Efficient nitrogen and phosphorus removal from wastewater by screening and constructing a novel *Chlorella* sp.-*Bacillus taeianensis* SD148BN12 consortium

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Abstract

The discharge of wastewater rich in nitrogen and phosphorus were posing a serious threat to ecological balance and human health. In the study, *Bacillus taeanensis* SD148BN12 with biosafety and capability of aerobic nitrogen removal by assimilation was selected to construct the *Chlorella* sp.-*Bacillus taeanensis* SD148BN12 consortium (M-B). Compared with single axenic *Chlorella* sp. and single *Bacillus taeanensis* SD148BN12, M-B improved the removal efficiencies of total nitrogen (91.89%) and total phosphorus (94.58%), the biomass yield (0.52 g/L), as well as the contents of carbohydrate and lipid after cultivation in synthetic wastewater. M-B had the high and stable nitrogen removal efficiency at temperature 20-30 °C, salinity 20-40‰, light intensity 50-200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, pH 6-9, C/N ratio 1-10, N/P ratio 4-20, N concentration 1-50 mg/L. The conspicuous removal efficiency of nitrogen and phosphorus from actual marine wastewater could be realized by M-B. Meanwhile, the synergistic interaction between *Chlorella* sp. and strain SD148BN12 was demonstrated by the characteristics of cell growth, photosynthetic activity and material exchange. The study provided an economical and environmentally friendly strategy for efficient removal of nitrogen and phosphorus from wastewater (including the neglected aquaculture wastewater) by establishing a hopeful microalgae-bacteria consortium.

1. Introduction

Excessive nitrogen (N) and phosphorus (P) contents in water environment have become a worldwide problem, resulting in eutrophication, impacting the ecological balance (Mohsenpour et al. 2021) and finally threatening human health. In addition to the discharge of industrial wastewater and domestic sewage, without enough attention, intensive aquaculture was also a main reason for the increase of N and P contents in water environment (Yang et al. 2017). In intensive aquaculture system, only a small fraction of the feeds was converted to the biomass of aquatic animals, of which only about 25% of N and P were utilized (Zhang et al. 2020b). Inorganic N (especially ammonia and nitrite with the toxicity to animals) and P from residual feed and animal waste released and accumulated in the water, which led to water quality deterioration and aquatic animal disease (Mohsenpour et al. 2021). After a production cycle, the aquaculture wastewater was often discharged into adjacent water without proper treatment, exacerbating a series of water quality problems (Malibari et al. 2018, Mohsenpour et al. 2021). Therefore, it was urgent to optimize and popularize the technologies of N and P removal from wastewater, of which, biological treatment have become an attractive choice due to the low cost, high efficiency and less secondary pollution (Hou et al. 2021).

Bacteria were the conventional basis in the biological treatment of wastewater. Recently, the bacteria with capability of heterotrophic nitrification-aerobic denitrification (HNAD), by which ammonium ($\text{NH}_4^+\text{-N}$), nitrite ($\text{NO}_2^-\text{-N}$), and nitrate ($\text{NO}_3^-\text{-N}$) were mainly converted to gas and removed, have been largely applied to wastewater treatment under aerobic conditions (Zhao et al. 2019). However, the N removal processes of HNAD could cause the loss of N and the production of greenhouse gas such as N_2O (Zhang et al. 2020a). Studies have shown that some bacteria removed inorganic N mainly by assimilating N into cells, avoiding N loss and harmful intermediate accumulation. A study showed that the *Pseudomonas putida* Y-9 was able to remove 99.36% of $\text{NH}_4^+\text{-N}$ and 82.00% of $\text{NO}_3^-\text{-N}$ and the above N sources were mainly absorbed by the bacteria through assimilation rather than lost as gas (Huang et al. 2021b). Marine bacterium *Vibrio* sp. Y1-5 were able to achieve N removal efficiency as good as HNAD through assimilation, with no risk of detrimental intermediate (Li

et al. 2017). Nonetheless, there were rare reports on the efficient assimilation in wastewater treatment. In addition, most of bacteria with N removal capability were isolated from industrial or domestic sewage, and such bacteria might not function safely and stably in the aerobic aquaculture environment with high salinity and relatively low N concentration (Su et al. 2021). In consequence, the acquisition of efficient and safe in-situ bacteria removing N by assimilation would provide a novel, reliable and environmentally friendly strategy for removal of N in wastewater and pollution control of N in aquaculture.

Microalgae with the capability of N and P removal was another common organism used in wastewater treatment (Su 2021), making up for the deficiency of inefficient P removal for most bacteria with N removal capability. Studies on microalgae-bacteria consortia revealed a mass of interactions between the two microorganisms. Material exchange was considered as one of the main interactions of microalgae-bacteria consortia: the aerobic bacteria consumed O₂, decomposed organic matter, produced CO₂ and released growth hormone, vitamins or other microalgal growth factors; the microalgae assimilated inorganic N, P and CO₂ to synthesize biomass, in addition, released O₂ and extracellular organic matter (Ferro et al. 2019, Saravanan et al. 2021). Based on the above synergistic relationship, some of microalgae-bacteria consortia as effective water purification system achieved the simultaneous removal of N and P and the production of microalgae biomass. Compared with traditional treatment using single microalgae or single bacteria, microalgae-bacteria consortia could adapt to more complex environments and achieve higher removal efficiency of N and P, as well as biomass yield (Saravanan et al. 2021). According a study about the treatment of dairy-derived liquid digestate, the removal efficiency of N, P and the growth rate of microalgae in co-culture system of *Chlorella vulgaris* and activated sludge were higher than that in single *Chlorella vulgaris* system (Feng et al. 2020). Another study showed that *Chlorella* sp. improved the removal efficiency of total phosphorus to 98.78% and had a higher biomass yield by culture with wastewater-borne bacteria (Liu et al. 2017a). Many studies like above showed that the consortiums constructed by mixing bacteria and microalgae had excellent performance of wastewater treatment, but the consortiums might be uncontrollable in actual application due to the complex biological composition (Wang et al. 2020). Therefore, relevant studies began to focus on the combinations of individual microalgae and individual strain to ensure the stability of the symbiotic consortiums, explore the mechanisms of efficient synergy and improve the benefits of wastewater treatment (Ji et al. 2018). However, there were few studies on screening specific bacteria and constructing a stable microalgae-bacteria consortium to efficient N and P removal and biomass production in wastewater, let alone reports on aquaculture wastewater with high salinity (Li et al. 2018). Filling the gaps would provide a new insight for resourceful treatment of wastewater.

In the study, the *Bacillus taeanensis* SD148BN12 isolated from aquaculture ponds and the *Chlorella* sp. were screened to construct a new microalgae-bacteria consortium that could improve N and P removal efficiency and biomass yield effectively. The biosafety and aerobic N removal capability of strain SD148BN12 were identified. The respective function and interactions of strain SD148BN12 and *Chlorella* sp. in M-B was explored and the effect of environmental factors on the N removal efficiency of M-B were assessed. The application of M-B to remove N and P in mariculture water and mariculture wastewater showed good results. The study provided a potential view for combining in-situ bacteria and microalgae to remove N, P and recover resource simultaneously from wastewater. Furthermore, it provided a reference for the environmentally-friendly treatment of aquaculture wastewater.

2. Materials And Methods

2.1 Microalgae, bacteria and media

The microalgae species *Chlorella* sp. (*Chlorella* sp. strain MACC/C95) was provided by the Laboratory of Applied Microalgae Biology, Ocean University of China, China and cultured in f/2 medium (Lananan et al. 2014). The light intensity, temperature and light/dark cycle were controlled at $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 25°C and 12 h/12 h, respectively.

In order to obtain an axenic isolate of *Chlorella* sp., 100 mg/L of each of the following antibiotics were administered: ampicillin, streptomycin, kanamycin sulfate and gentamycin sulfate. The *Chlorella* sp. was inoculated in the f/2 medium containing mixed antibiotics at a ratio of 10% for 5 days and then transferred to the f/2 medium without antibiotics for 8 days. After repeated twice, the *Chlorella* sp. culture was streaked on f/2 agar plates to obtain single microalgal strains. Then the isolates were transferred into the f/2 liquid medium without antibiotics for further culture and the *Chlorella* sp. culture was coated on LB agar plates and cultured at 28°C for one week. Non-contamination was demonstrated by the absence of bacteria on the LB agar plates. The axenic *Chlorella* sp. strains were stored in f/2 medium and sub-cultured every 14 days for all subsequent experiments.

The 36 strains with the capability of N removal were preserved by the Laboratory of Environmental Physiology of Aquatic Animals, Ocean University of China, China. These strains were respectively isolated from *Litopenaeus vannamei* ponds located in Shandong, Guangdong and Hainan Province, and stored at -80°C in 25% glycerol solution. The 36 strains cultured at C/N=10, N concentration=10 mg/L, 28°C and 160 rpm, could remove more than 50% of $\text{NH}_4\text{-N}$ in 48 h. Before experiments, the bacteria were cultured in LB medium at 28°C , 160 rpm for 24 h.

The heterotrophic nitrification medium (HNM), denitrification media (DM) and simultaneous nitrification and denitrification medium (SNDM) were used to measure the capability to N removal of strain SD48BN12. The HNM contained (per liter): glucose 0.25 g, $(\text{NH}_4)_2\text{SO}_4$ 0.0471 g, $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ 0.05 g, $\text{FePO}_4 \cdot 4\text{H}_2\text{O}$ 0.01 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.25 g. The DM based on the HNM replaced $(\text{NH}_4)_2\text{SO}_4$ with NaNO_3 0.493 g (DM-1), KNO_3 0.0722 g (DM-2), respectively. The SNDM based on the HNM increased glucose to 0.75 g and replaced $(\text{NH}_4)_2\text{SO}_4$ with 3 N sources: $(\text{NH}_4)_2\text{SO}_4$ 0.0471 g, NaNO_3 0.493 g and KNO_3 0.0722 g.

A synthetic wastewater medium (SWM) containing $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ was used to cultivate microalgae-bacteria consortiums. The SWM contained (per liter): glucose 0.25 g, NH_4Cl 0.0382 g, NaNO_2 0.0246 g, NaNO_3 0.0304 g, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 0.0101 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.25 g. In addition, 1 mL of trace metal solutions were added to 1000 mL of the above solution: $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 3.15 g/L, Na_2EDTA 4.36 g/L, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 0.18 g/L, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.022 g/L, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.01 g/L, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.0098 g/L, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.0063 g/L. All the HNM, DM, SNDM and SWM were prepared with filtered seawater (salinity $\approx 30\text{‰}$, $\text{pH} \approx 8.1$, total nitrogen $< 0.01 \text{ mg/L}$), adjusted the initial pH to 7.5, and autoclaved before using.

2.2 Screening of the strains co-cultured with *Chlorella* sp.

In the experiment, 36 strains with N removal capability were co-cultured with *Chlorella* sp.. The microalgae-bacteria consortiums with higher removal performance of N were screened by measuring the N removal efficiency of the microalgae-bacteria combinations. *Chlorella* sp. cultured for 8 days and strains cultured for 24

h were washed and resuspended with aseptic SWM, adjusted the cell density, and added into 250 mL conical flasks containing 100 mL SWM. The initial cell densities of *Chlorella* sp. and strains in SWM were 5×10^5 cell/mL and 5×10^5 CFU/mL, respectively. The culture system with only *Chlorella* sp. was applied to compare, and all the culture systems were incubated at $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 25 °C, 140 rpm, under a 12 h/12 h light/dark cycle for 8 days. The samples were taken from each flask to determine the contents of $\text{NH}_4^+\text{-N}$, NO_2^- -N and cell density periodically.

2.3 Identification, biosafety assessment and N removal performance of strain SD148BN12

The strain of *Bacillus taeanensis* SD148BN12 (Genbank accession number: MN988751.1) was selected by the co-cultivation of microalgae and bacteria for subsequent experiments (Huang et al. 2020a). Strain SD148BN12 was isolated from the water samples in mariculture ponds of *Litopenaeus vannamei* located in Dongying, Shandong Province and carried out genetic identification in the previous research for the Laboratory of Environmental Physiology of Aquatic Animals. The 16S rRNA gene sequence of strain SD148BN12 was compared with the reported gene sequences in GenBank database by Basic Local Alignment Search Tool (BLAST). A phylogenetic tree was constructed on MAGE 7.0 software with the neighbor-joining method. Morphological, physiological-biochemical characteristics and of the strain were also determined (Zhang et al. 2021a).

Strain SD148BN12 was inoculated on the agar plate with 5% goat blood (Qingdao Hope Bio-Technology Co., LTD, China), and the hemolytic activity of the strain was determined by observing the existence of the hemolytic circle. In order to measure the antibiotic sensitivity of strain SD148BN12, according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) disk diffusion method, the strain was coated on Mueller-Hinton agar plates and the antimicrobial susceptibility disk (Hangzhou microbial reagent co. LTD, Hangzhou, China) was placed on the surface of the mediums, then the diameter of the inhibition zone was determined after incubation.

After 24 h culture, 15 mL bacterial culture of strain SD148BN12 was obtained, centrifuged and removed supernatant, washed with the same amount of sterile filtered seawater, and the strains were resuspended. The bacterial suspension was severally transferred into 100 mL HNM, DM-1, DM-2, SNDM placed in 250 mL conical flasks at 1% (v/v) ratio. All the flasks were continuously oscillating cultured at 160 rpm with the temperature of 28 °C for 48 h. The samples were withdrawn every 4 h and the cell density (OD_{600}) as well as the content of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^+\text{-N}$, $\text{NO}_3^+\text{-N}$, intracellular nitrogen (Intracellular N) and total nitrogen (TN) were measured.

2.4 Evaluation of M-B for N, P removal performance

The *Chlorella* sp. cultured for 8 days and the *Bacillus taeanensis* SD148BN12 cultured for 24 h were washed and resuspended with aseptic filtered seawater, adjusted the cell density, and added into 250 mL conical flasks containing 100 mL SWM synchronously to form the M-B. Both of the *Chlorella* sp. (cell/mL) and strain SD148BN12 (CFU/mL) were at initial cell density of 5×10^5 . Incubator parameters were set as temperature of 25 °C, light intensity of $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, shaking speed of 140 rpm and light/dark ratio of 12/12h. The culture system with same amount of *Chlorella* sp.-only (M-O) and the system with same amount of strain SD148BN12-only (B-O) were used to contrast. Samples were taken to analysis every 2 days.

The following indicators were measured: the cell growth and biomass production parameters included the dry cell weight (DCW), the contents of protein, carbohydrate and lipid, the chlorophyll-a concentration and the number of viable bacteria; the photosynthetic activity parameter included the maximal photochemical efficiency (Fv/Fm); the water quality parameters included the concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$, total nitrogen (TN), total phosphorus (TP) as well as the pH value and dissolved oxygen (DO) content.

2.5 Effects of different factors on N removal efficiency of M-B

The effects of factors including temperature, salinity, light intensity, pH value, C/N ratio, N/P ratio, N content on the N removal capacity of M-B were assessed by single-factor experiment. The basic conditions of the experiment were set as follows: *Chlorella* sp. after 8 days cultivation and strain SD148BN12 after 24 h cultivation were prepared into concentrated cell suspension with aseptic SWM, and added into 100 mL SWM served in 250 mL conical flasks simultaneously with the initial density of 5×10^5 cell/mL (CFU/mL). The incubation conditions were set at 25 °C, 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 140 rpm, 12 h light/12 h dark with the salinity of 30‰, pH of 7.5, C/N ratio of 5, N/P ratio of 10, N concentration of 20 mg/L and culture cycle of 8 days. The individual factor was adjusted according to the following designs: temperature was set at 15, 20, 25, 30, 35, 40°C; salinity was set as 0‰, 10‰, 20‰, 30‰, 40‰, 50‰ (adjusted by the addition of NaCl and dilution with pure water); light intensity was set as 12.5, 25, 50, 100, 150, 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$; pH value was set as 6, 7, 7.5, 8, 8.5, 9; C/N ratio was set as 0, 1, 2.5, 5, 10, 15 (the N concentration was stayed at 20 mg/L and the glucose content was changed); N/P ratio was set as 4, 5, 7, 10, 20, 40 (the N concentration was stayed at 20 mg/L and the $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ content was changed); N concentration was set as 1, 2.5, 5, 10, 20, 50 mg/L. The M-O and B-O with the same culture conditions were used to contrast.

2.6 Real mariculture water and wastewater treatment by M-B

The mariculture water (MW-1) was taken from the intensive *Litopenaeus vannamei* culture ponds. The physicochemical characteristics of MW-1 were shown as follows: TN, 3.99 ± 0.14 mg/L; TP, 0.70 ± 0.01 mg/L; $\text{NH}_4^+\text{-N}$, 2.08 ± 0.08 mg/L; $\text{NO}_2^-\text{-N}$, 1.11 ± 0.03 mg/L; salinity, 30‰; pH, 7.78 ± 0.11 . The mariculture wastewater (MW-2) were effluents of shrimp ponds. The physicochemical characteristics of MW-2 were shown as follows: TN, 13.10 ± 0.19 mg/L; TP, 2.04 ± 0.05 mg/L; salinity, 30‰; pH, 7.81 ± 0.13 . The *Chlorella* sp. suspension (1×10^8 cell/mL) and strain SD148BN12 suspension (1×10^8 CFU/mL) were simultaneously added into conical flasks containing 100 mL MW-1 and conical flasks containing 100 mL MW-2. The inoculated cell density ratios of *Chlorella* sp. (cell/mL)-to-strain SD148BN12 (CFU/mL) were set as $10^4:10^4$, $10^5:10^5$, $10^6:10^6$. The culture systems were incubated at 25 °C, 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 140 rpm, 12 h light/12 h dark. Samples were taken from each container to measure the concentrations of N and P in the water.

2.7 Analytical methods

2.7.1 Determination of cell density

Bacterial density was determined as follows: OD₆₀₀ values were measured at 600 nm through a spectrophotometer. The McFarland turbidity of bacterial suspension was measured by bacterial nephelometer (TA-2XJ, Beijing, China). The number of viable bacteria was measured by the flat colony counting method. The microalgal density measurement used the hemocytometer method with an optical microscope.

2.7.2 Determination of biomass

The biomass yields of B-O, M-O and M-B were indicated by DCW. The samples were filtered through a dry filter of 0.22 µm pore size and dried at 105 °C to constant weight. DCW was expressed as the difference between the weight of the filter containing the sample after drying and the weight of the filter before using (Ji et al. 2018).

The biomass composition including protein, carbohydrate and lipid as well as chlorophyll-a in B-O, M-O and M-B were measured. The Biomass composition including protein, carbohydrate and lipid were extracted and quantified in accordance with the methods described by previous studies (Li et al. 2018): the protein content of the extracts was determined by Lowry's method (Lowry et al., 1951), with the bovine serum albumin as the standard, the carbohydrate content was measured by phenol sulfuric acid colorimetric method (Monsigny et al., 1988), using the glucose as the standard; the lipid content was determined by the methanol-chloroform method (Bligh and Dyer, 1959). The chlorophyll-a was extracted by heating 90% methanol according to previous studies (Wang et al. 2020), and quantified absorbance by an ultraviolet spectrophotometer (UV-2000, Shanghai, China). With 90% methanol as a control, the chlorophyll-a concentration was calculated by the formula (Wang et al. 2019, Wang et al. 2020): Chlorophyll-a (mg/L) = $16.82 \times A_{665} - 9.28 \times A_{652}$.

2.7.3 Determination of photosynthetic activity

The microalgal photosynthetic activity was indicated by Fv/Fm, which was measured by a modulated chlorophyll fluorometer (Junior-PAM, Heinz Walz GmbH, Effeltrich, Germany) and samples had a 20 min dark period in ambient conditions in the laboratory before determination (Wang et al. 2019).

2.7.4 Determination of water quality

The supernatant separated from samples after centrifuging at 4000 ×g for 15 min at 4 °C was used to measure the concentrations of NH_4^+-N , NO_2^--N , NO_3^--N , TN and TP by the standard method (Huang et al. 2017). The intracellular N content was the subtraction for TN of inoculated media and TN of inoculated media after centrifugation. The soluble organic N concentration in the solution was calculated by deleting NH_4^+-N , NO_2^--N , NO_3^--N from total soluble N at the end of cultivation (Huang et al. 2017). N or P removal efficiency was calculated as $(C_0 - C_1)/C_0 \times 100\%$ (C_0 : the initial concentration, C_1 : the final concentration). The pH value and dissolved oxygen content of solution were measured by pH tester and dissolved oxygen tester.

2.8 Statistical methods

The non-seeded media were as controls and all the experiments were repeated three times. The data was expressed as means ± standard deviations. One-way analysis of variance was used to analysis the differences among groups with SPSS 24.0. The $p < 0.05$ meant the differences were significant statistically. Statistical analysis, calculation and drawing of experimental data were performed by Excel software.

3. Results

3.1 Screening of the strain co-cultured with *Chlorella* sp.

The 36 strains were co-cultured with the *Chlorella* sp. for screening combinations that could remove N from water rapidly (Fig. S1). The microalgae-bacteria consortiums cultured for 4 days were analyzed, and 10 groups of

microalgae-bacteria consortiums more than 95% of $\text{NH}_4^+\text{-N}$ removal efficiency were screened, in which the bacteria promoted or did not inhibit the growth of the *Chlorella* sp.. Groups of M-B12, M-B35 and M-B36 had higher $\text{NO}_2^-\text{-N}$ removal efficiency and *Chlorella* sp. cell density, of which group M-B36 achieved the $\text{NO}_2^-\text{-N}$ removal efficiency of 75.36% and *Chlorella* sp. density of 2.72×10^7 cell/mL respectively. After 8 days of culture, 5 groups of microalgae-bacteria consortiums had more than 95% of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ removal efficiency and higher *Chlorella* sp. density than M-O. In group M-B36, the removal efficiency of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ were over 99%, while the *Chlorella* sp. cell density (5.52×10^7 cell/mL) was highest, and was 1.56 times as much as that of M-O. The strain SD148BN12 used in M-B36 was selected to construe a new microalgae-bacteria consortium.

3.2 Identification, biosafety assessment and N removal performance of strain SD148BN12

The strain SD148BN12 grew on LB agar plates as white, opaque, circular colonies with smooth surfaces and regular peripheries. Based on the results of the physiological and biochemical tests, strain SD148BN12 was positive for gram-staining, indole production, catalase activity, amylase activity, nitrate reductase activity, while negative for Voges-Proskauer (V-P) test, Methyl-Red (M.R) test, ammonia production test, gelatin liquefaction test and urease activity. The glucose oxidation fermentation test showed that the strain was fermentation type. The partial sequence analysis of the 16S rDNA of strain SD148BN12 revealed that the proximities of 16S rRNA between the strain SD148BN12 and *Bacillus taeanensis* strain BH030017 was 98.19%. The phylogenetic tree further confirmed the homologies of strain SD148BN12 with some other phylogenetically related strains (Fig. 1). Therefore, the strain SD148BN12 was tentatively identified as *Bacillus taeanensis* SD148BN12.

During the culture process of strain SD148BN12 on the blood agar plate, hemolysis circle was not observed, suggesting that the strain had no hemolysis activity (Fig. S2). Antibiotic susceptibility was determined to characterize the resistance of bacterial strains. Of the 21 antibiotics tested (Table 1), the inhibition zone diameter of sulfisoxazole was 0 mm, which indicated that the strain SD148BN12 was resistant to the drug. The strain showed high antagonism to streptomycin, gentamicin, erythromycin, ampicillin, bacitracin, ofloxacin, norfloxacin, ciprofloxacin, tetracycline with the inhibition zone diameter was less than 10 mm. Beyond that, the strain had a high degree of sensitivity to the other 11 antibiotics.

The N removal performance and N balance of strain SD148BN12 cultured in different N sources medium for 48 h were shown in Fig. 2 and Table 2. In the HNM, the $\text{NH}_4^+\text{-N}$ concentration in solution decreased from 10.13 to 0.85 mg/L, with nearly no accumulation of $\text{NO}_2^-\text{-N}$ and 0.30 mg/L accumulation of $\text{NO}_3^-\text{-N}$. The maximum removal rate of $\text{NH}_4^+\text{-N}$ was 1.13 mg/L/h. As well, in DM-1, the concentration of $\text{NO}_2^-\text{-N}$ decreased from 10 to 1.13 mg/L, with 0.56 mg/L accumulation of $\text{NH}_4^+\text{-N}$ and 0.53 mg/L accumulation of $\text{NO}_3^-\text{-N}$. The maximum removal rate of $\text{NO}_2^-\text{-N}$ was 0.84 mg/L/h. In DM-2, the concentration of $\text{NO}_3^-\text{-N}$ decreased from 10 to 1.15 mg/L, with nearly no accumulation of $\text{NO}_2^-\text{-N}$ and 0.79 mg/L accumulation of $\text{NH}_4^+\text{-N}$. The maximum removal rate of $\text{NO}_3^-\text{-N}$ was 0.58 mg/L/h. In SNDM, the concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ decreased from 10 to 0.59, 0.78 and 2.63 mg/L. When 3 N sources coexisted, $\text{NH}_4^+\text{-N}$ was used firstly because of its ability to bind to biomolecules directly. 63.93% of the initial N was removed from water and stored in thallus as intracellular N. According to the N balance, 63-66% of the initial N was removed from water under different N sources by strain SD148BN12, and the removed N was stored into cells, without the release of nitrogenous

gases. Hence, strain SD148BN12 was able to remove N rapidly from water through assimilating inorganic N into intracellular N.

3.3 Wastewater treatment and growth performance of M-B

3.3.1 N and P removal efficiency

The N removal performance of the *Chlorella* sp.-*Bacillus taeanensis* SD148BN12 consortium was shown in Fig. 3a and Fig. 3b. Within 8 days of culture in M-B, the concentration of TN decreased from 21.54 to 2.76 mg/L, and the concentration of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$ decreased to 0, 0.08, 0.74 mg/L, respectively, which was significantly superior to M-O and B-O ($p<0.05$). After 8 days, the N removal rates of M-B tended to stagnate and 91.88%, 99.97%, 98.46% and 91.74% of TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ were removed from the water at last, almost no $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ accumulated. In B-O, strain SD148BN12 grew faster during the first 4 days of culture, the $\text{NH}_4^+\text{-N}$ content decreased from 10.23 to 1.31 mg/L, but only 28.82% of TN was removed by strain SD148BN12. In M-O, when 3 kinds of N sources coexist, the *Chlorella* sp. also used $\text{NH}_4^+\text{-N}$ firstly, the $\text{NH}_4^+\text{-N}$ content reached the lowest at 0.084 mg/L on 6th day, but when the *Chlorella* sp. used $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ as N sources, a small amount of $\text{NH}_4^+\text{-N}$ was accumulated. At last, 75.34%, 99.76%, 98.92% and 55.45% of TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ were removed in M-O.

The removal performance of P was shown in Fig. 3c. The removal efficiency of TP in M-B was always significantly higher than the other ($p<0.05$). On 8th day, the TP concentration decreased from 2.04 to 0.19 mg/L, 94.58% of TP was removed by M-B, which the TP removal efficiency in M-B was 2.07 and 4.51 times that of B-O and M-O.

3.3.2 DO and pH levels

The dissolved oxygen (DO) concentration in both M-B and B-O decreased rapidly on 2 day (Fig. 3d). With the photosynthesis of *Chlorella* sp., the concentration of DO in M-B gradually increased from 2.56 mg/L to 5.19 mg/L. In M-O, the DO concentration was always higher than the initial value, and the maximum DO concentration reached 8.30 mg/L on 8 days. The DO content of M-B was between that of M-O and B-O, changing the low DO level in B-O.

The pH values of the three conditions all increased during the culture process (Fig. 3e). The pH value of B-O had a small variation range and lower than 7.95. The pH value of M-O continued to rise from 7.50 to 9.51, while the pH value of M-B increased to 8.98 eventually. The presence of strain SD148BN12 changed the high pH in M-O.

3.3.3 Cell growth and biomass accumulation

The results measured chlorophyll-a concentration and Fv/Fm indicated that the co-culture of *Chlorella* sp. and strain SD148BN12 promoted the growth and photosynthetic activity of *Chlorella* sp. (Fig. 4a and Fig. 4b). After 8 days of culture, the chlorophyll-a concentration of M-B reached 4.24 mg/L and was 1.62 times that of M-O. The Fv/Fm in M-B increased to 0.74 and then decreased, which was always higher than that of M-O.

With the enhancement of photosynthetic activity for *Chlorella* sp. cells in M-B, the capacity of biomass production the consortium was increased significantly. On 8 days, the dry cell weight (DCW) of M-B reached

0.52 g/L, higher than the sum of M-O (0.23 g/L) and B-O (0.10 g/L), which remained stable over the next 6 days (Fig. 3a). The DCW continued to increase in M-O, while that gradually decreased after reaching the peak on 4 days in B-O. The number of viable bacteria in B-O increased rapidly, reaching a peak of 4.53×10^7 CFU/mL on the 4th day, and then decreased to 2.31×10^7 CFU/mL continuously (Fig. 4c). In contrast, the viable bacteria number had a more gradual upward or downward trend in M-B, and the maximum viable bacteria number (4.16×10^7 CFU/mL) appeared on the 6th day and remained higher than that of B-O thereafter.

The protein, carbohydrate and lipid contents of microorganism in the N removal process were shown in Fig. 5. After culture, the protein content in M-B increased to 23.32% of DCW, which was slightly higher than that M-O ($p > 0.05$). The carbohydrate and lipid contents increased to 19.31% and 36.06% of DCW in M-B, improving by 27.72% and 39.88% respectively compared with those in M-O.

3.4 N removal performance of M-B under different environmental factors

M-B could remove more than 70% of TN, 99% of NH_4^+ -N and 98% of NO_2^- -N at the temperature of 20-30 °C, salinity of 20-40‰, light intensity of 50-200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, pH of 6-9, C/N ratio of 1-10, N/P ratio of 4-20, N concentration of 1-50 mg/L. TN removal efficiency in M-B was higher than M-O and B-O under following environmental factors (Table S1).

Temperature. As shown in Fig. 6a, at 20-30 °C, NH_4^+ -N-RE (NH_4^+ -N-RE) and NO_2^- -N removal efficiency (NO_2^- -N-RE) of M-B exceeded 99% and TN removal efficiency (TNRE) and DCW went up as the temperature, with no significant difference ($p > 0.05$). At 15 and 40 °C, N removal and biomass production performance of M-B were retarded ($p < 0.05$).

Salinity. As shown in Fig. 6b, at high salinity (20-40‰), NH_4^+ -N-RE and NO_2^- -N-RE surpassed 99%. At 30‰, TNRE and DCW were higher than other salinities. The lower salinity (0-10‰) was not conducive to N removal, and N removal and biomass production performance fortified as the salinity within the range. As the salinity was 0, M-B was significantly inhibited and the TNRE was reduced to 13.39% ($p < 0.05$).

pH. As shown in Fig. 6c, at pH of 7-9, TNRE, NH_4^+ -N-RE and NO_2^- -N-RE of M-B exceeded 85%, 99% and 98%, respectively, with no significant difference ($p > 0.05$). At pH of 6, the TNRE decreased significantly but still reached 80.73% ($p < 0.05$).

Light intensity. As shown in Fig. 6d, when the light intensity was 50-200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, there were no significant differences among the treatment groups ($p > 0.05$). TNRE, NH_4^+ -N-RE, NO_2^- -N-RE and DCW maintained at high levels, over 88%, 99.5%, 99.5% and 0.52 g/L respectively. Below 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, higher light intensity improved the DCW and N removal efficiency of M-B ($p < 0.05$).

C/N ratio. As shown in Fig. 6e, when C/N ratio was 2.5-10 mg/L, there were no significant differences in biomass production and N removal among different treatment groups ($p > 0.05$), with the highest N removal efficiency and DCW at 5. Higher (15) and lower (0.1) C/N ratios inhibited DCW and TNRE ($p < 0.05$).

N/P ratio. As shown in Fig. 6f, When N/P ratio was at 4-20, high N removal efficiency and DCW could be achieved by M-B. TNRE and DCW reached the highest at the N/P ratio of 7. When N/P ratio was at 40, the N removal capacity was significantly reduced ($p<0.05$).

N concentration. As shown in Fig. 6g, when the N concentration was 1-10 mg/L, the TNRE was more than 92%, 99%, 99%, respectively. When the N concentration increased to 20 mg/L, the TNRE decreased to 86.43%. When the N concentration increased to 50 mg/L, the TNRE decreased to 75.33%, and the NH_4^+ -N-RE and NO_2^- -N-RE were still close to 100%. At the N concentration of 1-50 mg/L, DCW of M-B increased significantly with the rise of N concentration ($p<0.05$).

3.5 N and P removal of M-B in real mariculture water and wastewater

The MW-1 treatment performance by M-B was shown in the Fig. 7a, 7b, 7c and 7d. After 14-day continuous treatment, NH_4^+ -N concentration were decreased from 2.01 to 0.02, 0, 0 mg/L, and NO_2^- -N were reduced from 1.11 to 0.75, 0.01, 0 mg/L when the inoculum cell density of the *Chlorella* sp. (cell/mL) / strain SD148BN12 (CFU/mL) were $10^4:10^4$, $10^5:10^5$, $10^6:10^6$ (group 10^4 , 10^5 , 10^6), respectively. Meanwhile, in the group of 10^4 , 10^5 , 10^6 , there were 67.11%, 87.06%, 89.83% of TN and 59.38%, 70.98%, 79.03% of TP removed from water. The increase of initial inoculation density was beneficial to improve the N and P removal efficiency. The MW-2 treatment performance by M-B was shown in the Fig. 7e and 7f. After treatment, TN in group 10^4 , 10^5 , 10^6 were decreased from 13.1 to 1.50, 1.37, 1.22 mg/L, while TP were reduced from 2.04 to 0.16, 0.10, 0.11 mg/L. The higher the initial cell density, the higher the removal rates of N and P, but different treatment groups could achieve similar removal efficiency after 14-day treatment.

4. Discussion

In order to optimize the removal efficiency of N and P in wastewater treatment and control the N, P pollution caused by intensive aquaculture, 36 strains isolated from aquaculture environments were used to co-culture with *Chlorella* sp.. A strain, *Bacillus taeanensis* SD148BN12 with synergistic effect with *Chlorella* sp. on N removal was screened and the *Chlorella* sp.-*Bacillus taeanensis* SD148BN12 symbiotic consortium was constructed. M-B had excellent N, P removal performance and biomass accumulation under aerobic conditions. The study provided a promising microalgae-bacteria consortium for synchronous removal of N and P from wastewater, which could solve the N pollution effectively and make up for the vacancy of efficient biological P removal process in the aquaculture wastewater.

4.1 Biosafety of strain SD148BN12

Biosafety was important for the application of bacteria used in aquatic environment. In this study, strain SD148BN12 had no hemolytic activity on the blood agar plate, which indicated it had no hidden danger of producing hemolysin to cause membrane damage and cell cytolysis (Zhang et al. 2021a). In the test of antibiotic sensitivity, strain SD148BN12 had high sensitivity to most antibiotics, but was highly resistant to some quinolone, sulfonamide, tetracycline, aminoglycoside, macrolide antibiotics and bacitracin. It had been reported that above antibiotics were often detected in aquaculture systems (Gao et al. 2012, Liu et al. 2017b), and the long-term pressure from the antibiotics might cause strain SD148BN12 to carry the resistance genes

(Shahi et al. 2018). At present, most of the bacteria used in wastewater treatment had the indeterminate safety (Zhang et al. 2020b), and the evaluation of the hemolytic activity and antibiotic sensitivity of strain SD148BN12 preliminarily ensured non-pathogenicity and application feasibility of strain SD148BN12 in water environment.

4.2 N and P removal performance of M-B

In SWM with the C/N ratio of 5, M-B removed 87.19% of TN and 90.95% of TP within 8 days. Compared with the results of a *Microcystis aeruginosa*-*Bacillus licheniformis* consortium cultured in synthetic wastewater (Ji et al. 2018), M-B achieved similar N removal amount and higher P removal amount. In B-O, the maximum removal efficiency of strain SD148BN12 for TN and inorganic N were only 28.82% and 59.75%. However, in HNM, DM and SNDM with the C/N ratio of 10, the removal efficiency of TN and inorganic N were higher than 63% and 88% within 48 h respectively, which was comparable to the current reported denitrifier isolated from aquaculture systems (Yun et al. 2019, Zhao et al. 2019). Correspondingly, the N removed from water was assimilated into intracellular N, avoiding N loss and noxious gases of N_xO . According to the reports, the C/N ratio determining the energy supply played an important role for the N removal capability of bacteria (Hou et al. 2021, Su et al. 2021). The low C/N ratio in SWM might be the main reason to limit the N removal efficiency of strain SD148BN12 (Table S1), implying the removal of TN mainly depended on the *Chlorella* sp. in M-B. On the 8th day, the TN removal efficiency in M-B was higher than the summation of that in B-O (25.50%) and M-O (48.69%), suggesting that the interactions between strain SD148BN12 and the *Chlorella* sp. had positive effect on N removal efficiency of M-B. Likewise, the TP removal efficiency in M-B was higher than the summation of that in B-O (20.16%) and M-O (43.83%) on the 8th day. Due to the slightly P removal efficiency of strain SD148BN12, the P removal in M-B mainly relied on the uptake capacity of the *Chlorella* sp.. The similar researches agreed that bacteria growing much faster than microalgae played a major role in N and P removal in the early stage of experiment, but the contribution of bacteria to N, P removal was limited due to the finite nutrient (especially organic carbon) (Li et al. 2018, Liu et al. 2017a). The higher removal efficiency of N and P in M-B was primarily attributed to the conspicuous capability of N, P absorption for the *Chlorella* sp. enhancing by strain SD148BN12 (Liu et al. 2017a). In a study about co-culture of *Chlorella vulgaris* and *Bacillus licheniformis*, the presence of *Bacillus* increased the N removal efficiency of *Chlorella* from 29–78% (Liang et al. 2013), and another study combining Effective Microorganism (EM-1) and *Chlorella* sp. to treat aquaculture wastewater showed that the addition of EM-1 increased the TP removal efficiency of microalgae *Chlorella* sp. from 49.73–99.15% (Lananan et al. 2014), which proved the superiority for improving the N, P removal efficiency by co-culture of microalgae and bacteria. The co-culture mode of M-B provided a new approach to solve the common problems of the N, P control in wastewater treatment, such as: the removal capacity of bacteria was limited by the low C/N ratio, bacteria with efficient P removal performance were difficult to isolated (Zhang et al. 2020b), single microalgae systems needed relatively long treatment cycles and released excessive alkali resulting in high pH in effluent (Li et al. 2018, Peng et al. 2021). This study expanded the application prospect of the *Chlorella* sp. and strain SD148BN12, as well, it enriched the rare information on the efficient removal of N and P from saline wastewater.

4.3 Biomass production performance of M-B

Biomass analysis showed the *Chlorella* sp. were the main providers of DCW in M-B. After 8 days of culture, the DCW of M-B was higher than the sum of that of M-O and B-O, suggesting that co-culture with bacteria might improve the biomass yield of the *Chlorella* sp. (Liu et al. 2017a). In M-O, the contents of protein, carbohydrate and lipid in biomass were consistent with those of ranges of reported *Chlorella* species (Cao et al. 2020,

Guccione et al.,2014). Compared with M-O, M-B maintained reliable contents of protein and carbohydrate, furthermore, M-B increased the content of lipid greatly. This lipid content (36.06% of DCW) was on the high level for the reported lipid proportions of *Chlorella* species and microalgae-bacteria consortia (Guccione et al.,2014, Li et al. 2018, Rasouli et al. 2018). Therefore, M-B would provide valuable biomass by recycling N, P from wastewater and optimizing the biomass composition. Currently, emerging biotechnology called for coupling to wastewater treatment and biomass production and using resources in wastewater to produce high-value chemicals, animal feed and biofuels through microalgae-bacteria consortia (Ramanan et al. 2016, Saravanan et al. 2021). This research provided promising biomaterials for the sustainable production and development.

4.4 Characterization of interaction between microalgae and bacteria in M-B

The synergistic interactions between *Chlorella* sp. and strain SD148BN12 provided support for the efficient operation of M-B. Higher Fv/Fm showed higher photosynthetic activity and lower environmental stress of the *Chlorella* sp. under M-B (He et al. 2015). On the 8th day, the chlorophyll-a concentration and the cell density of *Chlorella* sp. in M-B were higher than that in M-O. These phenomena indicated that strain SD148BN12 had a positive effect on the photosynthetic activity of *Chlorella* sp., which enhanced the growth of *Chlorella* sp. and the removal of N, P. The dependence of CO₂/O₂ between microalgae and aerobic bacteria was considered to be the basic interaction for microalgae-bacteria consortia in wastewater treatment (Wang et al. 2020). In this study, differences of DO in M-O, B-O and M-B possibly implied that O₂ released by *Chlorella* sp. was provided for strain SD148BN12 (Lananan et al. 2014). In return, strain SD148BN12 would stimulate the *Chlorella* sp. activity by release CO₂ produced by respiration (Ferro et al. 2019). Furthermore, at later stage of the experiment, the live bacteria continued to decline in both M-B and B-O due to depletion of nutrients (especially the organic carbon) in SWM, but the number of viable bacteria in M-B was still higher than that in the B-O. It might be put down to the release of nutrients (such as dissolved organic carbon) by microalgae metabolism that allow more bacteria to survive. (Natrah et al. 2014, Peng et al. 2021). To sum up, the *Chlorella* sp. and strain SD148BN12 mainly showed cooperative interactions in cell growth, photosynthetic activity and material exchange, which was beneficial for M-B to remain efficient and stable in wastewater treatment.

4.5 Evaluation of environmental adaptability of M-B

The single-factor experiment demonstrated the superior adaptability of M-B to the changes of physical and chemical factors of water. When environmental parameters changed, different from the large fluctuation of removal performance in B-O and M-O, M-B could maintain a stable high N removal efficiency in a wider range of environmental parameters (Table S1). It was worth mentioning that M-B relieved the inhibition for single microalgae and single bacteria cultivation of N removal efficiency caused by the change of carbon, N and P contents and pH value in water. However, the ability of M-B to deal with the impacts of high temperature (40 °C), low temperature (15 °C) and low salinity (0-5‰) on N removal was still weak. It had been proved that temperature and salinity were the key factors affecting biological removal efficiency. In many similar studies, higher or lower temperature could decrease microbial enzyme activity and the lack of essential ions might inhibit microbial growth gravely (Su et al. 2021, Zhang et al. 2021b). According to studies, in most of mariculture environments, temperatures were higher than 20 °C (Chang et al. 2020, Kumlu et al. 2010), C/N ratios were lower than 5 (Huang et al. 2020b), N/P ratios were generally lower than 20 (Chang et al. 2020, Han et al. 2020, Hoang et al. 2020, Xu et al. 2019, Yang et al. 2017), pH values were at 7-9 (Su et al. 2021), as well as

the N concentrations were generally lower than 20 mg/L in pond water and effluent (Huang et al. 2021a). According to the results of this study, M-B could remove N considerably from medium and high salinity aquaculture water and wastewater, and almost completely eliminate $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ which were toxic to aquatic animals. It was reported that the N concentrations were close to 50 mg/L in high strength mariculture wastewater (Zhang et al. 2021b) and saline domestic sewage (Salmanikhas et al. 2016, Wu et al. 2017), according with the range of efficient N removal for M-B, which suggested that M-B also had potential application value in more types of wastewater treatment.

4.6 Application of M-B in real wastewater treatment

Different from synthetic media, the real wastewater had more complex chemical and biological composition, which might practically affect the survival and N and P removal performance of M-B (Zhang et al. 2021a). There was little reduction of N and P in the mariculture water and wastewater non-inoculated M-B, while there was a significant removal performance in mariculture water and wastewater inoculated the consortium, suggesting the inoculated M-B, rather than indigenous microorganism, played the major role in actual treatment. Studies proved that increasing the inoculum density in a certain range could improve the removal capacity of microorganisms (Li et al. 2018, Zhang et al. 2020b). In the study, the higher inoculation density of M-B was beneficial to establish a stable combination rapidly under different environmental conditions and improve the removal rate and efficiency of N and P. By comparison with some studies on aquaculture wastewater treatment by microalgae-bacteria consortiums, the consorts achieved the similar removal efficiency of N and P with lower light energy (Han et al. 2020). The results showed that M-B could remove N and P efficiently in the medium of real wastewater, which provided strong support for practical engineering applications.

5. Conclusion

A novel microalgae-bacteria symbiotic consortium of with higher N, P removal efficiency and biomass production was constructed by combining *Chlorella* sp. strain MACC/C95 and *Bacillus taeanensis* SD148BN12. The aerobic N removal capability by assimilation and biosafety of the strain SD148BN12 were identified to ensure its safe and eco-friendly application in aquatic environments. M-B had wide adaptation to environmental change and showed substantial removal performance of N and P in marine wastewater. The main interactions between the *Chlorella* sp. and strain SD148BN12 for N, P removal and cell growth were synergetic during cultivation. The study provided a reliable microalgae-bacteria consortium for the stable, efficient and resourceful treatment of wastewater, and enriched the research about removal process of N, P in aquaculture wastewater.

Declarations

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Author contributions

Liping Liu: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. Mengyu Zhang: Investigation, Formal analysis. Yun Li: Supervision, Conceptualization, Writing - review & editing. Luqing

Pan: Investigation, Formal analysis. Le Dou: Investigation. Zhaopeng Su: Investigation.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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All data generated or analyzed during this study are included in this published article.

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Tables

Table 1 Antibiotic susceptibility of *Bacillus taeanensis* SD148BN12 to different antibiotics.

Antibiotic class		Antibiotic content per disk	Inhibition zone diameter (mm)
Aminoglycosides	Streptomycin	10 µg	+
	Neomycin	30 µg	+++
	Gentamicin	10 µg	+
Amphenicols	Chloramphenicol	30 µg	+++
	Florfenicol	30 µg	++++
Cephalosporins	Cefotaxime	30 µg	++++
	Cefoperazone	75 µg	+++++
Furanes	Furazolidone	100 µg	+++++
Macrolides	Erythromycin	15 µg	+
	Roxithromycin	15 µg	+++
Penicillins	Penicillin	10 U	+++
	Ampicillin	10 µg	+
	Augmentin	20/10 µg	++++
Polypeptides	Bacitracin	0.04 U	+
Quinolones	Ofloxacin	5 µg	+
	Norfloxacin	10 µg	+
	Ciprofloxacin	5 µg	+
Sulfonamides	Sulfisoxazole	300 µg	-
Tetracyclines	Tetracycline	30 µg	+
	Doxycycline	30 µg	+++
Others	Fosfomycin	200 µg	++

-: Inhibition zone diameter of 0 mm;

+: Inhibition zone diameter between 10-15 mm, ++: Inhibition zone diameter between 16-20 mm;

+++: Inhibition zone diameter between 21-25 mm, ++++: Inhibition zone diameter between 26-30 mm;

+++++: Inhibition zone diameter between 31-35 mm.

Table 2 Nitrogen balance of *Bacillus taeanaensis* SD148BN12 under different nitrogen sources.

Media	Initial nitrogen concentration (mg/L)	Final nitrogen concentration (mg/L)					Lost nitrogen (%)
		NH ₄ ⁺ -N	NO ₂ ⁻ -N	NO ₃ ⁻ -N	Organic N	Intracellular N	
HNM	10.13±0.05	0.85±0.01	0.03±0.006	0.30±0.001	2.24±0.18	6.71±0.19	-
DM-1	10.19±0.03	0.56±0.01	1.13±0.04	0.53±0.07	2.03±0.14	5.95±0.10	-
DM-2	10.02±0.14	0.79±0.008	0.01±0.00	1.15±0.03	1.73±0.15	6.34±0.14	-
SNDM	30.05±0.08	0.59±0.03	0.78±0.06	2.63±0.18	1.84±0.20	19.21±0.13	-

Lost N = (Initial N – Final N)/Initial N × 100%.

Values were expressed as average ± standard deviation

Figures

Figure 1

Phylogenetic tree based on the partial 16S rRNA gene sequences of *Bacillus taeanensis* SD148BN12 using neighbor-joining method with bootstrap values of 1000 replications.

Figure 2

Nitrogen removal performance and cell growth of *Bacillus taeanensis* SD148BN12 under different nitrogen sources: (a) $\text{NH}_4^+\text{-N}$ as the single nitrogen source; (b) $\text{NO}_2^-\text{-N}$ as the single nitrogen source; (c) $\text{NO}_3^-\text{-N}$ as the single nitrogen source; (d) $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$ as mixed nitrogen source.

Figure 3

Nitrogen and phosphorus removal performance, biomass accumulation, and other water quality parameters of the *Chlorella* sp.-only (M-O), the *Bacillus taeanensis* SD148BN12-only (B-O), the *Chlorella* sp.-*Bacillus taeanensis* SD148BN12 consortium (M-B) in synthetic wastewater medium: (a) content of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$ and dry cell weight (DCW); (b) content of TN; (c) content of TP; (d) content of dissolved oxygen; (e) pH value.

Figure 4

Microorganism growth and photosynthetic activity of the *Chlorella* sp.-only (M-O), the *Bacillus taeanensis* SD148BN12-only (B-O), the *Chlorella* sp.-*Bacillus taeanensis* SD148BN12 consortium (M-B) in synthetic wastewater medium: (a) content of Chlorophyll-a; (b) maximal photochemical efficiency (Fv/Fm); (c) number of viable bacteria.

Figure 5

Biomass composition contents of the *Chlorella* sp.-only (M-O), the *Bacillus taeanensis* SD148BN12-only (B-O), the *Chlorella* sp.-*Bacillus taeanensis* SD148BN12 consortium (M-B) in synthetic wastewater medium after 14 days of cultivation.

Figure 6

TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ removal efficiency and dry cell weight (DCW) of the *Chlorella* sp.-*Bacillus taeanensis* SD148BN12 consortium (M-B) under different culture conditions after 8 days of cultivation: (a) temperature; (b) salinity; (c) pH; (d) light intensity; (e) C/N ratio; (f) N/P ratio; (g) N concentration.

Figure 7

The removal performance of (a) $\text{NH}_4^+\text{-N}$, (b) $\text{NO}_2^-\text{-N}$, (c) TN, (d) TP in mariculture water and (e) TN, (f) TP in mariculture wastewater with the inoculated density ratios of *Chlorella* sp. (cell/mL)-to- *Bacillus taeanensis* SD148BN12 (CFU/mL) as $10^4:10^4$ (10^4), $10^5:10^5$ (10^5), $10^6:10^6$ (10^6) and 0 (control).

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