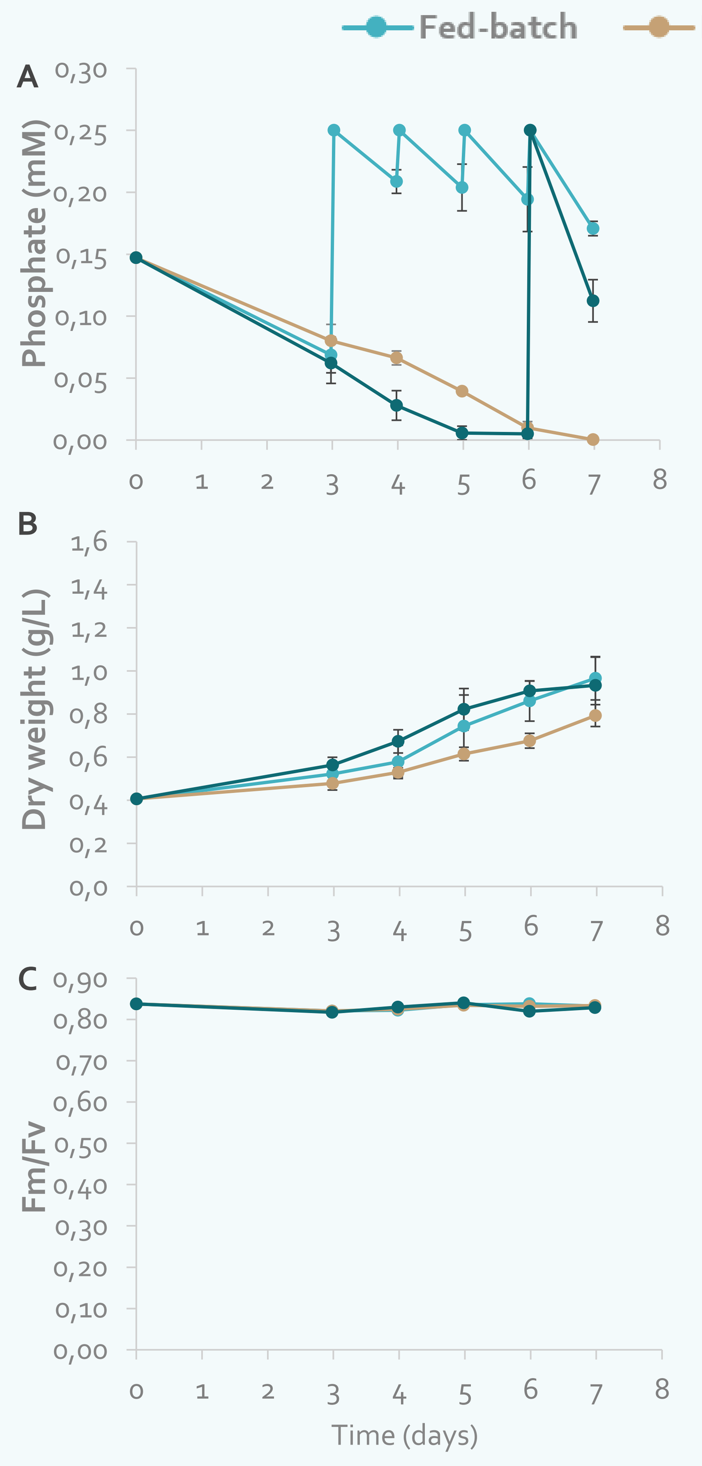

Fig. 3: *T. chui* metabolite induction by phosphate modulation throughout time: A) Nitrate concentration, B) Microalgae dry weight, and C) Maximum quantum yield of PSII (*n*=3)

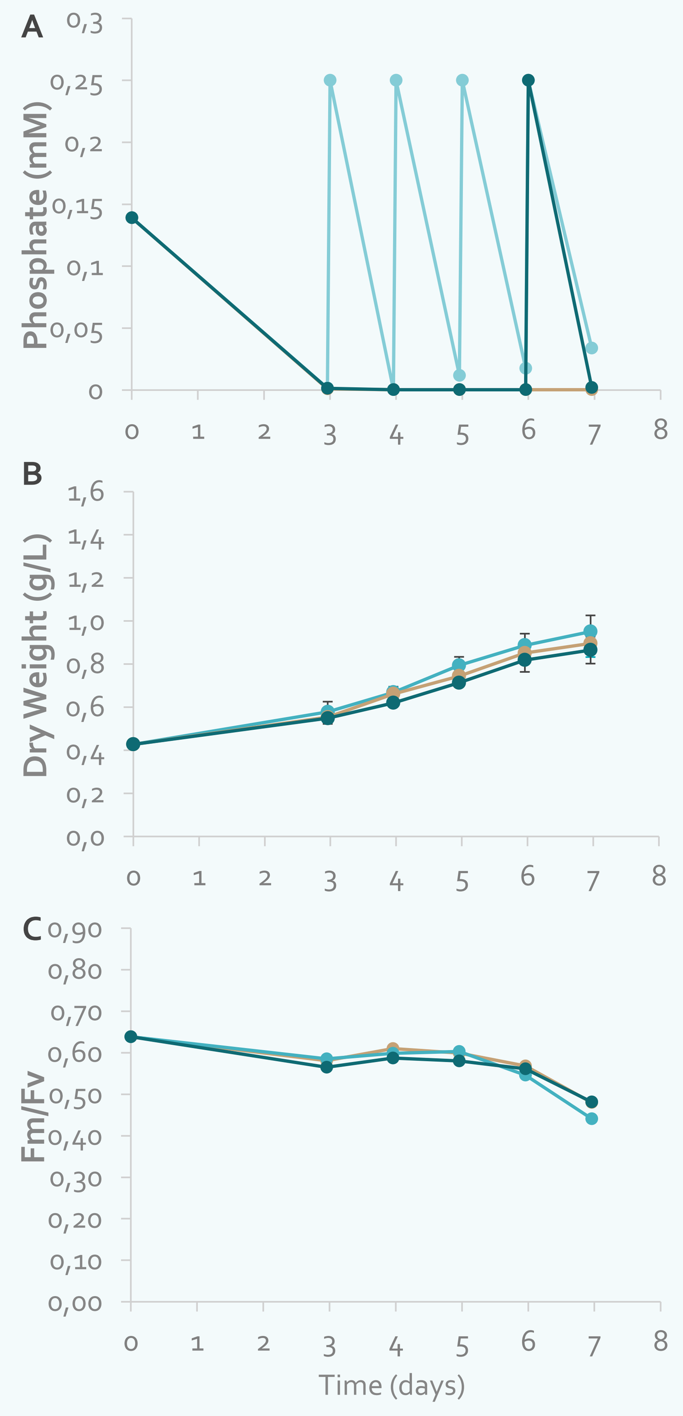

Fig. 4: *P. tricornutum* metabolite induction by phosphate modulation throughout time: A) Nitrate concentration, B) Microalgae dry weight, and C) Maximum quantum yield of PSII (*n*=3)

- Phosphate was fully consumed in 5 and 3 days in batch for *T. chui* and *P. tricornutum*, respectively. In contrary to *T. chui*, *P. tricornutum* fully depleted phosphate daily under fed-batch. No significant changes in growth were observed between conditions. After starvation, replenished cultures showed higher phosphate consumption than fed-batch showing phosphate limitation was ongoing although no stress was observed in PSII nor in growth.

Biomass samples of each species and each condition were sent to Algae4IBD partners to determine the effect of nutrient modulation on active compounds with potential against IBD

Take Home Message

- Growth was similar between all conditions per microalgae but *T. chui* revealed a higher specific growth rate than *P. tricornutum* in the nitrate trials
- *P. tricornutum* required higher levels of phosphate than *T. chui*
- There was a higher nutrient consumption after microalgae were grown under fed-batch mode

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Next steps

- Repeat induction strategies with other species with high potential against IBD
- The most productive species with IBD potential will be selected for scale-up
- Biomass will then be delivered for new product development against IBD

Bibliography

Peng, K.S., Wu, N., Cui, Z.W., Zhang, X.Y., Lu, X.B., Wang, Z.X., chen, D.D., Zhang, Y.A., 2020. Effect of the complete replacement of dietary fish meal by soybean meal on histopathology and immune response of the hindgut in grass carp (*Ctenopharyngodon idellus*). Vet. Immunol. Immunop. 221, 110009

Armstrong, F.A.J., 1963. Determination of nitrate in water by ultraviolet spectrophotometry. Anal. Chem. 35 (9), 10–12. https://doi.org/10.1021/ac60202a036, 10–12.