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Replacement of feed by fresh microalgae as a novel technology to alleviate water deterioration in aquaculture†

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The main aim of this work was to evaluate the feasibility of microalgae-assisted aquaculture and explore the relevant mechanisms. In this regard, our work explored the pollution problems in traditional aquaculture and studied the contribution of microalgae to eutrophication control, oxygen gas production and feed replacement. Besides, potential protection mechanisms of microalgae-assisted aquaculture were studied by bacterial community profile analysis and microscope observation. The results showed that microalgae performed well in nutrient assimilation and oxygen production, thus slowing down the eutrophication and preventing oxygen depletion in aquaculture. Study of the mechanisms revealed that microalgae-assisted aquaculture contained much fewer pathogens and a microalgal biofilm was formed to prevent the eutrophication caused by sludge degradation. It is expected that the findings in this work can support the further development of microalgae-assisted aquaculture and promote the industry upgrade.

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1 Introduction

Intensive aquaculture, in recent years, has developed all over the world, bringing with it a whole new set of environmental problems, particularly an increasing production of nutrient-rich wastewater.[1,2] Boopathy *et al.* (2007) reported that concentrations of total nitrogen (TN), total ammonia nitrogen (TAN) and chemical oxygen demand (COD) in aquaculture effluent could reach 395 mg L^{-1}, 101 mg L^{-1}, and 1201 mg L^{-1}, respectively, exceeding the limits of wastewater discharge standard.[3] According to the statistics of FAO, from 2000 to 2017, aquaculture production increased by 160.26% and aquaculture value increased by 373.48%. In the foreseeable future, aquaculture will undergo a tremendous growth, further increasing the risks of water pollution and threatening the ecological balance. Aquaculture with high stocking density is regarded as a main way to increase profitability,[4] but a large amount of wastewater is produced. Hence, the pollution caused by aquaculture with high stocking density merits more attention from researchers.

Most of previous studies focused on the post-treatment of effluent from aquaculture system by traditional wastewater remediation technologies, such as aerobic digestion, anaerobic fermentation, and flocculation.[5–7] The wastewater, however, is treated at the expense of high energy consumption and generation of secondary pollutants. For instance, according to the study of McCarty *et al.* (2011), the energy needs for wastewater treatment by aerobic digestion and anaerobic fermentation is about 0.6 kW h m^{-3}.[7] In addition, by traditional technologies, most nutrients in aquaculture wastewater are converted to greenhouse gas (CO_2 and CH_4) or sludge, which accelerate greenhouse effect or cause secondary pollution.[3,8] Hence, it is important to find out an eco-friendly way to control the pollution caused by aquaculture with high stocking density.

Microalgae, which perform well in nutrients assimilation and biomass accumulation, have been intensively studied in nutrients recovery from wastewater. Previous studies successfully employed microalgae in the treatment of various sources of wastewater in both lab-scale and pilot-scale experiments.[9,10] In a real-world application, it might be an eco-friendly way to culture microalgae for nutrients recovery from aquaculture wastewater and produce value-added biomass as fish feed. The value-added components, such as polyunsaturated fatty acids, astaxanthin, and carotene, in microalgae can improve the fish immunity. For example, immune response could be enhanced by using marine microalgae as alternative ingredients to dietary fish meal, suggesting an effective role of microalgal components as immunostimulant ingredients.[11] Recently, a novel concept of adding fresh microalgae into aquaculture system as an *in situ* method for wastewater treatment, oxygen gas generation, and aquaculture feed production was proposed.[2] Theoretically, this new concept has four main advantages: (1) *in situ* treatment of wastewater by microalgae-based nutrients removal to delay the water deterioration; (2) generation of oxygen gas to

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reduce the energy consumption of aeration; (3) yield of biomass to partly replace traditional aquaculture feed and lower the total cost of aquaculture; (4) exploitation of value-added components to improve fish immunity and prohibit the antibiotics abuse.[12,13] However, to our knowledge, few studies have provided experimental data to evaluate the feasibility of this novel concept.

This work focusing on the contribution of microalgae in aquaculture system to water quality control will provide reasonable answers to three questions: (1) What are the specific water pollution problems with the increase of stocking density in aquaculture? (2) What are the contributions of microalgae to the management of aquaculture with high stocking density? (3) In this novel aquaculture system, what are the protection mechanisms of microalgae in water quality control? The results of this work will not only prove the feasibility of this novel concept, but also solve the technical problems of aquaculture with high stocking density, promoting the sustainable development of microalgae-based eco-friendly aquaculture.

2 Materials and methods

2.1. Materials

Chlorella sp. used in this work for biomass production and water quality control was preserve in TAP medium, of which the components have been documented in our previous work.[14] Microalgae biomass was produced in a 250 mL flask filled with 100 mL medium, under continuous light (120 μmol photons per m^2 per s) at room temperature (25 ± 1 °C). The inoculum size of microalgae in flask was set as 0.25 g L^{-1}.

The larvae of bighead carp (length: 2.60 cm; weight: 0.49 g), a widely commercialized Asian carp, were cultured in glass containers at room temperature for experiment and aquaculture feed used in this work was obtained from fish farm (Nanchang, China). To support the microalgal photosynthesis in aquaculture system, 12 h light (80 μmol photons per m^2 per s) was provided for fish rearing each day.

2.2. Experimental design

This work, aiming at employing microalgae for water quality control in aquaculture with high stocking density and explaining relevant mechanisms, was carried out in three steps: (1) based on the changes of biochemical properties in conventional aquaculture, problems to fish rearing were studied; (2) effects of microalgae addition on water quality of aquaculture system were assessed and nutrients profile of algae grown in aquaculture system was analyzed; (3) bacterial community analysis and microscopic observation were conducted to reveal the potential mechanisms in micro-environment and explain the contribution of microalgae to water quality control.

All the experiments and tests in this work were performed in triplicate. The results were expressed as average value ± standard deviation.

2.3. Parameters measurement

2.3.1. Water quality measurement. Concentrations (mg L^{-1}) of chemical oxygen demand (COD), total nitrogen (TN), and total ammonia nitrogen (TAN), which reflect the eutrophication degree of aquaculture water body, were measured by using assay kits purchased from Hach (USA).[15]

Dissolved oxygen (DO) content and pH value were measured by using DO analyzer (Hach, USA) and pH analyzer (Sartorius, Germany).

2.3.2. Observation of micro-structure. Sludge, mainly consisting of feces and feed residues, in aquaculture was collected and subjected to vacuum dryer. Micro-structures of sludge were observed by using both optical microscope (OM) (Jiangnan, China) and scanning electron microscope (SEM) (Hitachi, Japan).

2.3.3. Bacterial community analysis. Aquaculture wastes collected from water body were subjected to total genomic DNA extraction, 16S rRNA gene amplicons, and subsequent sequencing at the High-throughput Sequencing Service Facility of Magigene Co., Ltd. (Guangzhou, China). The sequence reads were then clustered into operational taxonomic units (OTUs).

2.3.4. Analysis of biomass composition. Microalgae biomass was collected by centrifugation at 8000 rpm and dehydrated by vacuum dryer. Protein content and lipid content of biomass were measured according to our previous work.[14] Polyunsaturated fatty acids (PUFA) were quantified by using gas chromatography-mass spectrometry (GC-MS).[9] Pigments, including carotene and chlorophyll, were quantified according to the published method.[16,17]

2.4. Comparison of aquaculture systems

2.4.1. Water deterioration in aquaculture. Water deterioration of conventional aquaculture with fish cultured at different stocking densities (2, 4, 6, 8, 10 fish/100 mL) was studied by measuring water quality in 6 days. Daily feed input was set as 0.0025 g per fish and the time interval between feed addition and water quality measurement was 12 hour. Acute and chronic toxicities to fish were identified according to water deterioration.

In microalgae-assisted aquaculture with same stocking densities, fresh microalgae were added at the same amount to totally replace traditional aquaculture feed. The system was operated under continuous light at 120 μmol photons per m^2 per s. Water deterioration in microalgae-assisted aquaculture was measured to study the contribution of microalgae to the alleviation of acute and chronic toxicities.

2.4.2. Effects of microalgae addition on aquaculture. Fresh microalgae were added into aquaculture system at 0.0025 g per fish to replace traditional feed. The concentration of microalgae was calculated by measuring the total volatile suspended solids (TVSS) according to previous publication.[14] Eutrophication, oxygen depletion, and pH fluctuation of water sample were studied daily to evaluate the performance of microalgae-assisted aquaculture.

Contribution of microalgae to oxygen production and pH change in aquaculture with 8 fish/100 mL was studied by adjusting the daily addition amounts (0.0010, 0.0025, and 0.0050 g per fish) of microalgae. DO contents and pH values in water body were measured daily.

2.4.3. Study of the potential mechanisms. Sludge, which are the main eutrophication source in aquaculture, collected from both aquaculture systems were subjected to microscopic observation. Bacterial community profiles of sludge collected from traditional aquaculture system (Sample 1) and microalgae-assisted aquaculture system (Sample 2) were identified based on high-throughput sequencing analysis. Thus, potential mechanisms were proposed to explain the roles of microalgae in the micro-environment of aquaculture system.

3 Results

3.1. Problems in conventional aquaculture

3.1.1. Eutrophication. As shown in Fig. 1, during 6 days, the concentrations of COD, TN, and TAN increased by 147 mg L^{-1}, 35.3 mg L^{-1}, and 28.6 mg L^{-1}, respectively, in aquaculture with a stocking density of 6 fish/100 mL. Similar trends were also observed in aquaculture with other stocking densities, suggesting that eutrophication became a serious problem in the aquaculture system gradually with the extension of culture time. In addition to culture time, stocking density is another factor that impacts the eutrophication in aquaculture. For example, concentration of COD in low-stocking aquaculture (2 fish/100 mL) increased by 85.0 mg L^{-1} in 6 days while that in high-stocking aquaculture (10 fish/100 mL) increased by 342.5 mg L^{-1}. According to the study of Lu *et al.* (2019), aquaculture eutrophication is mainly attributed to the accumulation of feed residues and fish feces.[2] Therefore, in this work, with the increase of stocking density, higher addition content of feed residuals and faster accumulation of feces dramatically accelerated eutrophication.

Among the water quality parameters, TAN is one of the detrimental factors threatening fish survival. Bhakta (2006) reported that under certain conditions, the semi-lethal concentration of TAN to carp in 96 h was 20 mg L^{-1}, which can be regarded as a threshold for fish culture.[18] In this work, with the accumulation of organics in water body over 4 days, concentration of TAN exceeded this threshold, threatening the survival of fish and disturbing the ecological balance in aquaculture. Since the high concentration of TAN was mainly observed after 4 day culture, ammonia toxicity can be considered as a toxicity in aquaculture.

3.1.2. Oxygen depletion and pH fluctuation. Fig. 1(d) showed that the DO contents dropped by 52.85%, 93.39%, 98.08%, respectively, when the stocking densities were 6, 8 and 10 fish/100 mL in 6 days. The fast depletion of oxygen was observed in aquaculture with 8 and 10 fish/100 mL, where DO contents decreased to 1.12 mg L^{-1} and 0.36 mg L^{-1} after 2 day culture. Owning to the negative effects of low DO contents on fish behavior and metabolism, oxygen depletion is a serious problem that should be addressed in the aquaculture with high stocking density.

In aquaculture, unionized ammonia (NH_3) is highly toxic, whereas the ionized form (NH_4^+) is nontoxic or less toxic and

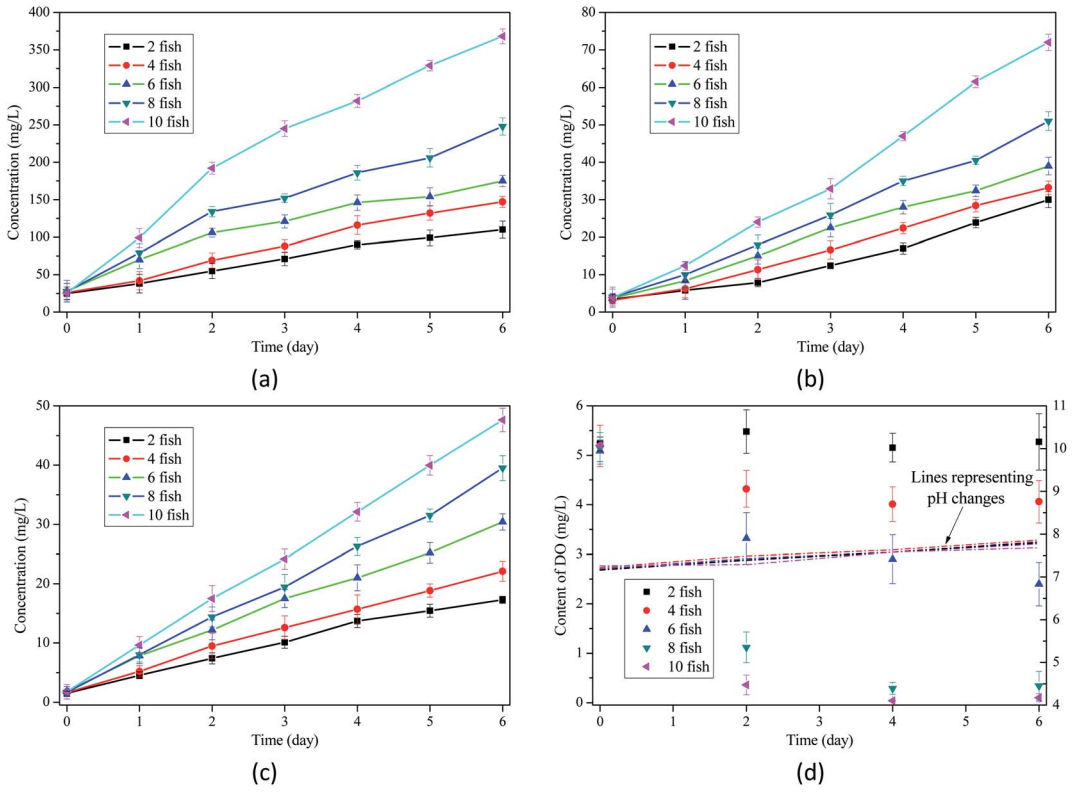

Fig. 1 Changes of water quality in conventional aquaculture: (a) concentration of COD; (b) concentration of TN; (c) concentration of NH_3–N; (d) values of pH and DO.

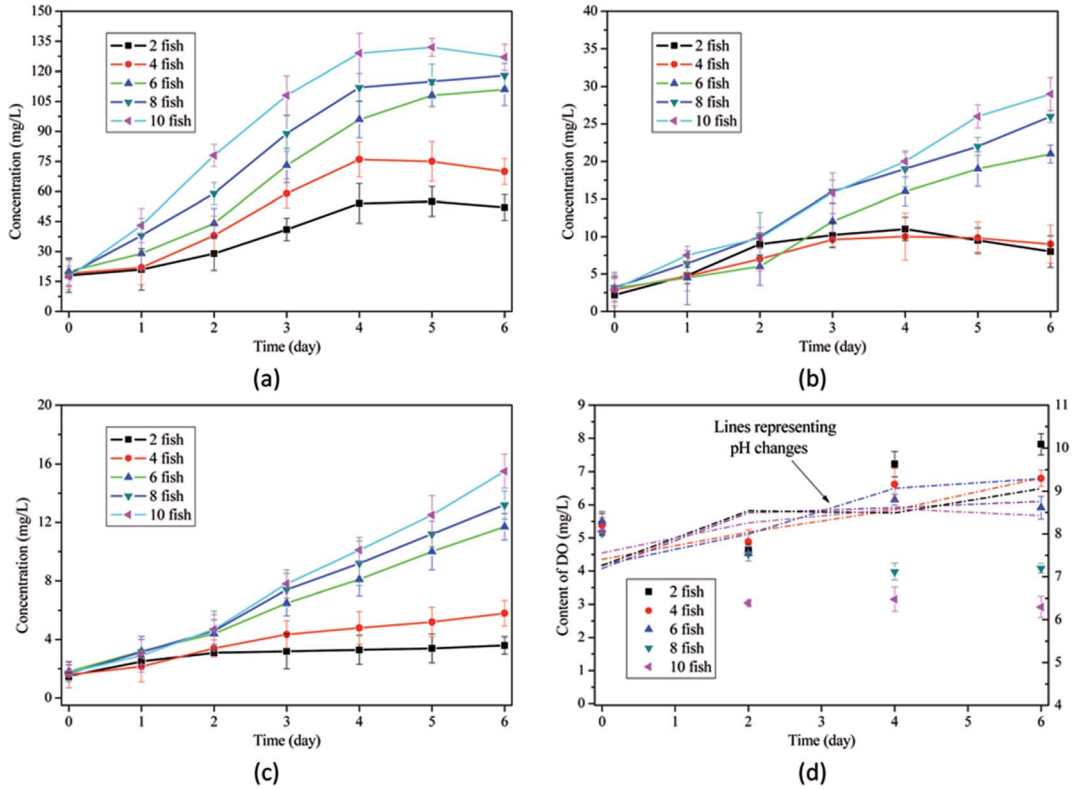

Fig. 2 Changes of water quality in microalgae-assisted aquaculture: (a) concentration of COD; (b) concentration of TN; (c) concentration of NH_3–N; (d) values of pH and DO.

the percentage of NH_3/NH_4^+ in TAN is closely related with the pH value of aqueous phase.[19] Thus, to reduce the toxicity of aquaculture eutrophication to fish, higher percentage of NH_4^+ in TAN is expected. Weakly acidic environment favors the hydrolysis of ammonia and increases the percentage of ammonium in TAN, creating a better condition for aquatic animals. In aquaculture with different stocking densities, the fluctuation of pH value, which stably ranged between 7.16 and 7.86, would not dramatically change the ratio of NH_3/NH_4^+ in TAN. Therefore, pH fluctuation is not a big concern in the management of aquaculture.

3.2. Alleviation of water deterioration by microalgae

3.2.1. Effects of microalgae on water quality. Fig. 2 indicated that the replacement of traditional feed by fresh microalgae did not totally prohibit the eutrophication of water body, but effectively reduced the eutrophication rate. For example, in aquaculture with 2, 4, 6, 8, and 10 fish/100 mL, the increase of COD concentrations were reduced by 51, 70, 56, 122, and 233 mg L^{-1} during 6 day culture (Table S1†). Similar phenomena were observed in the analysis of TN and TAN. As shown in Fig. 2(c), at the end of 6 day culture, TAN concentrations in aquaculture with 2, 4, 6, 8, and 10 fish/100 mL were 3.6, 5.8, 11.7, 13.2, and 15.5 mg L^{-1}, respectively, much lower than the semi-lethal concentration of TAN (20 mg L^{-1}) reported by Bhakta.[18] Therefore, the replacement of traditional feed by fresh microalgae is a feasible way to suppress the eutrophication and prevent the ammonia toxicity.

Fig. 2(d) indicated that the microalgae-based aquaculture had higher pH values (8.42–9.31) than traditional aquaculture by the end of 6 day culture. The main reason is that photosynthetic metabolism of microalgae uptake HCO_3^- from aqueous

Table 1 Nutrition of traditional aquaculture feed and microalgae biomass

Item	Traditional aquaculture feed	Microalgae biomass
Crude protein (g/100 g DW)	42.0 ± 2.8	50.6 ± 3.2
Crude lipid (g/100 g DW)	3.1 ± 1.4	24.5 ± 4.5
PUFAs (% of fatty acids profile)	—	42.3
Carotene	—	2.52 ± 0.28
Chlorophyll (mg g^{-1} DW)	—	21.3 ± 1.9

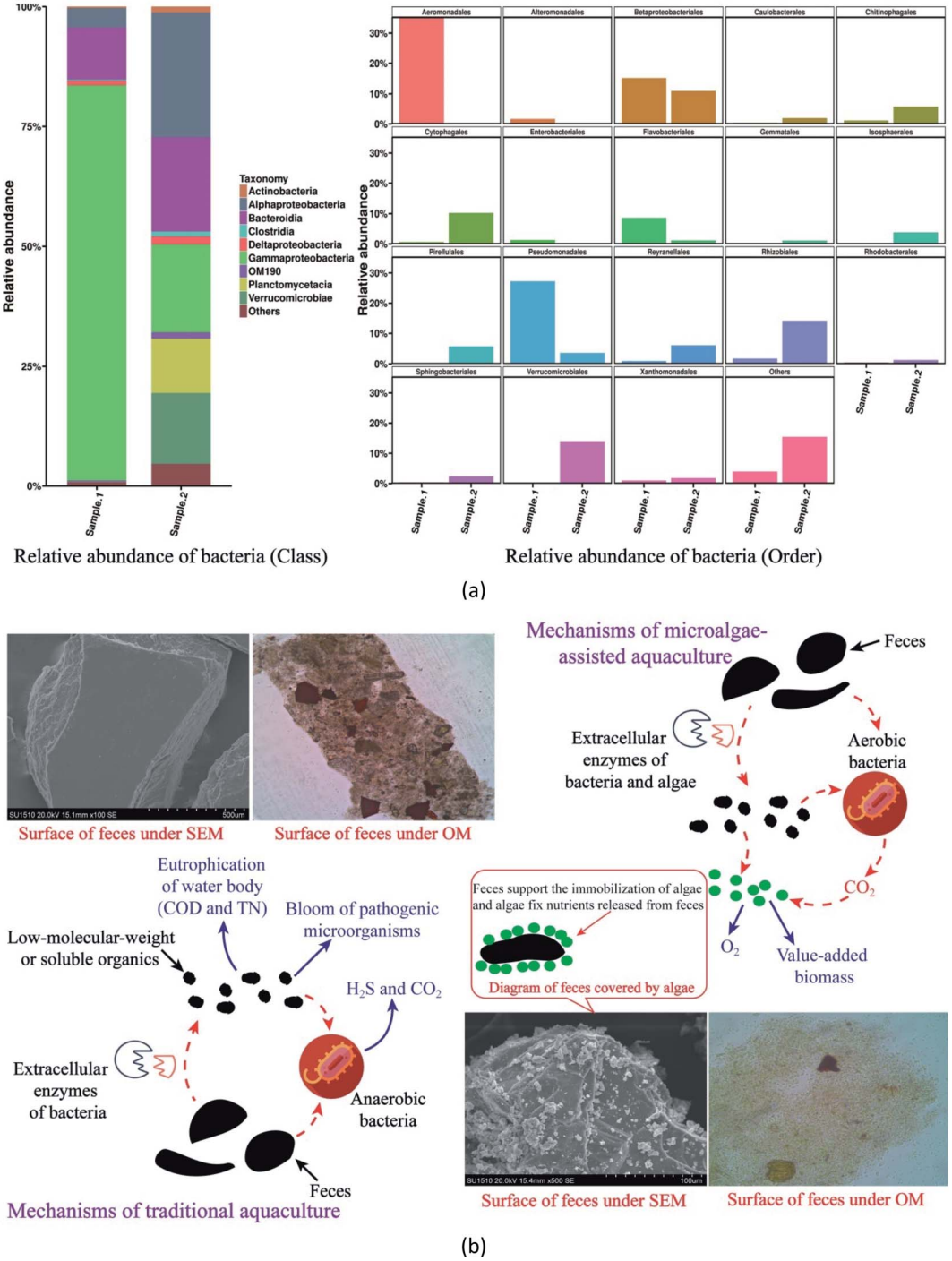

Fig. 3 Potential mechanisms in micro-environment: (a) bacterial community profile in aquaculture; (b) sludge in aquaculture.

phase, at the same time, H^+ is continuously consumed with the conversion of HCO_3^- to CO_2.[20] With the consumption of H^+, the increase of pH value was observed. As a result, the equilibrium between NH_3 and NH_4^+ may be disturbed and higher percentage of NH_3 in TAN would threaten the survival of aquatic animals. Hence, given the toxicity of NH_3, it is hypothesized that microalgae addition amount might be an important concern in aquaculture.

Microalgae also favored the maintenance of DO content in aquaculture system. As discussed above, oxygen depletion is an acute toxicity to fish survival in aquaculture with high stocking density. In this work, the increase of DO content was observed as long as the stocking densities did exceed 8 fish/100 mL. In aquaculture with high stocking density (8 and 10 fish/100 mL), the reduction of DO content decreased to 1.06 and 2.28 mg L^{-1} in 6 day culture with the addition of microalgae (Table S1†). Hence, microalgae addition can be a practically-feasible

method to replace or partly replace traditional aeration in aquaculture.

3.2.2. Assessment of the microalgae addition amount. The increase of pH value was observed in aquaculture with different addition amounts of microalgae, but excessive addition of microalgae (Aquaculture 1) did not cause dramatically faster increase of pH value than other addition amounts (Aquaculture 2 and Aquaculture 3) (Fig. S1†). This result is not in accordance with the hypothesis mentioned above. The main reason for such a difference is that in aquaculture practice, microalgae, which have been proven to be a good feed source by previous studies,[12,21] can be consumed by fish continuously, thus reducing the biomass content in water body and limiting the photosynthesis effect. As a result, the increase of microalgae addition amount from 0.0025 g per fish to 0.0050 g per fish did not cause the dramatic increase of pH value.

It was observed that DO contents reached 10.39 and 5.43 mg L^{-1} by the end of 6 day culture when the addition amounts of microalgae were 0.0050 and 0.0010 g per fish, respectively, confirming that microalgae addition can greatly increase DO content in aquaculture system and reduce the risks of oxygen depletion.

3.2.3. Comparison of nutritional values. Comparison between traditional aquaculture feed and microalgae biomass showed that microalgae grown in aquaculture water body contained more protein and lipid, which are essential nutrients to fish growth, than traditional fish feed (Table 1). In addition, microalgae biomass are rich in polyunsaturated fatty acids, of which the intake can positively impact the fish metabolisms,[22] while traditional fish feed contains no or trace amount of PUFAs. Last but not the least, natural pigments, including carotene and chlorophyll, contained in microalgae can enhance the fish immunity and potentially reduce the risks of fish diseases.[23] Therefore, in terms of nutritional values, microalgae grown in eutrophic water body can be considered as a better choice than traditional feed for aquaculture practice.

3.3. Potential mechanisms in micro-environment

3.3.1. Bacterial community. Difference was observed in the aspects of bacterial count and bacterial community profile of samples obtained from traditional aquaculture system (Sample 1) and microalgae-assisted aquaculture system (Sample 2). Sample 2 had much less number of sequences than Sample 1, suggesting that microalgae-assisted aquaculture contained less bacteria than traditional aquaculture (Table S2 and Fig. S2†). The most possible reason for this phenomenon is that microalgae competed with other microbes in aquaculture system for nutrients, thus, the nutrient deficiency caused by microalgae growth partly prevented the bloom of bacteria.[2] The prevention of bacterial bloom, which intensively consumes oxygen by heterotrophic metabolisms, is one of the factors contributing to the solution of oxygen depletion in microalgae-assisted aquaculture.

At the level of class, percentages of Gammaproteobacteria in Sample 1 and Sample 2 were 82.50% and 18.41%, respectively (Fig. 3(a)). This result is in accordance with previous study which discovered that Gammaproteobacteria accounted for 84.6% of total bacterial community in traditional aquaculture. Gammaproteobacteria contain a variety of harmful pathogens, not only threatening the fish survival, but also bringing potential safety problems in food chain, creating a detrimental environment for fish rearing in traditional aquaculture.[24] On the contrary, microalgae-assisted aquaculture with much less Gammaproteobacteria should be more favourable for fish growth.

Fig. 3(a) showed that Alphaproteobacteria, at the level of class, were the dominant bacteria (26.01%) in Sample 2. To our knowledge, Alphaproteobacteria contain both pathogens and beneficial microbes. To specify the roles of Alphaproteobacteria in microalgae-assisted aquaculture, bacterial community profile has to be studied at the level of order. As shown in Fig. 3(a), Rhizobiales, an order of Alphaproteobacteria, were the dominant bacteria (14.18%) in microalgae-assisted aquaculture while traditional aquaculture contained few Rhizobiales (1.76%). The synergistic relationship between Rhizobiales and microalgae in nutrients assimilation has been reported by previous study.[25] In this work, since Rhizobiales accounted for 54.52% of Alphaproteobacteria in Sample 2, the high percentage of Alphaproteobacteria in Sample 2 would not be a problem threatening fish rearing in microalgae-assisted aquaculture. In addition to Rhizobiales, other dominant bacteria, Cytophagales and Verrucomicrobiales, at the level of order, are not typical pathogens in aquaculture.

Based on the analysis results of bacterial count and bacterial community profile, it is considered that microalgae in aquaculture system can improve the water quality and create a favorable environment for fish growth. With the inhibition of pathogenic bacteria, the morbidity of aquatic animals can be reduced to a lower level.[24] As a result, the abuse of antibiotics in aquaculture can be effectively avoided. On the contrary, in the traditional aquaculture, the diseases of aquatic animals caused by the bloom of pathogenic bacteria are usually treated by a variety of antibiotics. The abuse of antibiotics could not only cause antibiotics resistance in aquatic animals and microorganisms, but also result in ecological disasters in water body. Therefore, microalgae-assisted aquaculture is more favorable to fish rearing than traditional aquaculture.

3.3.2. Microalgal biofilm. It was observed that sludge in microalgae-assisted aquaculture was covered by microalgae while those in traditional aquaculture were directly exposed to the water environment (Fig. 3(b)). In traditional aquaculture, with the activity of both aerobic and anaerobic bacteria, unfavorable gas (H_2S and CO_2) can be produced in the eutrophic water body, threatening the survival of aquatic animals. Also, with the degradation of sludge, soluble or low-molecular-weight organics were directly released to the aquaculture system, accelerating the eutrophication process.[26] Last but not the least, without the competition of microalgae with other microbes for ecological niche, the risks of pathogens bloom in aquaculture can be improved.

With the formation of a well-performing protection mechanism, the serious deterioration of water body in microalgae-assisted aquaculture can be avoided, providing a reasonable

explanation for the data in Fig. 2. Fig. 3(b) revealed that soluble or low-molecular-weight organics formed with the degradation of sludge were assimilated by microalgae cells in the biofilm for value-added biomass synthesis. In addition, with the continuous production of oxygen gas by photosynthesis, the activity of anaerobic bacteria can be prohibited and the CO_2 released by aerobic bacteria can be fixed by algae. Beneficial microalgae can reduce the occurrence possibility of pathogens bloom in aquaculture (Fig. 3(a)). Therefore, the formation of microalgal biofilm played an important role in the well-performing protection mechanism of microalgae-assisted aquaculture.

4 Conclusions

It is concluded that: (1) oxygen depletion and eutrophication are main problems in conventional aquaculture with high stocking density; (2) microalgae can solve the problem of oxygen depletion through photosynthesis and slow down the eutrophication by assimilating nutrients; (3) microalgae in aquaculture prevent the bacterial bloom and change the bacterial community profile, creating a favorable environment for fish rearing; (4) microalgal film covered on the sludge plays an important role in the well-performing protection mechanism of microalgae-assisted aquaculture; (5) application of microalgae biotechnology is expected to solve some problems in aquaculture and realize the industry upgrade.

Conflicts of interest

There is no conflict to declare.

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References

1 R. Gothwal and T. Shashidhar, *Clean: Soil, Air, Water*, 2015, **43**(4), 479–489.
2 Q. Lu, P. Han, Y. Xiao, T. Liu, F. Chen, L. Leng, H. Liu and J. Zhou, *J. Cleaner Prod.*, 2019, **217**, 573–575.
3 R. Boopathy, C. Bonvillain, Q. Fontenot and M. Kilgen, *Int. Biodeterior. Biodegrad.*, 2007, **59**(1), 16–19.
4 K. Sahin, H. Yazlak, C. Orhan, M. Tuzcu, F. Akdemir and N. Sahin, *Aquaculture*, 2014, **418**, 132–138.
5 C. S. Lee, J. Robinson and M. F. Chong, *Process Saf. Environ. Prot.*, 2014, **92**(6), 489–508.
6 H. Liu, Q. Wang, Y. Sun, K. Zhou, W. Liu, Q. Lu, C. Ming, X. Feng, J. Du and X. Jia, *Bioresour. Technol.*, 2016, **211**, 6–15.
7 P. L. McCarty, J. Bae and J. Kim, *Domestic wastewater treatment as a net energy producer–can this be achieved?*, ACS Publications, 2011.
8 I. Irvan, B. Trisakti, V. Wongistani and Y. Tomiuchi, *Int. J. Sci. Eng.*, 2012, **3**(1), 32–35.
9 Q. Lu, J. Li, J. Wang, K. Li, J. Li, P. Han, P. Chen and W. Zhou, *Bioresour. Technol.*, 2017, **244**, 542–551.
10 S. A. Razzak, M. M. Hossain, R. A. Lucky, A. S. Bassi and H. de Lasa, *Renewable Sustainable Energy Rev.*, 2013, **27**, 622–653.
11 M. Messina, C. Bulfon, P. Beraldo, E. Tibaldi and G. Cardinaletti, *Aquaculture*, 2019, **500**, 660–669.
12 S. Hemaiswarya, R. Raja, R. R. Kumar, V. Ganesan and C. Anbazhagan, *World J. Microbiol. Biotechnol.*, 2011, **27**(8), 1737–1746.
13 A. Muller-Feuga *Handbook of Microalgal Cultures: Applied Phycology and Biotechnology*, Wiley Blackwell, West Sussex, 2nd edn, 2013, pp. 615–627.
14 Q. Lu, W. Zhou, M. Min, X. Ma, C. Chandra, Y. T. Doan, Y. Ma, H. Zheng, S. Cheng and R. Griffith, *Bioresour. Technol.*, 2015, **198**, 189–197.
15 Q. Lu, W. Zhou, M. Min, X. Ma, Y. Ma, P. Chen, H. Zheng, Y. T. Doan, H. Liu and C. Chen, *Bioresour. Technol.*, 2016, **201**, 33–40.
16 A. Hosikian, S. Lim, R. Halim and M. K. Danquah, *Int. J. Chem. Eng.*, 2010, 1–11.
17 J.-P. Yuan, F. Chen, X. Liu and X.-Z. Li, *Food Chem.*, 2002, **76**(3), 319–325.
18 J. N. Bhakta, *Electronic Journal of Biology*, 2006, **2**(3), 39–41.
19 Y. Collos and P. J. Harrison, *Mar. Pollut. Bull.*, 2014, **80**(1–2), 8–23.
20 Z. Chi, J. V. O'Fallon and S. Chen, *Trends Biotechnol.*, 2011, **29**(11), 537–541.
21 R. Cerezuela, F. A. Guardiola, J. Meseguer and M. A. Esteban, *Fish Physiol. Biochem.*, 2012, **38**(6), 1729–1739.
22 J. Sargent, L. McEvoy and J. Bell, *Aquaculture*, 1997, **155**(1–4), 117–127.
23 A. Mustafa, L. Randolph and S. Dhawale, *J. Appl. Aquacult.*, 2011, **23**(2), 136–146.
24 M. Broszat, H. Nacke, R. Blasi, C. Siebe, J. Huebner, R. Daniel and E. Grohmann, Mexico, *Appl. Environ. Microbiol.*, 2014, **80**(17), 5282–5291.
25 B. P. Hodkinson and F. Lutzoni, *Symbiosis*, 2009, **49**(3), 163–180.
26 H. Liu, Q. Lu, Q. Wang, W. Liu, Q. Wei, H. Ren, C. Ming, M. Min, P. Chen and R. Ruan, *Bioresour. Technol.*, 2017, **235**, 59–69.

