Development of advanced light-tolerant microalgae-nitrifying bacteria consortia for ammonia removal under strong light irradiation using light-shielding hydrogel

遮光ゲル担体を用いた強光下におけるアンモニア除去のための耐光性微細藻類-硝化菌共存系の開発

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SYNOPSIS

人間活動に起因する窒素含有廃水は、主に生物学的硝化脱窒法によって処理されている。しかし、硝化に必要な機械的曝気の高い運転コストが課題となっている。微細藻類-硝化菌共存系は、微細藻類の光合成により硝化菌に酸素を供給でき、曝気不要であることから省エネルギーな処理プロセスとして注目されている。しかし、硝化菌は一定以上の光強度で阻害を受けるため、屋外環境でのプロセス破綻が懸念される。そこで本研究では、ゲル固定化技術を用いて、耐光性をもつ微細藻類-硝化菌共存系の開発およびその評価を行った。まず、ゲル中に硝化菌のみを固定化し遮光材を添加した「遮光ゲル担体」を開発し、1600 µmol photons m^{-2} s^{-1}までの光照射下で硝化菌の光阻害抑制効果を確認した。遮光ゲル担体は、1600 µmol photons m^{-2} s^{-1}においても、暗条件と比較して顕著な低下なく高い硝化性能を維持した。次に、遮光ゲル担体を微細藻類-硝化菌共存系に適用した結果、1600 µmol photons m^{-2} s^{-1}であっても遮光ゲル担体は亜硝酸の蓄積なく完全硝化を達成し、硝化速度は分散状の約 9 倍であった。以上の結果に基づいて、強光下における微細藻類-硝化菌共存系のプロセス破壊メカニズムを提案した。さらに、異なるバイオマス比による影響を強光下で評価した結果、1:9（微細藻類 : 硝化菌）で 100%のアンモニア除去率を示し、強光下において高いバクテリア割合はアンモニア除去性能を向上させることが明らかになった。本研究で開発した耐光性微細藻類-硝化菌共存系を用いることで、日射強度の高い様々な地域でも、省エネルギー型の窒素含有廃水処理の実現が可能となると期待される。

Keywords: Microalgae-nitrifying bacteria consortia, Light-shielding hydrogel, Immobilization, Photoinhibition, Nitrification

Introduction

With the expansion and diversification of human activities, hotspots of high nutrient discharge causing eutrophication in aquatic environments are materializing worldwide [1]. For example, nitrogen-containing wastewaters such as sludge digestion liquid, landfill leachate, and agricultural wastewater [2] are commonly treated with biological nitrification-denitrification processes. However, it has been known that aeration for nitrification is an energy-intensive and costly operation and may account for 45–75% of energy consumption of the whole process [3]. Therefore, in many developing countries, the nitrification-denitrification method has not been implemented yet, and the development of a more economical and sustainable nitrification process is required. Using a consortia of microalgae and nitrifying bacteria has attracted attention owing to its advantages such as its energy- and cost-efficiency. Ammonia in wastewater is removed by two pathways: uptake by microalgae and nitrification by nitrifying bacteria. Nitrifying bacteria use O_2 supplied via microalgal photosynthesis for nitrification, thus eliminating mechanical aeration-associated costs, and thereby significantly reducing the total cost.

Nitrifying bacteria are generally more sensitive to light than microalgae. The specific growth rate of microalgae *Chlorella sorokiniana* increases up to 250 µmol photons m^{-2} s^{-1} [4]. Conversely, Merbt et al. (2012) reported that the growth inhibition of nitrifying bacteria, such as ammonia-oxidizing archaea (AOA) and ammonia-

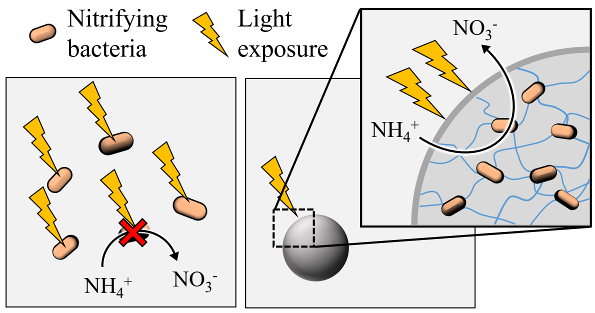

Fig. 1 Dispersed nitrifying bacteria and Light-shielding hydrogel under light irradiation.

oxidizing bacteria (AOB), starts at 60 µmol photons m^{-2} s^{-1} [5]. Moreover, they found that nitrite-oxidizing bacteria (NOB) are more susceptible than AOB to light. Despite the presence of sufficient oxygen in dispersed microalgae–nitrifying bacteria consortia, nitrification activity is reduced by 70% at 1600 µmol photons m^{-2} s^{-1} due to photoinhibition as compared to the dark condition [6]. Therefore, it is important to protect only nitrifying bacteria under intense light intensity conditions, such as sunlight. To mitigate the photoinhibition of nitrifying bacteria in intense light exposure, “light-shielding hydrogel” that entraps nitrifiers in carbon black-added alginate hydrogel beads was developed (Fig. 1). It is hypothesized that combining the light-shielding hydrogel with the microalgae-nitrifying bacteria consortia could establish a novel ammonia removal process capable of outdoor operation even under intense light irradiation. However, it is not clear if the proposed consortium using the light-shielding hydrogel can supply sufficient oxygen comparable to aeration at various light intensities. Further, the ammonia removal performance of microalgae-nitrifying bacteria consortia with light-shielding hydrogel needs to be examined in detail.

In addition, the effects of external factors such as C/N ratio [7], inoculum biomass ratio [8], and aeration time [9] have also been investigated to improve the treatment performance of the microalgae-bacteria consortia. Especially, the inoculum biomass ratio of microalgae and nitrifying bacteria is a crucial for effective ammonia removal owing to its different essential roles. Since it could be possible that the optimum biomass ratio in microalgae-bacterial consortia under intense light is different from those under low intensity light, to investigate the effect of the biomass ratio on the proposed "light-tolerant microalgae-nitrifying bacteria consortia" utilizing light-shielding hydrogels is essensial.

In this study, we first prepared light-shielding hydrogel and its nitrification performance was evaluated under a wide range of light intensity in a batch test. Next, establishment of a "light-tolerant microalgae-nitrifying bacteria consortia" in which light-shielding hydrogel was applied to a microalgae-nitrifying bacteria consortia was tried under intense light irradiation. Finally, the effect of inoculum biomass ratio on ammonia removal efficiency in the proposed consortia was examined.

Materials and methods

Preparing light-shielding hydrogel

Immobilized nitrifying sludge in the light-shielding hydrogel was prepared using alginate hydrogel by the following procedure: Nitrifying sludge was centrifuged at 3000 rpm for 10 min and obtained the sludge concentration of 7.9 g-SS L^{-1}. 2.0 wt% sodium alginate was dissolved in deionized water, and concentrated nitrifying sludge was added in the sodium alginate solution. Then, carbon black powder of 0.1 wt% was added to the mixture solution. The resulting mixture solution was dripped into 1.0 wt% calcium chloride solution. The hydrogel beads were formed immediately and then stored in calcium chloride solution at 25 ºC for 6 h. The resulting sample was referred to as "Light-shielding hydrogel". To examine the effect of immobilization and light-shielding separately, another type of immobilized nitrifying sludge without carbon black was formed and referred to as "Hydrogel". Suspended sludge without immobilization was used for comparison and was referred to as "Dispersion".

Light irradiation test for only nitrifying bacteria

The effect of light irradiation with different intensities on the nitrification properties of the resulting three sludge samples was examined by a batch test using serum bottles with an effective volume of 100 mL. Concentrated synthetic wastewater was diluted and added to each bottle, leading to the NH_4^+ concentration of 50 mg-N L^{-1}. The concentration of nitrifying sludge in each bottle was set to 0.5 g-SS L^{-1} for all the conditions. Pure oxygen gas was supplied to the bottles for 5 min to sufficiently saturate oxygen in the water, and then the bottles were sealed. The samples were irradiated using a customized

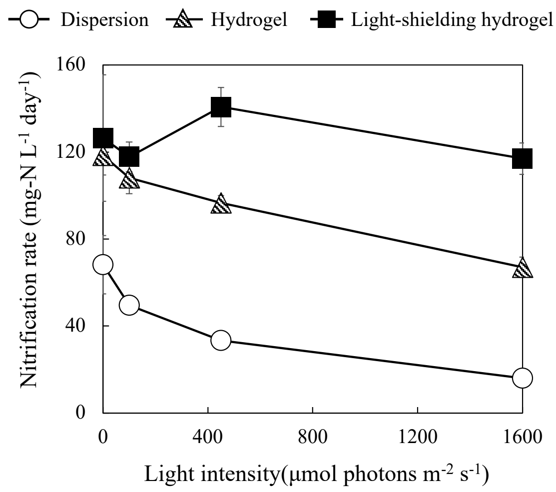

Fig. 2 Influence of light intensity on nitrification rate.

LED light irradiation device. The incident light intensities were adjusted to 0 (as a control), 100, 450 and 1600 μmol photons m^{-2} s^{-1}. The control bottles (0 μmol photons m^{-2} s^{-1}) were sealed with aluminum foil to prevent exposure to the light. The samples were incubated in triplicate at 25±1 ºC for 12 h and were shaken at 180 rpm.

Microalgae-bacteria consortia using light-shielding hydrogel under high light irradiation

To establish the light-tolerant microalgae-nitrifying bacteria consortia, its ammonia removal and nitrification performance were evaluated under various light intensities. Three sludge samples as same as only nitrifying bacteria experiment were used. The sludge and microalgae *Chlorella sorokiniana* (NIES-2173) were added to achieve 0.5 and 0.3 g-SS L^{-1} in each bottle, respectively. Before sealing the bottles, nitrogen gas was supplied for 5 min to remove dissolved oxygen from the bottle. The initial pH was set to 8.0 adjusted using 1 M HCl. The reactor and NH_4^+ concentration were the same as mentioned above in the test for nitrifying bacteria. The incident light intensities were adjusted to 0 (as a control), 100, 450 and 1600 μmol photons m^{-2} s^{-1}. The samples were incubated in triplicate at 25±1ºC for 24 h and were shaken at 180 rpm.

Different inoculum biomass ratio in microalgae-bacteria consortia using light-shielding hydrogel

The effect of different inoculum biomass ratio on ammonia removal performance was examined. As nitrifying sludge conditions, light-shielding hydrogel and dispersion were selected. Light irradiation was set under 500 μmol photons m^{-2} s^{-1} of light intensity and 12h/12h of light/dark cycle. The sludge and microalgae *Chlorella sorokiniana* (NIES-2173) were added to achieve the total biomass concentration of 0.3 g-SS L^{-1} in each bottle. The biomass ratio was adjusted to 6 conditions; 10:0, 9:1, 7:3, 5:5, 1:9 and 0:10 (microalgae: nitrifiers). The initial pH was set to 8.0 using 1 M HCl. The reactor and NH_4^+ concentration were the same as mentioned above in the test for nitrifying bacteria. The samples were incubated in triplicate at 25±1ºC for 24 h and were shaken at 180 rpm.

Results and discussion

Photoinhibition of nitrifying bacteria

Fig. 2 shows the relationship between light intensity and nitrification rate. The nitrification rate in the dispersion decreased monotonously as the light intensity increased. The nitrification rate in the hydrogel also decreased with the increase in light intensity, but it was higher than that in the dispersion at all light intensities. Whilst it is worth noted that the nitrification rate was hardly affected by strong light irradiation up to 1600 μmol photons m^{-2} s^{-1} in the light-shielding hydrogel. The nitrification rates of the dispersion, hydrogel, and light-shielding hydrogel at 1600 μmol photons m^{-2} s^{-1} were 16.0, 67.0, and 117 mg-N L^{-1} day^{-1}, respectively. This result shows that addition of carbon black into hydrogel enhanced light stress tolerance of immobilized nitrifiers.

Microalgae-nitrifying bacteria consortia under intense light irradiation

The pH values increased to 8.0–11.4 for all the light-irradiated conditions. However, they were maintained or decreased for the control (0 μmol photons m^{-2} s^{-1}). In microalgae-nitrifying bacteria consortia, pH values varied depending on the balance between photosynthesis and nitrification. pH increase occurs due to the CO_2 capture, and the decrease is due to nitrification of ammonia. Nitrifying bacterial activity is inhibited by approximately 40–80% at pH 9.0 [10]. Moreover, the biomass productivity of the microalgae *Chlorella sorokiniana* is significantly reduced at pH 9.0 as compared to pH 8.0 [11]. High pH values (> 11.0) were observed only in the light-irradiated "dispersion" conditions, which exceeded the level of inhibition for both microalgae and nitrifying bacteria. This suggests that the inhibition of nitrification by light irradiation was severe in the dispersion, but it was reduced in both the immobilized conditions.

In a consortia, DO values also varied depending on the balance between photosynthesis and nitrification. Regardless of sludge conditions and light intensity, relatively high DO concentrations (> 2.0 mg L^{-1}) were observed for the light-irradiated conditions as compared to the control (dark) condition. This suggests that sufficient photosynthesis was induced by light irradiation.

The ammonia removal efficiency and nitrogen mass balance at the experimental endpoint for each condition are shown in Fig. 3. Ammonia removal efficiencies were maximum at 100 μmol photons m^{-2} s^{-1} for all conditions, that is, 94.1%, 100%, and 100% for the dispersion, hydrogel, and light-shielding hydrogel, respectively. The low ammonia removal efficiency under dark conditions was attributed to essentially no contribution of nitrification owing to the lack of oxygen supply from the microalgae. The ammonia removal efficiency subsequently decreased with increasing light intensity for all sludge conditions, reaching 74% at 1600 μmol photons m^{-2} s^{-1} even for the light-shielding hydrogel. From the results of nitrogen mass balance, NO_2^--N accumulated at ≥ 100 μmol photons m^{-2} s^{-1} for dispersion. Hydrogel exhibited almost no NO_2^--N accumulation at 100 and 450 μmol photons m^{-2} s^{-1}, whereas 8.8% accumulation was observed at 1600 μmol photons m^{-2} s^{-1}. Whilst for the light-shielding hydrogel condition, no NO_2^--N accumulation was observed even at 1600 μmol photons m^{-2} s^{-1}. This suggests that the light-shielding hydrogel prevented the photoinhibition of both AOB and NOB and thus demonstrated its effectiveness.

Based on the obtained results of process breakdown in the dispersion condition in this study, a possi

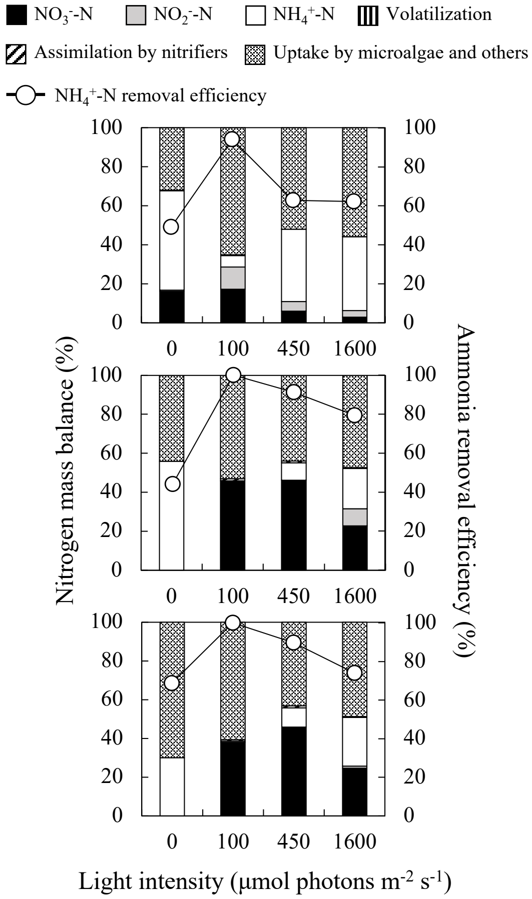

Fig. 3 Nitrogen mass balance and ammonia removal efficiency. (Top) Dispersion; (Middle) Hydrogel; (Bottom) Light-shielding hydrogel. Error bars indicate the standard deviations of triplicate samples.

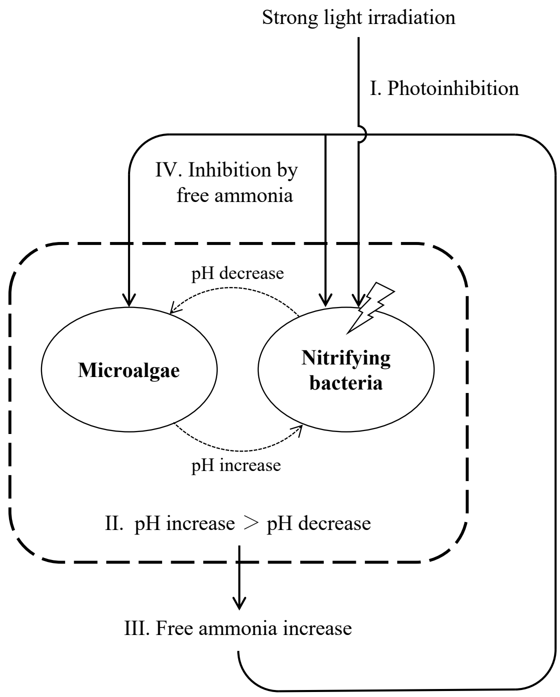

Fig. 4 Breakdown of the consortia system by strong light irradiation. I–IV shows the time phase of the proposed process breakdown.

-ble mechanism for the breakdown of microalgae-nitrifying bacteria consortia under intense light irradiation was proposed (Fig. 4). When the light intensity is an appropriate value in dispersed microalgae–nitrifying bacteria consortia, a neutral pH can be maintained. This is because the pH-decreasing effect derived from nitrification by nitrifying bacteria and the pH-increasing effect derived from CO_2 capture by microalgae can be balanced. Alternatively, a pH increase may occur when the light intensity is increased to a level at which nitrifying bacteria begin to experience photoinhibition. Such imbalance between the two effects on pH would lead to the breakdown of the process. This is explained according to the following mechanism. First, intense light irradiation inhibits nitrification (Fig. 4-I), and this weakens the pH-decreasing effect and allows the pH-increasing effect derived from microalgae to become dominant (Fig. 4-II), thereby causing a pH increase. High pH conditions in wastewater readily lead to increased FA concentrations (Fig. 4-III), which inhibits the activity of nitrifying bacteria and microalgae (Fig. 4-IV). In this mechanism, photoinhibition plays an essential role as a trigger, with subsequent FA inhibition becoming more severe. These integrated effects ultimately lead to process breakdown. The understanding of these mechanisms can help to improve treatment performance in continuous outdoor operation. In contrast, since light-shielding hydrogel can mitigate photoinhibition of nitrifying bacteria, the use of light-shielding hydrogel can effectively prevent process breakdown even under intense light intensity.

Effect of inoculum biomass ratios

The influence of bacteria proportion in microalgae-bacteria consortia on ammonia removal efficiency obtained from this study and previous studies was shown in Fig. 5. In this study, the maximum ammonia removal efficiencies for both dispersion and light-shielding hydrogel were 70.5% and 100% at 1:9 (90% bacterial proportion), respectively. The use of light-shielding hydrogel had the highest ammonia removal efficiency because the pH in the reactor remained neutral throughout the experiment, allowing the microalgae to uptake ammonia without pH inhibition. The optimum biomass ratio was determined to 1:9 which resulted in the highest ammonia removal efficiency in this experiment. Almost 50–60% of ammonia removal efficiencies were achieved for other biomass ratios. The lower ammonia removal efficiencies for other biomass ratios can be attributed to two factors. At 0:10 (100% bacterial proportion), the reason of lower ammonia removal efficiency is the lack of oxygen without microalgae for both sludge conditions. For the bacteria proportions between 0 and 50%, high pH throughout the experimental period in the reactor resulted in low ammonia removal efficiencies. Although other previous studies have reported no change in ammonia removal efficiency for different bacteria proportions, it could be due to their low light intensity as compared to the high light intensity in this study. Optimum bacteria proportion (biomass ratio of microalgae to bacteria) under different light intensity needs to be studied in more detailed in the future.

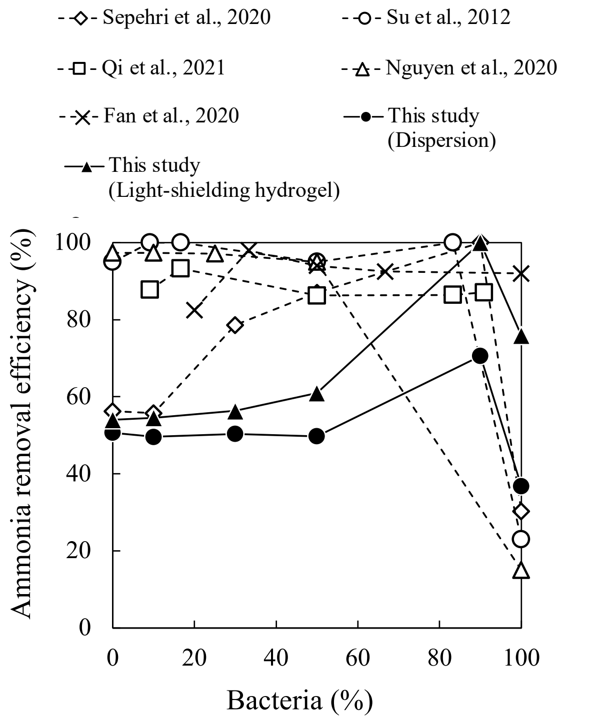

Fig. 5 Influence of bacteria proportion in microalgae-bacteria consortia on ammonia removal efficiency

Summary

To establish advanced light-tolerant microalgae-nitrifying bacteria consortia, first, light-shielding hydrogel was developed in which nitrifying bacteria were immobilized in alginate hydrogel incorporating carbon black powder. The nitrification performance for light-shielding hydrogel was evaluated under the light intensity up to 1600 µmol photons m^{-2} s^{-1}, resulting in maintaining high nitrification rate even under intense light irradiation. Second, light-shielding hydrogel and microalgae were combined without aeration under various light intensity. Using the light-shielding hydrogel, the ammonia removal efficiency achieved 74%, and the NO_2^--N accumulation was prevented at 1600 µmol photons m^{-2} s^{-1}. The possible mechanism for the breakdown of microalgae-nitrifying bacteria consortia under intense light irradiation was proposed. Third, the inoculum biomass ratio affected the light-tolerant microalgae-nitrifying bacteria consortia under intense light irradiation, and the optimal biomass ratio was 1:9 (microalgae: nitrifiers) in which 100% of ammonia removal efficiency was achieved. The developed light-shielding hydrogel appeared suitable for application with microalgae–nitrifying bacteria consortia to treat ammonia-containing wastewater under the conditions of intense light irradiation. It is further expected that the proposed method will contribute to the practical application of microalgae–nitrifying bacteria consortia in various countries that experience high sunlight intensity due to their location in the sunbelt areas.

References

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