Optimization of an organic medium for industrial production of a protein-rich *Arthrospira platensis*

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Introduction

The world population is expected to increase by 2 billion by 2050, leading to a boost in demand for agricultural products by at least 50% [1],[2]. This increase will result in competition for natural resources, increased greenhouse gas emissions, and further deforestation and land degradation.

Arth*rospira platensis* is a protein-rich cyanobacterium approved as food, containing all the essential amino acids and high levels of minerals, and pigments. It is a sustainable food product, since it can grow in non-potable water on non-arable land [3].

Microalgae cultivation must follow agricultural trends, currently moving towards organic food production since consumers associate this practice as a more safe, sustainable, and healthy alternative.

In this context, an organic medium suitable for the industrial production of *A. platensis* was optimized using a design of experiment methodology under lab-scale conditions.

Methodology

Two types of tests were performed to optimize culture media: one-variable-at-a-time (OVAT), to test the salt need and to compare the different commercial organic media, and Design of Experiments (DOE). The DOE was performed in Design Expert® software where a 3 Factor Box Behnken design with 4 center points was used for the RSM methodology.

The assays were performed at lab-scale, in 35-mL reactors with constant light, temperature and aeration.

All the ingredients used in the organic medium followed the norms of the Regulation (EU) 2021/1165.

Results and discussion

A. platensis was grown with different organic media against an inorganic control (based on Zarrouk medium). The organic media were supplied in fed-batch and supplemented with 3 gL^{-1} of commercial salt.

Table 1 – Media characterization.

Medium solution	N Source	[N] (mM)	N/P ratio	Suplementation		
				Iron	Micronutrients	Sodium
Inorganic	NO_3	30	11.5	✓	-	$NaHCO_3$
MNBio (2020)	NH_4	2	7.6	✓	✓	NaCl
MNBio (2021)	NH_4	2	11.3	✓	✓	NaCl
MNBio (N)	NH_4	2	37.9	✓	✓	NaCl
Residue	Complex	Unk	-	-	-	NaCl

Table 2 – Global productivities of *A. platensis* in the different media.

Global Productivity	Biomass ($gL^{-1}day^{-1}$)	Protein ($mg\ L^{-1}day^{-1}$)	Phycocyanin ($mg\ L^{-1}day^{-1}$)
Control	0.234±0.014^a	101.5±8.6^a	12.1±1.5^c
MNBio(2020)	0.128±0.006^b	42.2±7.2^b	8.2±2.0^c
MNBio(2021)	0.160±0.017^{ab}	81.4±21.9^a	19.6±3.6^b
MNBio(N)	0.183±0.015^{ab}	93.0±20.3^a	27.8±4.0^a
Residue	0.043±0.041^c	31.9±26.3^b	2.1±1.8^d

- MNBio(N) allowed to obtain the highest biomass, protein and phycocyanin productivities, being chosen to the optimization step.

Optimization through Design of Experiements

The optimized variables were the salt concentration (A) (3-16 g L^{-1}), supplementation of micronutrient (B) (0-2.2 mL L^{-1} of MNBio (N)) and iron (C) (0-1.6 µM). The MNBio (N) was added as needed to maintain an NH_4 concentration between 0.4 and 2 mM.

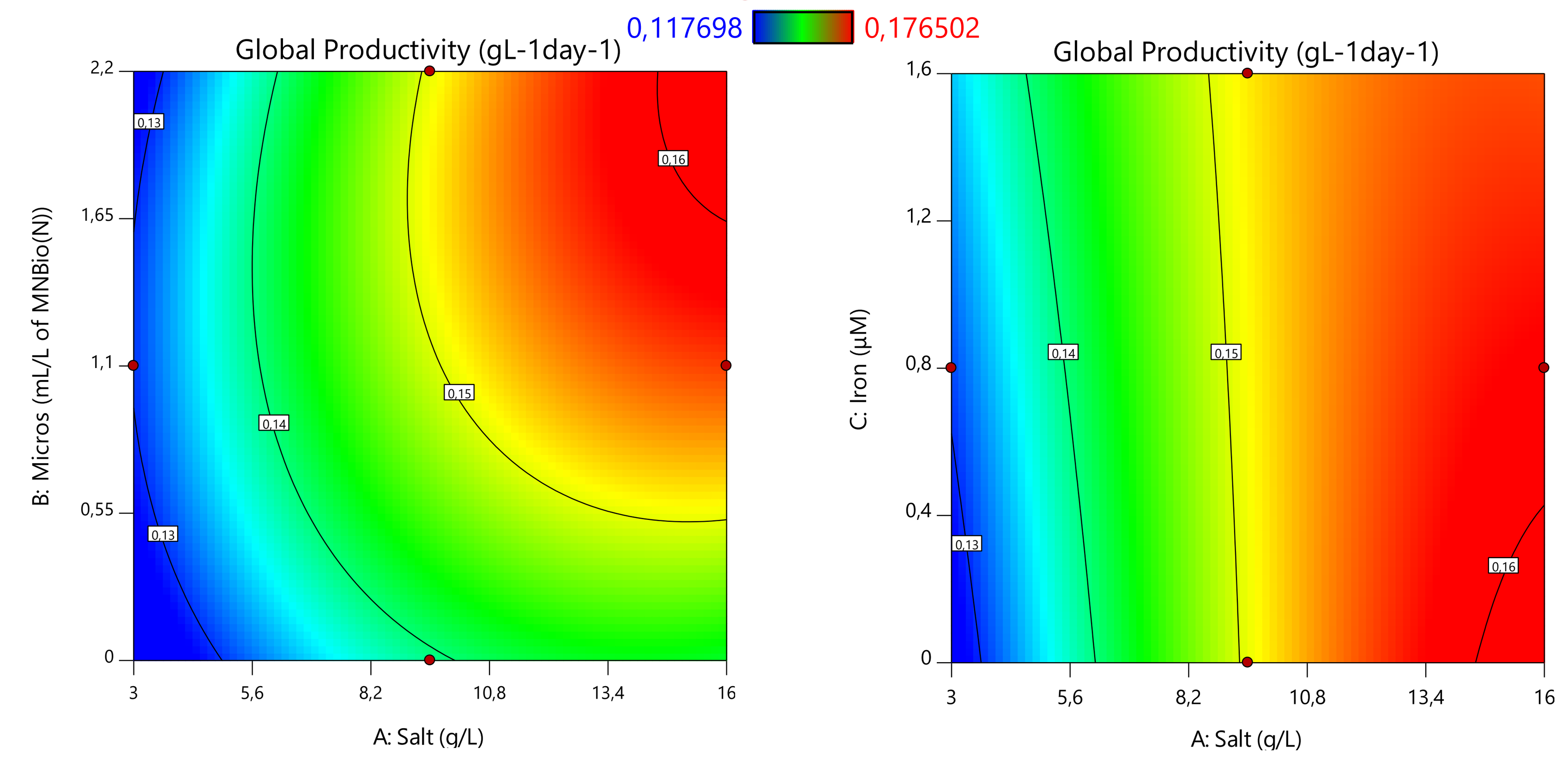

Figure 1 – Variations on global productivity (GP) obtained through the model GP(g L^{-1} day^{-1})=0.1130+0.0042 A-0.0090 B-0.0065 C-0.0006 AB-0.0006 AC -0.0001 A^2-0.0038 B^2, R^2=0.9738, adjusted R^2=0.9508 and a predicted R^2=0.7860. [Iron]=0 µM (left) and [Micronutrient]= 2.2 mL L^{-1} (right).

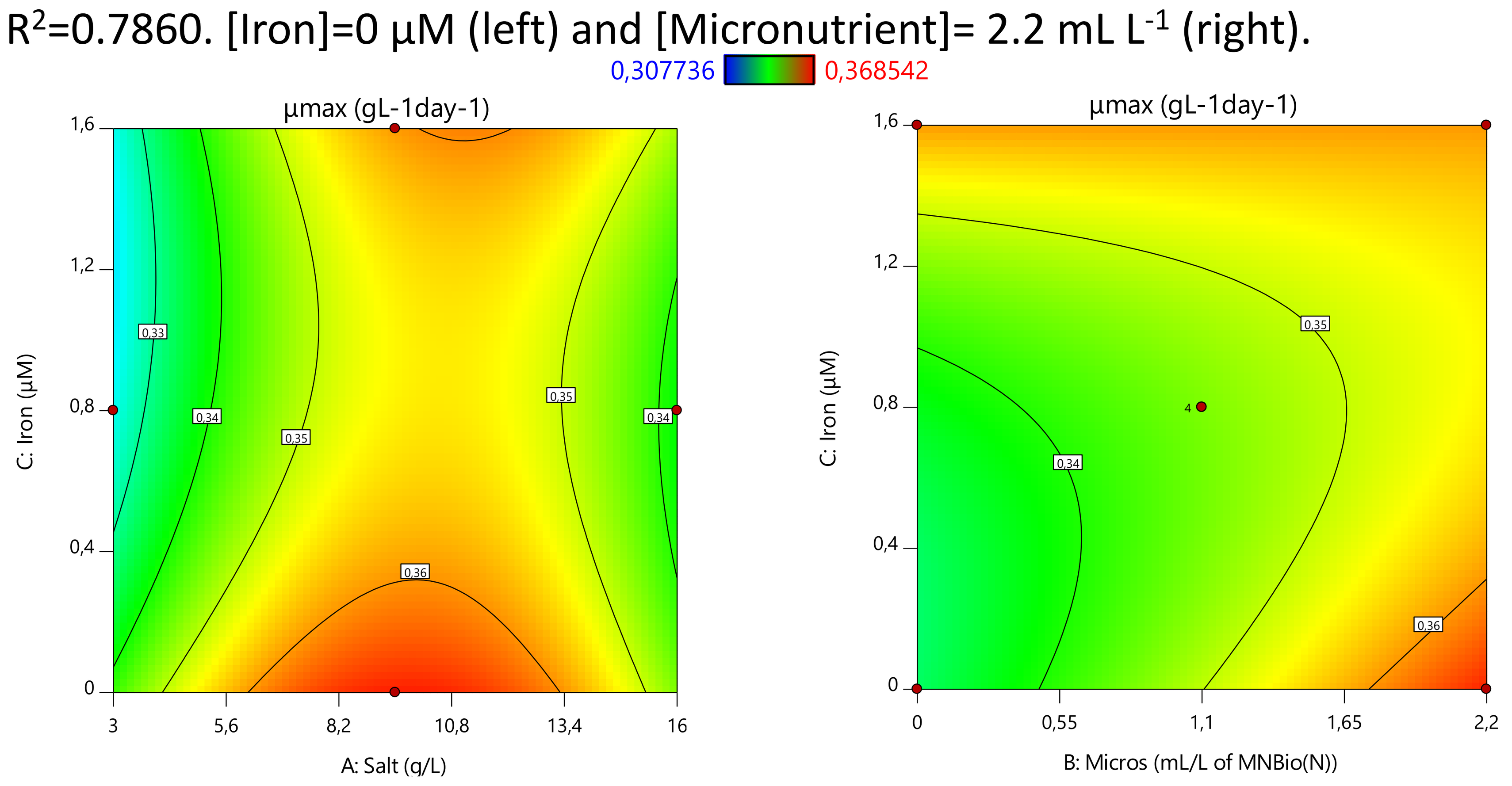

Figure 2 – Variations on the μ_{max}obtained through the model μ_{max}=0.2807+0.0107 A+0.0157 B-0.016 C+0.0010 AC-0,0097 BC-0.00056 A^2+0.0139 C^2, R^2=0.9502, adjusted R^2=0. 9067 and a predicted R^2=0.79220. [Micronutrient]= 2.2 mL L^{-1} (left) and [Salt]=9.5 g L^{-1} (right).

Conclusions

- From the studied media, MNBio(N) is the one that allows obtaining the highest biomass, protein, and phycocyanin productivities.
- To maximize the global productivity and μ_{max} *A. platensis* should be grown using MNBio (N) with a low ammonium content supplied by fed-batch, a concentration of salt between 11 and 13 g L^{-1} micronutrient supplementation close to 2.2 mL L^{-1} of MNBio (N) and without iron supplementation.

Future work

- Analyse the protein content of the runs and create a model.
- Validate the results in outdoor flat pannels

References

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