Bioresource Technology 267 (2018) 492–501

Contents lists available at ScienceDirect

Bioresource Technology

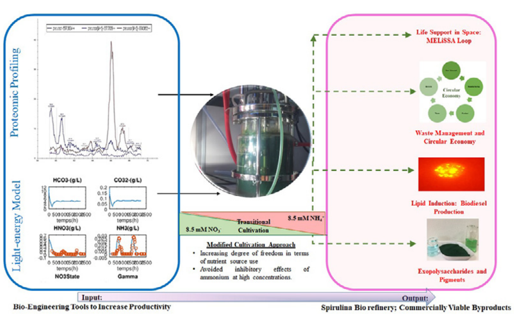
journal homepage: www.elsevier.com/locate/biortech

Assessment of transient effects of alternative nitrogen sources in continuous cultures of *Arthrospira* sp. using proteomic, modeling and biochemical tools

Neha Sachdeva[a], Giuseppe Giambarresi[a], Laurent Poughon[b], Juan Carlos Cabrera[c], Baptiste Leroy[a], Christophe Lasseur[d], Claude-Gilles Dussap[b], Ruddy Wattiez[a,*]

[a] Department of Proteomic and Microbiology, Research Institute of Biosciences, University of Mons, Mons 7000, Belgium
[b] Institut Pascal, Université Clermont Auvergne, CNRS, SIGMA Clermont, 4 avenue Blaise Pascal, Aubière 63178, France
[c] Biotechnology Unit, Materia Nova, Ghislenghien, Belgium
[d] European Space Agency, ESA/ESTEC, Noordwijk 2200, The Netherlands

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Arthrospira sp.
Alternative nitrogen source
Continuous cultivation
Proteomics
Light-energy transfer

ABSTRACT

The ability of cyanobacterium *Arthrospira* sp. to assimilate waste nitrogen sources (ammonium and urea) makes it an important candidate for wastewater management. The aim of this work was to evaluate a cultivation approach based on continuous-transitional-feeding regime (nitrate-ammonium-nitrate) in a photobioreactor to assess the effects of ammonium salts on *Arthrospira* sp. PCC 8005 metabolism. Using a comprehensive biochemical, proteomic and stoichiometric profiling of biomass, this study demonstrated that the proposed cultivation approach could increase the proteins and pigments yields by 20–30%, compared to the respective yields obtained from wild-type *Arthrospira* sp. strain A light-energy-transfer model was used to predict the biomass and oxygen productivities of *Arthrospira* sp. cultivated under transitional-feeding regime. 95 ± 2% match was observed between the experimental and simulated productivities. This study thus opened new avenues for use of ammonium rich wastewater for commercial production of high value pigments, biofuel and bioplastics using *Arthrospira* sp.

1. Introduction

The genetic robustness of cyanobacteria *Arthrospira* sp. and their versatility to adapt to a wide range of environmental conditions (pH, temperature, nitrogen sources, etc.) have increased their popularity with therapeutic, food, nutraceutical, waste water management, biofuel and various other commercial sectors (Zhou et al., 2017; Smetana et al., 2017; Shirazi et al., 2017; Jiang et al., 2015; Leema et al., 2010;

* Corresponding author at: Department of Proteomics and Microbiology, 20, Place du Parc, University of Mons, Mons 7000, Belgium.
E-mail address: Ruddy.Wattiez@umons.ac.be (R. Wattiez).

https://doi.org/10.1016/j.biortech.2018.07.062
Received 4 June 2018; Received in revised form 11 July 2018; Accepted 12 July 2018
0960-8524/

Spolaore et al., 2006). Due to the wide range of applications, the global Spirulina (*Arthrospira* sp.) market is expected to reach the value of USD 1.8 million by the year 2026 (Persistence Market Research, 2017).

Though sodium nitrate ($NaNO_3$) is the mostly commonly used nitrogen (N hereafter) source for commercial cultivation of *Arthrospira* sp., the use of low-cost alternative N sources such as ammonium salts (NH_4^+ hereafter) could increase the economic viability of *Arthrospira* sp. production (Carvalho et al., 2004). NH_4^+ salts are not only cost effective but also metabolically preferred N sources for cyanobacterial cultivation (Rodrigues et al., 2010; Carvalho et al., 2004). Furthermore, with the depletion of fresh water resources and the burning issue of waste water management (rich in $NH_{4)}^+$, exploring the potential of using NH_4^+ rich waste water as the alternative N source for commercial *Arthrospira* sp. cultivation could aid in environmental remediation. Despite of these advantages, NH_4^+ salts are rarely used for cyanobacterial cultivation because of the lower biomass productivity and high pH associated inhibitory effects (Markou et al., 2016; Markou et al., 2014; Rodrigues et al., 2010; Carvalho et al., 2004).

The advantages associated with controlled feeding (fed batch or continuous cultivation) of nutrients to the photobioreactor (PBR) had been extensively reviewed in literature (Carvalho et al., 2004; Sassano et al., 2010; Markou, 2015). Interestingly, most of these studies conducted with the aim to evaluate the effect of different concentrations of NH_4^+ salts on productivities of photosynthetic cells (batch mode culture, pH ≥ 9.5), overlooked to factor in the effect of high pH on the NH_4^+/ammonia (NH_3) equilibrium (pKa = 9.25, temperature range 25–30 °C) in the cyanobacterial cultures. Sassano et al. (2010) conducted a study to evaluate the effect of different concentrations of NH_4Cl (vs KNO_3) on concentration and composition of *Arthrospira* sp. biomass, under fed-batch conditions (no pH control). The study reported decreased biomass productivities at NH_4Cl concentration > 5.0 mM. Since the pH was not controlled in this study, the lower productivities could have been the result of N starvation in the culture caused due to the loss (≥80%, pH > 9.2) of NH_4^+ ions as NH_3 (Collos and Harrison, 2014). Similar trends were reported by Deschoenmaeker et al. (2017) in their batch study, wherein the *Arthrospira* sp. fed with 6.0 mM NH_4^+ exhibited higher N uptake rates than the cells fed with 6.0 mM NO_3^-, potentially due to the high pH induced loss of NH_4^+ as NH_3.

At pH > 9.25 (temperature 25–30 °C), > 80% of NH_4^+ ions have been reported to be lost (gas-off) as gaseous NH_3 (Collos and Harrison, 2014). High NH_3 concentration had been further reported to have inhibitory effect on photosynthetic machinery of cells. Dai et al. (2008) investigated the effects of high NH_3 concentrations on Photosystem PSII of cyanobacterial cells and reported that NH_3 inhibits PSII activity by changing the photochemistry of oxygen evolving complex (OEC). Being a structural analog of water molecule, NH_3 can easily attach to oxidized OEC (see Supplementary data) and hence reduce the overall photosynthetic activity of cells. Dai et al. (2008) quantified this effect in terms of fluorescence (Fv/Fm) which was seen to decrease under high NH_3 concentrations. Markou et al. (2016) reported that gaseous NH_3 also inhibits the PSI activity, by damaging the reaction centers (RC) of thylakoid membrane. Since these RCs are the main sites for energy efflux and ATP generation, any damage caused to RCs changes photochemistry of the cells. Thus in summary, the prevalence of high pH in cyanobacterial cultures could lead to: (1) loss of NH_4^+ ions to gaseous NH_3, leading to the onset of nutrient starvation; (2) presence of dissolved NH_3 concentrations in the liquid phase, leading to metabolic inhibitions.

Arthrospira sp. have developed robust regulatory pathways for N metabolism (see Supplementary data). The exogenous N sources are sequestered intracellularly with the help of membrane transporters and are metabolized to simpler NH_4^+ form before being incorporated into GOGAT pathway. These chemical reactions are catalyzed by enzymes and are regulated by transcription operons (Deschoenmaeker et al., 2017). The N source used for *Arthrospira* sp. cultivation, thus has a significant impact on the metabolic and proteomic profile of the cells. Most of the previous proteomic studies on *Arthrospira* sp. had been limited to the use of NO_3^- salts. Deschoenmaeker et al. (2017) investigated the effect of N source transition (NO_3^- to NH_4^+, concentration 28 mM) on the proteomic profile of *Arthrospira* sp. The authors reported lower abundance of cyanophycin (N reserve compound) related proteins and higher abundance of N stress (depletion) related proteins under NH_4^+ regime.

The versatility of *Arthrospira* sp. to assimilate both organic (urea, glutamate, etc.) and inorganic (NH_4^+, nitrate) N sources, had earned this oxygenic cyanobacterium an important place in the field of life support in space (LSS) (Cornet et al., 1995). European Space Agency (ESA) through its MELiSSA (Micro Ecological Life Support System Alternative) project, aims to harness the oxygenic and waste recycling potential of *Arthrospira* sp. PCC 8005 (*Arthrospira* sp. henceforth) for production of edible biomass and oxygen (O2) for manned space missions. One of the main objectives of the MELiSSA loop is to improve the closure and simplicity of the loop, by bypassing the oxidation process (NH_4^+ to NO_3^-) and use the crew waste (urea, NH_4^+) as the N sources for *Arthrospira* sp. cultivation.

The present study aimed to evaluate the effect of transition between the two N sources (NH_4^+ and NO_3^- salts) and variable cultivation parameters (N source transition, N concentration 8.5 mM, pH 8.5 and temperature 36 °C) on O_2 productivity, stoichiometric yields, metabolic, proteomic, biochemical profile of *Arthrospira* sp. biomass. The biochemical and metabolic results were used to upgrade the light-energy transfer model (Cornet and Dussap, 2009) in order to facilitate accurate prediction of cyanobacterial growth and productivities in presence of two transiting N sources. This study thus made the pioneering attempt to develop a knowledge model and evaluate the impact of different N sources on the biochemical profile of the *Arthrospira* sp. The novelty of the study lies in the comprehensive profiling of *Arthrospira* sp. using stoichiometric, mathematical, biochemical, metabolic and proteomic tools. This study, thus, opens new avenues for the use of fluctuating stream of (waste) N sources for commercial production of photosynthetic microorganisms.

2. Materials and methods

2.1. Cyanobacterial strain cultivation, photo-bioreactor setup and growth profile

The stock culture of *Arthrospira* sp. strain PCC 8005 was photoautotrophically maintained in 250 mL Erlenmeyer flasks in Cogne Modified Zarrouk medium (Cogne et al., 2003) at 30 °C (pH 8.5, 8.5 mM $NaNO_3$) on rotary shaker (140 rpm) under continuous illumination of 60 µmol photons $m^{-2} s^{-1}$. Each liter of Cogne Modified Zarrouk medium contained 1.0 g of NaCl, 0.03 g of $CaCl_2$, 1.0 g of K_2SO_4, 0.08 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of K_2HPO_4, 10.5 g of $NaHCO_3$, 7.6 g of Na_2CO_3, 0.08 g of EDTA, 0.01 g of $FeSO_4 \cdot 7H_2O$, and 1 mL per liter of the trace element solution containing (per liter) 0.23 g of $MnCl_2 \cdot 4H_2O$, 0.11g of $ZnSO_4 \cdot 7H_2O$ and 0.03 g of $CuSO_4 \cdot 5H_2O$.

A radially illuminated 2.0 L cylindrical double jacketed (Biostat®, Sartorius AG, Germany) PBR was used for the cultivation of photoautotrophic cultures (both batch and continuous mode) of cyanobacteria (see Supplementary data). Incident flux of 300 ± 50 µmol photon/m^2/s (Li-193SA; Li-Cor BioSciences, USA) was maintained using halogen lamps (Philips, 20 W, 12 V) and the agitation of 150 rpm was achieved with Rushton turbine. The pH (Mettler Toledo, InPro 3250) was automatically maintained at 8.5 with HCl (0.5 N) and NaOH (1.0 N). Turbidostatic regime under the continuous run was maintained with a dilution rate of 0.2 per day using Cogne Modified Zarrouk medium (8.5 mM $NaNO_3$ or NH_4Cl, pH 8.5). The PBR was continuously purged with 99.9% pure nitrogen gas at flow rate of 50 mL/min. Culture samples were collected every 24 h under axenic conditions and the biomass was recovered on filter (0.2 µm Sartorius