International Biodeterioration & Biodegradation 135 (2018) 9–18

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Enhancement of wastewater treatment efficiency through modulation of aeration and blue light on wastewater-borne algal-bacterial consortia

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ARTICLE INFO

Keywords:
Supplementary aeration
Algal-bacterial consortia
Blue-light
Diauxic growth
NOB inhibition

ABSTRACT

Since few studies have investigated the nitrification and assimilation of nitrogens by algal-bacterial consortia, this study aimed to evaluate the effects of supplementary aeration and blue light on nitrogen removal and biomass growth of algal-bacterial consortia in real domestic wastewater. When blue light was weakly irradiated (500 μmol $m^{-2} s^{-1}$), it was found that supplementary aeration enhanced ammonia removal from 38.5% to 96.3% and algal growth from 72.5 mg algae L^{-1} to 345.3 mg algae L^{-1} by providing oxygen for nitrification and inorganic carbon for photosynthesis of microalgae. It was also observed that ammonia was consumed first and then nitrate produced by nitrification was assimilated, indicating that diauxic growth of consortia on nitrogen sources occurred. Thus, it was expected that nitrogen removal could be enhanced by lowering nitrification and denitrification loads. Moreover, intense blue light was found to accumulate nitrite by selective photoinhibition of nitrite oxidizing bacteria (NOB) of which c-type cytochrome is known to be photo-bleachable at 408 nm. From these results, it was concluded that favorable conditions for growth and nitrogen removal by algal-bacterial consortia in real wastewater could be established by controlling aeration and light intensity.

1. Introduction

Microalgae are particularly attractive for wastewater treatment because they produce molecular oxygen that is utilized for nitrification and degradation of organic matters by bacteria, and, in turn, the carbon dioxide produced by bacteria is used for microalgal growth (Vergara et al., 2016). Besides, microalgae are capable of assimilating large amounts of nutrients (NH_4^+, NO_3^-, PO_4^{3-}) and absorbing heavy metals (Aziz and Ng, 1993). In this context, algal-bacterial consortia have been studied for decades with aims to remove organic and inorganic contaminants from wastewater (Maza-Márquez et al., 2014). Despite of their low operating costs and environmental impacts, algal-bacterial consortia systems for wastewater treatment have been rarely applied at full scales (Craggs et al., 2012). In practical aspects, hard-to-control factors such as light availability, temperature variation, and fluctuating characteristics of real wastewater are still remained challenges to scaling-up of algal-bacterial systems.

For algal-bacterial consortia, photosynthetically produced oxygen could be major factors affecting the organic removal and nitrification in large scale processes. When light is, however, insufficient due to mutual shading, absence of light at night time, and unstable sun light, supplementary aeration might be possible alternative to the organic removal and nitrification. Mechanical aeration supplies oxygen and carbon dioxide to algal-bacterial consortia, therefore, enhancing effect of the nitrification and micro algal growth could be possible. Moreover, aeration might be inevitable for scaling up photo-bioreactor because light transmittance is limited in large reactors. Ouellet-Plamondon et al. (2006) investigated the effect of mechanical aeration on the nitrogen removal in wetlands where microalgae and bacteria co-existed and found that aeration improved the removal of organic matters and nitrogen, especially in winter.

At the same time, light is also an influencing factor on the activity and growth of nitrifying bacteria. It was known that blue light enhanced the algal growth and could be a more important regulatory

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https://doi.org/10.1016/j.ibiod.2018.07.008
Received 1 February 2018; Received in revised form 5 July 2018; Accepted 12 July 2018

factor on photo respiratory consumption of NO_3^--N than other lights (Azuara and Aparicio, 1983; Das et al., 2011). Meanwhile, Merbt et al. (2012) reported that light completely inhibited nitrite production and that *de novo* synthesis of ammonia monooxygenase was required after the culture was exposed to the visible light. Likewise, Guerrero & Jones (1996a) reported that blue light did not inhibit the activity of ammonia oxidizing bacteria (AOB) but the activity of nitrite-oxidizing bacteria (NOB). Thus, blue light seemed to enhance algal growth but selectively inhibit the activity of NOB. Numerous studies have been focused on NOB inhibition based on dissolved oxygen (DO) concentration, temperature, sludge retention time, substrate concentration, and intermittent aeration with an aim to achieve partial nitrification (*i.e.* NOB inhibition) related to a decrease in oxygen consumption for nitrification (Ge et al., 2014; Thanh et al., 2013). Few studies, however, reported the partial nitrification using algal-bacterial consortia with illumination of blue light onto real wastewater. Therefore, it is reasonable to assume that artificial light illumination and aeration might be possible solutions to enhance nitrification and algal growth in algal-bacterial consortia, particularly when light intensity is not sufficient for algal growth. Furthermore, combined effects of aeration and light intensity have not been studied extensively.

Considering the large-scale cultivation of algal-bacterial consortia, microalgal preference for the nitrogen sources (*i.e.,* ammonium or nitrate) is important. All studies published to date, however, focused on a single species and its preferred nitrogen source (Sanz-Luque et al., 2015), and nitrogen sources for growing wastewater-borne algal-bacterial consortia remain veiled.

In this study, wastewater-borne algal-bacterial consortia were grown in real wastewater to investigate the effects of aeration and light on nitrogen removal, oxygen production, BOD removal, and growth of microalgae and bacteria. Particularly, concentrations of nitrogen species in the culture of algal-bacterial consortia were closely monitored to understand the behaviors of nitrogen sources. Blue light was selected as a light source with an aim to monitor NOB inhibition and the uptake of NO_3^--N by algal-bacterial consortia growing in real wastewater. Furthermore, Changes of bacterial community and physiological responses of nitrifying bacteria to increased blue light intensity are also presented.

2. Materials and methods

2.1. Induction of algal-bacterial consortia

The wastewater used in this study was collected from a drum screen with 75 μm mesh in a domestic wastewater treatment plant located in Yongin, Korea and contained 292.8 ± 29.4 mg L^{-1} of biochemical oxygen demand (BOD), 144.4 ± 30.1 mg L^{-1} of suspended solids (SS), 52.4 ± 4.7 mg L^{-1} of total nitrogen (TN), 52.2 ± 4.6 mg L^{-1} of total Kjeldahl nitrogen (TKN), 34.8 ± 3.1 mg L^{-1} of ammoniacal nitrogen (NH_3-N), 0.25 ± 0.1 mg L^{-1} of NO_x^- -N (sum of nitrite and nitrate nitrogen), 6.9 ± 0.5 mg L^{-1} of total phosphate (TP), and 194.4 ± 5.0 mg L^{-1} of alkalinity as $CaCO_3$. To induce wastewater-borne algal-bacterial consortia, this wastewater was added to a photo bioreactor with a 12.6 L of effective volume and irradiated at the rate of 500 μmol $m^{-2}s^{-1}$ for 24 h at room temperature from two sides of the reactor with light-emitting diode (LED) sticks (25W per stick, Sungkwang, Korea) emitting blue light. From the second day, 25% of the effective volume were drained for 10 min and filled with fresh wastewater for 10 min. The mixed liquor was irradiated at 500 μmol $m^{-2}s^{-1}$ for 1420 min while agitated at 150 rpm. Therefore, the reactors were operated at photo-sequencing batch reactor (PSBR) mode with 10 min of fill, 1420 min of reaction, and 10 min of withdrawal every day. The algal-bacterial consortia were stabilized at 500 ± 100 mg L^{-1} of TSS and pH 7.2 ± 0.6 after 2 months of cultivation. The grown algal-bacterial consortia were used to analyze the O_2 production, species diversity, and nutrient removal in separate PSBRs.

2.2. PSBR set-up and operation

For investigating effects of aeration and blue light on oxygen production, growth, and nitrogen removal by algal-bacterial consortia, three (P)SBRs were used. Each reactor was made of an acrylic cylinder (internal diameter of 20 cm, height of 50 cm and total volume of 15.7L) and was agitated mechanically at 150 rpm using an impeller (Fig. S1). Two PSBRs with a working volume of 12.6 L each in which algal-bacterial consortium was cultivated were illuminated by blue light-emitting LEDs at light intensity of 500 μmol $m^{-2}s^{-1}$ with two LED sticks and 1000 μmol $m^{-2}s^{-1}$ with four LEDs, respectively. The third SBR reactor containing activated sludge (AS) without microalgae was continuously aerated at a rate of 0.1 vvm (volume to volume per minute) without illumination of blue light. Here after, PSBRs illuminated by blue light at 500 μmol $m^{-2}s^{-1}$ and 1000 μmol $m^{-2}s^{-1}$ and uni-illuminated SBR with continuous aeration were labeled as “Weak”, “Intense”, and “AS”, respectively.

The algal-bacterial consortia (200 ± 15 mg TSS L^{-1}) grown in the induction culture and activated sludge (200 ± 10 mg TSS L^{-1}) obtained from a domestic wastewater treatment plant located in Yongin, Korea were inoculated into 12.6 L of wastewater contained in PSBRs and SBR, respectively, and cultivated at 25 °C. Fill-react-and-draw was sequenced in a 24 h cycle, and each cycle consisted of 5 min fill with 3.15 L of domestic wastewater, 23 h 50 min react, and 5 min draw. Thus, sludge retention time (SRT) and hydraulic retention time (HRT) of the reactors were identically 4 days. The withdrawn mixed liquor suspended solids (MLSS) was settled in a separate sedimentation vessel for an hour, and the supernatant was used for physicochemical analyses. Weak and Intense PSBRs were not aerated for the first 52 days (Phase 1) and then aerated for next 43 days (Phase 2). SBR without light illumination was continuously aerated during the entire cultivation period.

2.3. Bacterial community analysis by next-generation sequencing (NGS)

Three MLSS samples were collected from both PSBRs and SBR at 91 days in Phase 2. For bacterial community analysis, DNAs were extracted from these samples, and emulsion-based PCR (emPCR) and NGS were carried out as described previously (Kang et al., 2018). Briefly, the bacterial genomic DNA was extracted from 10 mL of each sample using Power soil extraction kit (MoBio Laboratories, Carlsbad, CA, USA). 20 ng of extracted DNA were used for PCR amplification. As primers, the bacterial-universal primer 27F (5′ GAGTTTGATCMTGGCTCAG 3′) and 800R (5′ TACCAGGGTATCTAATCC 3’) were used for Fast Start High Fidelity PCR System (Roche, Indianapolis, IN, USA). The emPCR for clonal amplification of the purified library was carried out using the GS-FLX plus emPCR Kit (454 Life Sciences, Branford, CT, USA). The experiments were repeated at least three times. Pyro Sequencing was performed by Macrogen (Seoul, Korea). Obtained sequences were analyzed at phylum and family levels.

2.4. Batch tests on nitrification and photosynthesis in aerated condition

In order to evaluate nitrifying and photosynthetic capabilities of microalgal-bacterial consortia in the presence of aeration (0.1 vvm), PSBRs were operated at batch mode for 192 h. For nitrification experiments, NH_4Cl and $NaHCO_3$ were added to the real wastewater to increase concentrations of ammonia and alkalinity to 63 mg NH_3-N L^{-1} and 500 mg $CaCO_3$ L^{-1}, respectively. Additionally, the wastewater was filtered using a GF/C (Whatman, U.K.) filter to minimize any side-effects of particulates contained in the real wastewater. For examining the effects of light intensity, consortia biomass was taken from Phase 2 of each PSBR, centrifuged at 3000 rpm for 5min, and resuspended in distilled water. This washing processes was repeated three times. AS was also prepared as a reference biomass following the washing procedure. The concentration of resuspended consortia and AS were