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Cyanophycin production in turbidostat cultivation of cyanobacteria under phosphorus limitation on synthetic urine media

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ABSTRACT

The cyanobacterium *Synechocystis* sp. PCC6803 is capable of using ammonium as nitrogen source which is the main nitrogen compound present in source-separated urine. Under phosphorus limiting conditions, *Synechocystis* has the ability to accumulate nitrogen in form of cyanophycin, an interesting polymer for the industry of bioplastics with a wide range of potential applications in biotechnology and agroindustry. We studied the accumulation of cyanophycin in *Synechocystis* cultivated in nutrient supplemented synthetic urine using ammonium as nitrogen source with different concentrations of phosphorus in batch and turbidostat cultivation. A decrease in biomass yield on light was observed in the turbidostat cultivation under strong phosphorus limitation, accompanied by overproduction of carotenoids and accumulation of cyanophycin. *Synechocystis* accumulated increasing amounts of cyanophycin reaching 10% of dry weight in form of cyanophycin in batch mode cultivation, and 7% in turbidostat cultivation. Higher accumulation of cyanophycin in both, batch and turbidostat cultivation, occurred at the lowest phosphate availability, biomass productivity decreased under strong phosphorus limitation. Mild phosphorus limitation allowed both cyanophycin and biomass production in turbidostat cultivation, and gave the higher titers in batch cultivation and the highest productivity in turbidostat cultivation. Strong phosphorus limitation decreased productivity of both biomass and cyanophycin as well as the nitrogen content in the biomass.

1. Introduction

Urine is an important source of nutrients; it contains 70% of the nitrogen and 40% of the phosphorus loads that end up in household wastewater [1]. At the same time, urine is a promising medium for cultivation of photosynthetic biomass. The cyanobacterial strain *Synechocystis* sp. PCC6803 (from here on *Synechocystis* 6803) is able to grow on ammonium, which is the main nitrogen source in (source-separated) urine. This strain is also known to accumulate reserve products of industrial interest such as polyhydroxybutyrate (PHB) [2] and cyanophycin [3]. Cyanophycin is particularly interesting; it can be used as a chemical platform for the production of a wide range of nitrogen containing chemicals otherwise produced from industrially fixed ammonia and oil, for example nylon-4,6 and acrylonitrile [4]. It is also possible to use cyanophycin for production of polyaspartic acid, an alternative to oil based poly acrylic resins [4].

Cyanophycin is a dipeptide of arginine and aspartic acid that is non-ribosomically synthesized in an ATP-dependent elongation reaction [5] as a carbon and nitrogen storage [6]. The accumulation of cyanophycin occurs under stress conditions induced by limitation of phosphate, potassium [3], sulfate or light [7] as well as by the inhibition of the ribosomal activity by addition of chloramphenicol [8]. During phosphorus limitation, the wild type of *Synechocystis* 6803 accumulated cyanophycin up to 18% of the biomass as dry weight (DW) and reached titers up to 0.35 g $CP \cdot L^{-1}$. These results were observed using nitrate as nitrogen source and under a light intensity of 70 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ in batch cultivation in photobioreactors (PBRs) [9]. Under these conditions the productivity of cyanophycin was 28 mg $CP \cdot L^{-1} \cdot d^{-1}$ [9]. In another study, high-density batch cultivation of *Synechocystis* 6803 under phosphorus starvation reached a 10% cyanophycin content and a titer of

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0.6 g CP·L^{-1} [10]. Also light limitation can trigger cyanophycin accumulation [8] and light and nutrient co-limitation often occurs in microalgae and cyanobacteria cultures [11]. However, the effect of phosphorus and light co-limitation on the accumulation of cyanophycin has not been studied yet.

When microalgae are cultivated on urine, phosphorus limitation occurs easily due to the high nitrogen-phosphorus molar ratio (N:P) of urine >30:1 compared to that of microalgae <22:1 [12,13]. Algal biomass generally contains around 1% (w/w) phosphorus in cultivation under nutrient balanced conditions where light is the only growth limiting factor [14]. *Synechocystis* 6803 phosphorus content ranges from 1.2% to 1.9% during balanced growth [15,16] to 0.1% under phosphorus starvation [9]. Phosphorus depletion in the growth medium does not affect *Synechocystis* 6803 biomass immediately; it continues to grow utilizing the intracellular phosphorus pool. Cyanophycin starts to accumulate when the phosphorus quotum of the biomass is below 0.4% and this continues until the phosphorus content reaches 0.1%, at which cyanophycin accumulation and growth stop [9]. Based on this, and assuming a 10% quotum of nitrogen in the biomass, *Synechocystis* 6803 biomass could reach N:P molar ratios up to 221:1, much higher than those of microalgae grown on urine [9]. This suggests the possibility to recover nutrients from urine in the form of cyanobacteria biomass and cyanophycin.

Most of the studies towards cyanophycin accumulation in *Synechocystis* 6803 used nitrate as nitrogen source. This makes sense, since ammonium can result in ammonia toxicity depending on the pH and also allows the ingrowth of nitrifying bacteria, which makes it more difficult to maintain axenic cultures. In the case of urine, after storage, >90% of the nitrogen is present as ammonium [17] and the direct use of ammonium for biomass production avoids the energy requirements of a nitrification process. The few studies of cyanophycin accumulation in *Synechocystis* 6803 with ammonium as the nitrogen source report values up to 3% of DW under conditions of phosphorus starvation [3], and a transient accumulation of up to 7% in another strain of the same genus *Synechocystis* sp. PCC6308 (Previously called *Aphanocapsa* 6308, from here on *Synechocystis* 6308) [18].

In contrast to nitrate, ammonium can be directly incorporated into the carbon-skeletons of organic molecules through the glutamine synthetase-glutamate synthase pathway (GS-GOGAT) [19,20]. Therefore, cyanophycin accumulation on ammonium seems to be energetically advantageous over nitrate since it does not require reduction of nitrate to ammonium. This was observed in nitrogen starved cultures of *Synechocystis* 6308 which assimilated extracellular nitrogen into cyanophycin faster when nitrogen was replenished using ammonium than when using nitrate [21].

Nutrient recovery and cyanophycin production rates with cyanobacteria are highly dependent on biomass productivity which is determined by the light availability inside the culture. Dense cultures are required for efficient nutrient recovery, but also in dense cultures the self-shading of the cells creates a light gradient that results in dark zones. These dark zones cause productivity loses due to cellular maintenance and respiration [22]. These losses can be diminished by continuous dilution of the photobioreactor, feeding the cultures in such a way that the darkest zone of the photobioreactor (opposite side to the light source) is reached by the light intensity needed to compensate for cell maintenance and respiration [23]. This operation mode, called turbidostat, also eliminates the risk of washout in case of nutrients and light co-limitation as feeding is done only when the biomass is actively growing.

This study addresses the effects of phosphorus and light co-limitation on the accumulation of cyanophycin by cultivating *Synechocystis* 6803 in batch mode under different levels of light and phosphorus. *Synechocystis* 6803 was also cultivated in turbidostat mode under different levels of phosphorus limitation to determine whether cyanophycin can be efficiently produced in a continuous mode photobioreactor by using phosphorus limitation as sole strategy to trigger cyanophycin accumulation. The study was done using an ammonium based medium mimicking diluted urine.

2. Materials and methods

2.1. Media

Synechocystis 6803 was precultivated in synthetic urine based on the composition of 10 times diluted human urine and supplemented with iron, magnesium and trace elements (Table 1). All media were sterilized by filtration through an autoclaved 0.22 μm pore size filter (Sartopore® Capsule 0.2 μm, Sartorius AG, Göttingen, Germany) into an autoclaved bottle or directly into a sterilized photobioreactor.

2.2. Inoculum

Synechocystis sp. PCC6803 was obtained from the Pasteur Culture Collection (PCC) of Cyanobacteria (Paris, France). Axenic pre-cultures for inoculation of the experiments on the effect of combined light and phosphorus limitation were derived from the PCC inoculum and cultivated on 10 times diluted synthetic urine (Table 1) under a light intensity of 200 $\mu mol_{photon}\cdot m^{-2}\cdot s^{-1}$ of warm white LED Lights SL 3500 (PSI Photon Systems Instruments, spol. s.r.o., Czech Republic). These cultures were pre-cultivated in 500-mL-Erlenmeyer flasks in 200 mL of synthetic urine (Table 1) in an Infors Multitron HT incubator (Infors, Switzerland) with orbital shaking at 120 rpm, in a 2.5% CO_2 enriched air atmosphere and at a controlled temperature of 35 °C. The axenic precultures for inoculation of the experiments on phosphorus starvation in turbidostat mode were cultivated in 250-mL-Erlenmeyer flasks in 100 mL of filter sterilized synthetic urine (Table 1). The precultures were cultivated in a climate chamber with constant shaking at 90 rpm, a temperature of 25°C and a 16/8 day night cycle with an irradiance of 50–60 $\mu mol_{photon}\cdot m^{-2}\cdot s^{-1}$ coming from the top in a 2.5% CO_2 enriched air atmosphere. The precultures were refreshed every week.

2.3. Cultivation conditions

2.3.1. Effect of combined light and phosphorus limitation in batch cultivation

We defined different phosphorus concentrations in the medium to reach 4 different phosphorus availability onsets:

Table 1
Elemental composition of synthetic urine in mg·L^{-1}.

Element/ compound	10× diluted synthetic urine trace elements supplemented (mg·L^{-1})
NH_4^+-N	500
PO_4^{3-}-P	30[a]
K	95
SO_4^{2-}-S	33.4
Ca	9.81
Fe	2.48[b]
Mg	9.89
Mn	0.50
B	0.50
Mo	0.15
Zn	0.05
Cu	0.02
Co	0.00001[c]
EDTA·Na_2Mg	36.8
pH	7.5
N:P	276:1
MOPS[d]	15–30 mM

[a] In the turbidostat experiments no phosphorus was added to the medium, instead, this was supplied in form of 1–2 M phosphoric acid solution.

[b] Added as $FeCl_3$ instead of Ferric Ammonium Citrate.

[c] Added as $CoCl_2$ instead of $Co(NO_3)_2$.

[d] pH buffer, added only for flask cultures.

1. (Bulk liquid) phosphorus depletion ($q_P = 1.0\%–0.4\%$); defined as the moment in which the concentration of phosphorus in the bulk liquid after removing the biomass is below the quantification limits of our equipment (<0.1 mg P·L^{-1}) and it is assumed zero.
2. Phosphorus limitation Level 1 triggering cyanophycin accumulation ($q_P = 0.4\%–0.1\%$)
3. Phosphorus limitation Level 2 using half of the phosphorus does of Level 1 triggering cyanophycin accumulation ($q_P = 0.4\%–0.1\%$)
4. Critical phosphorus limitation halting growth ($q_P = 0.1\%$). The defined phosphorus concentrations were 30 mg P·L^{-1} as reference experiment and 6.0, 3.0 and 1.5 mg P·L^{-1} as phosphorus limited experiments.

To achieve these four onsets the cultures were harvested at different timepoints as presented in Table 2.

The cultures were sampled daily to estimate the level of phosphorus limitation based on the biomass concentration and on the initial phosphorus concentration in the medium (Table 2).

These experiments were done in two separate batch cultivations under two different light intensities, i.e. 200 and 400 μmol$_{photon}$·m^{-2}·s^{-1} of cool white LED Lights SL 3500 (PSI Photon Systems Instruments, spol. s.r.o., Czech Republic). Each batch cultivation consisted of four sets of three replicate flasks, one set per concentration of phosphorus, completing twelve flasks. The flasks of each batch cultivation were inoculated using a homogeneous mix of precultures to achieve the total volume of inoculation needed for an initial optical density of 0.3 at 750 nm (OD_{750}) (approximately 0.07 g DW·L^{-1}). The cultures were cultivated in 200 mL of medium in 500-mL-Erlenmeyer flasks in an air atmosphere enriched by approx. 2.5%CO_2 at 35°C, with orbital shaking of 120 RPM. The cultures of the experiments at 400 μmol$_{photon}$·m^{-2}·s^{-1} were cultivated for the first 12 h under a light intensity of 200 μmol$_{photon}$·m^{-2}·s^{-1}, to avoid photoinhibition. After this period, the light intensity was increased to 400 μmol$_{photon}$·m^{-2}·s^{-1}. Photosynthetic growth using ammonium as nitrogen source results in the release of protons decreasing the pH. Therefore, the pH was buffered by addition of 30mM MOPS and maintained at values above 6.5, by occasional manual addition of a sterile solution of 1M NaOH to avoid growth limitation by low pH.

2.3.2. *Continuous experiment turbidostat mode*

Synechocystis 6803 was cultivated in a flat panel photobioreactor Labfors 5 Lux (Infors HT, Switzerland, 2010) with a volumetric capacity of 1.8 L and a light path of 0.02 m (Fig. 1). The experiment was performed under axenic conditions at a light intensity of 200 μmol$_{photon}$·m^{-2}·s^{-1} of warm white LED, temperature of 35°C and pH control by automatic addition of a sterile solution of 1M NaOH. The feeding of medium was automatically controlled by the photobioreactor as a response to the light measured at the back of the reaction vessel. The control was set to keep the light transmittance at 5 μmol$_{photon}$·m^{-2}·s^{-1}, this value was selected close to the compensation value for *Synechocystis* 6803 of 6.36 μmol$_{photon}$·m^{-2}·s^{-1} reported by [9]. The excess of broth was continuously harvested through a chemostat tube at the top of the photobioreactor controlling the level of the liquid. The harvested broth was pumped by an independent peristaltic pump into a bottle placed on top of a balance that was continuously recorded (Fig. 1). The dilution rate was calculated as the change in weight of the accumulated harvest daily.

Table 2
Harvest points and phosphorus onsets.

P in medium (mg P·L^{-1})	Harvest time criteria	Biomass quotum (q_P)	Phosphorus onset
30	When DW > 3.0 g DW·L^{-1}	1.0%–0.4%	(Control) medium phosphorus depletion
6.0	When DW > 3.0 g DW·L^{-1}	0.4%–0.1%	Phosphorus limited growth Level 1
3.0	Simultaneous with Level 1	0.4%–0.1%	Phosphorus limited growth Level 2
1.5	When 3 days of stable DW	0.1%	No growth due to phosphorus limitation

Initially the photobioreactor was fed with synthetic urine containing sufficient nutrients to support nutrient balanced growth up to a concentration of 3 g DW·L^{-1} as control medium. It was known from previous experiments that a turbidostat photobioreactor under 200 μmol$_{photon}$·m^{-2}·s^{-1} and light intensity at the back of the photobioreactor of 5 μmol$_{photon}$·m^{-2}·s^{-1} would maintain a biomass concentration of approximately 1 g DW·L^{-1}. Therefore, the phosphorus load was more than sufficient to support growth assuming a biomass phosphorus quotum of 1.0% (w/w) (Table 1). This guaranteed that the biomass was growing under sufficient nutrient availability. The photobioreactor was allowed to stabilize under these conditions and after reaching a steady state, defined as 3 consecutive days of a stable dilution rate, the volumetric biomass productivity of the photobioreactor fed with control medium was determined. In the phosphorus starvation phases the feed was switched to phosphorus free medium and the supply of phosphorus was done separately as a concentrated solution of phosphoric acid by an independent pump (Fig. 1). This allowed to control the supply of phosphorus independently and to limit the cultures growth in function of the phosphorus availability. Four different phosphorus starvation phases were established, denominated P Starve 1, 2 and 3. The phosphorus supply during P Starve 1, 2 and 3 was 0.54%, 0.27% and 0.13% of the volumetric biomass productivity determined in the control phase respectively. P Starve 1 had phosphorus availability just above the cyanophycin accumulation threshold of 0.4% based on the biomass productivity in our control phase. P Starve 2 and 3 had phosphorus availability within the cyanophycin accumulation range 0.1%–0.4%. At the same time, sufficient amounts of the other nutrients were added in the medium fed by the turbidostat control.

2.4. *Sampling and samples processing*

The feed pump of the photobioreactor was switched off 5 min before sampling. Six samples were taken from the photobioreactor, two samples of 10 mL for dry weight analysis, and four samples of 15 mL for cyanophycin extraction and for storage for later analysis. These samples were centrifuged at 4255 ×*g* for 15 min; pellets and supernatant were stored in separated flasks at −20°C for later analysis. Additional 5 to 10 mL samples were taken in a separate tube for determination of optical density, cell count, and light absorption.

2.5. *Analytical methods*

Culture growth was followed by measuring optical density at 750 nm (OD_{750}) in a bench spectrophotometer (DR6000, Hach-Lange, USA). Additional ODs at 480, 620, and 680 nm were measured in the same equipment as information on the pigmentation of the biomass can be extracted from the ratio of absorbance at these wavelengths in relation to 750 nm where no pigment absorption takes place. The OD_{680}, OD_{620} and OD_{480} were used as an estimate of the chlorophyll-a, C-phycocyanin and carotenoid content respectively. To correct for scattering, the OD_{750} was subtracted and the result was divided by the OD_{750}. The samples were diluted 10× with demineralized water to fit the OD750 values within the linear range of the OD750 to dry weight (DW) correlation. Dry weight was determined by a 13 h freeze drying of pellets obtained after centrifugation. Cell counts and biovolume were determined by a Multisizer III (Beckman Coulter Inc., USA) using a 50 μm aperture tube. Samples were diluted in ISOTON II diluent. The cell diameters were calculated based on the biovolume assuming spherical cells.

The wavelength dependent light absorbance of the biomass was measured in a UV–vis/double beam spectrophotometer (Shimadzu UV-2600, Japan) equipped with an integrating sphere that minimizes light scattering (ISR-2600) using cuvettes with an optical path of 2 mm. The

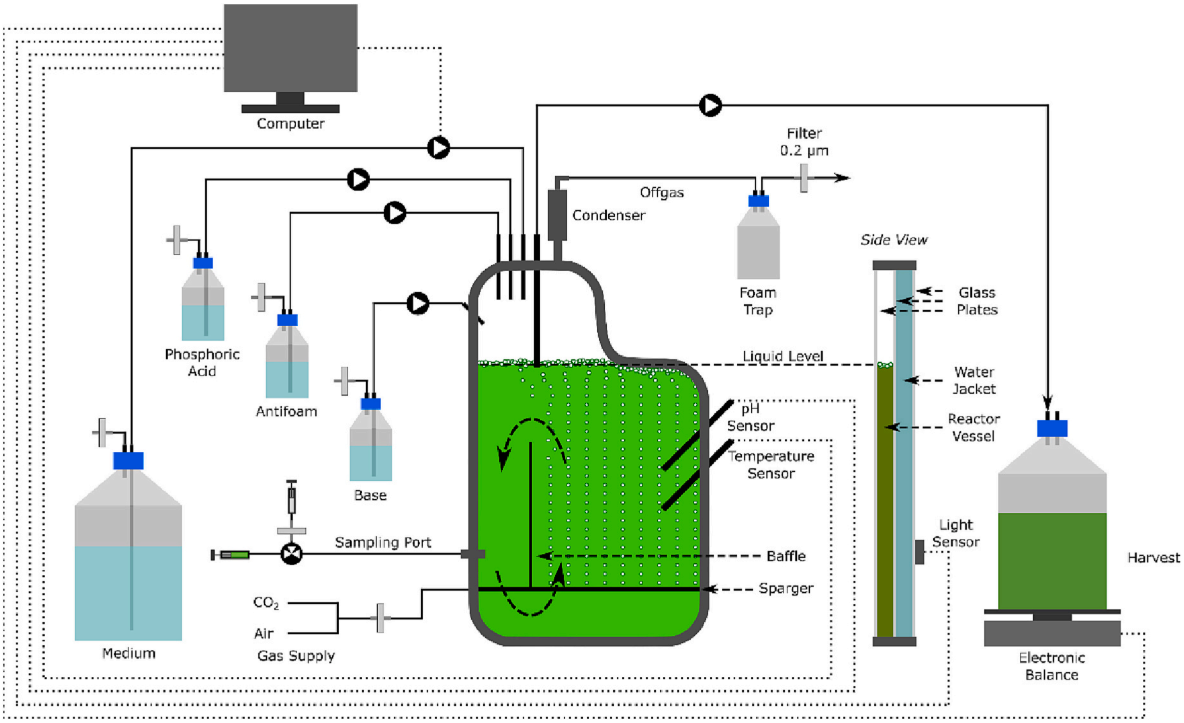

Fig. 1. Liquid phase diagram experiments p starvation in continuous mode.

average-dry-weight-specific optical cross section (ax, $m^2 \cdot g^{-1}$) was measured and calculated according to de Mooij et al. using the light absorbance of *Synechocystis* 6803 in the wavelengths between 400 and 750 nm with a step size of 1 nm [24].

Cyanophycin extraction and quantification were done in duplicate for each independent culture. Cyanophycin was extracted based on the method of [25] and quantified by the Sakaguchi reaction assay for arginine containing proteins developed by [26] with modifications of the procedure by [10].

2.6. Calculations nutrients quota and N:P molar ratio of biomass

No precipitation was observed in the flasks and ammonia volatilization could be neglected under the experimental conditions 35°C and pH 7.5 or below, therefore the changes in concentration of nitrogen and phosphorus in the medium are attributed to their assimilation into biomass. No abiotic nutrient losses were observed in this cultivation medium in a previous study [27] under the same conditions used in the present study. The quota of each nutrient (E) in the biomass $q_{E,X}$ (w/w) was calculated assuming that the change in concentration of each nutrient in the medium was due to assimilation into the biomass. The calculation was done as a mass balance for the nutrients in the biomass using Eq. (1),

$$q_{E,X} = \frac{C_{E,m,t_0} - C_{E,m,t_i}}{C_{X,t_i} - C_{X,t_0}} \tag{1}$$

where C_{E,m,t_0} and C_{E,m,t_i} are the concentrations (w/v) of the element in the medium at times t_0 and t_i respectively; and C_{X,t_0} and C_{X,t_i} are the concentrations (w/v) of the biomass at times t_0 and t_i respectively.

The N:P was calculated using Eq. (2),

$$N:P = \frac{n_{N,X}}{n_{P,X}} \tag{2}$$

where $n_{N,X}$ and $n_{P,X}$ are the moles of nitrogen and phosphorus per gram of biomass respectively, which were derived from their respective quotas by dividing by the molecular weight of each element.

3. Results and discussion

3.1. Effect of light and phosphorus co-imitation on cyanophycin content in batch cultivation

Synechocystis 6803 was cultivated on 10 times diluted synthetic urine in batch mode with phosphorus concentrations of 30.0, 6.0, 3.0 and 1.5 mg $P \cdot L^{-1}$ under two light intensities 200 and 400 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ to determine the effect of different levels of phosphorus and light co-limitation on the accumulation of cyanophycin in the biomass. Under both light intensities the reference cultures (cultivated with 30 mg $P \cdot L^{-1}$) exhibited similar growth curves (Fig. 2). In our batch experiments, the reference cultures were harvested during the linear growth phase at biomass concentrations of 4 g $DW \cdot L^{-1}$. These cultures maintained linear growth and stable biomass productivities of 0.8 and 0.6 g $DW \cdot L^{-1} \cdot d^{-1}$ ($R^2 = 0.99$) during 5 and 8 days under 400 and 200 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ respectively. These cultures did not have significant productivity losses due to light limitation caused by cells self-shading. Note that the cultures on 400 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ were exposed to 200 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ during the first 12 h. The phosphorus limited cultures diverted from the growth curve of the reference cultures at different time points indicating phosphorus limited growth, this occurred earlier at lower phosphorus doses (Fig. 2). Before the observed productivity decline the phosphorus limited cultures cultivated with 6.0, 3.0 mg $P \cdot L^{-1}$ had biomass productivity similar to the reference cultures during the linear phase. The cultures cultivated with 1.5 mg $P \cdot L^{-1}$ diverted from the growth curve of the reference cultures at an earlier stage of growth with much lower productivities of 0.13 and 0.31 g $DW \cdot L^{-1} \cdot d^{-1}$ under 400 and 200 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ respectively. Under higher light intensity, phosphorus limitation had a stronger negative effect on the biomass. The cultures under 400 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ had a larger decrease in productivity due to phosphorus limitation than those under 200 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ (Fig. 2C).

The cultures were harvested at different biomass concentration to analyze them under different phosphorus limiting onsets (Table 2). The cultures on 30.0 and 6.0 mg $P \cdot L^{-1}$ were harvested at a biomass concentration of 3.9 ± 0.2 g $DW \cdot L^{-1}$, and those cultivated with 3.0 mg $P \cdot L^{-1}$

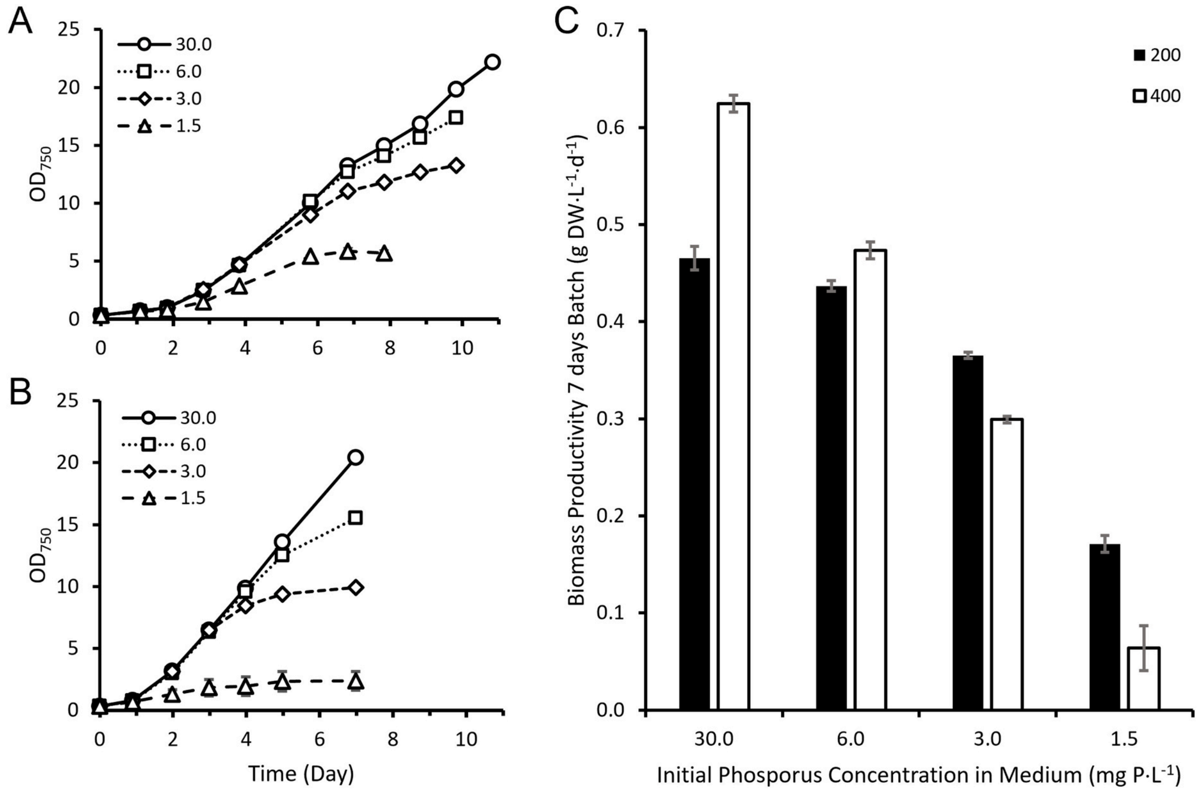

Fig. 2. Growth curves of *Synechocystis* 6803 cultivated on medium containing 30, 6.0, 3.0 and 1.5 mg P·L^{-1} under a light intensity of (A) 200 µmol$_{\text{photon}}$·m^{-2}·s^{-1}, and (B) 400 µmol$_{\text{photon}}$·m^{-2}·s^{-1}; and (C) biomass productivity during 7 days of batch cultivation calculated based on the OD to DW correlation. All values are averages of three biological replicates, the error bars are covered by the markers if not visible.

were harvested at a biomass concentration of 2.7 ± 0.3 g DW·L^{-1}. The cultures on 1.5 mg P·L^{-1} that were expected to reach a stationary phase were harvested after 3 days of stable biomass concentration at 1.30 ± 0.09 g DW·L^{-1} and 0.55 ± 0.17 g DW·L^{-1} under 200 and 400 µmol$_{\text{photon}}$·m^{-2}·s^{-1} respectively (Fig. 3A).

At both light intensities more cyanophycin accumulated in the cultures with the lower phosphorus availability; at the same time the lowest cyanophycin content was observed, as expected, in the reference experiments. In the cultures under 200 µmol$_{\text{photon}}$·m^{-2}·s^{-1} the accumulation of cyanophycin reached values of 2.0 ± 0.1% and 5.7 ± 0.9% of biomass dry weight in form of cyanophycin after 10 days when cultivated with 6.0, 3.0 mg P·L^{-1} respectively and 6.6 ± 0.6% after 8 days when cultivated with 1.5 mg P·L^{-1}; while the reference experiment (on 30 mg P·L^{-1}) accumulated the lowest value 1.5 ± 0.6% after 11 days (Fig. 3C). A similar trend was observed under 400 µmol$_{\text{photon}}$·m^{-2}·s^{-1}, reaching 1.4 ± 0.2%, 3.8 ± 0.3% and 10.3 ± 4.2% in the phosphorus limited cultures with 6.0, 3.0 and 1.5 mg P·L^{-1} respectively and only 1.1 ± 0.3% in the reference experiment; all after 7 days.

The difference in cyanophycin content observed between the cultures cultivated with 30.0 and 6.0 mg P·L^{-1} was rather small. This indicates that the cultures started with these concentrations did not reach a level of phosphorus starvation sufficient to trigger a significant accumulation of cyanophycin. This was expected in the control cultures, but not in the cultures on 6.0 mg P·L^{-1}, which slowed down growth diverting from linear growth due to phosphorus limitation already after 7 and 4 days under 200 and 400 µmol$_{\text{photon}}$·m^{-2}·s^{-1} respectively (Fig. 2).

The cultures on 30.0 mg P·L^{-1} under both light intensities, 200 and 400 µmol$_{\text{photon}}$·m^{-2}·s^{-1}, did not show growth limitation due to a lack of phosphorus but exhibited linear growth associated with light limitation. These cultures were harvested at a similar biomass concentration (4.0 ± 0.2 g DW·L^{-1}) and therefore it is clear that those at 200 µmol$_{\text{photon}}$·m^{-2}·s^{-1} had a lower light availability than those at 400 µmol$_{\text{photon}}$·m^{-2}·s^{-1}. This lower light availability did not result in a significantly higher cyanophycin accumulation. On the contrary, the difference was marginal, giving a higher cyanophycin content of 1.5 ± 0.2% at 200 µmol$_{\text{photon}}$·m^{-2}·s^{-1} compared to 1.1 ± 0.3% at 400 µmol$_{\text{photon}}$·m^{-2}·s^{-1} respectively. Previous studies suggested that lower light intensities resulted in higher total amount of cyanophycin, however the effect on the amount of cyanophycin in the biomass that they reported were also marginal [7,8].

Cyanophycin production is often associated to the transition from exponential or linear growth to a stationary phase. In our results, the highest contents of cyanophycin were found in the biomass that was already in stationary phase i.e. in the cultures cultivated on an initial phosphorus concentration of 1.5 mg P·L^{-1} (Fig. 3B). The highest titer however was achieved in the cultures cultivated on 3.0 mg P·L^{-1} under both light intensities (Fig. 3C), giving a maximum value of 0.18 g CP·L^{-1} under 200 µmol$_{\text{photon}}$·m^{-2}·s^{-1}; these cultures were in the transition from linear to stationary phase (Fig. 2). This is significantly lower than the 0.35–0.60 g CP·L^{-1} reached in other studies with nitrate instead of ammonium as the nitrogen source [9,10]. Differences in the cultivation techniques used in these studies make it difficult to define the reason of such different titers. For instance, the value of 0.60 g CP·L^{-1} was achieved under gradually increasing light up to 950 µmol$_{\text{photon}}$·m^{-2}·s^{-1} [10], much higher than the 400 µmol$_{\text{photon}}$·m^{-2}·s^{-1} used in our batch experiments. It is also possible that the energy cost of compensating for some degree of free ammonia inhibition could be reflected in such lower titer.

Under lower light intensity it is more likely to have simultaneous biomass growth and cyanophycin accumulation than under higher light intensity, this can be observed in the higher titers of the cultures at 200 µmol$_{\text{photon}}$·m^{-2}·s^{-1} than in the cultures at 400 µmol$_{\text{photon}}$·m^{-2}·s^{-1} (Fig. 3C). Phosphorus limited growth at an earlier stage in the cultures at 400 µmol$_{\text{photon}}$·m^{-2}·s^{-1}, resulting in an earlier shift of the nitrogen metabolism from protein production to cyanophycin production, as a consequence this cultures had lower biomass concentration with a

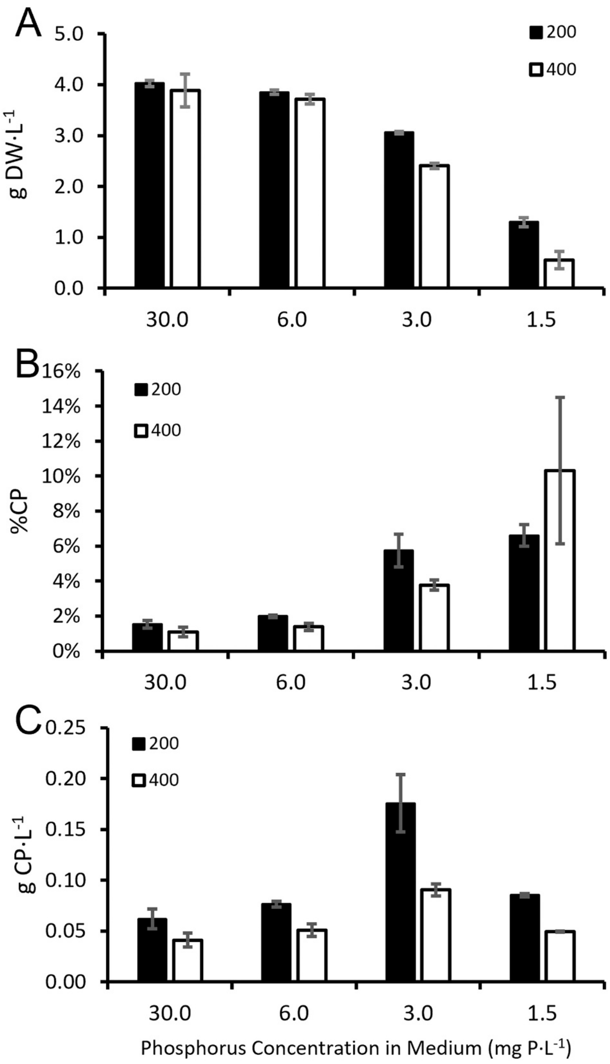

Fig. 3. Biomass concentration (A), cyanophycin content in biomass as dry weight basis (B) and cyanophycin titrant at the time of harvest (C) of *Synechocystis* 6803 cultivated on medium containing 30.0, 6.0, 3.0 and 1.5 mg $P \cdot L^{-1}$ under a light intensities of 200 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ and 400 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$. All values are averages of three biological replicates, the error bars are covered by the markers if not visible.

higher content of cyanophycin. Differently, the cultures at 200 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ had a delay in this shift, which allowed higher cyanophycin titers. This is confirmed by the biomass concentration at the stationary phase in the cultures cultivated on 1.5 mg $P \cdot L^{-1}$, which was lower under 400 than under 200 mg $P \cdot L^{-1}$ $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ (Fig. 3A). In phosphorus limited cultures, the excess of light energy absorbed that cannot be used for growth must be channeled by the cells towards either photosystem repair, dissipation as heat or alternative metabolic routes; otherwise the excess energy can cause photodamage [28,29]. The effects of light and phosphorus limitation suggest an optimum combination of phosphorus dose and light intensity to trigger the production of cyanophycin without limiting excessively biomass growth.

3.2. Nitrogen and phosphorus consumption in phosphorus limited batch cultivation

Although phosphorus got depleted from the bulk medium in all batch experiments, *Synechocystis* 6803 continued to grow utilizing its internal phosphorus pool and continued to take up nitrogen (Fig. 4). This resulted in an increase of the (calculated) N:P molar ratio of the biomass to values as high as 111:1 (Supplementary material Fig. S1). In the control cultures the N:P molar ratio ranged between 19:1 and 32:1 (Fig. S1, Supplementary material). This flexibility of *Synechocystis* 6803 to adapt its N:P ratio is very different from e.g. the microalgae *Chlorella sorokiniana* with reported values between 10:1 and 23:1 in batch cultivation in media with increasing N:P ratios [13].

Synechocystis 6803 shows a dynamic pool of phosphorus ranging from 0.13% to 3.80%. (Fig. S2, Supplementary material). In our phosphorus limited cultures, the phosphorus quota decreased exhibiting an asymptotic behavior towards minimum values of 0.15 ± 0.01% and 0.13 ± 0.01%. At these values growth stopped due to the lack of phosphorus. Minimum phosphorus content of 0.1% was previously reported for *Synechocystis* 6803 [9].

On the first two days of cultivation, the ammonium-nitrogen consumption was not reflected in biomass production, resulting in a nitrogen conversion up to 400 mg N·g DW produced^{-1} (Fig. S2, Supplementary material). The nitrogen quota calculated at the end of the experiments was lower the phosphorus availability and ranged between a very high value of 17.8% to a low of 2.5%. As a comparison balanced growth should results in a nitrogen content of 10% [30]. The ammonium-nitrogen consumed during the first two days of cultivation was not transformed into nitrite nor nitrate; and other nitrogen species such as nitrous oxide or dinitrogen gas are unlikely to occur under the experimental conditions and if present these would represent a marginal fraction of the consumed ammonium. Cyanophycin itself contains 24% of nitrogen and its accumulation only cannot explain such a high nitrogen content in *Synechocystis* cells. An accumulation of 10% cyanophycin for example would represent only 2.4% of the nitrogen present in the cells of *Synechocystis* 6803. The reason behind the wide range of nitrogen quota observed in our dynamic batch experiments therefore remains unclear.

3.3. Cyanophycin accumulation due to phosphorus starvation in turbidostat cultivation

Cyanophycin production is mostly studied in batch experiments [7,9,31,32]. A continuous process offers the possibility of a higher biomass productivity by optimizing light use, this can be achieved by maintaining an optimal biomass concentration in the photobioreactor, minimizing dark zones under a given light availability [23]. A recent study achieved a maximum cyanophycin productivity of 27 $mg_{CP} \cdot L^{-1} \cdot d^{-1}$ in continuous cultivation in a phosphorus limited chemostat photobioreactor using nitrate as nitrogen source [33]. Phosphorus limitation triggers the accumulation of cyanophycin [3], however high levels of phosphorus limitation decrease biomass productivity, resulting in the risk of washout. A continuous process operated in turbidostat mode allows (1) operating photobioreactors under light and phosphorus co-limitation while eliminating the risk of washout and (2) independently controlling light regime and phosphorus availability. In this study, we explored the possibility of continuously producing cyanophycin using solely phosphorus limitation while maintaining a constant light regime. As the titers obtained in the batch experiments suggested a delicate tradeoff between biomass and cyanophycin formation depending on the availability of phosphorus, we expected a similar tradeoff in a continuous process, but at higher productivities.

Synechocystis 6803 was cultivated in a turbidostat photobioreactor in ammonium based 10 times diluted synthetic urine, the production of cyanophycin was induced by decreasing the level of phosphate supply in four steps resulting in four different phases of limitation; Control, P Starve 1, 2 and 3. The dilution rate and cyanophycin content in the biomass expressed fluctuations during the periods between the stable phases, especially on the phosphorus loads of 2 and 1 mg $P \cdot L^{-1} \cdot d^{-1}$ (Fig. 5). This seems to be a consequence of the method used to control the feeding of fresh medium to the photobioreactor. The feeding was

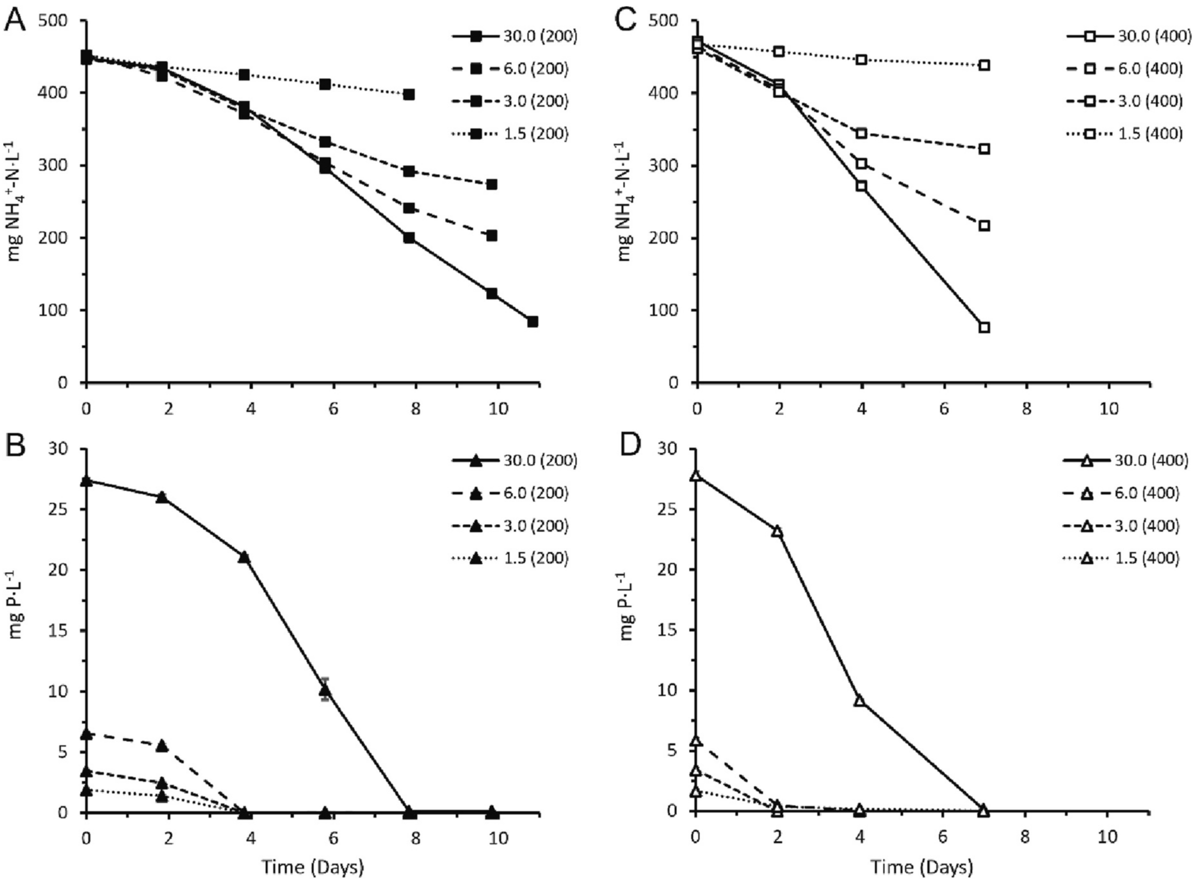

Fig. 4. Nitrogen and Phosphorus concentrations in the medium during cultivation of *Synechocystis* 6803 on medium containing 30, 6.0, 3.0 and 1.5 mg P·L^{-1} under a light intensity of 200 μmol$_{photon}$·m^{-2}·s^{-1} (A and B) and 400 μmol$_{photon}$·m^{-2}·s^{-1} (C and D). All values are averages of three biological replicates; the error bars are covered by the markers if not visible.

done in function of the light intensity measured at the back of the culture. The feeding pump was controlled by a script with 30 sec evaluation time that increased or decreased the speed of the feeding pump by 5% every time the light intensity was below or above the set point (5 μmol$_{photon}$·m^{-2}·s^{-1}). The sensitivity of the light meter was 1 μmol$_{photon}$·m^{-2}·s^{-1}, being this the minimum change in value that would trigger a change in the feeding of medium. Since biomass growth decreased at lower phosphorus availability, a change in light transmittance of 1 μmol$_{photon}$·m^{-2}·s^{-1} took longer time to occur and consequently, the speed of the fresh medium feeding pump took longer to change. This resulted in periods of up to 30 min without feeding of fresh medium during which phosphorus was being added to the photobioreactor at a constant flow. As a consequence, the availability of phosphorus had larger fluctuations during the phosphorus limited periods. More precise feeding of the photobioreactor could result in more stable cyanophycin content in the biomass. This was observed in chemostat photobioreactors as result of constant feeding [33].

We suspect that cyanophycin accumulation is a very dynamic process leading to large fluctuations in its content especially during the transition and acclimation period between two levels of phosphorous limitation [34]. Despite these fluctuations, we will compare the general trends observed in the stable phases summarized in Fig. 6.

The dilution rate of the photobioreactor increased during the first 20 days in the control phase reaching a maximum value of 1.23 ± 0.05 d^{-1} between days 24 and 28 (Figs. 5 and 6 where the average results of the distinct phases are summarized). These days were the phosphorus abundant period and used as control condition to determine the phosphorus supply in the phosphorus limited phases. During the control period the biomass concentration stabilized at 0.61 ± 0.02 g DW·L^{-1} giving a volumetric biomass productivity of 0.75 ± 0.05 g DW·L^{-1}·d^{-1}. Based on this biomass productivity and based on the assumptions also used in the experimental design of the batch experiments, we aimed to limit biomass growth by decreasing the phosphorus supply to values below 1% (w/w) of the biomass DW productivity during the control phase i.e. 0.52%, 0.26% and 0.13%. This resulted in phosphorus loads of 4, 2 and 1 mg P·L^{-1}·d^{-1} during P Starve 1, 2 and 3 phases respectively much lower than the 37 mg P·L^{-1}·d^{-1} during the control phase. The phosphorus load was changed on days 28, 50 and 69 for the phosphorus starvation phases P Starve 1, 2 and 3 respectively (Fig. 5). After day 46 the photobioreactor operation was stable and the period between days 50 and 56 was assumed as the steady state of P Starve 1 phase, on day 56 the phosphorus load was halved and allowed to stabilize under the new phosphorus supply. Steady state of P Starve 2 phase was assumed during days 64 and 69. On day 69 the phosphorus load was halved once again for the last phase of phosphorus starvation P Starve 3 (Fig. 5).

During the steady state phases, the dilution rate of the photobioreactors is considered equivalent to the biomass specific growth rate as it is a completely stirred tank reactor (CSTR) and that the specific light absorption cross-section is constant under stable conditions. The cyanophycin content of the biomass during the (Control) phase was 1.13 ± 0.03%, but this increased to higher values at lower phosphorus supply rates giving values of 3.51 ± 0.32%, 5.17 ± 1.98% and 7.31 ± 1.62% cyanophycin on a dry weight basis during P Starve 1, 2 and 3 respectively (Fig. 6B). This was expected for P Starve 2 and 3 on the basis of the findings of Trautman et al. [9], but not for P Starve 1 as the phosphorus load was higher than 0.4 % of the volumetric biomass productivity of the control phase. Phosphorus starvation also negatively affected the specific growth rate, which decreased with the lower phosphorus loads

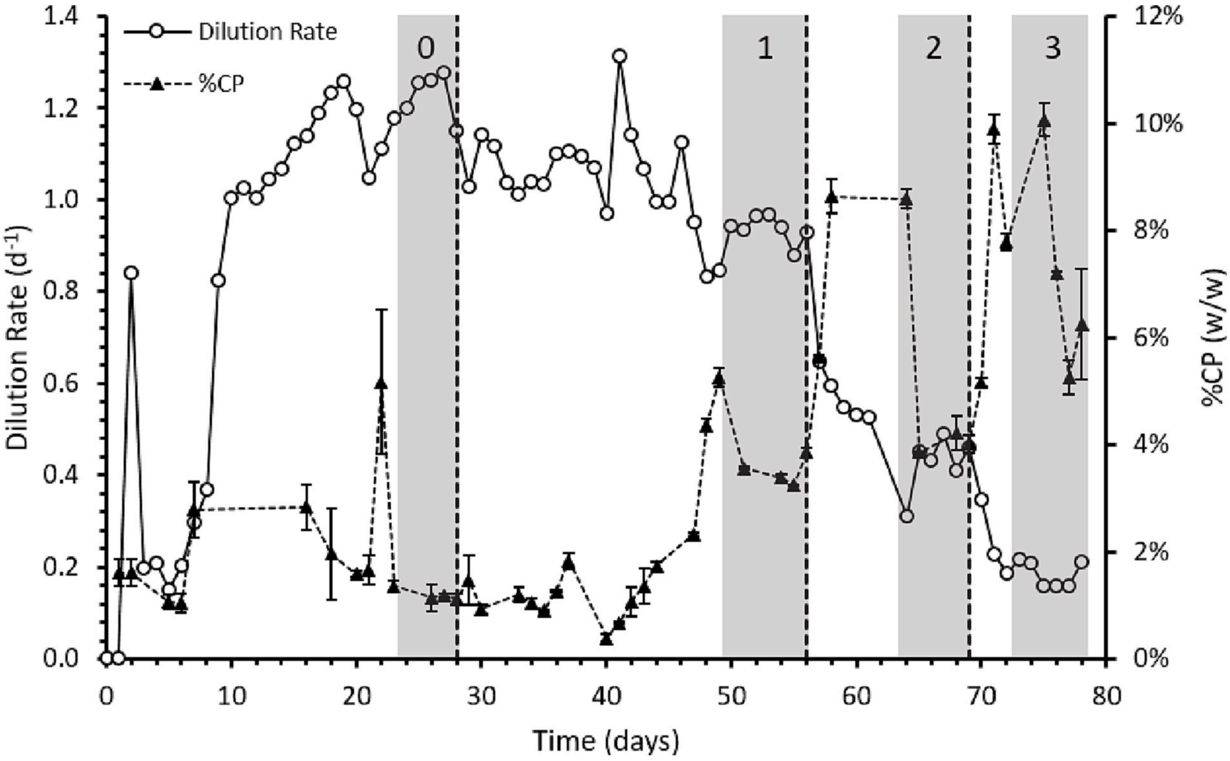

Fig. 5. Dilution rate and CP content of *Synechocystis* 6803 cultivated in turbidostat mode under a light intensity of 200 $\mu mol_{photon}\cdot m^{-2}\cdot s^{-1}$ on phosphorus loads of 37, 4, 2 and 1 mg $P\cdot L^{-1}\cdot d^{-1}$. Grey areas are considered steady state under control phase (0) and (1, 2 and 3) phosphorus limited phases respectively. Cyanophycin values are averages of duplicate samples processed independently.

giving 1.23 ± 0.05, 0.94 ± 0.03, 0.43 ± 0.06 and 0.19 ± 0.03 d^{-1} during the Control, P Starve 1, 2 and 3 phases respectively (Fig. 6A).

The volumetric biomass productivity was similar on the control and P Starve 1 phases with values of 0.75 ± 0.05 $g_{DW}\cdot L^{-1}\cdot d^{-1}$ and 0.84 ± 0.10 $g_{DW}\cdot L^{-1}\cdot d^{-1}$, respectively. However, during P Starve 2 and 3, the volumetric biomass productivity decreased to values of 0.46 and 0.23 $g_{DW}\cdot L^{-1}\cdot d^{-1}$ respectively (Fig. 6C). Biomass productivity was higher than in our batch experiments (0.6 g $DW\cdot L^{-1}\cdot d^{-1}$ at 200 $\mu mol_{photon}\cdot m^{-2}\cdot s^{-1}$).

The highest volumetric cyanophycin productivity was 29.6 ± 3.9 $mg_{CP}\cdot L^{-1}\cdot d^{-1}$ in phosphorus starvation phase P Starve 1 but decreased to 22.7 ± 4.9 and 16.9 ± 3.3 $mg_{CP}\cdot L^{-1}\cdot d^{-1}$ under stronger phosphorus limitation in P Starve 2 and 3, respectively (Fig. 6D). Trentino et al., reached a similar productivity of cyanophycin of 30 $mg_{CP}\cdot L^{-1}\cdot d^{-1}$ with *Synechocystis* 6803 in a chemostat photobioreactor under phosphorus starvation. Trautmann et al., also reached a similar productivity of 28 $mg_{CP}\cdot L^{-1}\cdot d^{-1}$ in batch experiments with a genetically engineered strain of *Synechocystis* 6803 denominated BW86 (*Synechocystis* BW86 from here on) using nitrate as nitrogen source. *Synechocystis* BW86 mutation results in a modification on the signaling PII protein that unlocks the arginine metabolic pathway causing the overproduction of CP in the cells [9]. As the phosphorus load supplied to the photobioreactor decreased, the photobioreactor stabilized at higher biomass concentrations (Fig. 6E), which suggests that phosphorus starvation had an effect on the amount of light absorbed per unit of mass of biomass and implies changes in pigmentation that we discuss later. The biomass concentration was 0.61 ± 0.02, 0.91 ± 0.08, 1.14 ± 0.09 and 1.36 ± 0.19 $g_{DW}\cdot L^{-1}$ during the steady state of the control, P Starve 1, 2 and 3 phases respectively.

Analogue to what was observed in the batch experiments, the conditions giving the highest cyanophycin content did not correspond with the highest volumetric cyanophycin productivity. A mild degree of phosphorus starvation allowed both, biomass growth; observed in significant volumetric biomass productivity; and cyanophycin accumulation in P Starve 1 (Figs. 6C and 7D). A higher degree of phosphorus starvation did not only trigger the accumulation of cyanophycin but also limited the volumetric biomass productivity, therefore cyanophycin productivity decreased as well, as observed in P Starve 2 and 3 (Figs. 6C and 7D).

The yield of biomass on light was similar under phosphorus replete conditions in the control phase and under mild phosphorus starvation in P Starve 1 giving values of 0.89 ± 0.06 and 1.00 ± 0.11 g $DW\cdot mol_{photon}^{-1}$. Similar yields of biomass on light under balanced conditions of 0.71–0.97 g $DW\cdot mol_{photon}^{-1}$ were achieved with *C. sorokiniana* cultivated in chemostat photobioreactors on urine [35]. Perhaps the lower values observed in the cultivation on urine are due to a higher level of ammonia inhibition as result of a higher light intensity.

Stronger phosphorus starvation in P Starve 2 and 3 decreased the yield of biomass on photons 38% and 69% with respect to the value in the control phase (Fig. 6F) showing that a large fraction of the light energy absorbed by the culture was not invested in biomass growth including cyanophycin accumulation. This is due to an imbalance caused by an excess of light energy absorbed with respect to the low phosphorus available for growth. The excess of light energy absorbed must have been channeled towards either photosystems repair; alternative metabolic routes; dissipated as heat or fluorescence through carotenoids.

3.4. Effects of phosphorus starvation in turbidostat cultivation

3.4.1. Phosphorus starvation decreases biomass nitrogen content

The nitrogen content in the biomass decreased from 10.7% under phosphorus replete conditions to 6.0% at the lowest phosphorus availability (Fig. 7). Cyanophycin does not necessarily increase the nitrogen content of cyanobacteria biomass although it might be the case under other conditions. The lower nitrogen content in phosphorus starved biomass could be due to a lower protein content as a consequence of the lack of phosphorus. The lower nitrogen content under phosphorus

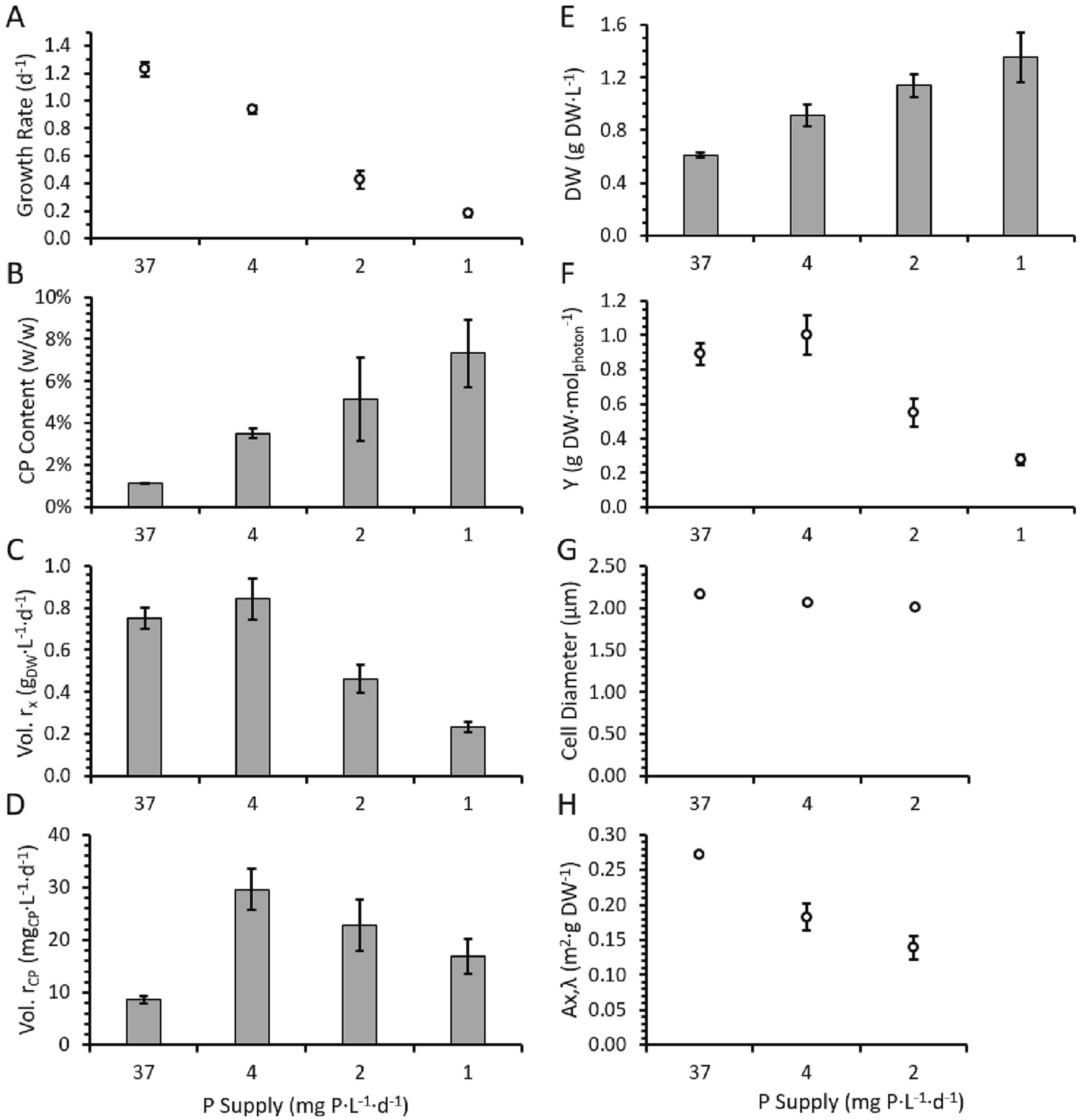

Fig. 6. Specific growth rate, cyanophycin (CP) content, biomass and cyanophycin volumetric productivity, dry weight (DW), biomass yield on photons (Y), Cell Diameter and spectrally averaged cross-section of absorption (Ax,λ) of *Synechocystis* 6803 cultivated in turbidostat mode under a light intensity of 200 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ on phosphorus loads of 37, 4, 2 and 1 mg $P \cdot L^{-1} \cdot d^{-1}$. Data points are averages of at least four consecutive days of stable cultivation.

limitation also implies the need for up to 40% higher biomass concentration to achieve full recovery of nitrogen by cultivation of *Synechocystis*.

As the nitrogen content in the biomass decreases at lower phosphorus availability, and the cyanophycin content increases, the share of intracellular nitrogen in form of cyanophycin increases from 3% under phosphorus replete conditions to 30% under the lowest phosphorus availability (Fig. 8).

3.4.2. Changes in cells size and pigmentation

Synechocystis 6803 cell diameter was 2.17 ± 0.02 µm under phosphorus replete conditions and decreased to 2.06 ± 0.02 and 2.01 ± 0.01 µm in P Starve 2 and 3 respectively (Fig. 6G). Decrease in cell size is a reported physiological strategy to cope with nutrient limitation; this strategy helps cells with maintaining or even increasing nutrient uptake rates by conserving transporter abundances per unit of area [36].

Decreased pigmentation due to phosphorus limitation was observed in both, batch and turbidostat experiments (Figs. 6H, 9 and Supplementary material Figs. S3 and S4). This was accompanied by a relative increase in the carotenoid content as compared to chlorophylls as well as a decrease of phycocyanin as compared to chlorophyll (Fig. 9).

In the batch experiments, the estimated content of pigments had similar patterns in the reference cultures and the cultures on 6.0 and 3.0 mg $P \cdot L^{-1}$ under both light intensities 200 and 400 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$. The most severe phosphorus starvation at 1.5 mg P L^{-1} resulted in lower content of C-phycocyanin and chlorophyll *a* in the cultures on 200 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ (Supplementary material Fig. S3). In the cultures at 400 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$, C-phycocyanin and chlorophyll *a* were absent after day 3 and this was accompanied by lower content of carotenoids in the cultures on 400 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ (Supplementary material Fig. S3). The chlorophyll-a content starts decreasing earlier the lower initial phosphorus concentration in the cultures under 400

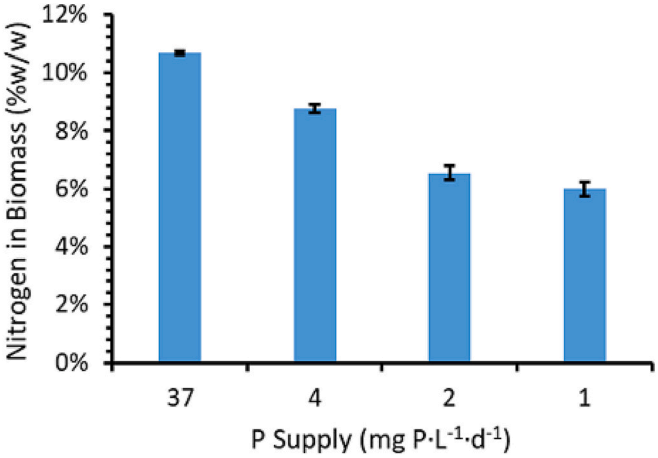

Fig. 7. Nitrogen content in *Synechocystis* Biomass Determined by Elemental Analyser. The values are averages of at least 3 datapoint at different sampling times during the defined steady states.

$\mu mol_{photon}\cdot m^{-2}\cdot s^{-1}$.

In the turbidostat cultivation, the changes in pigmentation were confirmed in the cross-section of absorption of the biomass, which had different spectral distribution patterns depending on the phosphorus availability (Fig. 9). Peaks of absorption were observed at wavelengths of 440, 620 and 680 nm corresponding to the main pigments present in *Synechocystis* 6803, carotenoids, C-phycocyanin and chlorophyll-a respectively (Fig. 9). The spectral distribution was normalized to the absorption at 680 nm. In the normalized spectra, the peak of absorption of C-phycocyanin decreased with respect to the peak of chlorophyll-a as phosphorus availability decreased while the peak of carotenoids increased (Fig. 9). Similar phenomena were observed in the cyanobacterium *Nostoc* sp. PCC7118 cultivated under phosphorus starvation [37]. Carotenoids play a protective role against photoinhibition in microalgae. Photoinhibition occurs when photosystems harvest excess of energy during imbalanced conditions as in the case of high light intensities and nutrients limitation.

As consequence of the changes in pigmentation, the spectrally averaged cross section of absorption decreased 23% and 49% in the P Starve 1 and 2 phases compared to the P Abundant phase (Fig. 6H). As the operation as turbidostat maintained the light absorbed by the cultures as a whole constant, the biomass concentration increased in the phosphorus starvation phases compensating for the decrease in the cross section of absorption (Fig. 6E).

3.5. Implications

The highest aerial biomass productivity in our experiments was 17 g $DW\cdot m^{-2}\cdot d^{-1}$ giving a very low titer of 0.03 g $CP\cdot L^{-1}$. Under a high level of phosphorus starvation, a much larger titer of 0.10 g $CP\cdot L^{-1}$ was achieved, at the cost of a lower biomass aerial productivity. The levels of phosphorus starvation needed for an increased titer decreased biomass aerial productivity between 30% and 60% in both batch and turbidostat cultivation. The highest cyanophycin titer of achieved with the wild type of *Synechocystis* 6803 was 0.6 g $CP\cdot L^{-1}$ in a batch high density cultivation setup with increasing light intensity between 50 and 950 $\mu mol_{photon}\cdot m^{-2}\cdot s^{-1}$ under sterile conditions [10]. It is important to note that our turbidostat experiments were done at 200 $\mu mol_{photon}\cdot m^{-2}\cdot s^{-1}$, a significantly lower light supply compared with real conditions e.g. 600 $\mu mol_{photon}\cdot m^{-2}\cdot s^{-1}$ as an average of a good summer day in the Netherlands averaging the light intensity over 24 h. Such higher light intensities would allow higher biomass concentrations, for example, operating a photobioreactor at higher biomass concentrations, around 4.5 g $DW\cdot L^{-1}$, which seems to be possible on ten times diluted urine based on its average nutrient content. Assuming a full nitrogen and phosphorus recovery from the urine by incorporation into the biomass and an accumulation of 10% of cyanophycin in *Synechocystis* 6803 dry biomass, the nitrogen fraction recovered in form of cyanophycin would represent 2.4%–3.0% of the nitrogen present in the cell dry mass. This means that 24%–30% of the nitrogen recovered from the source separated urine would be already in form of a useful product that could be used in a nitrogen circular economy.

Cyanophycin production with cyanobacteria using phosphorus limitation as cyanophycin accumulation strategy still needs a solution to compensate for the negative impact of phosphorus limitation on biomass productivity and titers. One possibility is the design of a two phase process. Phase one can be optimized for biomass productivity on light, avoiding nutrient limitation and allowing higher biomass concentrations as well as efficient biomass growth and nutrient recovery. Phase two can be optimized for cyanophycin accumulation using an efficient cyanophycin accumulation trigger such as phosphorus limitation.

4. Conclusions

Phosphorus starvation caused an imbalance between the light absorbed and the light utilized for growth; this was accompanied by a

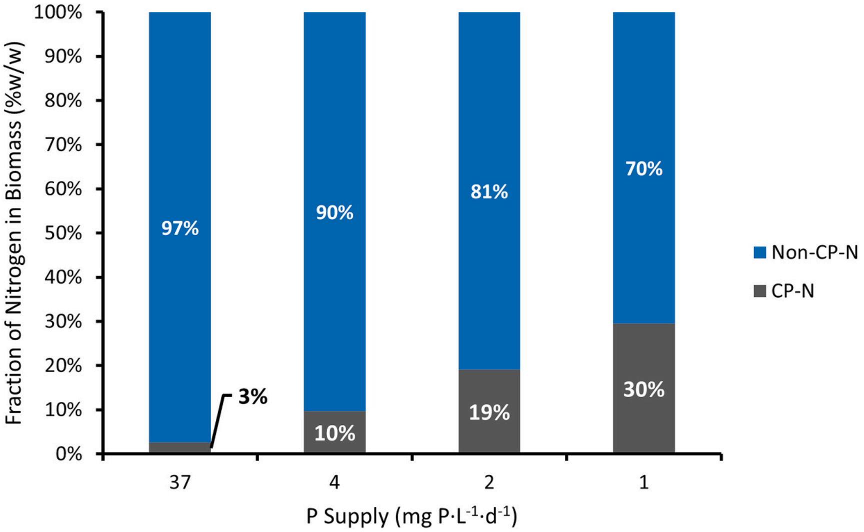

Fig. 8. Fraction of nitrogen present in Synechocystis biomass in form of Cyanophycin.

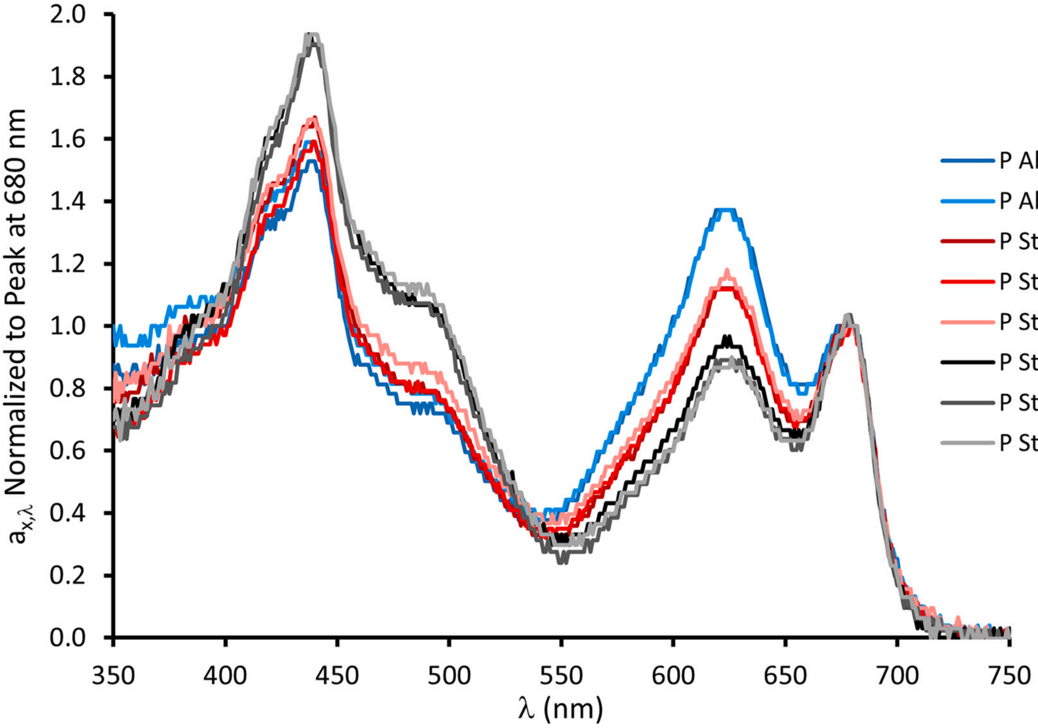

Fig. 9. Pigment content relative to the control phase of *Synechocystis* 6803 cultivated in turbidostat mode under a light intensity of 200 $\mu mol_{photon} \cdot m^{-2} \cdot s^{-1}$ on phosphorus loads of 37, 4 and 2 mg $P \cdot L^{-1} \cdot d^{-1}$, P Abundant, P Starve 1 and P Starve 2 phases respectively. A, B and C indicate the different sample replicates, taken in different days of each phase.

relative increase in the carotenoid content as compared to chlorophylls as well as a decrease of phycocyanin as compared to chlorophyll.

Synechocystis 6803 accumulated higher amounts of cyanophycin under stronger phosphorus starvation reaching values up to 10% of dry weight in form of cyanophycin in batch mode cultivation, and 7% in turbidostat mode cultivation. Higher cyanophycin content in biomass did not result in higher cyanophycin productivities. Higher productivities and higher titers were obtained under mild phosphorus limitation, which allowed both, biomass and cyanophycin production. The severe impact of phosphorus limitation on biomass productivity resulted in low cyanophycin titers, which are prohibitive for efficient cyanophycin production at a larger scale.

CRediT authorship contribution statement

Sebastian Canizales was responsible for the conception and design of the study, for the collection and assembly of data, analysis and interpretation of the data, and writing the article.

Loes Hilderink and Merab Apkhazava were responsible for the collection and assembly of data.

Hardy Temmink was responsible for the conception and design of the study, critical revision of the article for important intellectual content, obtaining funding for the study, and for the final approval.

René Wijffels was responsible for the critical revision of the article, and for the final approval.

Marcel Janssen was responsible for the conception and design of the study, analysis and interpretation of the data, drafting and critical revision of the article, and for the final approval.

The authors Sebastian Canizales, and Marcel Janssen take responsibility for the integrity of the work as a whole, from inception to finished article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2023.103143.

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