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Introduction

The increasing production of wastewater and the lack of sufficient protein supply worldwide are two major challenges caused by rapid population growth and increasing environmental awareness (Li et al., 2023). The utilization of wastewater streams to produce single-cell protein (SCP) can be a promising method to solve the above problems simultaneously. Purple non-sulfur bacteria (PNSB) are a promising SCP mediator due to their unique metabolic features, mostly thanks to their high biomass yields (Capson-Tojo et al., 2020). In addition, PNSB can exhibit tolerance to high organic loads and utilize the organic constituents present in the wastewater for growth (Lu et al., 2019). Furthermore, in comparison to many other microbial SCP sources, PNSB biomass contains value-added biomolecules, such as carotenoids, bacteriochlorophyll, coenzyme Q10, and a broader scope of essential amino acids (Zhi et al., 2020), which improve the nutritional value of SCP. Light plays a critical role on PNSB growth, being the energy source during phototrophic metabolism. Light factors include light intensity, photoperiod, and light spectrum. Previous studies on the impact of light regulation on PNSB have primarily examined its effects on biomass growth and pigment production (Zhou et al., 2015; Kuo et al., 2012). It has been found that light regulation has a significant impact on PNSB metabolism, especially on carbon and nitrogen assimilation. SCP, as a major component of PNSB organisms, is susceptible to the influence of the light environment. However, comprehensive studies on light strategy regulation of SCP synthesis in wastewater systems with photosynthetic bacteria are currently lacking. This study investigates the effects of light intensity, photoperiod and light spectrum on biomass production, SCP synthesis, pollutant removal, nutrient transformation efficiency and the correlation between these factors in a PNSB wastewater resource recovery system.

Materials and Methods

Artificial sugar wastewater was used in the experiment, made from glucose (1.6 g/L), malic acid (1.6 g/L), sodium acetate (1.6 g/L), ammonium sulfate (1 g/L), potassium dihydrogen phosphate (0.2 g/L), and magnesium sulfate heptahydrate (0.1 g/L). The initial concentrations of chemical oxygen demand (COD) and ammonium nitrogen (NH_4^+-N) were 4000 and 250 mg/L, respectively. The initial pH was adjusted to 7.0 using HCl (0.5 mol/L) and NaOH (0.5 mol/L) solution.

The inoculated PNSB was a strain of *Rhodopseudomonas palustris* (*R. palustris*) and was purchased from the China BeNa Culture Collection. The initial inoculum of *R. palustris* had a concentration of 360 mg/L (dry weight). All experiments were conducted on a 2.5 m × 1.2 m × 1.5 m (length × width × height) experimental stand. The setup included an illumination system (either incandescent lamps or LEDs), batch photobioreactors (1,000 mL high-silica glass bottles with a working volume of 600 mL) and a magnetic stirrer (operating at 120 rpm) for mixing. Blackout curtains were used to create light-tight enclosures between different treatment groups to prevent interference between light sources. Each group consisted of three replicates. Three different lighting factors were modified, namely light intensity, photoperiod and light spectrum. To control for a single variable, the light intensity was maintained at a constant level of 120 μmol/m^2/s in the experiments involving photoperiod and light spectrum (see Table 1).

Table 1. Experimental design of light supply strategies

Light strategies	Control groups	Experimental groups
Light intensity (μmol/m^2/s)	0	40, 80, 120, 160, 200, 240

Photoperiod (L/D) *	24/0	3/21, 9/15, 12/12, 15/9, 18/6
Light wavelength (nm)	Incandescent lamp (380~780)	White light (380~780), Red light (557~711), Yellow light (542~665), Blue light (404~528), Green light (542~665)

* L corresponds to light (illuminated); D corresponds to dark (non-illuminated). The values indicate the number of hours for L and D

Results and discussion

The results indicated that, under conditions of 120 μmol/m²/s light intensity, L/D equal to 18/6, and incandescent light, *R. palustris* exhibited the highest biomass concentrations and daily bacterial production, reaching 1,140.5±19.7 mg/L and 0.32±0.02 g/L/d, respectively. This represents an increase of 17.06% ~ 93.21% and 54.43%~299.93% compared to the L/D of 24/0, 3/21 and 9/15, respectively ($P<0.05$). Moreover, under 120 μmol/m²/s light intensity, L/D equal to 3/21, and incandescent light, *R. palustris* displayed the highest protein content, at 67.47%. This protein content was significantly greater, showing an increase of 21.96%~44.54% compared to all other experimental groups ($P<0.05$). Additionally, COD and NH_4^+-N removal efficiencies reached 72.03%~78.40% under these optimal conditions (L/D = 18/6), showing an increase of 20.26%~40.97% compared to other experimental groups (L/D = 3/21) ($P<0.05$). Correlation analysis revealed significant negative associations between light intensity and light spectrum with protein content and concentration, respectively. Conversely, photoperiod exhibited a significant positive correlation with protein concentration (Fig. 1). Consequently, photoperiod is an effective regulatory strategy for enhancing the production of SCP by *R. palustris*. This research provides innovative methods and insights for enhancing SCP synthesis in wastewater systems using photosynthetic bacteria.

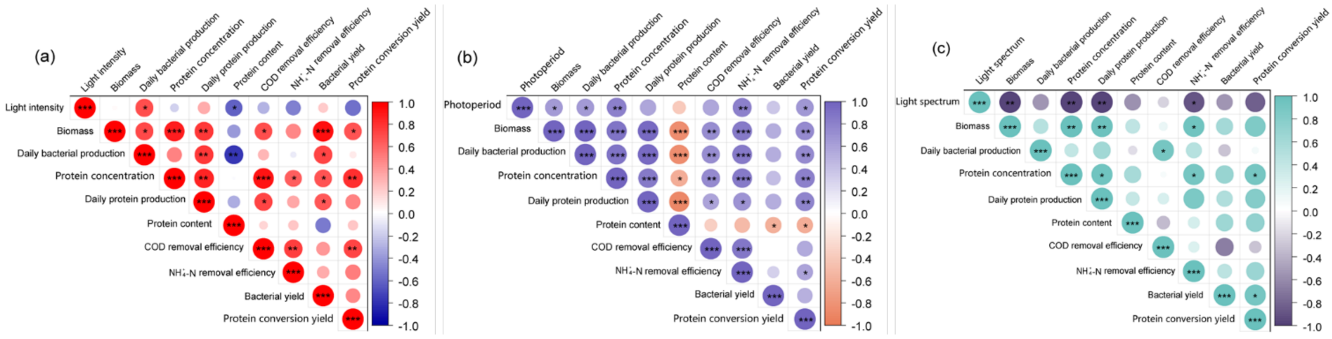

Fig. 1. Correlation analysis under three light supply strategies: (a) light intensity; (b) photoperiod; (c) light spectrum (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$)

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