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Embedding photosynthetic biorefineries with circular economies: Exploring the waste recycling potential of *Arthrospira* sp. to produce high quality by-products

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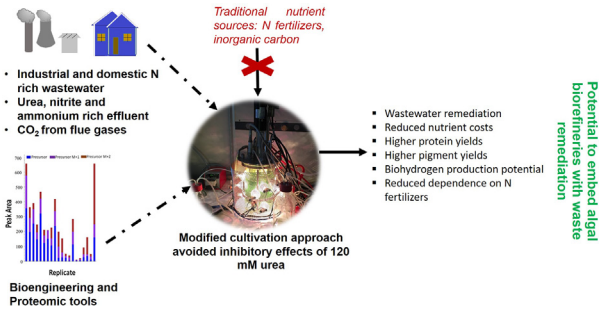
GRAPHICAL ABSTRACT



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ABSTRACT

This study was conducted with the aim of embedding circular economies (waste recycling) with photosynthetic biorefineries, for production of commercially viable by-products. Since nitrogen source constitute the major input costs for commercial *Arthrospira* sp. production, the use of nitrogen rich wastewater for *Arthrospira* sp. cultivation could significantly reduce their production costs. This study evaluated the effects of high concentrations (8.5–120 mM) of alternative nitrogen sources (urea, ammonium and nitrite) on the biochemical, pigment and proteomic profile of *Arthrospira* sp., under batch and continuous conditions. *Arthrospira* sp. cells fed with urea were quantified with modified biochemical and proteomic profile compared to the nitrate fed cells. No inhibitory effect of urea was observed on the biomass even at 120 mM. Nitrite fed cells exhibited comparable biochemical and proteomic profiles as nitrate fed cells. These results clearly indicated at the possibility of using urea rich wastewater streams for profitable cultivation of *Arthrospira* sp.

1. Introduction

The photosynthetic cyanobacteria *Arthrospira* sp., have played an important role in the evolution of human civilization. From being a major food source for ancient civilizations (Spolaore et al., 2006) to their current roles in synthesis of high-value nutraceuticals/pigments (Zhou et al., 2017; Leema et al., 2010), bioplastics (Zeller et al., 2013), biofuels (Shirazi et al., 2017) and wastewater treatment (Jiang et al., 2015), have truly brought this photosynthetic cyanobacterium at the forefronts of curating environmentally sustainable living for humans.

The rise in human population increased the water pollution and depletion of natural resources, making it a necessity to recycle and

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reuse resources everywhere. Photosynthetic microorganisms like *Arthrospira* sp., could play a role in this task. These cyanobacteria use light, nitrogen (N hereafter) and carbon dioxide (CO_2) as nutrients to produce high value products. However, the high costs associated with production and procurement of the required nutrients, in particular N source, reduce the economic viability of large scale *Arthrospira* sp. production. Harnessing the potential of *Arthrospira* sp. as a photosynthetic biorefinery, capable of utilizing low cost waste N (ammonium and urea) sources (Deschoenmaeker et al., 2017) for the simultaneous production of high value co-products (bioplastics, biofuel, pigments, etc.) could lower their production costs. Slade and Bauen (2013) conducted a life cycle assessment study and compared the algal biomass production costs in a raceway pond (RP) and an ideal tubular photobioreactor (PBR). The authors reported a respective reduction of 22.22% (RP, 1.6 € kg^{-1} to 0.3 € kg^{-1}) and 42.2% (tubular PBR, 9.0 € kg^{-1} to 3.8 € kg^{-1}) in the overall algal biomass production cost when the conventional fertilizers were replaced with locally sourced N-rich wastewater for algal cultivation.

European Space Agency (ESA) through its MELiSSA project has been studying the potential of *Arthrospira* sp., to recycle crew waste (urine and CO_2) and produce edible biomass and oxygen (O_2) for space missions (Farges et al., 2008). Currently the MELiSSA loop uses nitrate produced by nitrification of ammonium (from crew compartment) as the N source for *Arthrospira* sp. cultivation. However, this process is highly energy extensive and consumes valuable O_2. Therefore, ESA is currently working on bypassing this nitrification step in the MELiSSA loop and evaluating the potential of directly using ammonium and urea (from crew waste) for cyanobacterial cultivation. The MELiSSA loop, is thus a perfect example of photosynthetic biorefinery based circular economy prototype, wherein the crew waste is aimed to be recycled as nutrients (N and CO_2) source for *Arthrospira* sp. cultivation. MELiSSA scientists have been working on various engineering tools (improved PBR designs and light-energy-transfer models) to improve the system performance and yields (Cornet et al., 1995; Cornet and Dussap, 2009). ESA has also been evaluating the potential of MELiSSA project for wastewater management and food production in African countries.

Depending on the effluent source (domestic, industrial or agricultural), wastewater streams contain varying concentrations of ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) and urea (Zhang et al., 2018; Zhou et al., 2017; Jiang et al., 2015, Anaga and Abu, 1996). Urea is the main constituent of domestic and municipal wastewater. The average urea content in human urine varies between 83.3 and 150 mM (Chang et al., 2013). Although multiple literature reports are available evaluating the effect of NH_4^+ and NO_3^- salts on biochemical and proteomic profile of *Arthrospira* sp. (Deschoenmaeker et al., 2014, 2017; Depraetere et al., 2015; Markou et al., 2015), only a few studies had been undertaken to evaluate the effect of urea (and direct urine) on *Arthrospira* sp. growth. Avila-Leon et al. (2012) evaluated the effects of different urea concentrations (0.5 and 5 mM) and dilutions rates (0.04–0.44 day^{-1}) on the biomass productivity of *Arthrospira platensis* cultivated in a continuous PBR. The authors reported the highest biomass productivity of 201.6 mg/L/day at 0.5 mM feeding concentration and 0.44 day^{-1} dilution rate. The authors also reported that urea was inhibitory to the cells at 5.0 mM at dilution rate $\geq$0.16 day^{-1}, possibly due to system washout. Sassano et al. (2004), on the other hand, investigated the effect of urea (4.58–12.08 mM) on growth yield and bioenergetics profile and of *Arthrospira* sp., under fed-batch feeding regimes and reported the highest biomass productivity of 75 mg/L/day (at the end of 15 days). Chang et al. (2013) evaluated the potential of using direct urine (120 fold diluted urine, urea concentration $\leq$2.7 mM) for *Arthrospira* sp. cultivation. In addition to urea; NH_4^+, NO_3^- and NO_2^- salts are the other main N components present in wastewater streams. To the best of our knowledge, no study had been reported evaluating the effect of NO_2^- salts on growth profile of *Arthrospira* sp. Thus in order to effectively embed the *Arthrospira* sp. cultivation with circular economy for recycling of N rich wastewater streams and producing high quality by-products (biorefineries), it would be essential to study the biochemical, growth and proteomic profile of *Arthrospira* sp. under various concentrations of these different N sources.

This study was undertaken with the following objectives: (a) evaluate the effects of NH_4^+, urea and NO_2^- (as NO_3^- salt alternatives) at high concentrations (up to 120 mM) on the biochemical, pigment and growth profile of *Arthrospira* sp., under both batch and continuous conditions, (b) evaluate the effect of alternative N sources on the proteomic profile of *Arthrospira* sp., especially the proteins involved in N and C metabolism and photosynthetic machinery of the cells, (c) evaluate the potential of using urea and NH_4^+ rich wastewater (and direct urine) for *Arthrospira* sp. cultivation, (d) improving the overall system performance by using a modified cultivation approach and hence increase the overall productivity and economic viability of the bioprocess and (e) evaluating the potential of embedding N rich wastewater remediation with production of high value coproducts from *Arthrospira* sp. (biorefineries).

The study results clearly highlighted the potential of using urea and NO_2^- as cheaper alternatives to NO_3^- salts for *Arthrospira* sp. cultivation. The *Arthrospira* sp. cells fed with urea were quantified with modified biochemical (protein, cyanophycin) and photosynthetic (higher pigment content) profile, when compared to the cells fed with NO_3^- salts of similar N concentration, under both batch and continuous conditions. The proteomic profiling of the urea fed cells indicated at the higher abundance of the proteins involved in N metabolism and hydrogenases (linked to hydrogen production). NO_2^- salts fed biomass exhibited comparable biochemical and photosynthetic profile as observed in the biomass fed with NO_3^- salts of similar N concentration. The present study thus clearly highlighted the potential of using urea rich wastewater for profitable production of biomass, proteins, biohydrogen and high value pigments. The study results could thus be used as the preliminary step stones towards the creation of photosynthetic biorefineries by embedding *Arthrospira* sp. cultivation with circular economies based N waste recycling.

2. Materials and methods

2.1. Cyanobacterial strain and photo-bioreactor setup and cultivation

The stock culture of *Arthrospira* sp. strain PCC 8005 (*Arthrospira* sp. hereafter) was maintained in 250 mL Erlenmeyer flasks on Cogne Modified Zarrouk medium (Cogne et al., 2003) in photosynthetic growth chamber (MLR-352H-PE, Panasonic, Japan) at 30 °C, 140 rpm shaking and continuous illumination of 60 μmol photons m^{-2} s^{-1} (Li-193SA; Li-Cor BioSciences, USA).

The batch experiments were conducted under same cultivation conditions as described for the stock culture. Depending on the experiment design, the flasks (in triplicates) were maintained in Cogne Modified Zarrouk medium (starting pH 8.5) with four different N sources, viz., NH_4^+, NO_3^-, NO_2^- and urea; each at five different total N concentrations of 8.5 mM, 15 mM, 30 mM, 60 mM and 120 mM.

The continuous cultivation, was undertaken in a 2.0-L cylindrical double jacketed (Biostat®, Sartorius AG, Germany) radially illuminated (300 ± 50 μmol photon/m^2/s) PBR, maintained at 36 °C. The continuous feeding regime was maintained with a dilution rate of 0.2 day^{-1} using Cogne Modified Zarrouk medium, containing 30 mM (total N) urea or NO_3^-. The pH (InPro 3250, Mettler Toledo, USA) was continuously controlled at 8.5 using HCl (0.5 N) and NaOH (1.0 N).

2.2. Biomass productivity and oxygen productivity

The optical density (at 750 nm, OD_{750}) was used for monitoring the culture growth profile (Deschoenmaeker et al., 2017). The growth rate of the batch cultures was calculated as a differential of OD_{750} at time t_0 (first day of experiment) and time t, as follows:

$$Growth\ rate(per\ day) = \frac{OD_{750}t - OD_{750}t_0}{t - t_0} \quad (1)$$

The samples (biomass and supernatant) from both the batch and continuous experiments were harvested every 24 h and stored at −20 °C for further biochemical analysis. The lyophilised biomass (Dry Cell Weight; DCW) was weighed and used to calculate the biomass concentration (g/L). This concentration was further used to calculate the biomass productivity (g/L/day) as follows:

$$Biomass\ productivity(g/L/day) = Biomass\ Concentration(g/L)X\ dilution\ rate \quad (2)$$

For PBR runs, the O_2 productivity (g/L/day) was calculated by measuring the % O_2 (Analox Gas Analyser 9212, Analox Sensor Technology, UK) and gas flow rate (GT1355 Sho-Rate G, Brooks Instruments, USA); as follows:

$$O_2\ productivity(g/L/day) = 0.76\frac{Outlet\ Gas\ Flowrate\ (mol/h) \times \text{Outlet mol fraction of } O_2(\%)}{Total\ reactor\ volume(L)} \quad (3)$$

2.3. Biochemical and proteomic analysis

The total carbohydrate, total protein and total lipid contents in the biomass were quantified by the protocols described by Deschoenmaeker et al. (2017). The total cyanophycin (CP) content in biomass were estimated by method described by Allen et al. (2005). The total chlorophyll (as sum of chlorophyll *a* and chlorophyll *b*) content was estimated by colorimetric method reported by Sesták (1971). The total carotenoid content was determined by the assay described by Liechtenthaler (1987). The total phycobiliprotein content in biomass, (as sum of C-phycocyanin, allophycocyanin and phycoerythrin), was quantified by the assay reported by Patel et al. (2006). All biochemical analyses were performed at the end of 7.0 days. Proteomic analysis, sample preparation and SWATH-MS data acquisition were performed as described by Deschoenmaeker et al. (2017).

2.4. Residual nitrogen content and nitrogen uptake rate

Spectrometric (HELIOS-ZETA UV–VIS, Thermo Scientific, USA) assays described by Ivančič and Degobbis (1984), Sachdeva et al. (2016a), Bar et al. (2011) and Chae et al. (2004) were used to quantify the residual NH_4^+, NO_3^-, urea and NO_2^- ion concentrations, respectively in the supernatant.

The N uptake rate (NUR); described as number of moles of N utilised by per mg of biomass per day, was calculated as per the following equation:

$$NUR\left(\frac{\frac{mol}{mg}}{day}\right) = \frac{(N_0 - N_i)}{t \times DCW} \quad (4)$$

where N_0 and N_i, were respectively the residual N (mM) concentrations on Day 0 (starting) and Day 7; t (days) was duration of experiment (i.e., 7.0 days) and DCW (mg) was the dry cell weight of biomass.

2.5. Statistical analyses

Software *GraphPad Prism* (version 6.0, GraphPad Softwares, USA) was used for the graphical interpretation of biochemical data. All the biochemical and proteomic samples were analysed in triplicates. The results of biochemical analysis were reported as their means and standard deviation of the three replicates, calculated using MS Excel, 2007. For proteomic analysis, only the protein quantified with at least two peptides and exhibiting a modification of relative abundance (fold change) of minimum 50% (≥1.5 or ≤0.66, *p-value* < 0.05) were considered to be relevant.

3. Results and discussions

3.1. Effect of culture medium pH on ammonium salts

NH_4^+ salts (≥3 mM) had been reported to be inhibitory to *Arthrospira* sp. growth (Markou et al., 2015; Rodrigues et al., 2010). Most of the previous studies that had reported the inhibitory effects of NH_4^+ salts were conducted under uncontrolled pH conditions. At pH > 9.25, NH_4^+ ions (pKa of 9.25) are known to gas-off as gaseous ammonia (NH_3). At pH 8.5, about 10% of NH_4^+ is present as free NH_3 and this percentage increases to ≥80% at pH > 9.25 (Collos and Harrison, 2014). Higher concentration of free NH_3 in the medium had been reported to irreversibly inhibit the functioning of cyanobacterial photosystems (PS). Since free NH_3 is a structural analog of water molecule, it can easily bind to the oxidized oxygen evolving complex of PS II (Dai et al., 2008). This replacement alters the photochemistry of PS II and inhibits its activity. High concentration of free NH_3 inside the thylakoid membrane, had been further reported to damage their reaction centers which inhibits the PS I activity of the photosynthetic cells (Markou et al., 2016). Deschoenmaeker et al. (2017) reported that the inhibitory effects of high concentration NH_4^+ salts could be avoided under controlled cultivation conditions of PBR (feeding rate and pH).

Therefore, in order to better understand the effect of pH on chemical stability of NH_4^+ ions in Zarrouk medium, a 72 h batch study was conducted on sterile and non-inoculated (no cyanobacterial cells) Cogne Modified Zarrouk medium containing 8.5 mM NH_4^+. To evaluate the effect of the increase of pH during *Arthrospira* sp. cultivation on NH_4^+ ion stability, the study was conducted at five different pH of 7.5, 8.5, 9.5, 10.5 and 11 (see Supplementary data). The flasks incubated (without cyanobacterial cells) at starting pH of 9.5, 10.5 and 11 registered a drop in the NH_4^+ content by 7.9, 6.9 and 12.4%, respectively within 2.0 h of incubation. More importantly, a respective decrease of 56.2, 52.7 and 66.9% in NH_4^+ content was observed after 72 h of incubation under sterile conditions. On the other hand, NH_4^+ ion levels in the flasks incubated at starting pH of 7.5 and 8.5, were seen to decrease only when the flask pH exceeded 9.25. This experiment demonstrated that even in the absence of cyanobacteria, NH_4^+ was quickly disappearing from culture medium causing N depleted conditions. This observation thus should to be taken into consideration when evaluating the previously reported inhibitory effects of NH_4^+ salts (Markou et al., 2015; Rodrigues et al., 2010). It could be that the exhaustion of N resources (caused by gassing of NH_4^+ ions at pH > 9.25) had caused a decrease in the productivity of the cyanobacterial culture in these studies.

3.2. Effect of nitrogen sources and concentrations on growth, biochemical and proteomic profile of Arthrospira sp. under batch conditions

In order to avoid NH_4^+ loss in culture medium before cyanobacterial inoculation, batch experiments were performed at a starting of pH 8.5. Analyses were conducted at five different (total) N concentrations of 8.5 mM, 15 mM, 30 mM, 60 mM and 120 mM (Table 1) of each of four N sources i.e., NH_4^+, NO_3^-, NO_2^- and urea. The samples (in triplicates) were monitored for their growth rate, N uptake rate (NUR) and biochemical composition (lipid, protein, carbohydrates, pigments and cyanophycin) for 7.0 days. The cells cultivated with ≥15 mM NH_4^+ salts did not survive beyond 72 h and hence, the results for NH_4^+ concentrations ≥15 mM were not reported here.

3.2.1. Effect of alternative nitrogen sources and concentration on growth profile

Average growth rates (per day) of 0.04, 0.06, 0.06 and 0.06 were observed for *Arthrospira* sp. when cultivated with 8.5 mM of NH_4^+,

Table 1
Effect of different nitrogen sources and their concentration on the average growth rates, nitrogen uptake rates, biochemical (total lipid, total protein and total carbohydrates), pigment (total chlorophyll and total phycobiliprotein) and cyanophycin content of the *Arthrospira* sp. PCC 8005 biomass cultivated under batch conditions (starting pH 8.5 and temperature 30 °C)*.

Nitrogen source	GR (day^{-1})	NUR (moles/mg/day)	TL*Δ (%)	TC*Δ (%)	TP*Δ (%)	Chl_{tot}* (mg/L)	PCB_{tot}* (g/L)	CP* (g/L)
8.5 mM total nitrogen								
NH_4^+	0.04	1.3	19.4 ± 2.9 e^{-2}	30.9 ± 1.1 e^{-2}	44.4 ± 1.2 e^{-2}	0.6 ± 5.0 e^{-2}	*ND*	*ND*
Urea	0.06	0.4	15.4 ± 3.7 e^{-2}	24.5 ± 8.9 e^{-2}	49.8 ± 5.0 e^{-2}	3.6 ± 3.7 e^{-2}	0.3 ± 3.0 e^{-2}	1.5 ± 1.7 e^{-1}
NO_3^-	0.06	0.1	11.4 ± 5.9 e^{-2}	21.4 ± 1.2 e^{-2}	50.2 ± 1.4 e^{-2}	17.8 ± 1.3 e^{-2}	0.5 ± 5.0 e^{-2}	0.8 ± 0.6 e^{-2}
NO_2^-	0.06	0.1	12.4 ± 1.1 e^{-2}	22.9 ± 6.8 e^{-2}	49.5 ± 8.4 e^{-2}	14.1 ± 3.8 e^{-2}	0.4 ± 4.0 e^{-2}	0.7 ± 1.6 e^{-2}
15 mM total nitrogen								
NH_4^+	*Not applicable*							
Urea	0.06	1.6	15.1 ± 5.0 e^{-2}	18.4 ± 3.9 e^{-2}	51.4 ± 7.4 e^{-2}	15.8 ± 4.3 e^{-2}	0.4 ± 1.0 e^{-2}	4.9 ± 1.7 e^{-2}
NO_3^-	0.11	1.1	10.6 ± 1.4 e^{-2}	17.5 ± 9.1 e^{-2}	50.8 ± 4.9 e^{-2}	14.9 ± 1.6 e^{-2}	0.4 ± 6.0 e^{-2}	3.2 ± 2.8 e^{-2}
NO_2^-	0.11	0.8	10.8 ± 8.4 e^{-2}	17.7 ± 7.9 e^{-2}	50.3 ± 8.2 e^{-2}	14.8 ± 3.9 e^{-2}	0.4 ± 7.0 e^{-2}	2.4 ± 1.9 e^{-2}
30 mM total nitrogen								
NH_4^+	*Not applicable*							
Urea	0.14	1.5	9.6 ± 8.7 e^{-2}	19.4 ± 5.9 e^{-2}	57.9 ± 1.0 e^{-2}	68.1 ± 6.6 e^{-2}	0.8 ± 5.1 e^{-2}	20.8 ± 2.3 e^{-2}
NO_3^-	0.14	0.9	8.7 ± 1.0 e^{-2}	18.7 ± 8.1e^{-2}	55.2 ± 4.0 e^{-2}	66.9 ± 4.0 e^{-2}	0.7 ± 6.2 e^{-2}	5.8 ± 4.9 e^{-2}
NO_2^-	0.14	0.7	9.1 ± 1.1 e^{-2}	19.3 ± 4.9 e^{-2}	52.7 ± 5.0 e^{-2}	59.3 ± 3.9 e^{-2}	0.8 ± 4.7 e^{-2}	4.5 ± 2.8 e^{-2}
60 mM total nitrogen								
NH_4^+	*Not applicable*							
Urea	0.21	3.8	8.7 ± 5.2 e^{-2}	18.1 ± 7.3 e^{-2}	62.1 ± 2.9 e^{-2}	99.7 ± 5.2 e^{-2}	1.6 ± 1.6 e^{-2}	27.0 ± 3.1 e^{-2}
NO_3^-	0.21	2.1	8.9 ± 7.8 e^{-2}	19.1 ± 1.9 e^{-2}	59.9 ± 5.3 e^{-2}	96.9 ± 3.8 e^{-2}	1.5 ± 1.0 e^{-2}	21.8 ± 6.1 e^{-2}
NO_2^-	0.20	1.3	8.8 ± 3.8 e^{-2}	19.5 ± 6.4 e^{-2}	57.6 ± 9.4 e^{-2}	94.9 ± 3.2 e^{-2}	1.8 ± 4.1 e^{-2}	20.8 ± 4.3 e^{-2}
120 mM total nitrogen								
NH_4^+	*Not applicable*							
Urea	0.14	8.5	6.6 ± 1.9 e^{-2}	18.2 ± 5.9 e^{-2}	65.1 ± 8.3 e^{-2}	134.2 ± 3.1 e^{-1}	2.5 ± 3.7 e^{-2}	53.6 ± 4.1 e^{-1}
NO_3^-	0.14	7.1	7.3 ± 5.8 e^{-2}	15.7 ± 8.1 e^{-2}	64.9 ± 7.6 e^{-2}	132.4 ± 1.6 e^{-1}	2.3 ± 1.4 e^{-2}	49.0 ± 7.6 e^{-2}
NO_2^-	0.14	3.6	7.8 ± 6.7 e^{-2}	17.3 ± 4.9 e^{-2}	63.7 ± 5.6 e^{-2}	131.7 ± 3.4 e^{-1}	2.5 ± 3.8 e^{-2}	43.1 ± 8.8 e^{-2}

Δ: Values were reported as the percentage of total dry cell weight of harvested biomass.
GR: Growth rate; *NUR*: Nitrogen Uptake Rate; *TL*: Total Lipid; *TC*: Total Carbohydrate; *TP*: Total Lipid; Chl_{tot}: Total chlorophyll (reported as sum of Chlorophyll *a* and Chlorophyll *b*); PCB_{tot}: Total Phycobiliprotein content (reported as sum C-phycocyanin, Allophycocyanin and phycoerytherin); *CP*: Cyanophycin content; *ND:* Not detectable.
* Values were reported as average of three replicates (n = 3) at the end of 7.0 days of the respective batch experiments.

NO_3^-, NO_2^- salts and urea, respectively (Table 1). The cultures fed with urea, NO_3^-, NO_2^- salts could reach an OD_{750nm} of 1.0 ± 0.2, but the flasks fed with 8.5 mM NH_4^+ could only reach a maximum OD_{750nm} of 0.4. The slower growth rates for the cells fed with 8.5 mM NH_4^+ (Table 1) could be attributed to early exhaustion of NH_4^+ ions in the culture. NH_4^+ concentration was indeed observed to decrease by > 50% of starting content by Day 3 (Fig. 1a). Indeed, a quick increase in pH (see Supplementary data) was observed in all cultures and would probably explain the quick decrease of NH_4^+ concentration in the culture. At Day 4, while the concentration of other N sources was still higher than 3.0 mM, NH_4^+ was almost exhausted in the culture medium, which could be corroborated with the decreased growth rate. This growth analysis further indicated that contrary to the previously reported inhibitory effects of NH_4^+ salts (Markou et al., 2015; Rodrigues et al., 2010) N source exhaustion could be responsible for the growth inhibition of *Arthrospira* sp. cells. The comparable growth profile of the cells in 8.5 mM NO_3^-, NO_2^- and urea subsets (Table 1), highlighted that urea and NO_2^- salts could be explored as alternative N sources for cultivation of *Arthrospira* sp. In order to further investigate the efficacy of urea and NO_2^- salts as alternative N sources for *Arthrospira* sp. cultivation; further batch experiments were conducted with higher total N concentrations of 15, 30, 60 and 120 mM.

At 15 mM total N concentration, similar growth rates was observed for the *Arthrospira* sp. cells cultivated with NO_2^- and NO_3^- (Table 1). An earlier exhaustion of urea in the culture was observed as compared with NO_3^-, NO_2^-, leading to the onset of N deplete conditions (Fig. 1b) and resulting in a global lower growth rate for urea condition as calculated at end of Day 7 of experiment (Table 1). These results indicated that 15 mM urea concentration was not enough to sustain the metabolic requirements of high density cyanobacterial culture.

At total N concentrations of 30 mM, 60 mM and 120 mM; all three N sources (NO_3^-, NO_2^- and urea) exhibited comparable growth rates (Table 1), indicating that urea and NO_2^- could be used as potential alternatives to NO_2^- without any inhibitory effects. However, the rate of N consumption (Fig. 1c–e) was found to be highest for urea followed by NO_3^- and NO_2^- which could be attributed to their variable N uptake rates, as discussed in the following section.

3.2.2. Alternative nitrogen sources uptake rate

At 8.5 mM concentration, highest N uptake rate (NUR) was recorded for NH_4^+ subset (1.3 mol/mg/day) followed by urea, NO_3^- and NO_2^- subsets; which exhibited NURs of 0.4, 0.1, 0.1 mol/mg/day, respectively (Table 1). However, due to the already discussed possible loss of NH_4^+ as NH_3, the high NUR for NH_4^+ would not be considered further.

The high NUR for 8.5 mM urea subset (vs NO_3^- and NO_2^-) could be corroborated with its higher growth rate (Table 1). Similar trends were observed for the higher concentration setups (15, 30, 60 and 120 mM) wherein the highest NURs were observed for the urea subsets (compared to NO_3^- and NO_2^- subsets of similar N concentrations). The high NURs for urea subsets (vs NO_3^-), despite of comparable growth rates (Table 1), confirmed that urea is more easily assimilated N source for *Arthrospira* sp. (Avila-Leon et al., 2012). Thus, its use as an energy and cost-efficient alternative to NO_3^- salts should be further explored for *Arthrospira* sp. cultivation. On the other hand, though the NO_2^- subsets exhibited comparable NURs (vs NO_3^-) at concentrations 8.5, 15 and 30 mM, lower NURs (vs NO_3^-) were observed at higher feeding concentrations of 60 and 120 mM N, despite of the comparable growth rates (Table 1). This variation could be attributed to the difference in the stoichiometric, bioenergetics and kinetic parameters of involved in metabolism of NO_2^- and NO_3^-, the discussion of which would require further analysis.

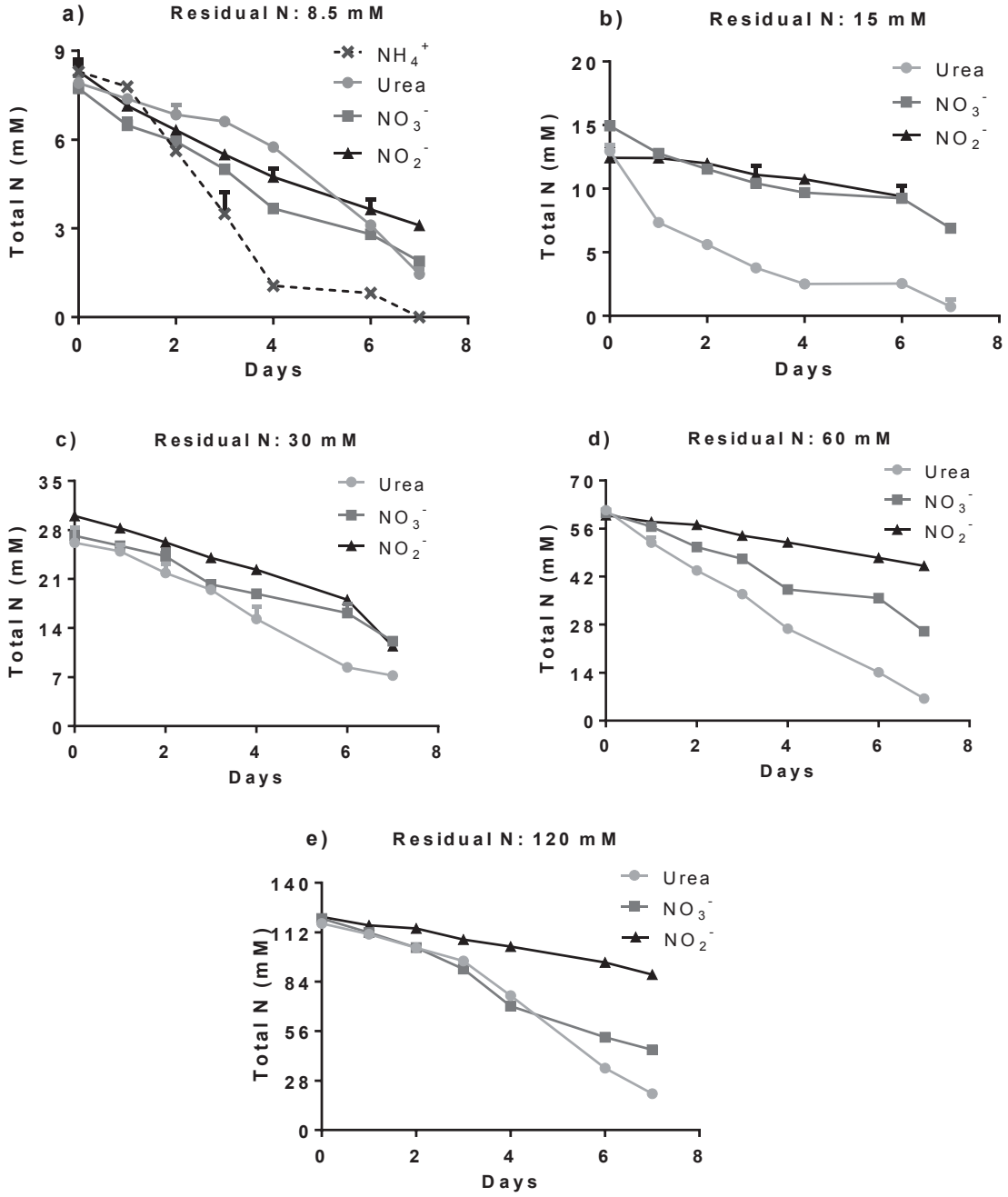

Fig. 1. Nitrogen (N) assimilation profile (as residual total nitrogen concentration in mM) of batch cultures of *Arthrospira* sp. PCC 8005 fed with different (initial) concentrations (a) 8.5 mM, (b) 15 mM, (c) 30 mM, (d) 60 mM and (e) 120 mM of ammonium, urea, nitrate and nitrite. All experiments were conducted in batch mode (flask) maintained at 30 °C and continuous illumination of 60 μmol photons $m^{-2} s^{-1}$.

3.2.3. Effect of alternative nitrogen sources and their concentrations on carbohydrate, lipid, protein and cyanophycin content

N starvation is known to alter the biochemical (lipid, protein, carbohydrate etc.) profile of the photosynthetic cells (Sachdeva et al., 2016b). The initial N starvation phase is known to increase the carbohydrate content of the cells by channeling the carbon source from protein to carbohydrate synthesis, before further diverting them to lipid/fatty acid synthesis under acute starvation (Wahidin et al., 2013). Additionally, the concentration of cyanophycin (CP); an endogenous N reserves found in cyanobacterial cells, could serve as an important biological marker to evaluate the extent of N starvation (Oppermann-Sanio and Steinbüchel, 2002).

The highest lipid and carbohydrate content (19.4 and 31%, respectively), lower protein content (44.4%) and absence of cyanophycin in 8.5 mM NH_4^+ subset (Table 1), indicated at the prevalence of acute N starvation in the culture. At all N concentration (to a lower extent at 120 mM), urea exhibited a higher CP content compared to NO_3^- and NO_2^- (Table 1). The higher CP content (and hence higher N reserves) in the urea subsets explained the probable reason behind the comparable growth rates of urea fed cells despite of their higher NURs (Table 1). At 8.5 and 15 mM urea subsets, it was interesting to note that the cells exhibited higher lipid content, which could be of interest for biofuel applications. At 15 mM, 30 mM, 60 mM and 120 mM N concentration, the respective urea subsets exhibited the highest total proteins contents, when compared to NO_2^- and NO_3^- subsets of similar N concentration (Table 1), indicating at better efficiency of urea at supporting the metabolic activity of *Arthrospira* sp.

An increase of 3.5 folds was observed in the CP content of 30 mM

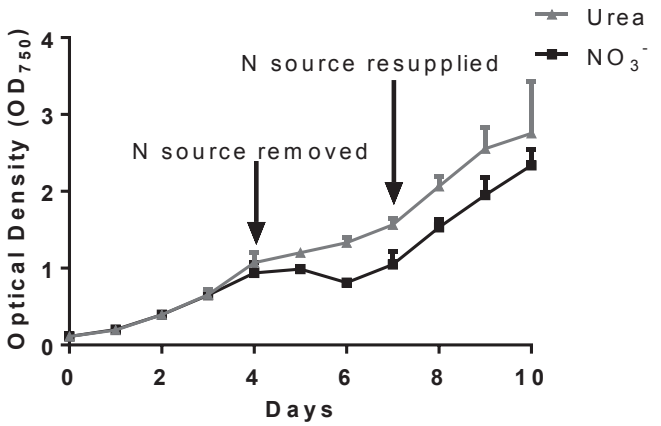

Fig. 2. Evaluation of the potential of nitrogen sources (urea and nitrate; 30 mM each) at creating endogenous nitrogen reserves in *Arthrospira* sp. PCC 8005 cells cultivated under batch conditions (starting pH 8.5, temperature 30 °C, 140 rpm and 60 μmol photons/m^2/s light intensity) and their ability to sustain the cyanobacterial growth (as optical density, OD_{750nm}) under absence/removal of nitrogen (N) source.

urea subset (vs 30 mM NO_3^- subset; Table 1). In order to further study if this marked increase in the CP content could help the cells to endure unstable environmental conditions, a nutrient starvation experiment was conducted. The cells were allowed to grow in N-supplied medium (under 30 mM urea and 30 mM NO_3^-) till they reached the exponential phase (Day 4; Fig. 2). On Day 4 the cells were harvested and transferred to N free medium, wherein they were left for 48 h. While the cells harvested from 30 mM urea subset kept growing in N free medium (Day 5- Day 7, Fig. 2), growth stopped in cells harvested from NO_3^- subset (Fig. 2). On Day 7, the cells (from respective urea and NO_3^- subsets) were transferred back to 30 mM urea and 30 mM NO_3^- (their original initial N sources). On the re-supply of respective N sources, an immediate increase in the OD_{750nm} (Day 8; Fig. 2) was observed for both subsets. This experiment demonstrated that the higher CP content in urea fed culture could help the cells to face a transient restriction in N supply. Since the composition of urea (and other N sources) in wastewater streams can significantly fluctuate on daily and seasonal basis, this observation could be considered as a major advantage towards using fluctuating wastewater streams for *Arthrospira* sp. cultivation.

3.2.4. Effect of alternative nitrogen sources and their concentrations on pigment content

Phycobiliproteins are a group of protein-chromophores, present in thylakoid membranes of photosynthetic cells and play a major role in the process of energy transfer during photosynthesis (Sala et al., 2018). The quantification of the total chlorophyll (Chl_{tot}) content in photosynthetic cells could help in evaluating their photosynthetic efficiency. This effectively translates into the efficiency of the N source in meeting the energy needs of the photosynthetic cells. Additionally, the pigments synthesized by photosynthetic cells play important role in nutraceutical and food industries, due to their antioxidant properties (Leema et al., 2010). Therefore, quantification of the content of various pigments like total Phycobiliproteins (PCB_{tot}) and Chl_{tot} content could be used as a biological important marker to not only evaluate the photosynthetic efficiency of cyanobacterial cells but also efficiency of N source to meet their nutrient needs.

The Chl_{tot} and PCB_{tot} contents were quantified at Day 7 in the biomass harvested from the different N conditions (Table 1). Lowest Chl_{tot} content of 0.6 mg/L and no PCB_{tot} were quantified in 8.5 mM NH_4^+ subset, which could be corroborated with the depressed cellular activity of the cells due to prevalence of acute N stress in the culture (Fig. 1a). Except for 8.5 mM, where urea showed unexpectedly lower chlorophyll content, no significant difference in Chl_{tot} and PCB_{tot} content was observed between NO_3^-, NO_2^- and urea subsets at higher N concentrations. These result again could provide the possibility to use any of those N sources for production of pigments from *Arthrospira* sp. cells without compromising on productivity.

3.2.5. Effect of nitrogen sources on proteomic profile

The 30 mM batch samples were selected for the proteomic analysis for two reasons; (1) to be consistent with the previous scientific studies on *Arthrospira* sp. that had been conducted at 28 mM NO_3^- concentration; and (2) to evaluate the probable cause for the 3.5 fold difference in CP content of the cells (urea vs NO_3^-) observed at 30 mM N concentration.

The proteomic profiling of the *Arthrospira* sp. biomass harvested from 30 mM of urea, NO_2^- and NO_3^- subsets, identified a total of 1421 proteins. Only the proteins quantified with at least two peptides, *p-value* < 0.05 and showing a minimum of 50% change in abundance (i.e., fold change ≥ 1.5 or ≤ 0.66) were considered to be statistically and biologically significantly regulated (see Appendix A for complete data set).

For the proteomic comparison between NO_3^- and NO_2^-, none of the proteins related to N metabolism were seen to be significantly affected by the change in N source. A few proteins involved in the functioning of photosynthetic machinery of the cells (C-phycocyanin, allophycocyanin, etc.) exhibited slightly higher abundance under NO_2^- conditions (see Supplementary data). The higher abundance of these protein under NO_2^- feeding conditions could be corroborated with the slightly higher total phycobiliprotein content of the biomass harvested under 30 mM NO_2^- conditions (Table 1).

For the proteomic comparison between NO_3^- and urea, a respective 2.1 and 2.5 folds higher abundance (Table 2) was observed for the proteins *Arg*H and *Arg*F (*ARTHROv5_30675|argH* and *ARTHROv5_12086|argF*). Since CP molecules are mainly composed of amino acid arginine (Oppermann-Sanio and Steinbüchel, 2002), these proteins are known to play an important role in CP synthesis. This observation was thus in line with the higher CP content previously observed in urea condition. Proteomic data also revealed that several proteins related to photosynthesis and photosystems exhibited higher abundance under urea conditions (Table 2). Interestingly, some hydrogenase related proteins: *HoxH*, *HoxY* and *HoxF*, were seen to be upregulated under urea conditions by 4.5, 6.25 and 3.0 folds, respectively. This observation would require further analysis and could be of major interest for bio-hydrogen production. The higher abundance of redox-potential and electron transfer (NAD^+) related proteins under urea fed cultures could be linked to (a) better efficiency of urea as a N source for cyanobacterial metabolism and (b) its better kinetics and bioenergetics than nitrate salts (Sassano et al., 2004). Additionally, some proteins involved in the N and C metabolism were seen to higher abundance under urea conditions (Table 2). This observation was in agreement with the higher NUR of the cells fed with urea was used as N source. Finally, two proteins related to the Kai circadian clock (*kaiA* and *kaiB*) of *Arthrospira* sp. were seen to be upregulated under urea feeding conditions (Table 2). Though *Kai* genes had initially been linked to the light/dark induced rhythmic phenomenon in photosynthetic cells, the role of N fixation (and related cellular processes) has recently been linked to the circadian oscillations in certain diazotrophic strains of cyanobacteria (Cohen and Golden, 2015). Since the cultures in the present study were maintained under continuous light and only the N source was changed, the activation of *kai* genes under these conditions would deserve additional scientific attention.

3.3. Effect of nitrogen sources and concentrations on growth profile of Arthrospira sp. under continuous conditions of photobioreactor

In order to further investigate the potential of *Arthrospira* sp. in large scale circular economies, it was necessary to replicate the batch-scale results at continuous level. Furthermore, it was also important to

Table 2
Effect of nitrogen source (nitrate on urea) on the proteomic profile of *Arthrospira* sp. PCC 8005, cultivated under batch mode and nitrogen concentration of 30 mM PBR under transition of nitrogen regime (*p value < 0.05, number of peptides > 1 and fold change ≥ 1.5 or ≤ 0.66*).

Peak mame	Protein function	Fold change (NO_3^- on Urea)	p-value	Number of peptides
Photosynthetic system				
ARTHROv5_11973|btpA	Photosystem I biogenesis protein btpA	$5.6\,e^{-1}$	$8.6\,e^{-3}$	3
ARTHROv5_41386|petH	Ferredoxin–NADP reductase (FNR)	$4.7\,e^{-1}$	$1.7\,e^{-2}$	6
ARTHROv5_61146	NADPH-dependent FMN reductase	$3.4\,e^{-1}$	$2.1\,e^{-2}$	6
ARTHROv5_11654	Putative thylakoid formation protein	$2.5\,e^{-1}$	$1.5\,e^{-2}$	2
ARTHROv5_60133|chlB	Light-independent protochlorophyllide reductase	$2.8\,e^{-1}$	$7.6\,e^{-3}$	3
ARTHROv5_41145|chlN	Light-independent protochlorophyllide reductase	$3.5\,e^{-1}$	$3.9\,e^{-2}$	5
Hydrogenase				
ARTHROv5_41299|hoxH	NAD-reducing hydrogenase	$2.2\,e^{-1}$	$5.2\,e^{-3}$	4
ARTHROv5_41298|hoxY	NAD-reducing hydrogenase	$1.6\,e^{-1}$	$2.4\,e^{-2}$	3
ARTHROv5_41295|hoxF	NAD-reducing hydrogenase	$3.3\,e^{-1}$	$2.6\,e^{-2}$	5
N metabolism and protein synthesis related				
ARTHROv5_60176|nthB2	Nitrile hydratase beta subunit	$1.4\,e^{-1}$	$6.7\,e^{-4}$	5
ARTHROv5_12133|glnA	Glutamine synthetase	$2.0\,e^{-1}$	$2.1\,e^{-3}$	5
ARTHROv5_40573	Putative Subtilisin-like serine protease	$3.1\,e^{-1}$	$2.2\,e^{-3}$	5
ARTHROv5_60175|nthA1	Nitrile hydratase alpha subunit	$1.2\,e^{-1}$	$6.9\,e^{-3}$	4
ARTHROv5_61026|thiC	Thiamine biosynthesis protein ThiC	$3.5\,e^{-1}$	$7.4\,e^{-3}$	5
ARTHROv5_30068|ureB	Urease subunit beta	$2.5\,e^{-1}$	$1.6\,e^{-2}$	2
Cyanophycin related				
ARTHROv5_30675|argH	Argininosuccinate lyase	$4.6\,e^{-1}$	$3.0\,e^{-2}$	5
ARTHROv5_12086|argF	Ornithine carbamoyl transferase	$4.0\,e^{-1}$	$3.1\,e^{-2}$	5
Carbon metabolism related				
ARTHROv5_20218|fmdA	Formamidase (formamide amidohydrolase)	$1.4\,e^{-1}$	$7.6\,e^{-4}$	4
ARTHROv5_11016|ilvY	Acetolactate synthase	$3.9\,e^{-1}$	$7.7\,e^{-4}$	5
ARTHROv5_10500|phaC	Poly(R)-hydroxyalkanoic acid synthase, PhaC subunit	$2.9\,e^{-1}$	$1.2\,e^{-3}$	4
ARTHROv5_61108|zwf	Glucose-6-phosphate dehydrogenase	$4.9\,e^{-1}$	$1.7\,e^{-3}$	6
ARTHROv5_10479	Putative Calvin cycle regulator CP12-like protein	$2.2\,e^{-1}$	$3.2\,e^{-3}$	3
ARTHROv5_10688|gltA	Citrate synthase	$2.4\,e^{-1}$	$5.7\,e^{-3}$	4
ARTHROv5_11455|pfkA2	6-phosphofructokinase I	$1.6\,e^{-1}$	$7.2\,e^{-3}$	5
ARTHROv5_50034	Putative glycosyltransferase, family 2	$2.7\,e^{-1}$	$7.9\,e^{-3}$	6
ARTHROv5_10719|rpiA	Ribose-5-phosphate isomerase A	$4.7\,e^{-1}$	$1.6\,e^{-2}$	4
ARTHROv5_61149	Putative glycosyltransferase, group 1	1.4	$1.8\,e^{-2}$	4
ARTHROv5_11761	Putative sugar kinase	$4.1\,e^{-1}$	$2.9\,e^{-2}$	4
ARTHROv5_61117|maeB	Malate dehydrogenase (oxaloacetate-decarboxylating)	$1.3\,e^{-1}$	$3.2\,e^{-2}$	4
Cellular health related				
ARTHROv5_60141|kaiB	Circadian clock protein kaiB	$3.4\,e^{-1}$	$5.5\,e^{-3}$	2
ARTHROv5_60140|kaiA	Circadian clock protein kaiA	$4.5\,e^{-1}$	$1.7\,e^{-2}$	2

monitor and compare the O_2 productivities obtained under urea feeding (vs NO_3^- salts), notably in order to evaluate potential of urea for MELiSSA Loop. Therefore, a continuous (controlled feeding and pH) PBR run was conducted to evaluate the effect urea (with respect to NO_3^- salts) on the O_2 productivity and biochemical profile of *Arthrospira* sp. The feeding concentration of 30 mM (NO_3^- or urea) was chosen for two reasons; (1) to be consistent with the 28–30 mM NO_3^- concentration conventionally used for the cultivation of *Arthrospira* sp. and (2) to evaluate the probable cause of 3.5 folds higher CP content in cyanobacterial biomass fed with 30 mM urea (batch cultivation).

3.3.1. Effect of nitrogen source transition on growth profile and nitrogen assimilation under continuous conditions

The PBR run was initially started under batch mode with 30 mM NO_3^-. The continuous mode was started on Day 6 once the cells had reached an optical density (OD_{750nm}) of 1.5 ± 0.2. The continuous feeding regime in PBR was maintained with feeding (dilution) rate of $0.2\,day^{-1}$. The transition to urea was made on Day 20 of the run.

N source transition (NO_3^- to urea) did have an impact on biomass productivity. 20% higher biomass productivity was recorded under urea regime (Fig. 3a). Average biomass productivities of 0.4 g/L/day and 0.5 g/L/day were quantified under NO_3^- and urea regimes, respectively (Table 3).

The *Arthrospira* sp. cells fed with 30 mM urea (batch experiment, Section 3.2) exhibited the highest NUR (Table 1). To investigate the effect of N transition on the N utilization profile of *Arthrospira* sp. under continuous mode, the residual N concentrations were quantified in the samples harvested under urea and NO_3^- regimes of the PBR (Fig. 3b). The culture accumulated much lower levels of residual N in the medium when cultivated with urea (5.14 mM, Day 24) when compared with NO_3^- (14.45 mM, Day 14). In other words, under continuous mode urea was utilized faster than NO_3^-, indicating at higher NUR for urea, as observed under batch conditions (Table 1). The fact, that the PBR accumulated a maximum of 5.14 mM of urea (one-sixth of feeding concentration of 30 mM; Fig. 3b) provided the basis of using higher concentration of urea (without inhibitory effects) for *Arthrospira* sp. cultivation with the present cultivation approach. This possibility of using higher concentrations of urea for *Arthrospira* sp. cultivation could be of a major advantage in the direction of treatment of urea (and possibly urine) rich effluent streams using *Arthrospira* sp.

3.3.2. Effect of nitrogen source transition on oxygen productivity and pigment content under continuous conditions

The MELiSSA project was conceived as a circular economy prototype with the objective of harnessing the N waste recycling potential of *Arthrospira* sp. and using it as a photosynthetic biorefinery for the production of edible biomass and O_2 for long term space missions (Farges et al., 2008). Therefore, from the perspective of MELiSSA project, the evaluation of the efficiency of different N sources in sustaining the photosynthetic health of the cyanobacterial cells was of primary importance. In order to evaluate the efficacy of urea (and eventually urine) for the cultivation of *Arthrospira* sp. from the perspective of

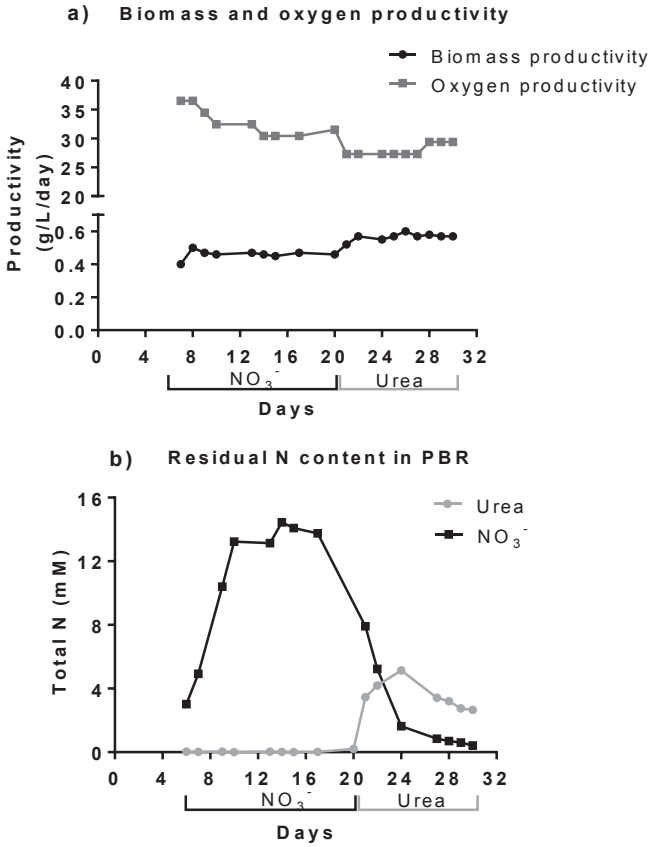

Fig. 3. Effect of nitrogen (N) source and transition on a) biomass and oxygen productivity (g/L/day), (b) N assimilation profile as residual N content (mM) and (c) oxygen productivity (g/L/day) of *Arthrospira* sp. PCC cells cultivated under controlled conditions PBR at 30 mM total N (urea or nitrate), pH 8.5 and 36 °C.

MELiSSA project, the culture operated under the two feeding N regimes of the continuous PBR run, was evaluated for its O_2 productivity and pigment content (Table 3).

The O_2 productivity observed under the urea regime (0.6 g/L/day) was 14.2% lower than the O_2 productivity (0.7 g/L/day) observed under the NO_3^- regime of PBR run (Fig. 3a, Table 3). The observed lower level of O_2 productivity under urea regime could be linked to stoichiometric difference (in terms of O_2 content) between urea and NO_3^-. In addition to the O_2 productivity, the pigment content of the photosynthetic cells is an important indicator of evaluating the efficiency of their photosynthetic machinery (Bandara et al., 2017). No significant differences were observed in the Chl_{tot}, PCB_{tot} and total carotenoid (Car_{tot}) contents of the biomass harvested under urea and NO_3^- regimes. Under batch conditions the 30 mM urea and NO_3^- subsets were respectively quantified with 68.1 mg/L and 66.9 mg/L of Chl_{tot} content (Table 1). These values were respectively lower by 57.7 and 60.2% than their corresponding value of 161.0 and 168.3 mg/L, observed under continuous conditions (Table 3). The higher Chl_{tot} content under continuous conditions could be attributed to the better availability of light and nutrient in the PBR due to better geometry and hydrodynamics. These results clearly indicated that (a) the productivity of the system could be increased by adopting a continuous and controlled feeding regime, as described under the present study conditions and (b) urea could be used as an efficient alternative to NO_3^- salts for the production of high value pigments from *Arthrospira* sp.

3.3.3. Effect of nitrogen source transition on biochemical profile under continuous conditions

The biomass harvested under the two N feeding regimes of PBR were quantified for their total protein, total carbohydrate and total lipid contents (as percentage of biomass). As observed for the batch experiments, the biomass harvested under continuous urea feeding regime, exhibited highest protein content. The total protein content of the biomass harvested under continuous urea regime was approximately 8.6% higher than the biomass fed with NO_3^- (Table 3). Similar trends were observed for the 30 mM batch experiments wherein the urea subset was quantified with 4.6% higher protein content than 30 mM NO_3^- subset (Table 1). Furthermore, the 70.9% total protein content quantified in the biomass fed with 30 mM urea (continuous regime) was comparable to the average protein content of 60–70% previously reported for *Arthrospira* sp. (Spolaore et al., 2006). The higher protein content quantified in the biomass harvested under urea feeding clearly indicated at the higher efficiency of urea at meeting the nutrient requirements of cyanobacterial cells under present cultivation parameters.

The total lipid and carbohydrate content of the cells (continuous PBR run) harvested under NO_3^- regime were found to be respectively higher by 21 and 18.2% than their corresponding values quantified under the urea regime (Table 3). The lower lipid and carbohydrate content in the biomass harvested under urea regime, coupled with their higher protein content indicated that urea is a better and efficient N source for supporting the metabolic needs of *Arthrospira* sp. On the other hand, for the batch experiments, the 30 mM urea and NO_3^- subsets exhibited comparable lipid and carbohydrate contents (Table 1), further indicating at the fact that indeed controlled-continuous feeding of nutrients could aid in improving the overall metabolic profile and productivity of the photosynthetic cells.

3.4. Practical applications and future perspectives

Despite of the many industrial, nutraceutical, medical and environmental advantages of *Arthrospira* sp., the mass acceptance of the cyanobacterial products has been hampered due their high production costs (Singh et al., 2017). Most of the commercial production of *Arthrospira* sp. is undertaken in raceway ponds (RP). Though the initial capital cost (equipment and energy costs) of these RPs are lower than the PBRs, but the lower biomass productivity, culture crashes due to

Table 3
Effect of different nitrogen sources (30 mM urea or nitrate) and transition on the average biomass productivity oxygen productivity, biochemical (total lipid, total protein and total carbohydrates) and pigment (total chlorophyll, total phycobiliprotein and total carotenoid) contents of the *Arthrospira* sp. PCC 8005 biomass cultivated under continuous conditions (pH 8.5, temperature 36 °C)* of photobioreactor.

NR	PB (g/L/day)	PO_2 (g/L/day)	TL*Δ (%)	TC*Δ (%)	TP*Δ (%)	Chl_{tot}* (mg/L)	PCB_{tot}* (g/L)	Car_{tot}* (mg/L)
NO_3^-	0.4	0.7	9.5	17.6	64.8	168.3	0.2	44.9
Urea	0.5	0.6	7.5	14.4	70.9	161.0	0.3	46.7

ΔThe values reported as the percentage of total dry cell weight (DCW) of biomass.
NR: Nitrogen Regime; *PB*: Biomass Productivity; *PO_2*: Oxygen Productivity; *TL*: Total Lipid; *TP*: Total Protein; *TPS*: Total Polysaccharide; *Chl_{tot}*: Total Chlorophyll (reported as sum of Chlorophyll *a* and Chlorophyll *b*; *PCB_{tot}*: Total Phycobiliprotein content (reported as sum C-phycocyanin, allophycocyanin and phycoerytherin); Car_{tot}: Total carotenoid content.
* Values reported as an average of all the timepoints of the respective transitions/nitrogen regime.

contamination and inefficient nutrient (light, CO_2, etc.) availability at bottom, reduce the overall economic viability of RPs. These shortcomings could be overcome by adopting a stringent bioprocessing approach and developing an energy and cost effective PBR. Consequently, Cornet and Dussap (2009) studied various PBR designs that could increase the overall productivity using an optimised light-transfer model. One of the PBR described by the authors was adopted in the present study to maximise the light use efficiency and increase the productivity.

Additionally, controlled and continuous feeding of nutrients to the PBR culture could help in improving the system performance by (a) preventing the possible inhibitory effects of pH and high N source concentration on photosynthetic cells and (b) avoiding the onset of N deplete conditions (and hence cellular stress) due to exhaustion of N source at the later stage of the culture. The present study thus highlighted the potential of using a modified cultivation approach that could aid in avoiding the inhibitory effects of urea and NO_2^-, even at high concentrations of 120 mM.

The ability of the *Arthrospira* sp. to grow at high concentrations (up to 120 mM) of urea (without inhibition) could provide the basis of using urea rich municipal wastewater (Rittstieg et al., 2001) for cyanobacterial cultivation, hence serving the purpose of N rich-wastewater recycling using photosynthetic cells. The use of locally available waste N sources had been reported to reduce the production costs of cyanobacterial byproducts (Slade and Bauen, 2013). Additionally, the concentrations of various N sources (urea, NH_4^+, NO_3^-, NO_2^-, etc.) in wastewater streams fluctuate on the basis of their effluent source, temperature, season, etc. The concentration and type of N source in the effluents can vary with source, temperature, season etc. Thus, the fact that the urea fed cultures were quantified with higher amount of cyanophycin (an important N reserve for cyanobacterial cells) and continued to grow even under N-free medium (removal of N supply), could offer a major advantage in using N-fluctuating wastewater streams for low cost cultivation of *Arthrospira* sp., without compromising on the overall productivity.

Urea fed cells were seen to have comparably better protein and pigment yields than the cells cultivated with NO_3^-.These biochemical results were supplemented with proteomic analysis of biomass fed with different N sources. The proteomic analysis further indicated that urea fed cells had higher abundance of the proteins related to N metabolism, PS II and pigment production. These results were in line with the results obtained on biochemical analysis of the cells. Additionally, hydrogenases, the proteins related to hydrogen production, were seen to be upregulated in urea fed cultures. This phenomenon could be harnessed for the production of bio-hydrogen. The accumulation of higher percentages of lipids and carbohydrates in the cells fed with low concentration ($\geq$15 mM) of urea (batch experiments) could provide the basis of using domestic and municipal wastewater for the production of biodiesel, bioethanol and renewable biopolymers from *Arthrospira* sp., which are of great economic importance for global economy. On the other hand, no significant variations were observed in the biochemical and proteomic profile were observed for the cells fed with NO_2^- (vs NO_3^- fed cells), which further indicated that NO_2^- rich effluents from meat and food industry could be used for low cost production of non-edible by-products from *Arthrospira* sp. Overall the different bioengineering, biochemical and proteomic tools used in the present study provides promising potential of coupling wastewater remediation with the creation of economically viable photosynthetic biorefineries.

4. Conclusions

This study, demonstrated the potential of using urea and NO_2^- salts, as cheaper alternatives to NO_3^- salts for *Arthrospira* sp. cultivation. The batch and continuous experiments established urea as better N source for cyanobacterial cultivation at concentrations up to 120 mM. While, highest protein and pigment content were quantified in urea fed biomass, comparable protein and pigment profiles were exhibited by biomass fed with NO_2^- and NO_3^- salts (of comparable N concentrations). This ability of *Arthrospira* sp. to assimilate high urea concentrations (up to 120 mM), without inhibitory effects, could help in embedding photosynthetic biorefineries with wastewater (urea/urine rich) remediation.

Conflict of interest

The authors hereby declare that they do not have any conflict of interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.biortech.2018.07.101.

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