# 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

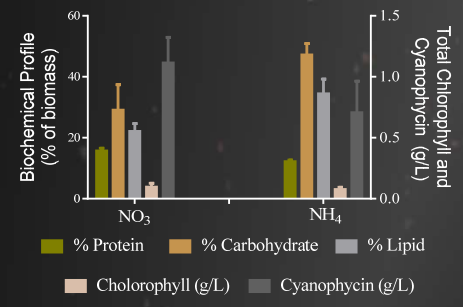


Fig. 1: Biochemical profile of *Arthrospira* sp. under $NH_4$ and $NO_3$ regimes.

***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$^{\S}$ *$^{\S}$ | | 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; $^{\S}$ 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

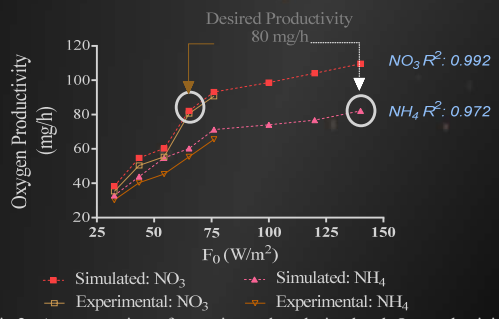


Fig.2: A comparative of experimental and simulated $O_2$ productivities under $NH_4$ and $NO_3$ regimes.

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

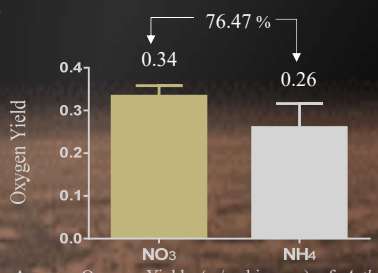


Fig.3: Average Oxygen Yields (w/w biomass) of *Arthrospira* sp. under $NH_4$ and $NO_3$ regimes.

***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.

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1. Cornet et al., 1995. Chem. Eng. Sci 2. Cornet and Dussap, 2009. Biotechnol. Prog. 3. Sachdeva et al., 2016. Bioresourc. Technol.2016.