Process Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

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

Effects of light-oxygen conditions on microbial community of photosynthetic bacteria during treating high-ammonia wastewater

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ARTICLE INFO

Keywords:
Photosynthetic bacteria
Microbial community
Light-oxygen conditions
High-ammonia wastewater

ABSTRACT

Photosynthetic bacteria (PSB) were reported to have great potential for nitrogen removal when treating high-ammonia wastewater. Light-oxygen conditions are the most important parameter; however, the role of the microbial community composition for PSB nitrogen removal remains unclear. This study focussed on the effects of three light-oxygen conditions on PSB performance and the microbial community during high-ammonia wastewater treatment. The results showed that light-oxygen conditions had a significant impact on nitrogen removal efficiency, microbial community diversity and composition. PSB under light-aerobic condition had the highest biomass, highest COD, NH_4^+-N, and NO_3^--N removal efficiency, and the highest community diversity; these results have not been reported before. The dominant genus in samples under light-aerobic condition was *Pseudomonas* (with a proportion of 58.23%). The correlation analysis showed that *Pseudomonas* was positively correlated with NH_4^+-N and NO_3^--N removal, with high correlation coefficients of 0.9. These results further revealed the underlying nitrogen removal mechanism of PSB in high-ammonia wastewater treatment.

1. Introduction

In the 1960 s, Kobayashi discovered that photosynthetic bacteria (PSB) have the ability to purify wastewater [1]. Since then, PSB wastewater treatment has developed significantly. Researchers have studied the application of PSB in treating various kinds of wastewater, such as soybean wastewater, domestic wastewater, dye wastewater, shrimp farm wastewater, poultry slaughterhouse wastewater, citric acid wastewater, and brewery wastewater [2–6]. These studies showed that PSB treatment technology could achieve high COD, nitrate, ammonia, and sulphide removal efficiency for different types of wastewater [7,8].

In particular, some studies reported the application potential of PSB for nitrogen removal from high-ammonia wastewater [9–11]. High-ammonia wastewater is a type of refractory wastewater, which is difficult to treat using conventional biological treatment. Ogbonna et al. reported that PSB could effectively remove ammonia at a rate of 20 mg/(L d) in wastewater containing 400 mg/L of ammonia [12]. Zhou et al. found that PSB could survive well in chicken manure wastewater with about 7000 mg/L of ammonia, while achieving an 83.2% ammonia removal efficiency [13]. In addition, 99.75% ammonia removal efficiency was achieved in aging biogas slurry with a low C/N ratio and high ammonia during PSB treatment [14]. During the PSB nitrogen removal process, nitrogen is removed by the metabolic processes of different strains of PSB. Therefore, microbial species and community diversity are the determining factors for directly controlling the nitrogen removal process and effects of PSB wastewater treatment. However, the relationship between microbial species, community diversity, and nitrogen removal were still unclear.

Light-oxygen conditions are the most important parameter for PSB wastewater treatment because they can affect the metabolic pathways of PSB [13,14]. For high-ammonia wastewater treatment, previous studies showed that different light-oxygen conditions had a distinct effect on the removal efficiency of pollutants and growth in PSB [13–15]. The optimal growth in PSB and wastewater treatment performance were achieved under micro-aerobic-light (4000 lx) condition in chicken manure wastewater treatment [13]. According to Hiraishi et al. [15], different light-oxygen conditions had significant impact on denitrifying enzyme activities in PSB wastewater treatment. Yang et al. reported that dark-aerobic condition was more conducive to NH_4^+-N removal than light-anaerobic condition for PSB in high-ammonia wastewater treatment [14]. From the above, it is evident that most studies of PSB focused on the effects of light-oxygen conditions on pollutants removal efficiency. To the best of our knowledge, there is no report on the effects of light-oxygen conditions on microbial community of PSB in

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https://doi.org/10.1016/j.procbio.2018.06.003
Received 16 March 2018; Received in revised form 5 June 2018; Accepted 9 June 2018

high-ammonia wastewater treatment. Yet, microbial communities are susceptible to different conditions during biological treatment [16–18].

For PSB wastewater treatment, the typical light-oxygen conditions are light-anaerobic condition and dark-aerobic condition. The reason for this is that PSB can grow well under either light condition or aerobic condition because of their two energy metabolic pathways, which are photophosphorylation and oxidative phosphorylation [19]. To distinguish the influence of the energy metabolic pathways of PSB and to save energy, previous studies focused on the effects of two typical light-oxygen conditions (light-anaerobic and dark-aerobic condition) on PSB wastewater treatment [20–23]. In order to conduct a thorough exploration of the effects of light-oxygen conditisons on PSB during high-ammonia wastewater treatment, three light-oxygen conditions were chosen in this study: light-anaerobic, dark-aerobic, and light-aerobic condition. To the best of our knowledge, the effect of light-aerobic condition on PSB wastewater treatment was rarely reported in previous studies.

The aim of this study was to explore the effects of three different light-oxygen conditions on treatment performance and microbial communities. The correlation between treatment performance and microbial populations of PSB was further analysed to reveal the underlying nitrogen removal mechanism.

2. Materials and methods

2.1. Materials

The PSB used in this study were complex bacteria that were isolated from a local river in Beijing, China (39°48′N, 116°24′E). The PSB were cultured in an improved RCVBN medium with 3000–4000 lux using a fluorescent lamp for 72–96 h before use [24].

The high-ammonia wastewater was livestock breeding wastewater from a livestock and poultry farm in Shandong, China. The initial concentrations of COD, NH_4^+-N, NO_3^--N, and chroma (dilution) were 4061.5 mg/L, 4431.4 mg/L, 89.9 mg/L, and 1785.7, respectively. The initial pH was 7.33. The wastewater was stored at 4 °C before use.

All chemical agents used in this study were from Sinopharm Chemical Reagent Co., Ltd.

2.2. Experimental setup

All experiments were conducted in 1000 mL glass flasks with 700 mL working volumes. Wastewater and PSB (70%/30%, v/v) were added to the reactors. The reaction temperature was room temperature. The hydraulic retention time was 96 h. Three light-oxygen conditions were set as follows.

a) Light-anaerobic condition: A 40 W incandescent lamp was used to keep light intensity around 2500–3000 lux. Dissolved oxygen was kept below 0.1 mg/L by flushing N_2 and sealing the bioreactors with parafilms.

b) Dark-aerobic condition: The bioreactors were covered with tinfoil to avoid light transmission. The aerobic condition was realised by putting the bioreactors into a shaker at 80 rpm. The dissolved oxygen (DO) was kept above 2.0 mg/L.

c) Light-aerobic condition: The light condition and aerobic condition were the same as those in a and b.

2.3. Analysis methods

Samples (8 mL every 24 h) were collected from each bioreactor and centrifuged at 11,000 rpm for 5 min to obtain a supernatant for detecting the concentrations of COD, NH_4^+-N, NO_3^--N, and chroma (dilution). NH_4^+-N was detected at 420 nm with a TU-1900 spectrophotometer (Beijing Purkinje General Instrument Co.), according to the Nesslerization method. COD and NO_3^--N were measured according to the APHA Standard Method. The pH value was detected using a pH tester. The collected PSB were used to measure biomass according to the method by Lu et al. [24].

2.4. Microbial community analysis

2.4.1. DNA extraction and PCR amplification

To analyse the microbial community composition, all samples were collected from bioreactors at 96 h and stored at −80 °C. DNA extraction was performed using the E.Z.N.A.® DNA Kit. DNA quality was checked using 1% agarose gel electrophoresis.

Polymerase chain reactions (PCR) were conducted in triplicate with 20 μL mixtures containing 4 μL of 5 × FastPfu Buffer, 0.8 μL of each primer (5 μM), 2 μL of 2.5 mM dNTPs, 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. For PCR amplification, the primers used in this study were 515 F (5′-GTGCCAGCMGCCGCGG-3′) and 907 R (5′−CCGTCAATTCMTTTRAGTTT-3′), which targeted the V4-V5 region of the bacteria 16S ribosomal RNA gene. Each sample for PCR reactions was performed at least three times. The purification and quantification procedures were conducted on the basis of previous studies [25].

2.4.2. DNA sequencing and data analysis

Amplicons sequencing was performed using an Illumina MiSeq PE250 platform (Shanghai Majorbio Bio-pharm Technology Co. Ltd., China). The raw FASTQ files were processed using QIIME (version 1.17). Operational units (OTUs) were clustered with a 97% similarity cut-off using UPARSE (version 7.1). The RDP Classifier against the Silva (SSU115)16S rRNA database was applied to analyse the taxonomy of the 16S rRNA gene sequence with a confidence threshold of 70%. The α-diversity index of samples under three light-oxygen conditions, namely the Shannon index, was calculated based on the average of three repeated measurements. The β-diversity was calculated by the weighted UniFrac distance matrix between three samples and was expressed by principal component analysis (PCA). A Spearman correlation analysis was used to evaluate the correlation between treatment performance and microbial populations of PSB.

2.5. Statistical methods

Three repetitions of each experiment were conducted. Three parallel measurements were performed to ensure the accuracy of data. Tukey's test was used to evaluate the significance level of the data, and the P value was below 0.05.

3. Results and discussion

3.1. Effects of light-oxygen conditions on treatment performance

For this high-ammonia wastewater, nitrogen removal was key because the NH_4^+-N concentration was above 4400 mg/L and the ratio of COD/NH_4^+-N was extremely low (0.9). In addition, this wastewater contained a high concentration of chromatic substances, with a chroma of 1786. Thus, this refractory wastewater was difficult to treat using conventional biological treatments. During PSB treatment, the growth in PSB and the removal of NH_4^+-N, NO_3^--N, COD, and chroma were investigated under three light-oxygen conditions. The results are shown in Fig. 1.

As shown in Fig. 1a, PSB biomass increased continuously under all three light-oxygen conditions, which illustrated that PSB could survive well in high-ammonia wastewater. In addition, it was evident that changes in PSB biomass showed a significant difference under the three light-oxygen conditions. The highest PSB biomass was achieved under light-aerobic condition at 96 h; under this condition, the biomass increased by 45.5%. This result showed that light-aerobic condition was more conducive to the growth in PSB than light-anaerobic or dark-aerobic condition, which was rarely reported in previous studies. Comparing light-anaerobic condition with dark-aerobic condition, it