



Recycling nutrients from greenhouse effluent with a stress-resistant cyanobacterial strain

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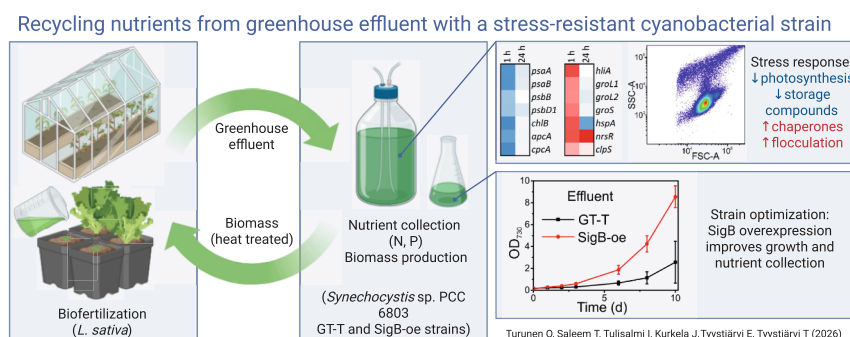
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HIGHLIGHTS

- *Synechocystis* grows and recycles nutrients from hydroponic greenhouse effluent.
- Greenhouse effluent induces stress, changing cell physiology and transcriptome.
- SigB overexpression improves growth, nutrient collection and stress resistance.
- Lettuce was successfully cultivated using *Synechocystis* biomass as a fertilizer.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Cyanobacteria
Biofertilizer
Wastewater treatment
RNA-seq
Bioremediation

ABSTRACT

Hydroponic agriculture is being increasingly utilized for its high productivity. Current wastewater treatment methods lead to loss of phosphate and nitrate, so a future circular economy calls for better methods to treat soilless systems' effluents, allowing reuse of nutrients. Cyanobacteria can use wastewaters as cultivation media and nutrient sources, atmospheric or anthropogenic CO₂ as a carbon source and artificial or sunlight for energy, while producing biomass and valuable products. This proof-of-concept study tests, at a laboratory scale, if the cyanobacterium *Synechocystis* sp. PCC 6803 can be used to remove nutrients, including nitrate and phosphate, from hydroponic greenhouse effluent (bioremediation), and if the collected *Synechocystis* biomass can later be used for biofertilization of lettuce (*L. sativa*). Cultivation in the greenhouse effluent triggered a broad stress response in *Synechocystis*: photosynthesis and cellular glycogen content decreased, cells flocculated, chaperone

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<https://doi.org/10.1016/j.biortech.2026.135937>

Received 7 April 2026; Received in revised form 17 September 2026; Accepted 23 September 2026

Available online 23 September 2026

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and photoprotection genes were upregulated and expression of micro- and macronutrient transporter genes altered. The GT-T control strain grew only slowly in the greenhouse effluent in high CO₂, and removal of nutrients was poor. When the more robust SigB-oe strain was used for bioremediation of the greenhouse effluent, formation of an extra RNA polymerase holoenzyme containing the stress-responsive SigB sigma factor altered gene expression, allowing fast growth and nutrient removal. Heat-treated cyanobacterial biomass could sustain the growth of lettuce seedlings for 21 days in a dose-dependent manner. Success in the use of *Synechocystis* biomass as a fertilizer, points to potential of cyanobacteria in a bioremediation-biofertilization cycle, warranting further studies.

1. Introduction

Over the last decades, agriculture has faced the increasing challenges of population growth, climate change and limitations in the supply of fertilizers (Kannan et al., 2022). Soilless agriculture and vertical farming can respond to these challenges by increasing crop productivity while reducing water, pesticide and land usage (Kannan et al., 2022; Richa et al., 2020). However, wastewater treatment is a problem in soilless hydroponic cultivation (Kannan et al., 2022; Richa et al., 2020). Hydroponic greenhouses produce wastewater in the form of discarded nutrient solution that is too saline or contaminated with pathogens for reuse (Mohammed, 2018; Samiotis et al., 2022). Closed hydroponic systems can decrease runoff by circulating the nutrient solution, but even then, production of contaminated or saline wastewater cannot be completely eliminated (Mohammed, 2018). Current methods for removal of nutrients from wastewaters, such as phosphorus collection by precipitation, do not recover nutrients, which leads to depletion of non-renewable natural resources and loss of economic potential (Li et al., 2019; Richa et al., 2020).

Phototrophic micro-organisms, including cyanobacteria, can perform biotechnological processes sustainably using light and CO₂ as energy and carbon sources, respectively. In addition to bioremediation, cyanobacteria can produce biomass for feed or fertilizer, as well as third generation biofuels or valuable chemicals. In recent years, understanding of cyanobacterial metabolism and development of production technologies has made clear both the potential and the bottlenecks of significant, industrial scale utilization of cyanobacteria (Lin et al., 2019; Novoveská et al., 2023; Victoria et al., 2024). Using waste streams as cultivation media combines the benefits of bioremediation with lower production costs and a smaller carbon footprint.

Cyanobacteria can collect a variety of compounds from wastewaters, including heavy metals (Ciani and Adessi, 2023), dyes, and nutrients including phosphate, nitrate and ammonium (Singh et al., 2019). Runoff fertilizers cause eutrophication, whereas remediation and reuse retain nutrients in a circular economy. Microalgae and cyanobacteria can be used as secondary or tertiary treatments for municipal, industrial, agricultural or aquaculture wastewaters, which also allows integration to existing infrastructure (Zamfirescu et al., 2024).

Gene technologies allow the development of more effective cyanobacterial strains for bioremediation. Genetic engineering of *Picosynechococcus* sp. PCC 7002 improved phosphate collection from dairy wastewater (Chavkin et al., 2025), and adaptive laboratory evolution of *Synechocystis* sp. PCC 6803 (from now on *Synechocystis*) increased its tolerance to synthetic wastewater (Hu et al., 2024). One putative strategy to enhance the tolerance of *Synechocystis* cells towards non-optimal growth conditions is to upregulate the expression of stress-resistance genes by engineer their transcription machinery (Stensjö et al., 2018). Until now, only few studies have tested this approach, but results from overexpressing the stress-tolerance-related sigma factor SigB (SigB-oe strains) are highly promising (Kaczmarzyk et al., 2014; Turunen et al., 2024). The *sigB* gene is upregulated in various stress conditions, including heat, salt, osmotic and oxidative stress (Hakkila et al., 2013; Nikkinen et al., 2012; Tuominen et al., 2006; Turunen et al., 2022, 2024). In SigB overexpression (SigB-oe) strains, an extra copy of the *sigB* gene has been added to *Synechocystis* cells, and SigB-oe cells

have shown to tolerate higher doses of butanol, and acclimate better to heat and chemically-induced oxidative stress than the wild type strain (Kaczmarzyk et al., 2014; Turunen et al., 2024). Furthermore, it was shown that the yield of heterologous protein production in SigB-oe host cells is higher than in wild-type host cells in non-optimal conditions, whereas no differences were measured in standard conditions (Turunen et al., 2024).

Microalgal biomass can be used as a sustainable alternative or supplement to mineral fertilizers (Coppens et al., 2016). Biofertilization has been studied with various species that can offer additional benefits such as soil aggregation by exopolysaccharides or biocontrol of pests, in addition to the direct effect of releasing nutrients from biomass (Dagnaisser et al., 2022). These effects depend greatly on the plant and biofertilizer species, e.g. *Synechocystis* increased germination of soybean more reliably than eukaryotic algae but had a smaller inhibitory effect against *Fusarium* (Ferreira et al., 2021).

Synechocystis is a widely studied model cyanobacterium with well-established techniques for metabolic engineering, used in numerous laboratory and pilot scale bioproduction studies (Satta et al., 2023). In bioremediation and biofertilization, however, *Synechocystis* is less established. In this study, we investigated if *Synechocystis* cells can be used as a biofertilizer to recirculate nutrients from nutrient-rich greenhouse effluent to new plants. This proof-of-concept study shows that nutrient recycling from the greenhouse effluent to new plants is possible via cyanobacterial biomass but also reveals the need for further studies to solve many practical issues before large scale utilization is possible.

2. Materials and methods

2.1. Strains and growth conditions

Synechocystis sp. PCC 6803 GT-T (Koskinen et al., 2023) was used as a control strain in this study. Constructions of all mutant strains have been described earlier: RNAP-His (Koskinen et al., 2016), SigB-oe (Turunen et al., 2024) and SigB-oe RNAP-His (Turunen et al., 2024). Strains were maintained on BG-11 agar plates in the standard growth conditions (32°C, ambient air, continuous illumination, photosynthetic photon flux density (PPFD) 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Antibiotics were supplemented on BG-11 plates sustaining RNAP-His (10 mg/L streptomycin, 20 mg/L spectinomycin), SigB-oe (10 mg/L nourseothricin) and SigB-oe RNAP-His (10 mg/L streptomycin, 20 mg/L spectinomycin, 10 mg/L nourseothricin) strains. If not otherwise stated, 30 mL liquid cultures were grown in 100 mL Erlenmeyer flasks with 90 rpm shaking. BG-11 medium buffered with 10 mM Hepes-NaOH to pH 7.5 was used as a control growth medium. The greenhouse effluent was sterilized by autoclaving, 121°C for 1 h, and used as growth medium with or without buffering with 3 mM Hepes-NaOH pH 7.5, as indicated. Cell cultures were grown in the standard conditions, or growth chamber air was enriched with 3% CO₂ in otherwise standard conditions (referred as high CO₂ conditions from now on), as indicated. Culture media used for high CO₂ experiments were equilibrated overnight in the CO₂-enriched growth chamber prior to the experiments. For some experiments, the greenhouse effluent was supplemented with additional nutrients, as indicated (Table A1). The optical density of cell cultures at 730-nm (OD₇₃₀) was set to 0.100, and OD₇₃₀ was monitored once a day with

Genesys 10S spectrophotometer (ThermoScientific). Samples of OD₇₃₀ ≥ 0.4 were diluted prior to measurement. For all growth experiments, three biological replicates were measured.

2.2. Nutrient analysis and properties of greenhouse effluent

Chemical and biological oxygen demand, suspended solids, and concentrations of N, P, Ca, Co, Fe, K, Mg, Mn, S and Zn of the greenhouse effluent (Robbe's Little Garden Ltd) were analyzed by Lounais-Suomen vesi- ja ympäristötutkimus Oy (Finland). Chemical and biological oxygen demand were analyzed according to standards ISO 15705:2002 and BOD7, SFS-EN ISO 5815-1:2019, respectively, suspended solids according to SFS-EN 872:2005, and the elements Ca, Co, Fe, K, Mg, Mn, S and Zn with inductively coupled mass spectrometry according to standards SFS-EN ISO 17294-1:2024, SFS-EN ISO 17294-2:2023, and SFS-EN ISO 15587-2:2002. Salinity was measured using Atago PAL-1 refractometer and pH with ThermoScientific Orion star A211 pH meter equipped with an 8302MNUMD electrode. To follow ammonium, nitrate, or phosphate removal of *Synechocystis* cultures, cells were removed from cultures either by centrifugation at 10 000 g for 5 min or by filtering through 0.22 μm PTFE filter (Millipore). The NO₃⁻ concentration was measured using Spectroquant® Nitrate Test (Sigma-Aldrich); the PO₄³⁻ concentration with Spectroquant® Phosphate Test (Sigma-Aldrich) and NH₄⁺ concentration with Spectroquant® Ammonium Test (Sigma-Aldrich) according to the manufacturer's protocols. Samples were diluted prior to measurements to obtain nitrate concentrations of 5–50 mg/L and phosphate concentrations of 1–8 mg/L. Absorbance values were measured with Shimadzu UV-1900 spectrophotometer. Nitrate and phosphate concentrations of effluent were measured daily from three biological replicates.

2.3. Western blotting

Cells were collected from 30 mL (OD₇₃₀ was 0.6) of cell culture by centrifugation (11 000 g, 5 min, 4°C) and washed with 600 μL of the STNE buffer (10 mM Tris-HCl pH 8.0, 0.4 M sucrose, 10 mM NaCl, 20 mM EDTA). The pellet volume of 150–210 μm acid washed glass beads (Sigma-Aldrich) were added, and cells were resuspended in 200 μL of the STNE buffer. Cells were lysed by vortexing (6 min, 4°C). Glass beads were removed by centrifugation (2x 3000 g, 5 min, 4°C) to obtain lysate containing a total protein fraction. Protein concentration was measured with BioRad Protein Assay (#5000114) according to manufacturer's protocol. Samples containing 50 μg total protein were solubilized with Laemmli buffer (10 min, 75°C) and proteins were separated on Mini-Protean TGX 4–15% acrylamide gels (BioRad). Proteins were transferred to Immobilon-P PVDF membrane (Millipore) by semi-dry transfer (BioRad Trans-blot) and detected by primary antibodies specific to each protein (Table A2). Primary antibodies were detected by goat anti-rabbit IgG (H + L) alkaline phosphatase conjugate (Zymed) and CDP-star Chemiluminescence reagent (Perkin-Elmer) and visualized on Super RX-N x-ray film (Fujifilm). Three biological replicates were analyzed, and relative protein amounts were quantified with FluorChem8000 software. Statistical analyses were done with unpaired two-tailed Student's *t*-test.

RNA-polymerase (RNAP) bound sigma factors SigA, SigB and SigC were quantified from RNAP-His (Koskinen et al., 2016) and SigB-oe RNAP-His (Turunen et al., 2024) strains according to Koskinen et al. (2016). Samples were collected from two 30 mL liquid cultures by centrifugation (11000 g, 5 min, 4°C), soluble protein extracted and the RNAP complexes purified with cobalt beads according to Koskinen et al., (2016). RNAP pulldown complexes collected from 0.44 mg of soluble protein were analyzed with Western blotting using custom antibodies as described above (Table A2). Three biological replicates were analyzed.

2.4. RNA sequencing

For standard growth conditions controls, cells were collected from 30 mL of GT-T and SigB-oe cultures (OD₇₃₀ was 0.6) grown in BG-11 medium in the standard conditions (32°C, ambient air, continuous illumination, PPFD 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$, shaking at 90 rpm). For greenhouse effluent treatments, cells were first grown in BG-11 medium in high CO₂ conditions and then collected by centrifugation at 5000 g for 5 min, and cell pellets were resuspended in the greenhouse effluent, OD₇₃₀ was adjusted to 0.6, and then treated for 0 h, 1 h or 24 h in high CO₂ conditions. Cells for RNA isolation were rapidly cooled by pouring cultures to centrifuge tubes containing 5 mL of –80°C ice, mixed until the ice melted, and collected by centrifugation at 11 000 g, 5 min, 4°C.

Total RNA was extracted using a hot phenol method. Cell pellet was washed with resuspension buffer (0.3 M sucrose, 10 mM Na-acetate pH 4.5, 60 mM Na-EDTA, pH 8.0), and cells were suspended in 250 μL of resuspension buffer and then 375 μL of lysis buffer was added, and then samples were extracted three times with phenol:chloroform (1:1): during the first extraction, the samples were treated at 60°C for 3 min. The samples were then extracted once with chloroform, and RNA was precipitated with 0.54 M LiCl and 68% ethanol overnight at –20°C. The RNA pellet was washed with ethanol, dried and resuspended in water. Ambion Turbo DNA Free kit was used to remove DNA from samples.

RNAseq was performed by BGI Tech Solutions (Hongkong) using the DNBseq sequencing platform. Three biological replicates of each treatment were analyzed. The sequencing depth varied from 12.0 M to 12.1 M reads between the individual samples. Sequences were trimmed from 100 bp to 89 bp with FastX to remove adapters. The sequencing data were analyzed using the web-based platform Chipster (version 4; CSC) (Kallio et al., 2011). FastQC was used to determine the quality of the sequencing data. Next, Bowtie2 (Langmead and Salzberg, 2012) was used to align reads to the reference chromosome sequence of *Synechocystis* sp. PCC6803 substrain GT-T and to the seven plasmid sequences of the substrain PCC-M (plasmids of GT-T have not been annotated). The reference data were retrieved from the NCBI database. The overall alignment rate for each sample was over 98%. The number of reads aligned to each gene was counted with HTSeq (Putri et al., 2022), and differential expression of the genes was calculated with DESeq2, assuming a negative binomial distribution for the reads and normalizing the data with DESeq sizefactors (Love et al., 2014). Differential expression analyses were done between GT-T grown in BG-11 and GT-T grown for 1 h or 24 h, as indicated, in the greenhouse effluent at 3% CO₂, and between SigB-oe and GT-T strains grown in BG-11 in ambient air, in BG-11 at 3% CO₂, and for 1 h or 24 h, as indicated, in the greenhouse effluent at 3% CO₂. The differential expression of genes was considered significant if their false discovery rate (Benjamini and Hochberg, 1995) was less than 0.05, and genes with two times or more (logFC ≥ 1) or 0.5 times or less (logFC ≤ -1) normalized counts in the target of the analysis than in the control were considered up or down-regulated, respectively.

2.5. Photosynthesis, chlorophyll and glycogen measurements

GT-T and SigB-oe strains were grown in BG-11 medium in high CO₂ conditions. After three days, cells were collected by centrifugation at 5000 g for 5 min, and resuspended either in BG-11 medium or in the greenhouse effluent (OD₇₃₀ was set to 0.6) and then cells were grown for 1d in high CO₂ conditions.

The light-saturated rate of photosynthetic oxygen evolution was measured with a Clark-type oxygen electrode from 1 mL cell culture (OD₇₃₀ was set to 0.6), supplemented with 0.5 mM 2,6-dichloro-p-benzoquinone and 0.5 mM ferricyanide, at PPFD 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Chlorophyll *a* was extracted with methanol and quantified spectrophotometrically as in Koskinen et al. (2023). For all experiments, three biological replicates were analyzed, and the mean of two technical replicates represented each biological replicate. Statistical analyses were done with unpaired two-tailed Student's *t*-test.

Glycogen content was measured following the protocol of Vidal and Venegas-Calderón (2019). Briefly, cells were pelleted from 0.5 mL of culture and the pellet was dried for 2 h at 30°C. Dried cells were suspended in 50 µL of distilled H₂O, treated with 24% KOH at 90°C for 30 min to lyse the cells and to isolate the alkali-stable glycogen from other macromolecules. After neutralization with glacial acetic acid, an enzymatic mix in 2.5 mM sodium acetate buffer (pH 5.2) was added. In this mix, amyloglucosidase hydrolyzed glycogen into free glucose, which was then colorimetrically quantified via a coupled reaction: glucose oxidase generated hydrogen peroxide, which subsequently oxidized o-dianisidine in a peroxidase-catalyzed reaction. Following a 30-min incubation at 37°C, sulfuric acid was added (4.8 N final concentration) to stabilize the colored product for optimal quantification. Absorbance was measured at 540 nm with Tecan Infinite M200Pro plate reader. Three biological replicates with three technical replicates in each were measured. Statistical analyses were done with unpaired two-tailed Student's *t*-test.

2.6. Flow cytometry

GT-T and SigB-oe strains were grown for 3 d in BG-11 medium in high CO₂ conditions, cells were collected by centrifugation at 5000 g for 5 min, resuspend in the greenhouse effluent (OD₇₃₀ set to 0.6) and treated for 1 d in high CO₂ conditions. Cultures were diluted to OD₇₃₀ = 0.10 and analyzed with a BD Bioscience LSRFortessa cell analyzer. Events were recorded for 30 s at a flow rate of 12 µL/min and visualized with FlowJo v10.10.1. Flowing Software 2.5.1 was used to gate standard sized cells grown in BG-11 and larger particles from the greenhouse effluent according to FSC-A and SSC-A (forward and side scattering area, respectively), and to count the populations. Three biological replicates were analyzed and statistical analyses were done with unpaired two-tailed Student's *t*-test.

2.7. Biofertilizer production and plant culture

For biomass production, the GT-T strain was cultured in 2 L bottles with air bubbling, in standard conditions, in 1.8 L volume of the greenhouse effluent to a final OD₇₃₀ of 2.5–3.5. Biomass was collected by centrifugation (5 min, 7000 g, 4°C). The cell pellet was resuspended in 50 mL of sterile water, biomass was heat-treated for 1 h at 100°C and stored at –20°C. One dose of *Synechocystis* fertilizer was defined as biomass equivalent to cells from 300 mL *Synechocystis* culture, OD₇₃₀ = 1. The dry mass of *Synechocystis* cells in one dose was 102 mg.

Four lettuce (*Lactuca sativa* var. *capitata*) seeds were sown in nutrient free coconut fiber in separate pots. Pots were either watered with 100 mL of tap water (H₂O control), tap water containing 1 or 2 doses of *Synechocystis* biomass or with 100 mL of the greenhouse effluent. Plants were grown at 23°C, 60% relative humidity, 160–170 µmol photons m^{–2} s^{–1}, 16 h/8h day/night rhythm, and further watered with tap water during the 21-d experiment. Growth was followed with photography.

3. Results and discussion

3.1. Greenhouse effluent limits growth of *Synechocystis*

To investigate the growth and nutrient collection potential of the model cyanobacterium *Synechocystis* sp. PCC 6803 substrain GT-T (hereafter GT-T) in greenhouse wastewater, greenhouse effluent from vertical farming of lettuce (*L. sativa*) was obtained from Robb's Little Garden Ltd. The effluent contained 155 mg/L phosphate, 1321 mg/L nitrate and 1.62 mg/L ammonium. The nitrate content was 121% and phosphate content 930% of those of BG-11, the standard growth medium for *Synechocystis* (Table A1). The high phosphate concentration also radically changed the molar N:P ratio from 100:1 in BG-11 medium to 13:1 in the greenhouse effluent. In addition, all other nutrients, except for cobalt, were found in much higher concentrations in the greenhouse

effluent than in the BG-11 medium (Table A1). The chemical and biological oxygen demand of the greenhouse effluent were 28 and 1.1 mg/L, respectively, suspended solids 1.7 mg/L, and salinity 4 ppt. Overall, the nutrient content of the greenhouse effluent from lettuce farming resembled those produced by farming other plants like tomato or cucumber (Mielcarek et al., 2019; Salazar et al., 2023; Samiotis et al., 2022). The effluent contained unspecified microbes, and it was sterilized to allow the growth of axenic GT-T cultures.

Unbuffered, sterilized greenhouse effluent could not support growth of GT-T cells (Fig. 1a). The pH of the effluent was only 5.0, which is too low for cyanobacterial growth. After buffering autoclaved greenhouse effluent with Hepes-NaOH, pH 7.5 (Hepes was also used to buffer BG-11), GT-T cells grew quite well (Fig. 1b), and removed 9% of the nitrate and 58% of the phosphate of the greenhouse effluent within 4 days (Fig. 1c).

In BG-11 medium, the growth of GT-T cells is greatly accelerated if the growth chamber air is enriched with 3% CO₂ (Kurkela et al., 2017), and nutrient removal efficiency was next tested in high CO₂ conditions. However, neither growth (Fig. 1d) nor nutrient removal (Fig. 1e) was faster than in ambient air, although normal high-CO₂-induced enhancement of growth was observed in BG-11 medium (Fig. 1d). During the high-CO₂ experiments, the pH of the buffered greenhouse effluent dropped first to pH 6.5, and increased back to pH 7.5 along with the cyanobacterial growth. Similar pH changes have been earlier observed in buffered BG-11 medium in high CO₂ (Kurkela et al. 2017). Thus, our results suggest that the composition of the greenhouse effluent restricted rapid growth in high CO₂.

3.2. *Synechocystis* cells adjust gene expression in greenhouse effluent

To understand the reasons behind the growth limitation in the greenhouse effluent, transcriptome changes were analyzed upon exposure of cells to the greenhouse effluent for 1 h or 24 h in high CO₂ conditions. Transcriptomes obtained after greenhouse effluent treatments were compared to the transcriptome of BG-11 grown cells. In total, 806 transcripts were differentially expressed after 1 h of exposure to the greenhouse effluent, whereas after the 24 h treatment, only 64 genes were differentially expressed (Fig. 2 and Dataset B1). Transcripts with known function showing the strongest responses to the greenhouse effluent are collected to Fig. 2, results of all genes are shown in Appendix B, and original data have been deposited to GEO (accession number GSE326740).

3.3. Greenhouse effluent induced transient downregulation of photosynthetic genes and reduced photosynthesis

Transferring cells from BG-11 medium to the greenhouse effluent induced downregulation of many photosynthetic genes. First, the transcripts of numerous components of photosynthetic light reactions were downregulated after 1 h, but most of them returned after 24 h to the same level as in the BG-11 grown cells (Fig. 2). These genes included twelve Photosystem I (PSI) genes, ten Photosystem II (PSII) genes, multiple ATP synthase genes, and the cytochrome *b₆/f* complex genes *petA* and *petC1*. In addition, ferredoxin isoforms *fed4* and *fed5* specific to low light (Poncelet et al., 1998) and isoforms *fed10* and *fed11* were downregulated. The *petC2* gene encoding a stress specific Rieske protein isoform (Tsunoyama et al., 2009) was transiently upregulated, whereas the *petE* gene encoding plastocyanin was upregulated both in 1 h and 24 h samples. Light-saturated PSII activity decreased in the greenhouse effluent in accordance with downregulation of light reaction genes (Fig. 3a).

Not only photosynthetic electron transfer genes but also light harvesting system genes reacted to the greenhouse effluent. The transcripts for the major phycobilisome proteins, *apc* and *cpc* were less abundant in the greenhouse effluent than in BG-11 medium after 1 h of treatment (Fig. 2). In addition, *cpcG1*, coding for a linker protein associating

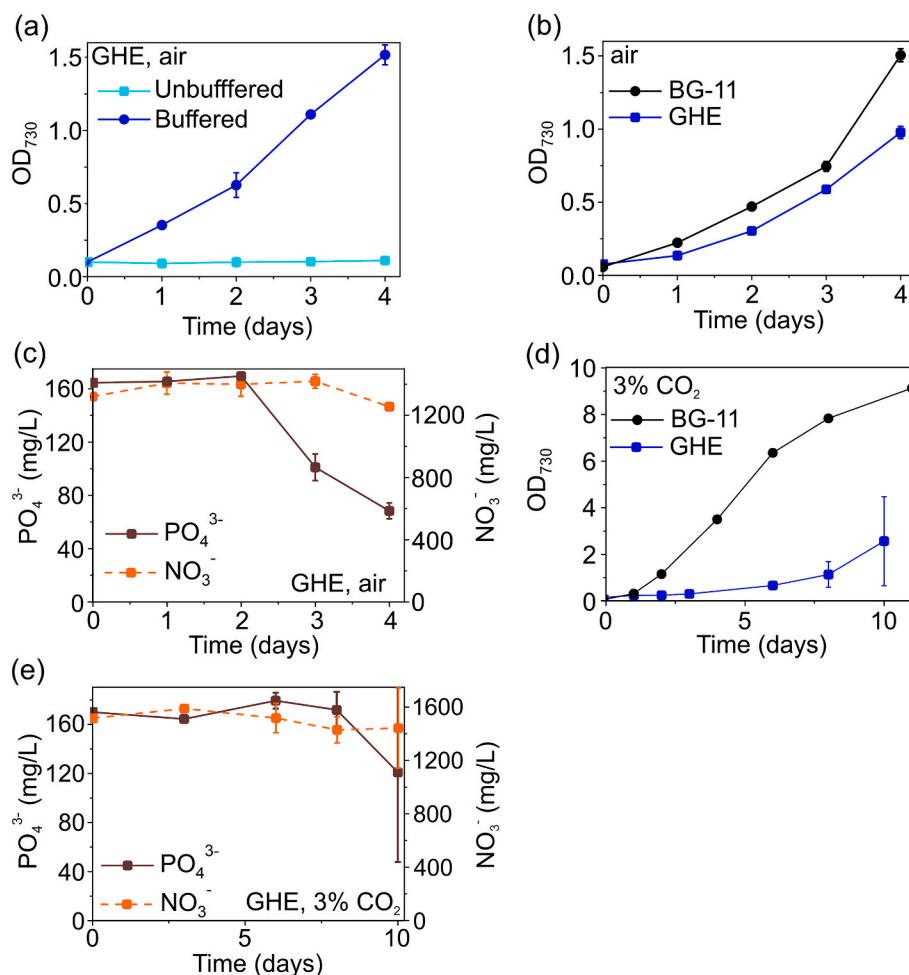


Fig. 1. Cyanobacterial growth and nutrient concentrations of the medium during cultivation of *Synechocystis* GT-T strain in BG-11 medium or in greenhouse effluent (GHE). (a) Growth in unbuffered and Hepes-buffered greenhouse effluent. (b) Growth in ambient air. (c) Nitrate and phosphate concentrations during growth in GHE in ambient air. (d) Growth in high CO₂ conditions. (e) Nitrate and phosphate concentrations during growth in GHE in high CO₂ conditions. Three biological replicates were measured, and the error bars denote SD.

phycobilisomes to PSII (Domínguez-Martín et al., 2022), and *cpcG2* and *cpcL* linker proteins, connected to light energy transfer from phycobilisome to PSI, were downregulated (Kondo et al., 2007; Watanabe et al., 2014). In addition to the downregulation of phycobilisome antenna genes, phycobilisome degradation related *nblA* genes were upregulated. NblA proteins are adaptors targeting phycobilisomes to proteasomal degradation (Baier et al. 2014). Together these gene expression changes suggest active reduction of the phycobilisome antenna during initial acclimation to the greenhouse effluent. The overall light capturing capacity was reduced, as many chlorophyll synthesis genes were strongly, albeit transiently downregulated, which led to 10% decrease in chlorophyll content (Fig. 3b).

The greenhouse effluent had a milder influence on carbon fixation genes than on light harvesting or electron transport genes. Among carbon concentrating mechanism genes, the *sbtAB* operon encoding the Na⁺/HCO₃⁻ symporter SbtA and the bicarbonate transporter regulator SbtB was upregulated. Simultaneous upregulation of *cupA* and *ndhF3* genes and downregulation of *ndhF4* suggest that the cells are replacing low-affinity NDH-1₄ with the inducible high-affinity NDH-1₃. Typically, both SbtA and NDH-1₃ complexes are strongly up-regulated in low CO₂ (Kurkela and Tyystjärvi, 2024), thus our results may indicate that inorganic carbon uptake is more inefficient from the greenhouse effluent than from the BG-11 medium.

3.4. Greenhouse effluent reduced growth and storage compound production

Fixed carbon is used for growth but can also be directed to storage compounds like glycogen or polyhydroxybutyrate, especially in conditions with a high C to N ratio (Damrow et al., 2016). Obviously, the greenhouse effluent limits growth, and in accordance with slow growth, numerous genes encoding proteins producing the cell wall components peptidoglycan (*mur*) or pili (*pil*) were down-regulated in the greenhouse effluent treated cells in comparison to cells grown in the BG-11 medium (Fig. 2). Additionally, pilus synthesis regulator (*syrcp2*) and cell division protein (*sepF*) genes were downregulated in the greenhouse effluent (Fig. 2).

Transcripts of the glycogen synthesis genes *glcP* (glucose transporter) and *glgC* (ADP glucose pyrophosphorylase) were downregulated, and simultaneously transcripts from glycogen utilization genes *glgP1* and *glgX1* were reduced in the effluent in comparison to the BG-11 medium (Fig. 2). In accordance with transcriptional changes, the glycogen content of the cells decreased by 30% upon treatment with the greenhouse effluent (Fig. 3c). The greenhouse effluent is rich in nitrogen, and simultaneous upregulation of nitrate reductase genes (*narB* and the cofactor genes *moe* and *moa*) and downregulation of photosynthetic activity suggest that glycogen metabolism reacts to the low C:N ratio of the greenhouse effluent.

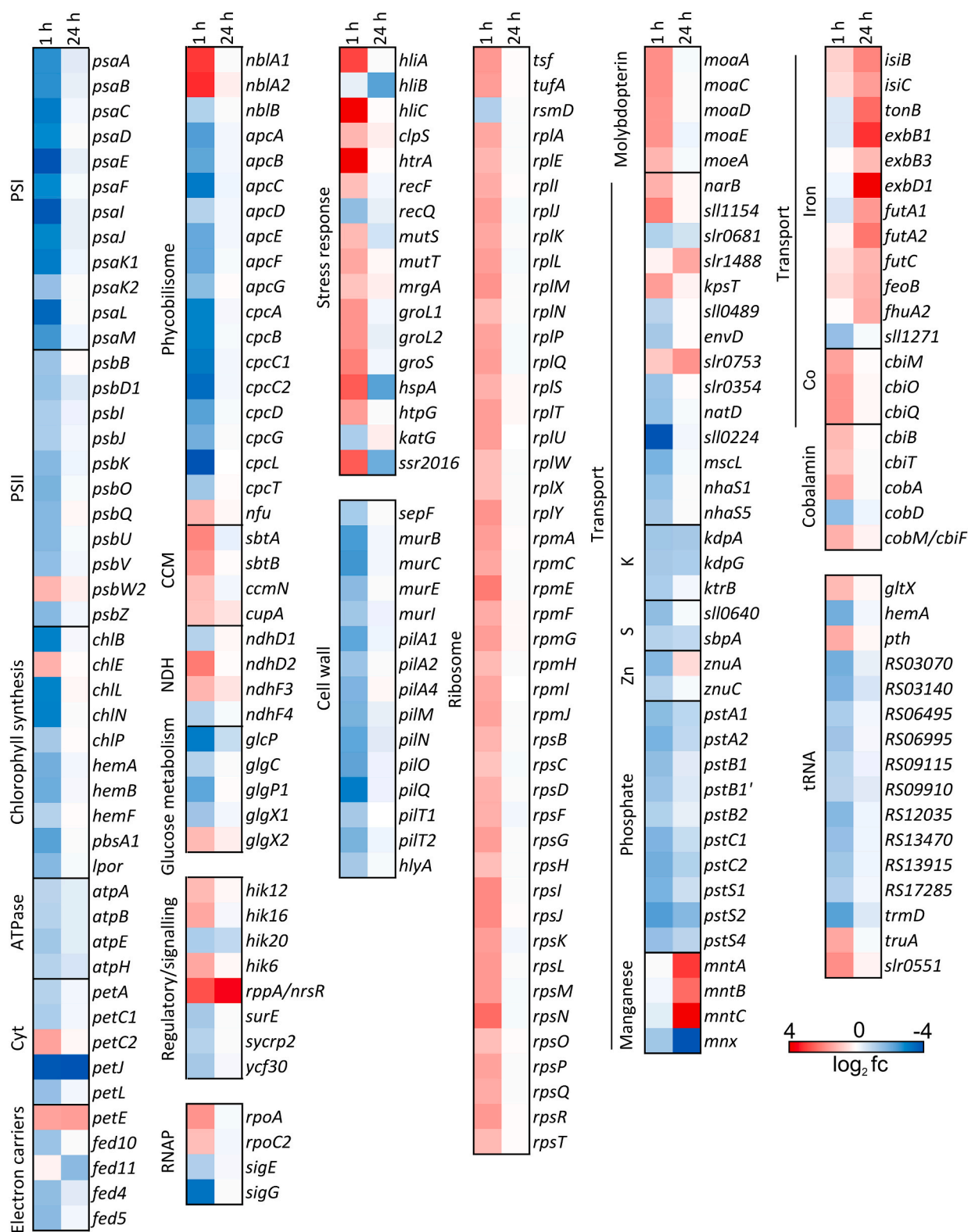


Fig. 2. Transcriptome changes of BG-11-medium-grown GT-T cells after 1 h or 24 h exposure to greenhouse effluent. Gene expression differences are shown with colors: blue, downregulation, red upregulation. Gene expression differences were measured with RNA sequencing. Differential expression was analyzed with DESeq2 and a gene was considered differentially expressed if the false discovery rate was less than 0.05 and the absolute value of log₂FC was higher or equal to 1. Three biological replicates of each treatment were analyzed.

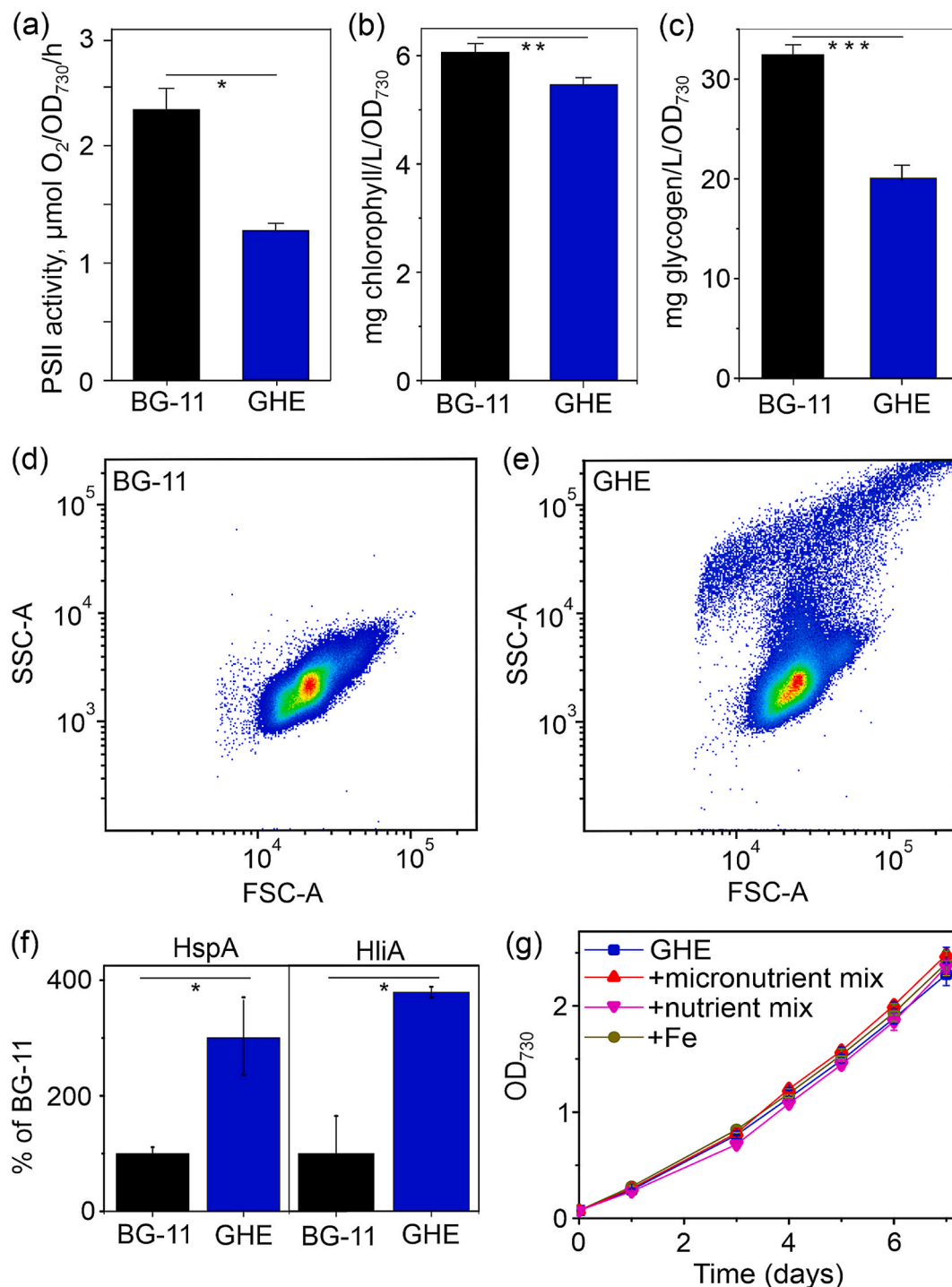


Fig. 3. Properties of *Synechocystis* GT-T strain in greenhouse effluent. (a) Light-saturated rate of PSII activity. (b) Chlorophyll *a* content of the cells. (c) Glycogen content. Flow cytometry analysis of culture grown in the BG-11 medium (d) or in the greenhouse effluent (e). (f) Amounts of HspA and HliA proteins detected by Western blotting. (g) Effects of supplementing the greenhouse effluent with Fe, micronutrient mix or with nutrient mix. *Synechocystis* cultures with initial OD₇₃₀ of 0.1, were grown in BG-11 medium or in the greenhouse effluent, as indicated, for 7 days. Three biological replicates were measured, and the error bars denote SD. Unpaired two-tailed Student's *t*-test was used in a, b, c, and f; ***, *P* < 0.001; **, *P* < 0.01; *, *P* < 0.05.

3.5. Greenhouse effluent induces aggregation of cells

Flow cytometry analyses revealed that in the BG-11 medium, GT-T cultures mainly contained single cells (Fig. 3d). After 3 days of growth in the greenhouse effluent, the number of larger and more complex particles had increased, indicating formation of cell aggregates (Fig. 3e, Table A2). The GT-T strain lacks thick pili just like all GT substrains (Koskinen et al., 2023), but retains the thin pili essential for flocculation

(Conradi et al., 2019). Upregulation of *hik12* in the greenhouse effluent might play an important role in triggering aggregate formation, as Hik12 is a part of a signaling cascade inducing biofilm formation in external stress conditions (Kobayashi et al., 2023). Furthermore, upregulation of the *kpsT* exopolysaccharide transporter gene might play a role in flocculation and induced protection against oxidative stress (Hu et al., 2022).

Aggregation of neighboring *Synechocystis* cells is a typical stress

response of *Synechocystis* culture in non-optimal environmental conditions (Jittawuttipoka et al., 2013; Turunen et al., 2022), and co-flocculation with a fungus, induced by a sublethal dose of erythromycin, has also been observed (Kaewbai-ngam et al. 2025). In *Synechocystis*, bright light induces cell aggregation, which reduces light absorption by providing shade (Turunen et al., 2022). Acclimation of *Synechocystis* cells to the greenhouse effluent clearly includes numerous strategies to reduce light capture even when cells are grown in low growth light conditions. Flocculation of cells increased shading, while a drop in pigment content and the down-regulation of light harvesting genes further suppressed light capture. Furthermore, light-protection-related *hliA* and *hliC* genes were upregulated (Fig. 2). In addition to the activation of the photoprotection mechanisms, general stress-response mechanisms were activated upon exposure to the greenhouse effluent. The stress-related DNA repair genes *recF*, *mutS*, *mutT* and *mrgA* were upregulated, and two protein-degradation genes, the *htrA* protease gene and the protease adapter gene *clpS*, were upregulated (Fig. 2). Furthermore, the chaperone genes *hspA*, *groL1-2*, *groS* and *hspG* playing roles in protein folding and degradation of misfolded proteins were transiently upregulated. Upregulation of the stress responsive *hliA* and *hspA* genes was confirmed at protein level (Fig. 3f, Fig. A1). Upregulation of chaperone genes together with ribosomal protein operons (Fig. 2), combined with a slow growth rate (Fig. 1d), might indicate an increased protein turnover rate in the greenhouse effluent compared to the BG-11 medium in high CO₂ conditions.

3.6. Collection of nutrients from greenhouse effluent

The phosphate concentration of the greenhouse effluent was 9.3-fold and nitrate concentration 1.2-fold as high as that of the standard BG-11 medium, which leads to a low N:P ratio in the effluent. Both phosphate uptake (*pst*) operons were downregulated after 1 h of effluent treatment, and remained downregulated also in 24 h samples (Fig. 2), suggesting that phosphate uptake genes are downregulated at the transcriptional level due to the high phosphate content of the medium. Nitrate transporter and metabolism genes showed only few changes, with only the nitrate reductase gene *narB* and nitrate-reductase-cofactor-producing *moa* operon being upregulated (Fig. 2).

In addition to the major nutrient transporters, also some micronutrient transporters reacted to the greenhouse effluent. Potassium, sulfate and zinc transporter genes were downregulated upon transfer of cells to the greenhouse effluent (Fig. 2), most probably responding to high levels of those nutrients in the greenhouse effluent (Table A1). In the case of potassium, also *hik20*, encoding a histidine kinase that signals extracellular potassium levels (Nanatani et al., 2015), was downregulated. Exposure to greenhouse effluent with low cobalt (Table A1) induced a transient upregulation of the cobalt transporter (*cblM*, *cblO*, *cblQ*) and cobalamin synthesis (*cbl* and *cob*) genes. The manganese import operon *mntCAB* was upregulated only after 24 h of greenhouse effluent treatment, and simultaneously the manganese exporter gene *mnx* was downregulated. Upregulation of the iron uptake genes *futA1*, *futA2*, *feoB*, the *tonB-exbB-exbD* system (Qiu et al., 2018) and the related *fhuA* gene, and upregulation of genes coding for the iron stress protein *isiC* and flavodoxin *isiB* may represent responses to iron limitation. The greenhouse effluent contained more elemental iron than BG-11 (Table A1), but the availability of iron may be compromised because of the high phosphate content of the effluent. Components of both Fe²⁺ and Fe³⁺ specific transport systems were upregulated (*feoB*, *futC*) (Mills et al., 2020).

Supplementing the greenhouse effluent with BG-11 nutrients by adding iron (in the form of ferric ammonium citrate), micronutrient mix (containing B, Mn, Zn, Mo, Cu, Co) or nutrient mix (containing NO₃⁻, Mg²⁺, SO₄²⁻, Ca²⁺ and the micronutrient mix) did not improve growth (Fig. 3g). Thus, growth restriction caused by the greenhouse effluent is unlikely due to limitation of cobalt, iron or other micronutrients. It should also be noted that the addition of the nitrate-containing nutrient

mix, which shifts the N:P balance towards the N:P ratio of the standard growth medium, did not improve growth. Furthermore, nitrate and phosphate levels remained constant as long as cyanobacteria grew very slowly (Fig. 1d and e), indicating that in the experimental time frame, these nutrients are lost from the effluent only via uptake by a growing cyanobacterial culture.

3.7. Addition of an extra copy of the stress-responsive *sigB* gene has only minor effects on gene expression in BG-11 medium

Since growth limitation of the GT-T control strain in the greenhouse effluent seems to be mainly linked to stress induction, performance of the stress resistant SigB-oe strain (Turunen et al. 2024) was tested. In the SigB-oe strain, the additional *sigB* gene is expressed under the strong *psbA2* promoter and replaces the *psbA1* gene in the genome of *Synechocystis* (Fig. 4a).

In addition to 3-fold upregulation of the *sigB* gene (Turunen et al., 2024), only the *slr1182* gene, located just downstream of the insertion site of the extra *sigB* copy (Fig. 4a), showed at least 2-fold upregulation in SigB-oe compared to the control strain in the standard growth conditions when cells were grown in BG-11 medium; no gene was significantly downregulated (Dataset B1). The upregulation of the *slr1182* gene was due to read-through transcription of the extra *sigB* gene. Formation of a transcription initiation competent RNAP-SigB holoenzyme did not differ between SigB-oe and the GT-T control strain in standard growth conditions (Turunen et al., 2024). Similarly, the amounts of the growth promoting RNAP-SigA (Imamura et al., 2003) and the growth restricting RNAP-SigC (Kurkela et al., 2025) holoenzymes did not differ between the strains in standard growth conditions (Turunen et al., 2024).

In 3% CO₂, the number of differentially expressed genes between the SigB-oe and GT-T strains increased to 41. Selected genes with known function are shown in Fig. 4b, and expression information of all genes is in Dataset B1. Four of the differentially regulated genes (*surE*, *phoA*, *slr1552*, *slr0685*) were downregulated, and 37 upregulated (Dataset B1). The biological functions of only few of the 37 upregulated genes have been confirmed (*nadM*, *rppA*, *petC2*, coding for a nicotinamide mononucleotide aminotransferase, a redox sensor affecting chlorophyll biosynthesis, and an alternative Rieske protein, respectively). Four genes (*psbA1*, *slr1182*, *slr1183*, *slr1184*) are most probably upregulated due to the read-through transcription of the inserted extra copy of the *sigB* gene (Fig. 4a), and 32 of the upregulated genes are located in plasmids. However, these gene expression differences did not influence on growth, as SigB-oe (Fig. 4f) and GT-T (Fig. 1d) grew similarly in the BG-11 medium in high CO₂ conditions.

3.8. Formation of additional RNAP-SigB holoenzyme in the SigB-oe strain allows faster growth and nutrient uptake from greenhouse effluent

The SigB-oe strain has been shown to tolerate heat, oxidative stress and organic solvents better than the control strain and to form extra RNAP-SigB holoenzyme in stress conditions (Turunen et al., 2024). Similarly, extra RNAP-SigB holoenzyme was formed in SigB-oe cells compared to the control strain upon greenhouse effluent treatment (Fig. 4c, Fig. A1), whereas the amounts of the growth restricting RNAP-SigC holoenzyme and the exponential-growth-related RNAP-SigA holoenzyme were similar in both strains (Fig. 4c, Fig. A1), suggesting that gene expression changes between the strains are mainly due to the formation of additional RNAP-SigB holoenzyme.

In ambient air, SigB-oe grew in the greenhouse effluent as well as in the BG-11 medium (Fig. 4d), unlike the control strain (Fig. 1b). During the 4-day growth experiment, SigB-oe was able to remove 7% of nitrate and 74% of phosphate from the effluent (Fig. 4e). In 3% CO₂, SigB-oe experienced a prolonged lag-phase compared to growth in the BG-11 medium, but thereafter SigB-oe cells grew exponentially, unlike the control strain (Fig. 4f). A similar initial drop of pH to 6.4 and subsequent

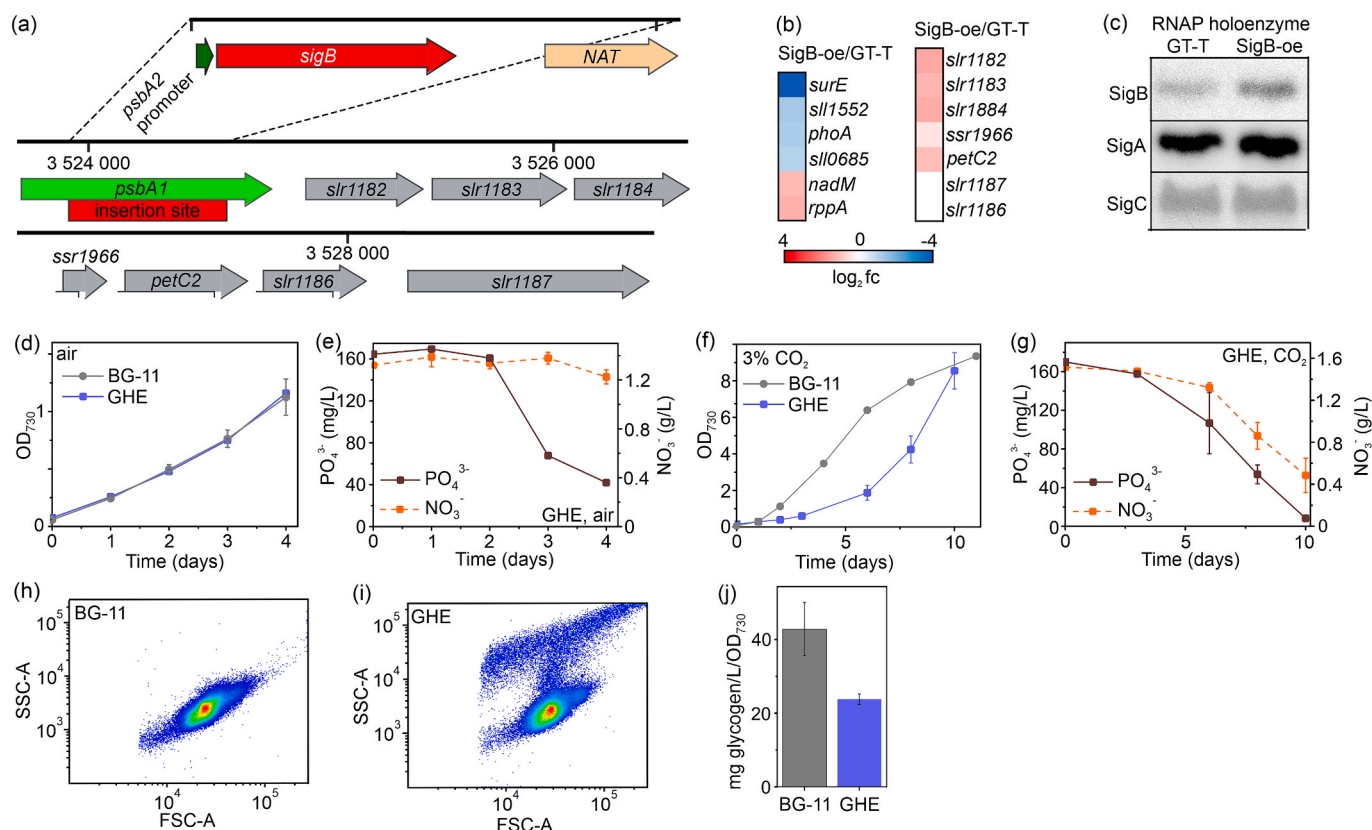


Fig. 4. Acclimation of SigB-oe strain to greenhouse effluent (GHE). (a) The SigB-oe strain construct, nourseothricin acyl transferase gene (NAT). (b) Comparison of the SigB-oe transcriptome to that of GT-T, cells were grown in BG-11 medium in high CO₂. (c) Content of SigB, SigA and SigC factors in RNAP holoenzyme in GHE exposed cells. (d) Growth of SigB-oe in ambient air. (e) Removal of nitrate and phosphate from GHE by SigB-oe cells in ambient air (f) Growth of SigB-oe in high CO₂ conditions (g) Removal of nitrate and phosphate from GHE by SigB-oe cells in high CO₂. Flow cytometry analysis of SigB-oe in BG-11 (h) and in GHE (i) Glycogen content of SigB-oe cells grown in high CO₂ in BG-11 or in GHE. Error bars denote SD of three independent biological replicates.

increase back to 7.5 was detected in SigB-oe strain as was detected in GT-T strain in high CO₂ conditions. SigB-oe cells were able to remove 68% of nitrate and 95% of phosphate in 10 days (Fig. 4g). The removal of phosphate was considerably faster than that of nitrate (Fig. 4e,g) during growth in the greenhouse effluent, and even the GT-T strain with strong growth restrictions removed a considerable amount of phosphate (Fig. 1). Phosphate is often the limiting nutrient for cyanobacteria, and they regularly show “luxury uptake” and storage of phosphate both outside and inside cells (Trautmann et al., 2016; Voronkov and Sinetova, 2019). On the other hand, the nitrate removal seems to be more directly connected to the growth rate (Figs. 1 and 4).

Despite an extended lag period of SigB-oe cells in the greenhouse effluent, after 10 days in high CO₂, the optical densities of the greenhouse-effluent-grown SigB-oe cultures had caught up with those of BG-11-grown cultures (Fig. 4f). This finding strongly suggests that some unidentified component(s) in the effluent act as stressors, leading to lasting growth suppression of the GT-T control strain. In the more robust SigB-oe strain, the growth limitation is only transient before the formation of the stress-resistance-inducing RNAP-SigB holoenzyme leads to improved stress acclimation. However, better acclimation did not prevent flocculation of SigB-oe cells in the greenhouse effluent (Fig. 4h,i). Greenhouse-effluent-grown SigB-oe cells were larger than the GT-T cells (Fig. 3e and 4i, Table A3) and contained 19% more glycogen (Fig. 3c and 4j).

3.9. Extended acclimation period of SigB-oe cells leads to superior acclimation to greenhouse effluent

To determine why SigB-oe acclimates to the greenhouse effluent

better than the GT-T strain, transcriptomes of the SigB-oe and GT-T strains were compared after 1 h and 24 h treatments in the greenhouse effluent in high CO₂ conditions. After 1 h of greenhouse effluent treatment, only few differences were detected between the control and SigB-oe strains, whereas after 24 h of effluent treatment, 237 genes were at least 2-fold up- or downregulated in SigB-oe compared to the control strain (Dataset B1). Selected differentially expressed genes with known functions are shown by category in Fig. 5.

Numerous light-harvesting and photosynthesis related genes were downregulated similarly in the SigB-oe strain as in GT-T after 1 h of greenhouse effluent treatment. However, many of those genes remained downregulated even after 24 h of greenhouse effluent treatment in the SigB-oe strain unlike in GT-T (Fig. 5). Light harvesting in SigB-oe may favor PSII, as the *cpcL* linker protein gene is even more strongly downregulated than in the control strain upon greenhouse effluent treatment (Fig. 5). This might be due to the downregulation of the *ccaS* and *ccaR* genes encoding regulators of the phycobilisome antenna (Hirose et al., 2008). Additionally, multiple stress-responsive genes (*hliA*, *hliC*, *hspA*) remained upregulated only in the SigB-oe strain after 24 h of greenhouse effluent treatment, while being similarly upregulated in both strains after 1 h of greenhouse effluent treatment. Some of the responses might be transmitted also via alternative sigma factors, as formation of extra RNAP-SigB holoenzyme affected the expression of the *sigE*, *sigF* and *sigG* genes (Fig. 5). SigE activates the expression of proteins functioning in carbon catabolic reactions (Osanai et al., 2011, 2005), and thus decreased expression of *sigE* may explain increased glycogen storages in SigB-oe. The *sigG* gene is an obligatory gene whose biological role remains unknown (Huckauf et al., 2000) and SigF controls expression of pilus genes and the secretion mechanism (Bhaya et al., 1999; Flores

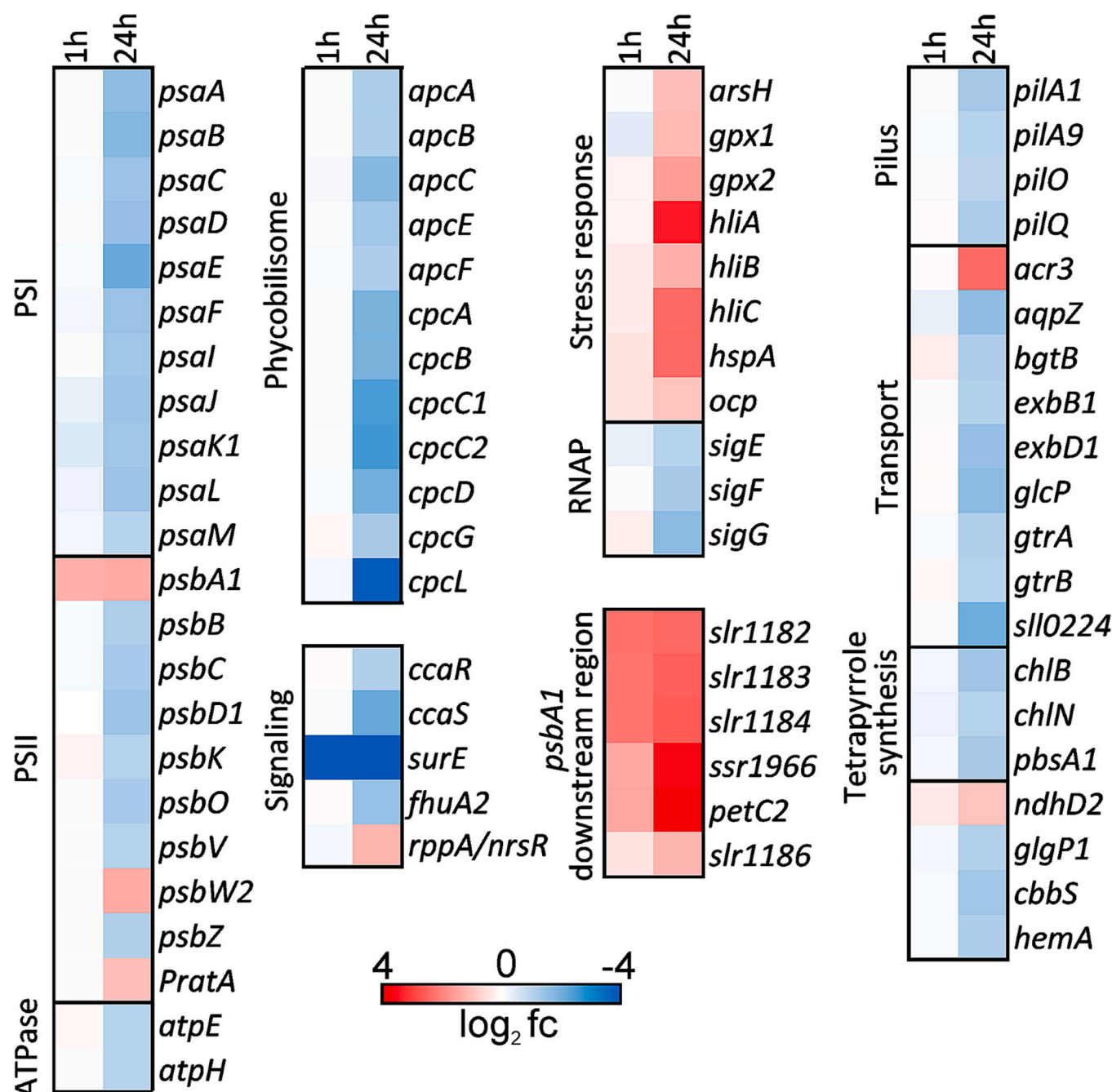


Fig. 5. Comparison of SigB-oe transcriptomes to those of GT-T after exposure to greenhouse effluent in high CO₂ conditions. Gene expression differences were measured with RNA sequencing. Differential expression was analyzed with DESeq2 and a gene was considered differentially expressed if the false discovery rate was less than 0.05 and the absolute value of log₂fc was higher or equal to 1. Three biological replicates of each treatment were analyzed.

et al., 2019).

Taken together, these results indicate that the overexpression of the *sigB* gene extends the transcriptional stress response state. A sustained stress response of SigB-oe fits well to a long lag phase observed in the growth of the SigB-oe strain in the greenhouse effluent (Fig. 4f). Obviously, an extended stress acclimation period is beneficial for SigB-oe, as these cells can fully acclimate to the greenhouse effluent, unlike the control strain. A short spike of SigB activity has been measured in the control strain in high temperature and salt stresses, where formation of RNAP-SigB complex has been shown to be beneficial (Koskinen et al., 2016; Nikkinen et al., 2012; Tuominen et al., 2006; Turunen et al., 2024).

In high CO₂, the *surE* gene was downregulated in SigB-oe. The SurE

protein is a 5'/3'-nucleotidase involved in inosine 5'-phosphate degradation, and known to be downregulated under nitrogen limitation and slow growth (Aguirre Von Wobeser et al., 2011), but its exact regulatory or metabolic role is not known. Genes *slr1182-slr1184*, located immediately downstream of the extra *sigB* gene (Fig. 4a), were upregulated in high CO₂. As discussed above, their upregulation in all studied conditions is most probably due to the read-through of the extra *sigB* copy. The *ssr1966* and *petC2* genes are especially upregulated after 24 h of greenhouse effluent treatment in the SigB-oe strain compared to the control strain. The *petC2* gene encodes an alternative isoform of the Rieske subunit of the cytochrome *b₆/f* complex, and has been shown to be mainly expressed in stress conditions (Tsunoyama et al., 2009), whereas Ssr1966 is predicted to be a membrane protein with unknown

function. In addition to genes with known functions, 98 of the differentially regulated genes in greenhouse-effluent-treated SigB-oe encode unknown or hypothetical proteins (Dataset B1). The actual roles of these genes in stress-responses and growth regulation remain to be elucidated. The molecular mechanisms controlling growth and carbon allocation in *Synechocystis* are only poorly understood (Kurkela et al. 2025), and this gene list might help to discover new regulators.

3.10. *Synechocystis* biomass can be used to fertilize lettuce

We also tested if *Synechocystis* biomass grown in the greenhouse effluent can be used as biofertilizer for lettuce. To produce the biofertilizer, GT-T was cultured in 2 L volume in the greenhouse effluent (Fig. 6a), and during three weeks of cultivation, the cyanobacteria removed 100% of the phosphate and circa 60% of the nitrate from the greenhouse effluent. After the growth period, cells were collected by centrifugation, the cell pellet was suspended in distilled H₂O, cells were killed by heat treatment and used as biomass in fertilization experiments.

Lettuce seeds were sown to nutrient free coconut fiber and then watered with only tap water, or with tap water mixed with *Synechocystis* biomass (102 mg or 204 mg of *Synechocystis* dry mass, as indicated) or with sterilized greenhouse effluent. Plants watered only with water grew only cotyledons (Fig. 6b). Plants fertilized with *Synechocystis* biomass, in turn, grew well, although upon a longer growth period, the plantlets

were smaller than plants fertilized with the greenhouse effluent (Fig. 6b). Biomass is considered a slow-release fertilizer (Mulbry et al., 2007), and based on present results and previous studies (Ferreira et al., 2021; Puglisi et al., 2020; Wuang et al., 2016) microalgal or cyanobacterial biomass is a suitable fertilizer for leafy vegetables. However, the amount and frequency of addition of a biofertilizer need to be further optimized to match the growth rates achieved using a commercial mineral fertilizer. In our experiments, two doses of *Synechocystis* biomass supported growth better than one dose, and most probably addition of a third dose for the last week would allow as fast growth as the greenhouse effluent.

3.11. Lessons learned from the laboratory proof-of-concept study

The results show that manipulation of *Synechocystis* transcription by overexpressing the stress-responsive SigB factor actually improves growth of *Synechocystis* cells in a non-optimal growth medium. Together with earlier findings showing benefits of SigB overexpression in high temperature and chemically induced stress conditions (Turunen et al. 2024), these results prove SigB-oe as an excellent candidate host strain for future bioproduction studies. In addition, the results prove that cyanobacteria can grow utilizing nutrients from the greenhouse effluent, and that the collected biomass can be used as a biofertilizer for plants. To our knowledge, this is the first study demonstrating a complete bioremediation-biofertilization cycle.

In the biorefinery context, biomass can be utilized as a fertilizer after extraction of more specific and higher value bioproducts. The remaining biomass may consist of cell residue after extraction, or whole cells after production of excreted or volatile compounds. A biofertilizer may also improve germination or stress tolerance of plants, which may further justify biofertilization in combination with traditional mineral fertilization (Chabili et al., 2024; El-Shazoly et al., 2025; Ferreira et al., 2021). *Synechocystis* is a model cyanobacterial species and a well-studied host for bioproduction, but the use of its biomass for biofertilization is relatively unstudied. In combination with bioremediation and bioproduction, the end use of *Synechocystis* biomass can become an integral part of a biorefinery value chain, as biofertilization can circulate the biomass back into the system. However, demand for further studies is obvious before the results of this proof-of-concept study can be applied to real life biorefineries.

An obvious question is whether the nutrient collection efficiency is good enough. Direct comparison of bioremediation efficiencies between different studies is difficult because the effluents vary, organisms, growth modes and cell densities are different, and illumination and temperature conditions vary between studies (for a recent review, see Zamfirescu et al., 2024). The removal of phosphate was essentially complete when SigB-oe was grown in high CO₂, whereas 40% of nitrate still remained after 10 days. During the last four days of culturing the SigB-oe strain in high CO₂ in the greenhouse effluent (Fig. 4d), decrease in nitrate concentration was 209 mg/(L x day), or 47.3 mg N-NO₃/(L x day). Earlier studies on nutrient removal from hydroponic greenhouse effluent with microalgae or cyanobacteria show roughly similar results as the present study. Salazar et al. (2021) reported complete removal of phosphate and 55.9% removal of N-NO₃ in a 5-day batch culture experiment with the green alga UHCC0027, whereas Borhan et al. (2026) report N-NO₃ removal efficiencies of 26.1–41.1 mg/(L x day) with the green alga *Scenedesmus* sp. NIVA-CHL 99 in a large photobioreactor run as a turbidostat in a Nordic greenhouse environment. *Scenedesmus* sp. NIVA-CHL 99 removed all nitrate and phosphate when a photobioreactor was run in batch mode under artificial illumination for 8–14 days (Salazar et al. 2023). *Synechococcus elongatus* PCC 7942 achieved 60% nitrate and 68% phosphate removal from rose hydroponic effluent in 20 days (Samiotis et al., 2022). Optimization of temperature and light conditions might further improve nutrient removal efficiency remarkably, as it is inextricably linked to growth of cells.

Our laboratory scale experiments revealed the high potential of

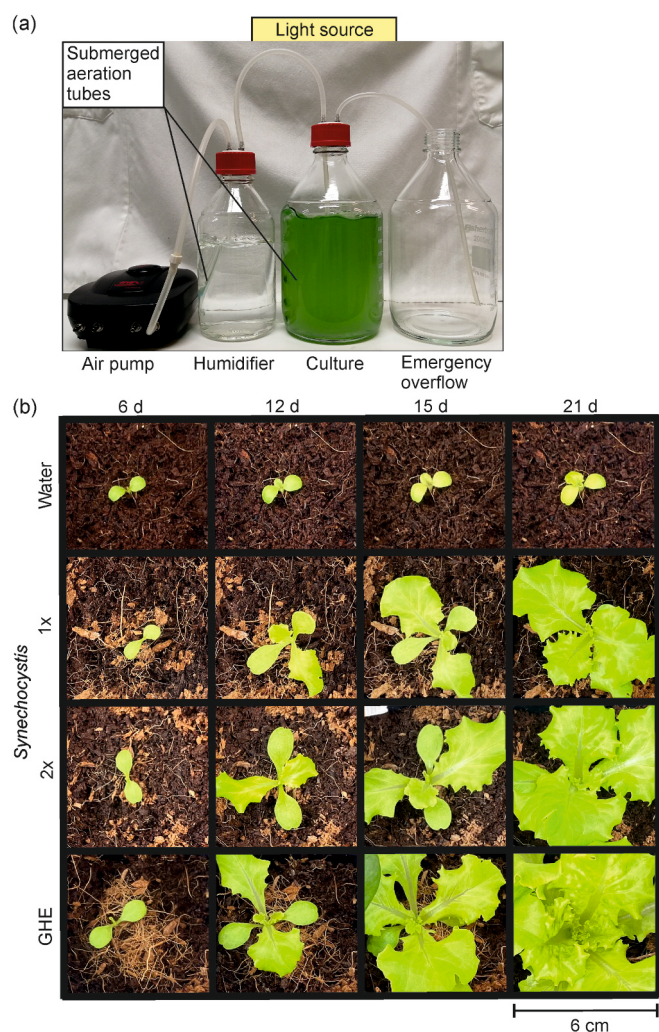


Fig. 6. *Synechocystis* as biofertilizer. (a) 2 L cultivation system. (b) Growth of lettuce using *Synechocystis* biomass as fertilizer.

cyanobacteria to circulate nutrients with a combined bioremediation and biofertilization strategy. However, further studies are required to improve cost efficiency of the system. Sterilization of microbes containing greenhouse effluent might not be avoidable, but filtration or UV-light treatment might provide more cost-efficient alternative for autoclaving. The pH values of greenhouse effluents vary, and some might be usable without pH adjustments. When pH adjustment is needed, additions of bicarbonate/NaOH and CO₂ can be used to replace expensive buffers. The optimal pH for bioremediation should also be tested, as typically cyanobacteria prefer alkaline conditions (Kurkela et al., 2017).

For biofertilization experiments, the results of this study just provide a starting point for actual system optimization. At least, dead and living biomass applied as wet biomass or as dried powder, and different pre-treatments prior to application should be compared. Dose and application frequency should be optimized, and properties of plants grown with a biofertilizer and a traditional mineral fertilizer should be compared. In addition, it would be interesting to know how soil microbes influence utilization of cyanobacteria as a biofertilizer and how much nutrient uptake from biomass varies between plant species.

4. Conclusions

The model cyanobacterium *Synechocystis* was successfully used to collect inorganic nutrients from greenhouse effluent and to support plant growth. The greenhouse effluent caused stress to *Synechocystis*, and only robust, stress tolerant SigB-oe strains showed the ability to grow fast in the greenhouse effluent in high CO₂ conditions, removing 65% of nitrate and 95% phosphate in 10 days. Comparison of transcriptomes and cellular responses of the *Synechocystis* control strain (poor acclimation to the greenhouse effluent) and SigB-oe strain (acclimates to the greenhouse effluent) shed light on how cyanobacteria can be protected against greenhouse-effluent-induced stress. The results of this proof-of-concept study show that improvement of the general stress tolerance of *Synechocystis* is beneficial for the bioremediation efficiency and provides a solid background for further studies aiming at efficient bioremediation at a large scale. Furthermore, the results show that cyanobacterial biomass grown in a greenhouse effluent can be used as a biofertilizer for lettuce.

CRedit authorship contribution statement

Otto Turunen: Writing – original draft, Visualization, Validation, Investigation, Funding acquisition, Formal analysis, Data curation. **Tayyab Saleem:** Investigation, Formal analysis. **Ida Tulisalmi:** Investigation, Formal analysis. **Juha Kurkela:** Validation, Supervision, Funding acquisition. **Esa Tyystjärvi:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Taina Tyystjärvi:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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.

Acknowledgements

This research was funded by NovoNordisk (grant NNF22OC007984), Finnish Cultural Foundation (grants 85232166 and 00260498), Research Council of Finland (grant no. 347172) and University of Turku graduate school. We thank Robbe's Little Garden Ltd for providing the hydroponic greenhouse culture media. Fin-BioFoundry and Photosyn are acknowledged for excellent research facilities. The live cell imaging and flow cytometry were performed in the Cell Imaging and Cytometry

Core of Turku Bioscience.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2026.135937>.

Data availability

Gene expression data have been deposited in GEO, accession number GSE326740. Other data will be made available on request.

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