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The effects of light regime on carbon cycling, nutrient removal, biomass yield, and polyhydroxybutyrate (PHB) production by a constructed photosynthetic consortium

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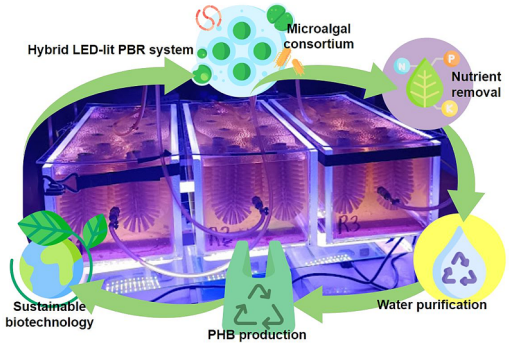
HIGHLIGHTS

- Three different light regimes were applied to a microalgal-bacterial consortium.
- High-intensity full-spectrum lighting increased PHB but decreased biomass yields.
- Medium-intensity blue-white light efficiently produced biomass and removed N and P.
- Low-intensity red light was the only net carbon–neutral treatment.
- Lipid content was unaffected by light regime.

GRAPHICAL ABSTRACT



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ABSTRACT

Microalgae can add value to biological wastewater treatment processes by capturing carbon and nutrients and producing valuable biomass. Harvesting small cells from liquid media is a challenge easily addressed with biofilm cultivation. Three experimental photobioreactors were constructed from inexpensive materials (e.g. plexiglass, silicone) for hybrid liquid/biofilm cultivation of a microalgal-bacterial consortia in aquaculture effluent. Three light regimes (full-spectrum, blue-white, and red) were implemented to test light spectra as a process control. High-intensity full-spectrum light caused photoinhibition and low biomass yield, but produced the most polyhydroxybutyrate (PHB) (0.14 mg g^{-1}); a renewable bioplastic polymer. Medium-intensity blue-white light was less effective for carbon capture, but removed up to 82 % of phosphorus. Low-intensity red light was the only net carbon-negative regime, but increased phosphorus (+4.98 mg/L) in the culture medium. Light

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spectra and intensity have potential as easily-implemented process controls for targeted wastewater treatment, biomass production, and PHB synthesis using photosynthetic consortia.

1. Introduction

Microalgal cultivation has been studied extensively for multiple industrial purposes, including carbon capture, resource recovery, and biomass production for upgrading to various value-added products (Yadav et al., 2019). Manipulating cultivation conditions to maximize resource use efficiency and growth rate is imperative towards the aim of economically viable microalgal production (Chang et al., 2022; Su, 2021). Recently, focus has shifted from microalgal monoculture towards mixed-species photosynthetic consortia. Photosynthetic consortia may contain multiple species of eukaryotic green algae, prokaryotic cyanobacteria, and non-photosynthetic members such as heterotrophic bacteria. Microorganisms co-exist in such consortia in nature, and therefore establish a dynamic equilibrium when co-cultured under conditions which emulate natural ecosystems (Kazamia et al., 2014; Zhang et al., 2020). These conditions include physical environment, light regime, and availability of carbon and nutrients. The physical environment of a photobioreactor (PBR) includes parameters such as flow rate, aeration, illumination, light penetration, and surface materials. Conventionally, PBRs were designed to discourage biofilm formation by using smooth surface materials for construction. Cultivation methods which immobilize microorganism cells (e.g. biofilms, and other forms of attached growth) have become more popular in recent years, as they can greatly improve harvesting efficacy (Rodrigues de Assis et al., 2020; Yu et al., 2020). Photosynthetic consortia are often better suited to forming biofilms, as they contain a diversity of species which can produce extracellular polymeric substances (EPS); the molecular scaffolding of biofilms (Chen et al., 2022; Xiao and Zheng, 2016). EPS provide further benefit to consortia members by accumulating valuable compounds via adsorption exterior to the cell surface (Cheah and Chan, 2021; Xiao and Zheng, 2016). For example, luxury uptake of phosphorus (P) within microalgal cells is a well-known phenomenon. Phosphorous storage within EPS and the effects of light availability upon bioconversion, however, have only recently been studied (Wu et al., 2021).

The effects of light regime on other microalgal biological processes, such as carbon metabolism, are better understood. Autotrophic metabolism occurs in the presence of light, utilizing inorganic carbon species, whilst heterotrophic metabolism occurs in darkness, and when organic carbon is available (Chang et al., 2022). Mixotrophic cultivation, facilitated by supplying both forms of carbon and a diurnal light cycle, is typically considered advantageous for biomass production (Pang et al., 2019). Recent work on the effects of light wavelength has demonstrated that light spectra may be just as important as light intensity and duration (Chang et al., 2022).

Additionally, manipulating light conditions can either stimulate or inhibit production and accumulation of valuable metabolites, such as lipids useful for biofuel synthesis (Chang et al., 2022; Iasimone et al., 2018), pigments such as astaxanthin (López-Sánchez et al., 2022), and biopolymers such as polyhydroxybutyrate (PHB) (Gracioso et al., 2021). Microalgal pigments are currently produced on the commercial scale, and efficient production of microalgal lipids has been studied extensively for decades. Biopolymers and bioplastics are relatively new, however, and deeper insight into the factors which control their production is required. PHB is a biopolymer of interest due to its similarity to polypropylene (Rueda et al., 2020). At present, most PHB on the market is produced by heterotrophic bacteria, which require constant oxygenation and organic carbon as substrates and generate CO_2 (Gracioso et al., 2021). Despite these drawbacks, some bacterial species are known to be capable of accumulating PHB, some of them up to 80 % cellular dry weight (Anderson and Dawes, 1990). Some microalgae, specifically prokaryotic cyanobacteria, are also known to produce and accumulate PHB; albeit at lower concentrations than heterotrophic bacteria in nature. Recent work has focused on enhancing PHB synthesis in cyanobacteria by manipulating environmental conditions and utilizing metabolic engineering tools. However, very little research has been conducted on the possibility of PHB-producing photosynthetic consortia. In principle, the limitations of heterotrophic bacterial PHB production (i.e. demand for oxygen and organic carbon) could be addressed by co-culturing these microorganisms with photosynthetic cyanobacteria. Cyanobacteria consume CO_2 during photosynthesis and produce the oxygen necessary for bacterial oxidation of organic carbon. Co-cultivation in wastewater containing both organic and inorganic carbon could satisfy the needs of both heterotrophic bacteria and cyanobacteria to effectively convert carbon into PHB, while simultaneously removing nutrients like nitrogen (N) and phosphorus (P), thus providing a wastewater treatment service (Bolan et al., 2009).

The aim of this study was to elucidate the effects of lighting with varying wavelengths and intensity on biomass production, PHB accumulation, and removal of carbon and nutrients by a constructed photosynthetic consortium. Of microalgal cultivation parameters, light spectrum and intensity can be readily manipulated to optimize growth. This work investigated how production of biomass and metabolites and assimilation of carbon and nutrients could be controlled using light. Three bottom-illuminated photobioreactors equipped with brush heads to encourage biofilm growth were subject to three light conditions, sequentially: full-spectrum (430 – 740 nm), blue-white (430 – 740 nm), and red (430 – 660 nm), with full-spectrum as the highest intensity (138 – 500 μmol m^{-2} s^{-1}), blue-white as medium intensity (84 – 324 μmol m^{-2} s^{-1}) and red as the lowest intensity (64 – 224 μmol m^{-2} s^{-1}). In keeping with recent work on enhancing microalgal biomass production and resource use efficiency, the cultivation systems were designed to facilitate hybrid cultivation (simultaneous attached/biofilm growth and conventional liquid suspension) of locally-sourced photosynthetic consortia. This consortium contained eukaryotic microalgae, filamentous and free-living cyanobacteria, and indigenous heterotrophic bacteria from ecological samples and living in the aquaculture effluent utilized as growth medium. This study demonstrates PHB synthesis by a photosynthetic consortium composed of indigenous wastewater microorganisms and microalgae and cyanobacteria sourced from the local environment, which is a phenomenon only sparsely reported in literature. The results reported herein underscore the significant impacts of light spectra and intensity on community function, and thus the ability to tailor photosynthetic consortia for different industrial purposes using light as a process control.

2. Materials and methods

2.1. Constructing and propagating a photosynthetic consortium

Sampling for local microalgae and cyanobacteria was conducted during the “midnight sun” months in Finland (13 July 2021, approximately 17:00) in a stormwater reservoir situated between a junkyard and active railway in Mikkeli, Finland, containing cyanobacteria like *Aphanocapsa* sp. and *Chlorella* sp. (GPS coordinates 61.701340, 27.288269). Additional filamentous cyanobacteria (mostly *Oscillatorian* genera) were sourced from a private freshwater aquarium; filamentous species are helpful in forming robust biofilms by exuding EPS. Both consortia were propagated separately in BG-11 media in glass bottles on a stir plate, under white fluorescent light in a laboratory fume hood at ambient temperature, for 6 weeks ahead of the experiment. The cultures were mixed by hand just prior to inoculation, and the aquaculture effluent used for cultivation was untreated (apart from sieving, Section

2.3) to allow native bacterial species to remain.

2.2. Photobioreactor design

Three experimental photobioreactors were constructed from transparent plexiglass (60 × 25 × 20 cm, 22 L working volume) and acrylic adhesive (Etra Oy, Mikkeli, Finland) and waterproofed with silicone (see supplementary materials). Clear plexiglass lids were fitted with nylon brush heads drilled into place (12 brush heads per reactor) to create a high surface area to volume ratio and maximize attached growth. Two outlets were placed at opposite ends of the reactors, and the flow through reactor tanks was facilitated by peristaltic pumps operating with a flow rate of 300 mL min^{-1}. One 15.5 × 25 cm plant growth LED light array per tank was placed 10 cm below the reactors, and operated with a 16:8 h light/dark cycle.

2.3. Wastewater preparation and cultivation conditions

Aquaculture effluent (ACE) from a recirculating aquaculture system (RAS) was collected from FinnForel Oy in Varkaus, Finland, and stored in the dark at 4 °C until the experiment. Prior to inoculation, the wastewater was equilibrated to room temperature and sieved sequentially (250 and 500 μm mesh) to remove large particulate matter and macro-invertebrates. After inoculation and over the course of each experimental period, the liquid medium and forming biofilms were monitored using light microscopy to assess biodiversity.

2.4. Experimental design

The experimental bioreactors were filled with ACE (pH 6.8) and inoculated with 150 mL prepared consortium stock at the beginning of each 14-day batch cultivation period. LED arrays provided illumination with three different settings, each a mix of different wavelengths at varying intensities. Three light spectra with three different intensities were tested; full-spectrum (FS, 430–740 nm, highest intensity), blue-white (BW, 430–740 nm, medium intensity), and red (R, 430–660 nm, lowest intensity) (Table 1). Before each cultivation period, after filling the PBR tanks with ACE but prior to inoculation, light intensity was measured with a Delta Ohm LP 471 PAR probe at the bottom (closest to the LED light arrays) and water surface (farthest from LEDs) of the PBR tanks. The range of light intensities were 138–500 μmol m^{-2} s^{-1}, 84–324 μmol m^{-2} s^{-1}, and 64–224 μmol m^{-2} s^{-1}, for FS, BW, and R, respectively. During the 14-day cultivation period, peristaltic pumps were run continuously to facilitate a constant medium circulation rate of 300 mL min^{-1}, and the LED arrays were run on a timer set for 16 h on and 8 h off. Before inoculation, at 24 and 48 h, and subsequently at 48 h intervals for the remainder of the 14-day cultivation period, 20 mL samples were withdrawn with a syringe and filtered through a 45 μm cellulose acetate filter. Samples were stored in the dark at 4 °C until chemical analyses after three consecutive cultivation periods.

2.5. Biomass quantification

After 14 days, the culture medium was emptied over a 50 μm aluminum sieve. The biofilms remaining on the brush heads were removed by hand using gloved fingertips, and from tank surfaces using a silicone spatula. Biomass harvesting required minimal force. Because the bulk of the biomass grew in biofilms, no suspended cells were harvested from the culture medium. 4–7 g wet biomass was aliquoted from each reactor for future analyses, and all remaining biomass was dried overnight in an oven at 60 °C and weighed.

Table 1
Spectral range and light intensities per treatment.

Treatment	Spectral range (nm)	Range of intensity (μmol/m^{-2}(−|-) s^{-1})	Range of intensity (converted to lux)
Full-spectrum	430 – 740	138 – 500	4,544 – 16,500
Blue-white	430 – 740	84 – 324	2,772 – 10,692
Red	430 – 660	64 – 224	2,112 – 7,392

2.6. Polyhydroxylbutyrate extraction and quantification

The method used for PHB quantification from algal consortia biomass is based upon the work of Abdo and Ali (2019), and chosen as a reliable and "greener" approach (i.e. no extreme temperatures, and therefore no unnecessary energy expenditure). The standard poly-3hydroxybutyrate was procured from Sigma-Aldrich. A PHB stock solution was prepared by dissolving 1 mg PHB per 1 mL chloroform, from which standard curve solutions were diluted to concentrations ranging from 100 to 0.001 mg L^{-1}. The stock solutions were first pipetted into Eppendorf tubes, then a measured volume of chloroform was added, and the tubes were capped tightly. The tubes were heated in a water bath at 65–70C° and vortexed. A 10 mL volume of concentrated sulfuric acid was added into the tubes, then sealed with caps and heated at 95–97C° in a water bath for 30 min to complete the conversion of PHB to crotonic acid and to fully evaporate the chloroform.

To extract PHB from experimental biomass, 0.5–1.0 g of dried, powdered biomass was weighed in a tube, then suspended in 3 mL of MilliQ purified water. Tubes were left to equilibrate overnight at ambient temperature, then homogenized by vortexing. A 2 mL volume of 2 M HCl was added to each tube followed by gentle shaking, then heating at 65° C in a water bath for 2 h. A 5 mL volume of chloroform was added to each tube and left overnight in a shaker at 150 rpm at ambient temperature to ensure complete conversion to crotonic acid. Tubes were centrifuged at 2000 rpm for 20 min, followed by the addition of 1 mL of chloroform. The samples were dried overnight in an oven at 40 °C. After drying, 10 mL of concentrated sulfuric acid was added to the tubes, which were then heated in a water bath at 98 °C for 30 min for PHB conversion to crotonic acid. PHB concentration was measured with UV spectrophotometry (Agilent Technologies UV–vis-NIR) at 235 nm, using concentrated sulfuric acid as a blank.

2.7. Lipid extraction and quantification

To quantify lipid content, a modified Bligh and Dyer approach (Bligh and Dyer, 1959) was employed, in which 100 mg of dried, powdered biomass was combined with 5 mL methanol and 2.5 mL chloroform in glass tubes, then vortexed for 1 min.

After vortexing, the tubes were sonicated for 30 min in a water bath sonication (Branson Ultrasonics, 15 × 15 × 29 cm, 5.7 L, 60 W, 40 kHz), after which solids had precipitated and the lipid-containing solvent phase was decanted into new tubes. The solid remainder in each tube was extracted a second time with half volumes of solvents (2.5 mL methanol and 1.25 mL chloroform). Vortexing and sonication were repeated, and the extraction solvents from first and second steps were collected and combined in a 50 mL glass bottle. A 4 mL volume of 1 % NaCl solution and 4 mL chloroform were added to each 50 mL bottle, shaken for 5 min, then centrifuged for 5 min at 6000 rpm. The chloroform phase was then pipetted off and filtered through Whatman GF/C glass microfiber filters into pre-weighed tubes and dried overnight in an oven at 60 °C. The lipid content from each sample was calculated by subtracting the weight of the tubes from the final weight after oven drying.

2.8. Chemical analyses and statistics

Filtered growth medium samples taken at 48 h intervals were analyzed for total nitrogen (TN), total carbon (TC), and total organic carbon (TOC) using an Analytik Jena multi N/C 2100S TC/TNb

Analyzer. Inorganic carbon values were obtained by subtracting TOC from TC values. Light microscopy was used to visually assess biodiversity and cell health of the inoculum culture, and the resulting biofilms after each cultivation period. Total dissolved phosphorus (P) and nitrate (NO_3^-) were measured using a Shimadzu LC-20AD SP ion chromatograph equipped with a Shodex IC SI-50 4E column. Three PBRs were constructed so that each light regime was tested in triplicate. Standard deviation between replicates was analyzed using GraphPad Prism software, and presented in Figs. 1-4.

3. Results and discussion

3.1. Effects of light regime on carbon cycling

Carbon cycling data during each light treatment illustrates the delicate nature of the relationship between heterotrophic bacteria and photosynthetic consortium members. At 24 h, inorganic carbon (C_i) increased in all reactors under all light spectra tested. Levels of C_i under BW and R light generally stabilized after 48 h and began to decline after 144 h (indicating photosynthetic consumption of C_i). However, under FS light, C_i concentrations rose slightly and continued to fluctuate for the duration of the experiment (Fig. 1). An increase in C_i indicates high levels of bacterial respiration, and possibly bacterial lysis of other consortium members. At 24 h, results indicate that all reactors experienced a bloom in heterotrophic bacteria, and, interestingly, only FS lighting prevented the establishment of a robust photosynthetic population to consume the C_i produced. It is likely that high-intensity, full-spectrum lighting caused photoinhibition of microalgae and cyanobacteria, inhibiting their growth and weakening their response to bacterial lysis.

While recent research on the relationship between light intensity and C assimilation found that higher light intensities (150 – 230 μmol m^{-2} s^{-1}) generally improved photosynthetic efficiency and C_i uptake, the results of the present study are less straightforward. Gao et al. (2022) conducted their study on a *Chlorella vulgaris* monoculture, rather than a mixed consortium, and the photoperiod was 12:12 on/off as opposed to the 16:8 regime reported here. Despite a testing similar range of light intensities, the present study found an overall increase in C_i across all treatments, indicating a combination of bacterial respiration of CO_2 and photoinhibition of photosynthetic consortium members, decreasing the overall rate of C_i consumption. Yadav et al. (2019) conducted a study operating at lower light intensities (50–100 μmol m^{-2} s^{-1}) and achieved much higher rates of CO_2 fixation. They reported a maximum CO_2 fixation rate of 187.65 mg L^{-1} d^{-1} at a 5 % CO_2 injection rate using a conventional liquid-suspension *Chlorella* monoculture. Another recent study

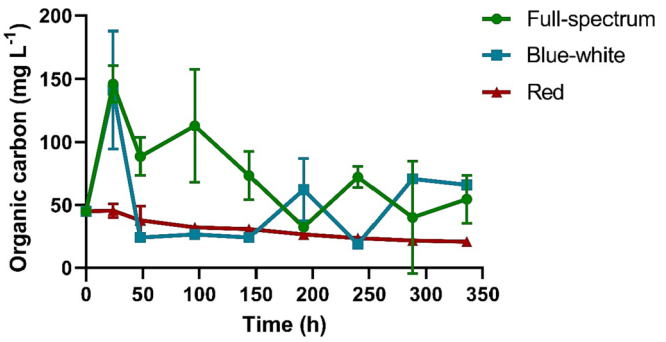

Fig. 2. Changes in total organic carbon (OC) in the culture medium over time across replicates (reactors 1, 2, and 3); under full-spectrum, blue-white, and red light regimes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Yuan et al., 2020) on biofilm cultivation of *Chlorella* and *Nannochloropsis* revealed that, while advantageous in many ways, biofilms can render microalgae more prone to photoinhibition. Yuan et al. (2020) reported that biofilms dissipate more light energy into heat as compared to cells in liquid suspension, which can lead to metabolic stress and cell damage caused by excess energy absorption. Considering that the upper limit of light saturation for most microalgal species is approximately 500 μmol m^{-2} s^{-1} (Su, 2021), it is reasonable to assume that the high-strength FS light treatment inhibited photosynthesis and prevented the establishment of a stable microalgal-bacterial equilibrium within the system, thus leading to oscillating carbon concentrations for the duration of the experimental treatment.

Organic carbon (OC) data further support the bacterial lysis interpretation. Under FS light, OC concentrations fluctuate for the duration of the cultivation period; with the only possible source of increasing OC being liberated as cellular components following bacteriolytic activity. Under BW light, after an apparent bacterial bloom at 24 h evidenced by spiked in both C_i and OC, OC fell below 30 mg L^{-1} for several days, and did not exceed 100 mg L^{-1} for the remainder of the cultivation period. In contrast to the previous two light regimes, under R light, maximum OC levels did not exceed 50 mg L^{-1} (OC maxima were 161.3 mg L^{-1} and 191.0 mg L^{-1} for FS and BW, respectively). R light resulted in stable consumption of OC after an initial spike at the beginning of the cultivation period (24 h in reactors 1 and 2, 48 h in reactor 3) (Fig. 2). This steady decline of OC under R light is the combined result of heterotrophic bacterial metabolism and mixotrophic consumption by microalgae, whereas the fluctuations under FS light reflect bacterial activity without

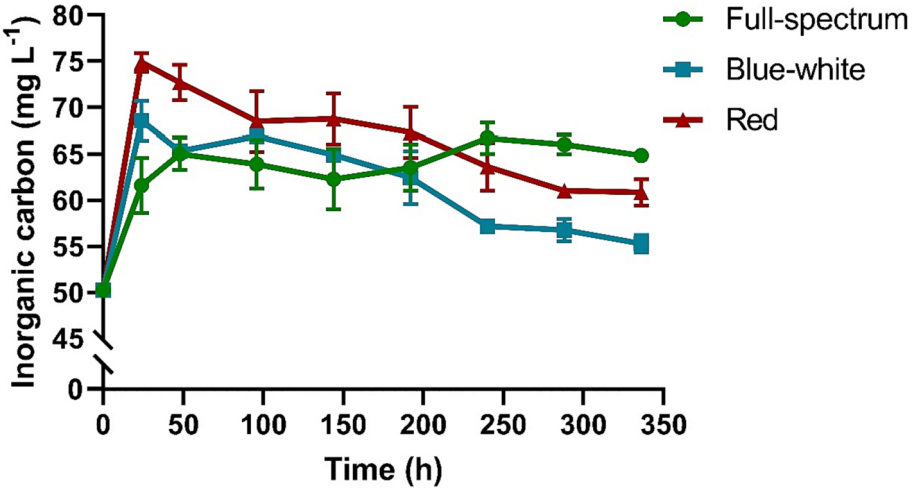

Fig. 1. Changes in total inorganic carbon (C_i) in the culture medium over time across replicates (reactors 1, 2, and 3); under full-spectrum, blue-white, and red light regimes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

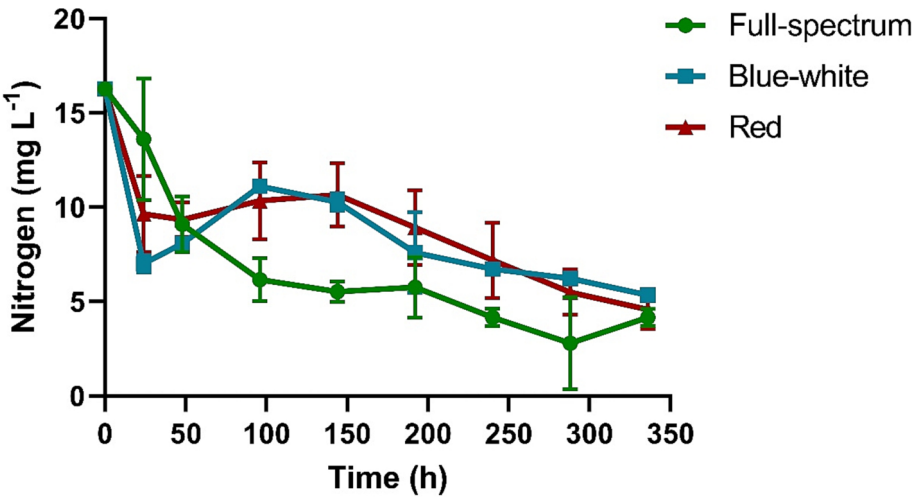

Fig. 3. Changes in total dissolved nitrogen (TN) in the culture medium over time across replicates (reactors 1, 2, and 3); under full-spectrum, blue-white, and red light regimes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

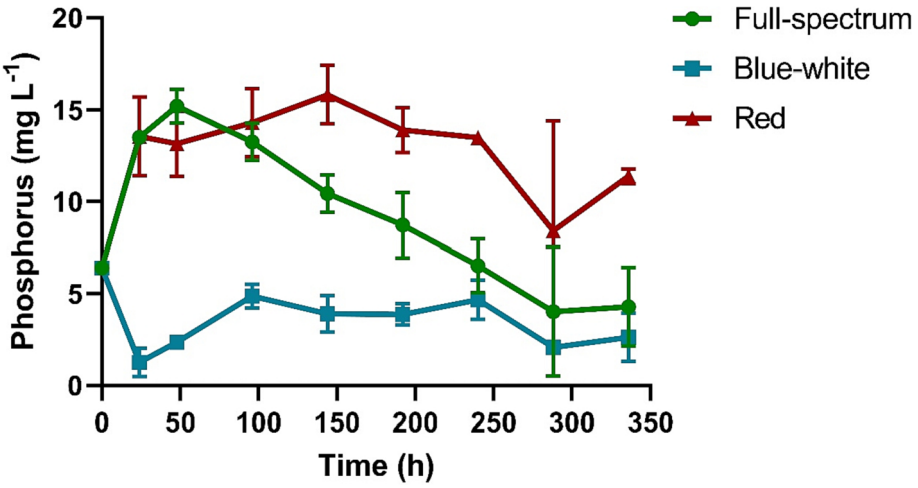

Fig. 4. Changes in total dissolved phosphorus (P) in the culture medium over time across replicates (reactors 1, 2, and 3); under full-spectrum, blue-white, and red light regimes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sustained populations of mixotrophic microalgae to consume OC. As this study was conducted using biofilm-forming species, exudation of EPS likely contributed to OC concentrations in the medium (Xiao and Zheng, 2016); however, the exact role played by EPS in organic carbon flux within this system remains unclear.

Importantly, the only carbon-negative condition achieved by this study was that of the red light treatment (Table 2). This result has important implications for future applications of photosynthetic consortia. Cyanobacteria are known to flourish under far-red light (Gisriel et al., 2022a,b), and therefore these spectra could be applied to encourage cyanobacteria dominance of a mixed consortium. Cyanobacteria can be highly valuable for production of non-lipid biofuels like ethanol (Sarma et al., 2016), as well as green biomass harvesting techniques such as bioflocculation of eukaryotic microalgae (Iasimone et al., 2021). Photobioreactor conditions (such as flow rate and nutrient balance) must be further optimized overall, however, in order to reach a net carbon-negative community equilibrium in this system.

Table 2
Average removal (mg/L) of carbon and nutrients under each treatment after 14 days of cultivation. Negative values indicate a net increase. FS: full-spectrum light, BW: blue-white light, R: red light.

Treatment	C_i (mg/L)	OC (mg/L)	TN (mg/L)	P (mg/L)
Initial concentrations (t0)	50.22	44.85	16.27	6.42
FS	−14.59	−9.68	12.11	2.22
BW	−5.03	−21.00	10.93	3.80
R	−10.62	24.13	11.69	−4.98

3.2. *Effects of light regime on nutrient flux*

3.2.1. *Nitrogen species*

Changes in light spectra clearly impacted nutrient cycling, especially with regard to nitrogen species. After 24 h, all reactors and all treatments (except one outlier; reactor 2 under full-spectrum light treatment) experienced a drop in total nitrogen (TN) concentration (Fig. 3). However, between time points 24 and 144 h, BW and R treatments showed a minor increase in TN, which began to decline again after 144 h; a result of an initial bacterial bloom and bacterial lysis and liberation of cellular constituents. All treatment conditions removed TN efficiently enough to satisfy EU wastewater discharge standards (for municipalities with a population range from 10,000 – 100,000, less than15 mg L^{-1}) within 24 h (Fig. 3) (European Commission, 2013), as a result of rapid bacterial

nitrification of ammonium to nitrite to nitrate, which is ultimately consumed by microalgae. Treatments BW and R evidence this further with a slight increase of total N after 48 h; N must remain in the system (rather than being lost through NH_3 volatilization) and anaerobic denitrification may be enabled by biofilm formation, as they can create low-oxygen environments below the biofilm surface. Although nitrate (NO_3^-) levels remained below detection for most of the experiment (data not shown), they spiked after the 192 h time point to nearly 20 mg L^{-1} (11.3–19.7 mg L^{-1} between 192 and 240 h) under BW light, and were rapidly depleted within 48 h. A similar but less dramatic spike was documented under R light, with NO_3^- concentrations reaching a maximum 6 mg L^{-1}, then declining before the end of the experiment. NO_3^- levels remained below detection limits for the entirety of the FS treatment, supporting the explanation of bacterial nitrification under BW and R light, but suggesting only partial nitrification (or rapid consumption of NO_3^- by eukaryotic microalgae) under FS light.

Recently, Ayre et al. (2021) reported that bacterial nitrification pathways were stimulated in co-culture with *Chlorella*. Using various dilutions of anaerobically-digested piggery effluent (ADPE) as growth medium, they examined N removal, functional genes, and biodiversity changes in a combined consortium of indigenous digestate bacteria and a *Chlorella*-dominated algal inoculum, as well as in two control conditions; a pure-culture digestate bacteria and the *Chlorella*-dominated inoculum without bacteria. Their results were striking; while the bacteria-only control culture demonstrated the highest removal rate of all nitrogen species (NH_4^+, NO_2^-, and NO_3^-), the mechanism identified was ammonia volatilization, by which free ammonia (NH_3) is emitted to the atmosphere (Ayre et al., 2021; Morales-Amaral et al., 2015). Conversely, Ayre et al. (2021) showed that when indigenous bacteria are co-cultured with a *Chlorella*-dominated photosynthetic consortium, NH_4^+ is quickly converted to NO_3^- via nitrification, which then remains in the system until it can be consumed and assimilated into biomass. Moreover, the *Chlorella*-dominated inoculum control (with no indigenous bacteria) was unable to remove any significant amount of nitrogen species from the system, presumably due to the lack of nitrifying bacteria oxidizing NH_4^+ to more bioavailable, less toxic NO_3^-. Finally, they attribute some of the mutually beneficial effect on nitrogen cycling to oxygen production; photosynthesis provides a stable source of oxygen for continuous bacterial oxidation of N species (Ayre et al., 2021). These results have important implications for controlling nitrogen pollution from intensive agriculture.

Another recent study focused on the effects of light regime on nitrification found that ammonium removal by a photosynthetic consortium was strongly affected by light intensity. Arun et al. (2021) tested four light intensities; 150, 500, 1500, and 2000 μmol m^{-2} s^{-1}, and reported NH_4^+ removal efficiencies of 100 %, 100 %, 6.2 % and −2.2 % for each condition, respectively. Light intensities above 500 μmol m^{-2} s^{-1} were detrimental to nitrification processes due to low availability of dissolved oxygen, a consequence of photoinhibition of photosynthetic consortium members. The bacterial species present (ammonium oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) were also prone to photoinhibition due to photooxidative damage to ammonia monooxygenase, a key enzyme which drives bacterial nitrification (Arun et al., 2021). The maximum light intensity tested in the present study was 500 μmol m^{-2} s^{-1}, although all treatments removed similar quantities of TN overall (Table 2). Arun et al. (2021) reported that nearly 80 % of ammonium in the system was assimilated into bacterial biomass, which may account for the efficient removal of N during this study across all light conditions. While microalgae were affected by photoinhibition during FS treatment in the present study, light intensities below 500 μmol m^{-2} s^{-1} did not appear to significantly impact bacterial activity.

3.2.2. Phosphorus

Phosphate removal results demonstrate efficient P uptake under FS and BW light, but P concentrations increased under the R light condition by an average of nearly 5 mg L^{-1} across replicates; from 6.42 mg L^{-1} to an average of 11.39 mg L^{-1} (Table 2). Only one replicate (reactor 1) during the BW light treatment achieved a final P concentration (1.16 mg L^{-1}) compliant with EU standards for wastewater discharge (Fig. 4) (European Commission, 2013). In this closed system, the only way for P to have increased is by cell lysis after luxury uptake of P by consortium members (Brar and Tolleson, 1975). Under all light conditions tested, initial concentrations of P were rapidly depleted during the first 48 h, inducing P stress. Bacteria have long been known to cause phytoplankton cell death under P-replete conditions, especially in the presence of organic carbon (Brussard and Riegman, 1998), which remained relatively high during the initial P consumption. A more recent study on the relationship between heterotrophic bacteria and cyanobacteria under P stress demonstrated the effect of active P-related enzymes released during bacterial cell lysis; alkaline phosphatase (APase) and other phosphatases remained active in the culture medium for days following lysis (Mine et al., 2021). These findings help to explain the steady increase in P concentrations in the media after the first sharp P increases under BW and R light during the present study.

Phosphorus has historically been considered a problematic nutrient in wastewaters, due to its role in the eutrophication of natural water bodies (De-Bashan and Bashan, 2004). Phosphorus is also considered as an “endangered” nutrient as natural mineral sources are finite, rapidly dwindling, and absolutely imperative for the continuation of modern agriculture as a fertilizer (Sengupta et al., 2015). Recycling P from various waste streams has thus been thoroughly investigated, using techniques such as adsorption (Loganathan et al., 2014; Ma et al., 2011), chemical precipitation (Karunanithi et al., 2015; Okano et al., 2013), biological uptake (Delgadillo-Mirquez et al., 2016), and, most recently, photosynthetic metabolism by microalgae and cyanobacteria (Solovchenko et al., 2020; Su, 2021).

Biofilm cultivation has demonstrated marked improvement on P uptake by microalgae, as compared with conventional liquid suspension. Shi et al. (2007) immobilized two species of microalgae (*Chlorella vulgaris* and *Scenedesmus rubescens*, cultivated separately) on a solid microporous substrate and assessed their ability to assimilate phosphate, ammonium, and nitrate from synthetic wastewater. They reported PO_4^{3-} removal efficiencies of 89 % and 90 %, for *C. vulgaris* and *S. rubescens*, respectively, during the first two days of cultivation. These removal rates are congruent with data on the same species cultivated in conventional liquid suspension, and improved somewhat upon previous microalgae immobilization studies (Shi et al., 2007). A subsequent study (Boelee et al., 2011) investigated the maximum nitrogen and phosphorus uptake capacities of microalgal biofilms cultured from an effluent settling tank of a Dutch wastewater treatment plant. The authors of this study reported reaching uptake maxima at loading rates of 1.0 g m^{-2} d^{-1}N and 0.13 g m^{-2} d^{-1}P, and demonstrated that this rate of removal met EU discharge standards of 15 mg L^{-1}N and 2 mg L^{-1}P (Boelee et al., 2011). Notably, the light intensity used in this study was 230 μmol m^{-2} s^{-1}, which roughly corresponds to the maximum light intensity under R light in the present study, and falls within the range of the BW light treatment intensity. The differences observed in this study between light treatments, compared with results reported by Boelee et al. (2011), as well as others, investigating the effects of light on nutrient removal by microalgae and photosynthetic consortia, indicate that spectral range of light provided may be as important as light intensity and photoperiod in promoting nutrient removal from wastewater (Delgadillo-Mirquez et al., 2016; Wu et al., 2021).

Conversely, another recent study (Rueda et al., 2022) investigated the effects of different environmental parameters (light, salinity, and nutrient levels) on PHB accumulation in *Synechocystis* sp. The reported a release of P into the medium during the accumulation phase (after N and P had been consumed during the prior growth phase), similar to the P spikes observed in the present study. Rueda et al. (2022) likewise attribute the release of P into the medium to cellular lysis and degradation of capsular polysaccharides. In contrast with the results of the

present study, they did not find strong evidence to support the effect of light intensity on P consumption. They tested two light intensities, 73 and 500 μmol m^{-2} s^{-1}, and reported that there was no significant difference in P assimilation between the two light conditions (1.26 and 1.1 mg P g^{-1} biomass accumulated during the growth phase under 73 and 500 μmol m^{-2} s^{-1}, respectively). In this case, the authors conclude that rapid depletion of nutrients, especially P, induced cellular stress and cell death, which is further supported by a decrease in biomass concentration corresponding with the liberation of cellular P into the medium. (Rueda et al., 2022). Furthermore, Rueda et al. (2022) were investigating osmotic stress in conjunction with light and nutrient-replete conditions, and these conditions may have simply created too harsh an environment for the cyanobacteria studied.

During the present study, visual analysis via light microscopy of the biofilm formed under R light indicated that cyanobacteria were more dominant in the consortium than in the other two light regimes. Many cyanobacterial species, as well as heterotrophic bacteria, are adept at accumulating P within the cell in response to environmental stimuli (Solovchenko et al., 2020). The underlying mechanisms of cyanobacterial phosphorus uptake is not well-constrained, and neither is the process by which they can alter their response to other dissolved nutrients and environmental conditions (Li and Dittrich, 2019). Levels of P under R light reach a striking maximum of 17 mg L^{-1} at 144 h, which is inconsistent with the apparent cyanobacterial bloom in all three PBR systems. Large amounts of heterotrophic bacteria were present in the untreated aquaculture effluent, and such an increase in P levels could be attributed to cell death of these bacteria when outcompeted by cyanobacteria in the consortium. It is possible that, given a longer cultivation period, photosynthetic members would have gradually assimilated the dissolved P liberated by bacterial cell death. In terms of carbon cycling, nutrient removal, and biomass production results altogether, BW condition is particularly interesting contrasted with R light. Blue-white light was less effective in terms of carbon capture (both inorganic and organic), and demonstrated a similar trend regarding N uptake. However, the BW treatment produced approximately 1.5 × more biomass than the R light condition, and proved most effective in P removal, despite similar increases (again, likely attributable to bacterial cell death) in P over time, with a maximum of 5.9 mg L^{-1}, nearly the initial P concentration, at 240 h (Fig. 4). Considering these data together, it is likely that BW light was more selective towards eukaryotic microalgae, whereas red light is known to select for cyanobacteria, which have evolved to take advantage of higher wavelengths not utilized by eukaryotic microalgae (Gisriel et al., 2022a,b). The effects of light spectra upon heterotrophic bacteria in photosynthetic consortia, however, are unknown and clearly consequential in terms of P removal, and must be elucidated in further studies.

3.3. Effects of light regime on biomass accumulation

Previous work has highlighted the impacts of light spectra and intensity upon microalgal biomass production (Chang et al., 2022; Iasimone et al., 2018). In the present study, analysis via light microscopy suggested that the FS treatment resulted in lower biodiversity than the other two light regimes, which impacted biomass yield. However, across all light treatments and PBR replicates, light microscopy showed a shift from the cyanobacteria-dominated inoculum towards a much more diversified biofilm, containing one dominant eukaryotic green alga and a bacterial "scaffold" of mixed filamentous and unicellular cyanobacteria and heterotrophic bacteria. The BW treatment, which produced the highest biomass yield, also appeared to have developed the most biodiverse community (see supplementary materials).

Photoinhibition by high-intensity, full-spectrum lighting is evidenced by the lowest biomass yields during the FS treatment (Fig. 5**a**). Given the carbon and nutrient cycling data presented in Section 3.2 (almost no nitrification to nitrate, fluctuating organic carbon, and steady depletion of phosphate), the biomass cultivated under the FS light

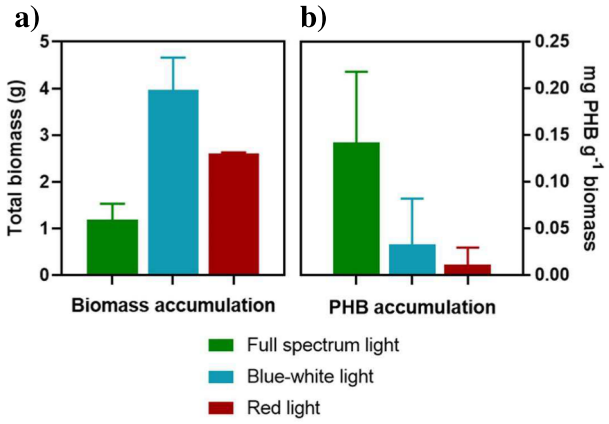

Fig. 5. Average a) biomass and b) PHB accumulated under each light regime after 14 d of cultivation. Error bars indicate standard deviation between three replicates (R1, R2, and *R*3).

condition was likely largely generated by eukaryotic microalgae. While BW light yielded the highest biomass values (Fig. 5**a**), it also had the highest variance (0.4771) between replicates (reactors). The BW treatment, however, resulted in the densest microalgal populations; evidenced by the highest rates of C_i and P uptake, as well as visual analysis via light microscopy. Higher densities of larger-celled eukaryotes and filamentous cyanobacteria will have contributed to the higher biomass yields; an average of 4 g after 14 d, corresponding to an overall average biomass accumulation rate of 0.28 g/L d^{-1}.

Red light yielded almost identical amounts of dry biomass from each independent bioreactor replicate; 2.6 g ± 0.3 g. Although this translates to an overall average production rate of ~ 0.19 g L^{-1} d^{-1}, the biological equilibrium achieved under red light shows promise for semi-continuous cultivation, rather than 14-day batch cultivation, given the low standard deviation observed compared with the two other light regimes. The consortia cultivated under red light demonstrated stable consumption of C_i, OC, and N, but P data was unusual in that it increased by an average of nearly 5 mg L^{-1} across all replicates. At Nordic latitudes, cyanobacteria and northern strains of eukaryotic microalgae are known to grow well under restricted light intensities and shorter photoperiods. A study of three Baltic cyanobacteria recently reported growth maxima at 190–280 μmol m^{-2} s^{-1} (Śliwińska-Wilczewska et al., 2019), roughly matching the higher light intensities in the R light regime of this experiment, and overlapping somewhat with the BW range. Positive selection for cyanobacteria rather than eukaryotic microalgae under the red light condition can partially explain these data, with smaller-celled cyanobacteria producing fewer grams of biomass per liter than their larger-celled eukaryotic counterparts.

Eukaryotic microalgae will undergo mixotrophic metabolism under lower light intensities; i.e. taking advantage of both organic and inorganic carbon sources. Multiple studies have shown a higher concentration (10 mg L^{-1}) of glucose in the growth medium, specifically, to be optimal for achieving high biomass yields from conventional eukaryotic microalgae under mixotrophic conditions (Li et al., 2014; Pang et al., 2019; Wan et al., 2011). In the present study, the initial concentration of organic carbon was markedly lower, with unaltered ACE containing less than 0.5 g/L. Future work aiming towards improving biomass yield under red light should target blending different types of wastewater, such that carbon species and other nutrients are well balanced without accruing high costs associated with conventional growth media preparation.

3.4. Effects of light regime on production of value-added compounds

3.4.1. Polyhydroxyburate (PHB) production

The highest levels of PHB accumulation were detected under the high-intensity FS light treatment (Fig. 5**b**), which strongly contrast with biomass production, carbon consumption, and nutrient removal results for this treatment. Previous work has likewise shown that light intensities approaching the upper limit tolerated by cyanobacteria (300 μmol m^{-2} s^{-1}) as the most effective for PHB production and accumulation, yielding 241 mg PHB L^{-1} (31 % dry weight), despite having a detrimental effect on biomass yield (Gracioso et al., 2021). The aforementioned study by Rueda et al. (2022) (Section 3.2.2) investigated the effect of two light intensities on PHB yield. They found that, after an initial growth phase (during which N and P were depleted in the medium), PHB production by *Synechocystis* sp. averaged 3.0 and 4.8 $\%_{VSS}$ after the accumulation phase, under 73 and 500 μmol m^{-2} s^{-1}, respectively (Rueda et al., 2022). The authors concluded that high light intensity (with the same maximum tested in the present work, 500 μmol m^{-2} s^{-1}) and osmotic stress stimulated PHB production in *Synechocystis* sp. (Rueda et al., 2022). In the present study, FS light within a range of 138 – 500 μmol m^{-2} s^{-1} produced the lowest biomass with an average yield of 1.19 g, yet PHB content was the highest in these samples with an average 0.142 mg g^{-1}. Under BW and R light regimes, PHB yields averaged 0.03 and 0.01 mg g^{-1}, respectively; nearly below the detection limit of the method employed (Fig. 5**b**).

In addition to the effects of light on PHB production, nutrient concentrations (N and P) are reportedly interlinked with PHB accumulation in cyanobacteria. Comparing a two-stage nitrogen-starved cultivation approach to conventional culture methods, Testa et al. (2022) reported that the final PHB concentration was 90 % lower in the conventional culture compared with the nitrogen-starved condition. Results from other studies have been more nuanced; Rueda et al., 2020) cultivated indigenous wastewater-borne microalgae (a mix of eukaryotes and cyanobacteria) in agricultural runoff as a source of N and P, and reported removal efficiencies of 99 % and 95 %, respectively, during continuous cultivation. Importantly, they note that the consortium was quickly dominated by cyanobacteria which outcompeted eukaryotic microalgae. They attribute their highest PHB yields (maximum 4.5 $\%_{VSS}$) to successfully selecting for cyanobacteria, the rapid depletion of N and P, and to optimizing carbon availability (the highest PHB levels corresponded with $NaHCO_3$ supplementation). Another study of mutant *Synechocystis* sp. PCC 6803 (*ΔSphU*, lacking a phosphate regulator) cultivated in shrimp farm wastewater reported a maximum PHB content of 32.48 % dry weight, and likewise attributed this high yield to nitrogen assimilation. They achieved nitrate, nitrite, and ammonium removal efficiencies of 80.10 %, 67.90 %, and 98.07 %, respectively, within a cultivation period of 14 d (Krasaesueb et al., 2019).

The results of the present study are congruent with findings in previous work on cyanobacterial production, demonstrating that there are tradeoffs between biomass production, PHB synthesis, and removal of carbon and nutrients.

3.4.2. Lipid content

In contrast with biomass and PHB production, lipid content was largely unaffected by differences in light regime. It had been hypothesized that perhaps light regime would favor eukaryotic microalgae under FS or BW light, and therefore produce some difference in total lipid content of the biomass. The average lipid contents for each treatment were 9.46 %, 9.10 %, and 9.60 % for FS, BW, and R light, respectively. These results reflect the dominance of cyanobacteria and heterotrophic bacteria within the consortium across light regimes, as well as the chemistry of the wastewater used as culture medium. Cyanobacterial biomass generally contains higher ratios of protein and carbohydrate to lipid, compared with eukaryotic microalgae. And, similar to the effect of nitrogen limitation upon cyanobacterial PHB accumulation, restricting N in growth media is well known to cause lipid accumulation in eukaryotic microalgae, while N abundance (present in the growth medium used in the present study, 16.27 mg L^{-1}), inhibits lipid production. Under these conditions and using a mixed consortium dominated by cyanobacteria, light had no significant impact on lipid production.

4. Conclusions

Light can manipulate the biological functions of photosynthetic consortia. Low-intensity red light was the only net carbon-negative treatment and reduced total nitrogen below EU discharge limits, but increased phosphorus; undesirable in water treatment. Medium-intensity blue-white light yielded the highest biomass and phosphorus removal. High-intensity full-spectrum light produced very low biomass, but the highest PHB overall. Targeted manipulation of environmental conditions can direct biological functions of consortia; e.g., towards biomass production or PHB accumulation, but not both simultaneously. Future studies elucidating the effects of light on community structure could further refine light spectra and intensity as a process control.

CRediT authorship contribution statement

Rebecca J. Wicker: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Heidi Autio:** Conceptualization, Methodology, Investigation. **Ehsan Daneshvar:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Binoy Sarkar:** Writing – review & editing. **Nanthi Bolan:** Writing – review & editing. **Vinod Kumar:** Writing – review & editing. **Amit Bhatnagar:** Supervision, Writing – review & editing.

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

The data that has been used is confidential.

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Appendix A. Supplementary data

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