# Response of *Arthospira* sp. PCC 8005 to modification of different nitrogen sources - A Case Study

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The European Space Agency, through its MELISSA project aims to harness the property of this photosynthetic cyanobacterium *Arthospira* sp. PCC 8005 to fulfill the basic human needs of oxygen and food production. This photosynthetic cyanobacterium essentially depends on light, carbon dioxide and nitrogen, in addition to few other micronutrients, for growth and metabolism. Preliminary studies using nitrate as the nitrogen source for cyanobacterial cultivation have shown promising results in terms of oxygen production. However, further research is required to demonstrate the efficacy of these results at the ground level (on actual consumers) and also to test the potential use of alternative/additional nitrogen sources such as ammonium and urea, with the aim to ultimately include human waste (urine) for the autotrophic cultivation of the cyanobacterium.

Ammonium ([NH4+]) (pKa 9.2) is known to change to gaseous ammonia ($[NH_3]$) at pH≥9.2[1]. $[NH_3]$ is known to poison the photosynthetic system of the cells[2]. Previous studies on *Arthospira* sp. using $[NH_4^+]$ as the nitrogen ([N]) source have indicated at its potential toxicity to the cells at concentrations higher than 3mM[1]. Nitrate ($[NO_3^-]$) is the most commonly used [N] source for *Arthospira* sp. cultivation, but is economically inviable. Furthermore, use of alternative [N] sources for the cyanobacterial cultivation could give a higher degree of freedom to the MELISSA project.

The present study attempts to study the growth profile of *Arthospira* sp. PCC 8005 cultivated in different [N] sources in addition/alternative to $[NO_3^-]$, with the aim to test the premise whether [NH4+] is toxic to cells at concentration greater than 3mM and eventually identify the optimal growth parameters (concentration & pH) wherein $[NH_4^+]$ is not inhibitory to *Arthospira* sp. PCC 8005 cultivation.

## Photobioreactor Study

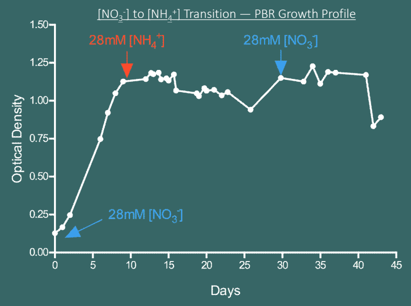


Fig.1 Growth profile of *Arthospira* sp. PCC 8005 in PBR on transition from 28mM $[NO_3^-]$ to 28mM $[NH_4^+]$, pH 8.5

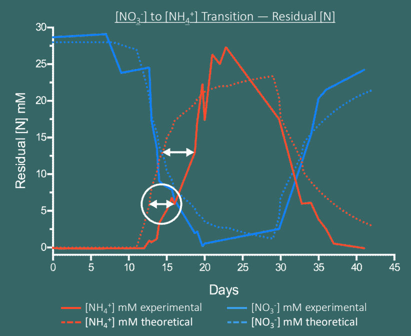


Fig.2. Experimental vs Modeled values of [N] assimilation in PBR from $[NO_3^-]$ to $[NH_4^+]$ in PBR, pH 8.5

The nutrient transition study in Photobioreactor (PBR) (28mM $[NO_3^-]$ to 28mM [NH4+], pH 8.5). Dilution rate and transition period (Fig.1) were defined through a fundamental (mathematical) model (Fig.2).The best fit between the experimental and theoretical concentrations of $[NH_4^+]$ was found around 8.5mM concentration (Fig.2) and the curve lines deviated at higher concentration with no mapping.

## Batch Study: Growth Profile

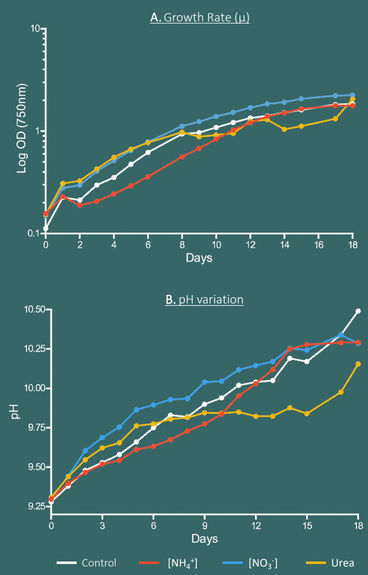


Fig.3 Growth rates & pH variations under batch cultivation of *Arthospira* sp. PCC 8005 under different [N] sources.

| Subset | Growth Rate (µ) (per day) | [N] Uptake Rate (per day) | [N] Assimilation Rate (per day) |
|---|---|---|---|
| 8.5mM $[NH_4^+]$ | 0.058 | 1.37 | 0.16 |
| 8.5mM Urea | 0.062 | 0.47 | 0.03 |
| 8.5mM $[NO_3^-]$ | 0.065 | 0.15 | 0.04 |
| Control (28mM $NO_3^-$) | 0.067 | 0.23 | 0.23 |

Table 1 Growth rate, [N] uptake and [N] assimilation rates of *Arthospira* sp. PCC 8005 under batch cultivation of different [N] sources

The batch study performed using 3 [N] sources: $[NH_4^+]$, Urea & $[NO_3^-]$, with starting concentration and pH (uncontrolled) of 8.5mM and 9.2 respectively. The growth and [N] uptake/assimilation rates were compared to 28mM $[NO_3^-]$ as control (Fig.3a, Table 1). $[NH_4^+]$ subset had the least growth rate (vs Urea & $[NO_3^-]$) but highest [N] uptake and assimilation rate, at pH>9.5 (Fig.3b); indicating that $[NH_4^+]$ is not harmful for the cells under these conditions.

## Batch Study: [N] Uptake Profile

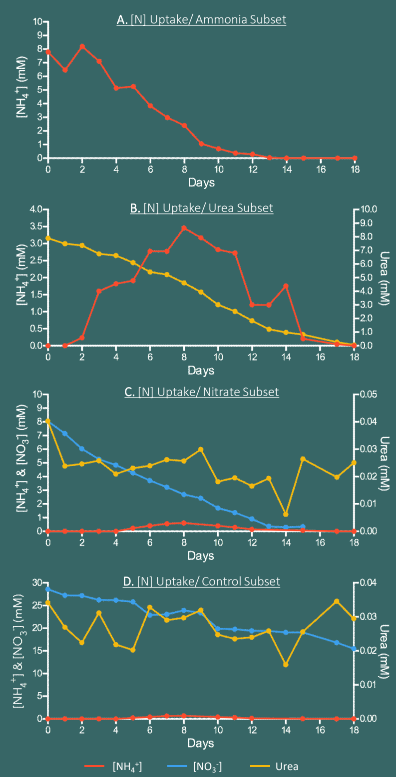


Fig.4 Assimilation trends of different [N] sources in batch mode

Cellular growth after the exhaustion of the nutrient sources can be attributed to presence of endogenous nutrient pool inside the cells. $[NH_4^+]$ has the highest uptake & assimilation rate (Table 1).
Presence of $[NH_4^+]$ in the Urea subset (Fig.4b) can be linked to hydrolysis of urea to $[NH_4^+]$ under alkaline conditions[3] (Fig.3b).
The cellular growth in presence of both Urea & $[NH_4^+]$ (Fig.4b) at approximately the approximately same concentration (Day8) shows that $[NH_4^+]$ doesn't inhibit cell growth.
Presence of trace amounts of Urea (Fig.4c, 4d) and Nitrite (data not shown) in $[NO_3^-]$ & Control subsets can be either due to the changes in the Arginine pathway[4] and/or nitrite (a byproduct of $[NO_3^-]$ metabolism) inhibition[5].

## Conclusions

1. *Arthosphira* sp. PCC 8005 can grow in Ammonium at concentration above 3mM without toxicity or inhibitory effect.
2. Ammonium has the fastest assimilation and uptake rate with respect to Urea and Nitrate as the nutrient source.
3. *Arthosphira* sp. PCC 8005 can assimilate trace amount of exogenous Urea and Nitrite during Nitrate assimilation.
4. Proteomics analysis to be performed to evaluate the underlying reason for exogenous Urea & Nitrite secretion.

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