Increasing Oxygen Productivity of *Arthrospira* sp. PCC 8005 using alternative nitrogen sources: A bioengineering and proteomic outlook.

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The BIORAT-2 project aims at reaching a new step in the development of the MELiSSA Loop by making it self-sufficient in meeting the oxygen (O_2) requirements and simultaneously increasing the degree of freedom, in terms of nitrogen (N) source use. Thus, in order to realize this objective and harness the potential of ammonium (NH_4) as an alternative N source for the cultivation of *Arthrospira* sp. PCC 8005; its effect was evaluated (with respect to nitrate; NO_3) on the O_2 productivity, biochemical and proteomic profile of the cyanobacteria.

Thus, a comprehensive meta-analysis (stoichiometric, proteomic and biochemical) was performed, to investigate the adaptation of *Arthrospira sp.* cells to fluctuating stream (transition) of alternative N sources (NH_4 and NO_3). The Photosim Model [1,2] was adapted to the BIORAT-2 conditions and used for the prediction of O_2 productivity under the two N regimes. The present study, evaluated the effect of transition between the two N sources (NH_4 and NO_3) on O_2 productivity, stoichiometric yields, metabolic, proteomic, biochemical profile of *Arthrospira* sp. PCC 8005 biomass. This study, thus not only focused on increasing the degree of freedom of the MELiSSA loop, but it also opened new avenues for the use of fluctuating stream of alternative N sources (from wastewater stream) for production of *Arthrospira* sp. PCC 8005, effectively bringing the MELiSSA technology as step closer towards embedding circular economies with photosynthetic biorefineries.

Biochemical Profile: Experimental Data vs Stimulated Data

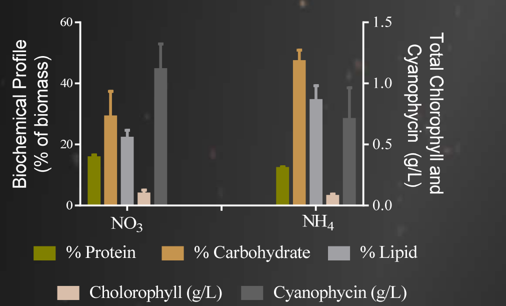

Fig. 1: Biochemical profile of *Arthrospira* sp. under NH_4 and NO_3 regimes.

Higher lipid and carbohydrate content could be attributed to the prevalence of N deplete (8.5 mM) conditions in the PBR[3].

Table 1: A comparative of biochemical and elemental composition of *Arthrospira* sp. PCC 8005 biomass cultivated under BIORAT-2$^{\Delta}$ conditions vs the parameters of Classical model (BIORAT-1) $^{\phi}$.

N Source	% Protein*	% Lipid*	% Carbohydrates¥ *§		Elemental Composition
			EPS	UA	
$^{\Delta}NH_4$	33.35	20.64	17.98	26.28	$CH_{1.81}O_{0.480}N_{0.17}$
$^{\Delta}NO_3$	37.38	12.19	12.56	10.01	$CH_{1.81}O_{0.48}N_{0.18}$
$^{\phi}$Photosim Model/ NO_3	48.07	9.6	25.52		$CH_{1.57}O_{0.459}N_{0.173}$

$^{\Delta}$: Cultivated in PBR at pH 8.5, 8.5 mM N (NH_4Cl or $NaNO_3$), 36 °C; $^{\phi}$: Cultivated at pH 9.5, 28 mM $NaNO_3$, 30 °C; *Reported as % of biomass; ¥ Value reported as sum of analyte in biomass and supernatant; EPS: Exopolysaccharide; UA: Uronic Acid.

Biomass cultivated with 8.5 mM NO_3 and NH_4 exhibited different elemental composition, higher lipid and exopolysaccharide content; indicating at the prevalence of N deplete conditions in the culture[3].

Oxygen Productivity and Yield : Experimental vs Simulated

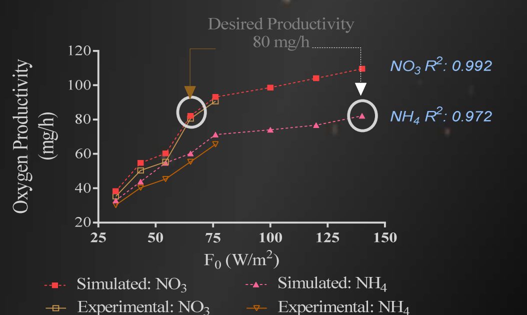

Fig.2: A comparative of experimental and simulated O_2 productivities under NH_4 and NO_3 regimes.

90 ± 2% match obtained between simulated and experimental values.

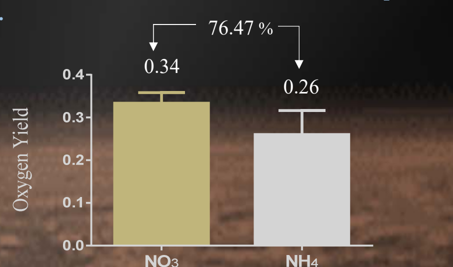

Fig.3: Average Oxygen Yields (w/w biomass) of *Arthrospira* sp. under NH_4 and NO_3 regimes.

76.47 % lower O_2 yield under NH_4 regime (vs NO_3) attributed to their stoichiometric difference.

N Regime and Proteomic Profile

Table 2: Effect of N regime (NO_3^- on NH_4^+) on the proteomic profile of *Arthrospira* sp. PCC 8005. *(p value <0.05, number of peptides > 1 and fold change ≥ 1.5 or ≤ 0.66).*

Protein Name and Function	Fold Change (NO_3 on NH_4)	Number of peptide	p value
ARTHROv5_60547(NAD(P) H-quinone oxidoreductase)	1.95	10	$6.7e^{-3}$
ARTHROv5_61031(Photosystem II protein Y)	6.05	2	$1.5e^{-2}$
ARTHROv5_10689 (NADH: ubiquinone oxidoreductase)	3.91	3	$3.4e^{-2}$
ARTHROv5_30863 (NADH dehydrogenase C1)	1.6	5	$3.9e^{-2}$

- Protein involved in the functioning of Photosystem (PS) I and II seen to be significantly impacted by the N source.
- Higher abundance of energy transfer related proteins under NO_3 regime were in line with the stoichiometric difference between NO_3 and NH_4.
- Higher abundance of PS II related proteins under NO_3 regime was in line with higher pigment and O_2 yield of biomass fed with NO_3.

Conclusions

- NH_4 can be used as alternative N source for *Arthrospira* sp. cultivation under controlled condition of PBR.
- Photosim Model can easily adapt to alternative N sources provided biochemical and elemental profile of biomass are known.
- Simulated and predicted values of O_2 productivity (90± 2% match) indicated that PBR can be successfully coupled with consumer chamber to meet the O_2 needs of rodent.

Acknowledgement: This research was supported by the European Space Agency (ELIPS 4: BIORAT-2), BELSPO and UMONS.

1. Cornet et al., 1995. Chem. Eng. Sci
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