Growth kinetics, oxygen production and nutrient assimilation of *Arthospira sp.* PCC 8005 under different nitrogen sources.

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The potential of autotrophic cyanobacteria like *Arthospira* sp. PCC 8005, to thrive and grow using waste nitrogen (both organic and inorganic), release oxygen and to be used as a food supplement has made it a very good candidate for the MELISSA loop. The BIORAT-2 project is essentially aimed at integrating the oxygen producing and nitrifying compartment of the MELISSA loop. This concept has been used as the basis of the present study with an additional objective to include use a mix of different nitrogen sources in oxygen producing compartment and eventually use urine as the nitrogen source for cyanobacterial cultivation.

The inhibitory/toxic effect of ammonium ([$NH4^+$]) (pKa 9.2) at concentrations higher than 3mM and pH above 9.2 ([$NH4^+$] is known to change to gaseous ammonia [NH_3] at pH≥9.2)[1] has limited its use for cyanobacterial cultivation. [NH_3] is known to poison the photosynthetic system of the cells[2]. Nitrate ([NO_3^-]) is the most commonly used nitrogen ([N]) source for *Arthospira* sp. cultivation which is converted to simpler [$NH4^+$], which is eventually be used by *Arthospira* sp. for their metabolism. Therefore the use of alternative [N] sources for the cyanobacterial cultivation could give a higher degree of freedom to the MELISSA project.

The present study evaluates the effect of different [N] sources on growth kinetics, oxygen production and nutrient assimilation profiles of *Arthospira* sp. PCC 8005 under the uncontrolled (flask) and controlled (Photobioreactor) conditions, with the aim to evaluate the maximum concentration of [$NH4^+$] sustainable (without any cellular toxicity/inhibition at pH greater 9.2) by the *Arthospira* sp.

Batch Study

Growth kinetics, [N] and [PO_4^-] Assimilation

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

*The higher [N] uptake rate of [NH_4^+] may be attributed to this convertion to gaseous [NH3] at pH>9.2

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

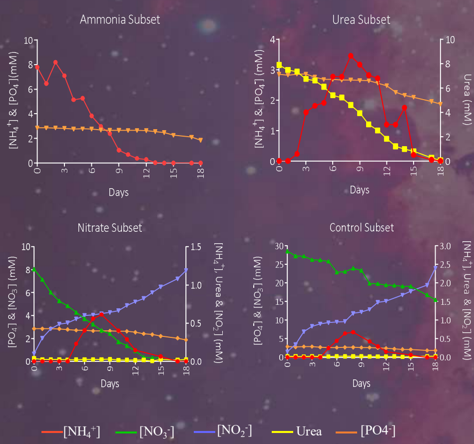

Fig.1 Residual [N] and [P] assimilation profile of *Arthospira* sp. under different [N] sources in batch mode

One set (in triplicate) was setup under batch conditions (flask) for each of 8.5mM [NO_3^-], Urea, [NH_4^+] along a with positive control of 28mM [NO_3^-] at a starting (uncontrolled) pH 9.5. The growth rate were all the sets were found to be similar. Though the highest [N] uptake rate was observed for [$NH4^+$] subset it could be due to the loss of [NH_4^+] to gaseous [NH_3] as the pH≥9.5 (Table 1). Therefore Urea exhibited the highest uptake and assimilation rates. Trace amount of Urea and [NO_2^-] were found to be present in the cultures cultivated with [NO_3^-] (Fig.1). Only minimal amount of [PO_4^-] was assimilated by the cyanobacteria (Fig.1).

Photobioreactor Study

Growth and Nutrient Kinetics

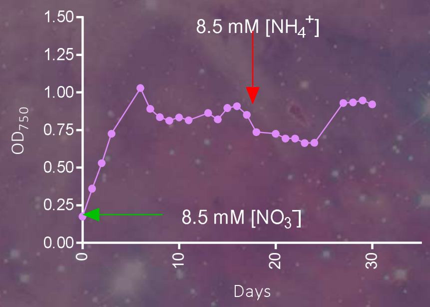

Fig.2 *Arthospira* sp. PCC 8005 growth profile under controlled conditions of PBR on transition from 8.5mM [NO_3^-] to 8.5mM [NH_4^+], pH 9.5

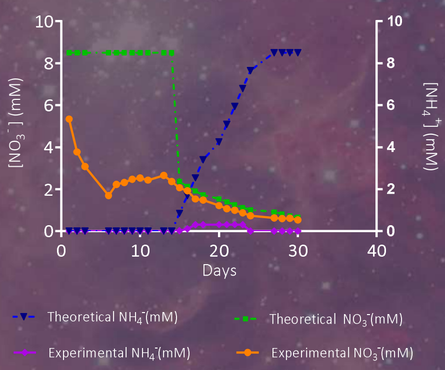

Fig.3 Experimental vs Theoretical values of [N] assimilation under transition from [NO_3^-] to [NH_4^+] in PBR, pH 9.5

A continuous PBR study was conducted to evaluate the effect of transition from 8.5mM [NO_3^-] to 8.5mM [$NH4^+$] (pH 9.5) on growth kinetics (Fig.2) under turbidostat conditions. Experimental [NO_3^-] assimilation rate (Fig.2) was observed to exactly overlap with theoretical rate of [NO_3^-] dilution, i.e. without any biological assimilation. Difference in the theoretical and experimental assimilation rate of [NH_4^+] could be attributed loss of [NH_4^+] to gaseous [NH_3] due to high pH (confirmed by another experiment, data not shown). The data clearly demonstrate inhibition of [NO_3^-] assimilation by [NH_4^+] (Fig.3).

Photobioreactor Study

[N], [PO_4^-] Assimilation and Oxygen Production

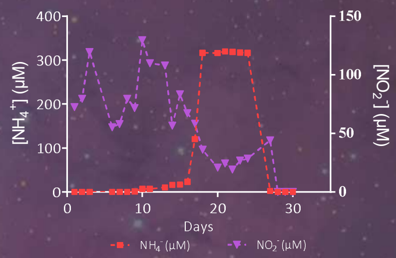

Fig.4 Residual [N] assimilation profile under transition from 8.5mM [NO_3^-] to [NH_4^+] in PBR, pH 9.5

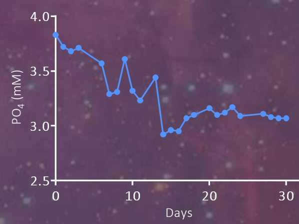

Fig.5 Residual [PO4-] assimilation profile under transition from 8.5mM [NO3-] to [NH4+] in PBR, pH 9.5

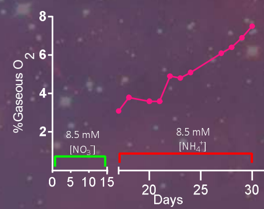

Fig.6 Exhaust Gaseous (%) Oxygen being produced under 8.5mM NH_4^+ cultivation

As under batch conditions trace amount of [NO_2^-] was found in PBR cultivations as well while being fed with [NO_3^-] (Fig.4) but not when culture was fed with [NH_4^+]. No depletion observed for [PO_4^-] in PBR (Fig.5) as was observed in batch conditions (Fig.1). The amount of oxygen (% gaseous) produced by the cyanobacteria (N_2 purging) cultivated under 8.5mM [NH_4^+] was quantitated (Fig.6). The initial amount was lower than previously reported[3] but increased unexpectedly in few days post transition and reached upto 18% by day 40 of cultivation (data not shown).

Conclusions

1. *Arthospira* sp. PCC 8005 can grow in [$NH4^+$] at concentration above 3mM without toxicity/inhibitory effect.
2. Ammonium was found to deplete fast due to its reduction (loss) to gaseous ammonia at pH≥ 9.5.
3. Urea exhibited the highest uptake and assimilation rate.
4. Nitrite was detected in the culture cultivated with nitrate.
5. Only trace amount of [PO_4^-] was utilized by *Arthospira* sp. PCC 8005 during (one month) cultivation.
6. Rate of oxygen production increased considerably during transition from nitrate to ammonium.

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1. Markou et al.; **Ammonia inhibition on *Arthrospira* platensis in relation to the initial biomass density and pH.** Bioresour Technol 2014 Microbiology 2014
2. Li et al.; Isolation and characterization of a novel ammonium overly sensitive mutant, amos2, in *Arabidopsis thaliana*. Planta 2012
3. Demey et al.; BIORAT: Preliminary evaluation of biological life support in space environment. SAE Technical Paper, 2000; DOI:10.4271/2000-01-2384