Dual-bioaugmentation strategy to enhance the formation of algal-bacteria symbiosis biofloc in aquaculture wastewater supplemented with agricultural wastes as an alternative nutrient sources and biomass support materials

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HIGHLIGHTS

- Bioaugmentation has the ability to generate algal-bacterial bioflocs effectively.
- Algal-bacterial bioflocs recycle pollutants in aquaculture wastewater.
- Implementation of agricultural wastes promotes algal-bacterial bioflocs.
- Bioaugmentation alters water quality and microbial community structure.
- Dual-bioaugmentation was most successful for biofloc formation and waste cycling.

GRAPHICAL ABSTRACT
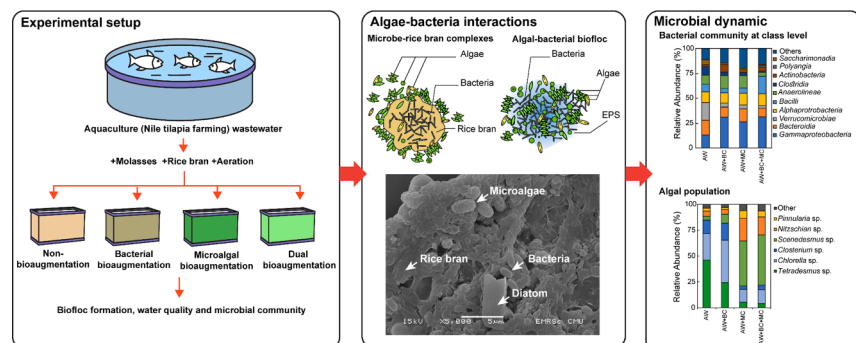
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ABSTRACT

This study performs an integrated evaluation of the formation and distribution of algal-bacterial bioflocs in aquaculture wastewater supplemented with agricultural waste, together with an assessment of their behavior in the microbial community and of the water quality of the system in which a new bioaugmentation strategy was applied. Results indicated that the dual bioaugmentation strategy via the consortium addition of bacteria and microalgae had the highest formation performance, providing the most compact biofloc structure (0.59 g/L), excellent settleability (71.91%), and a large particle diameter (4.25 mm). The fed-batch supplementation of molasses and rice bran, in terms of changes in the values of COD, NH_4^+, NO_3^-, and PO_4^{3-}, stimulated the formation

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of biofloc through algal-bacterial bioflocs and microbe-rice bran complexes within a well-established microbial community. These findings provide new insight into the influence of bioaugmentation on the formation of an innovative algal-bacterial biofloc.

1. Introduction

By 2050, the world's population will have increased by another 2 billion people, totaling 9.7 billion people, with the majority of this increase occurring in developing nations in Asia and Africa (Pekkoh et al., 2022). With this expansion in the population is a concomitant increase in the food demand. Food-producing industries are facing a tremendous challenge in meeting this increased demand for food given limited land resources. As such, aquaculture is expected to play a critical role in optimizing the use of different aquatic resources to produce a diverse range of foods via the incorporation of intensive cultivation procedures (Kumar et al., 2018). However, these intense cultivation techniques are connected with various environmental issues, including wastewater discharge, excessive feed consumption, contaminants, infectious diseases, and unfavorable environmental effects (Liu et al., 2019). Typically, aquaculture organisms only take up 20–30% of the nitrogen in their diet; the remainder is excreted and released into the water as polluting agents (ammonia and nitrite) and other waste products, which adversely affect water quality (Kumar et al., 2018; Liu et al., 2019). As a consequence, the water in these aquaculture ponds must be routinely exchanged, which leads to eutrophication and other anaerobic conditions in the neighboring watersheds (Addo et al., 2021). Therefore, innovative methods toward sustainable and environmentally friendly technologies are needed for improving pollution removal to ensure water quality for the increased productivity of aquaculture systems.

Innovative aquaculture technologies, such biofloc technology (BFT), have recently shown potential to increase productivity while preserving the environment and contributing to the well-being of society and the economy. Biofloc contains beneficial bacteria, fungi, algae, zooplankton, and protozoa that are bound together in a matrix with organic debris that is suspended in the water. These microbial communities are generated by supplying organic carbon to the aquaculture system and maintaining the C/N ratio at a constant level (Wang et al., 2015). Using BFT has three major advantages. First, bioflocs effectively decrease toxic nitrogen content in aquaculture ponds (Luo et al., 2017). Second, bioflocs provide an extra *in situ* food supply for aquaculture animals due to their small particle size (Addo et al., 2021). Third, microorganisms in biofloc degrade and consume food waste, farm animal feces, and secondary metabolites of pests, resulting in the efficient removal of aquaculture wastewater contaminants (Kumar et al., 2018). Hence, BFT is applied for a wide variety of aquaculture organisms worldwide. Despite BFT being a sustainable solution for aquaculture water treatment, its widespread acceptance faces a number of barriers. According to Betanzo-Torres et al. (2020) and Addo et al. (2021), developing a biofloc system with steady nutrient removal efficiency is slower than might be expected. As a result, the implementation of BFT in the field has encountered technological difficulties. Because of this, some aquaculture farms still use the traditional methods of changing the water in their aquaculture ponds, which leads to large amounts of wastewater.

To overcome the issues mentioned above, aquaculture wastewater can be treated with an algal-bacterial symbiosis-based biofloc system before being discharged into the environment. This method has been used successfully in previous studies for advanced treatment of wastewater from different sources due to its high efficiency in nutrient removal and recovery, as well as low energy consumption and carbon dioxide (CO_2) release (Wang et al., 2019; Fallahi et al., 2021; Tang et al., 2021; Vishwakarma et al., 2022; Wang et al., 2022). However, algal-bacterial biofloc creation depends on the symbiotic interaction between microalgae and bacterial communities. Through photosynthesis,

microalgae transform solar energy into chemical energy, producing oxygen (O_2) and nutrients for the bacteria that live in their biofloc. Bacteria are metabolically active and create CO_2 and growth factors that are beneficial to the growth of microalgae (Wang et al., 2022). In aeration conditions, the algal-bacterial biofloc is more efficient at removing toxic nitrogen and forming biofloc, but more energy-consuming than in non-aeration conditions (Shen et al., 2021). Tang et al. (2021) and Shen et al. (2021) found that this synergic interaction is complicated. These communities indirectly assist one another in the form of survival stress alleviation, growth enhancement, and nutrient accumulation. They also regularly interact to form the algal-bacterial biofloc. Unfortunately, several considerable barriers remain to the long-term functioning of algal-bacterial biofloc for the treatment of wastewater, which must be overcome.

To enhance the performance of algal-bacterial biofloc, researchers have used agricultural waste as the nutrients, such as molasses, rice, barley, or wheat straw, for microorganisms in the early stages of cultivation (Pacheco-Vega et al., 2018; Addo et al., 2021). In addition to serving as alternate nutrient sources, these nutrients function as biomass support materials, allowing microbes and particles to form complexes that aid in the production of algal-bacterial biofloc (Addo et al., 2021). However, researchers have not yet examined the impact of continuous supplementation using these wastes on algal-bacterial biofloc formation in wastewater treatment systems, which would provide useful information for continuous biofloc harvesting in the future. Furthermore, the synergistic interaction between algae and bacteria can be improved by adding specific strains of microbes with known characteristics. This technique, known as bioaugmentation, is often employed to increase the effectiveness of biological wastewater treatment (Liu et al., 2019). The addition of probiotic *Bacillus* spp. consortia inhibited pathogens (Mohammadi et al., 2021) and improved water quality (Dash et al., 2018), but did not have any significant effect on floc-forming bacteria communities (Miranda-Baeza et al., 2020). The addition of a microalgae-nitrifying bacteria consortium effectively promoted ammonia elimination and the creation of an algal-bacterial biofloc (Jiang et al., 2018). Additionally, when extracellular polymeric substances (EPSs)-generating microalgae are added to a liquid digestate containing native bacteria, a larger amount of algal-bacterial biofloc is produced, which is beneficial (Wang et al., 2019). EPSs are regarded as essential during the algal-bacterial biofloc formation process (Tang et al., 2021). The presence of native bacteria in unsterilized digestate wastewater can be used as a bacterial inoculum, and some of these bacteria negatively impact the formation of algal-bacterial biofloc by inhibiting algal growth (Wang et al., 2022). The use of aquaculture wastewater, however, may be able to overcome these constraints owing to the content of helpful bacteria that are involved in biofloc formation (Ferreira et al., 2021). However, little evidence is available on the combined addition of a probiotic *Bacillus* consortium and a microalgal consortium through a dual-bioaugmentation strategy using aquaculture wastewater supplemented with agricultural waste as nutrient sources and biomass support materials, which may result in a more suitable and continuous formation of algal-bacterial biofloc coupled with wastewater treatment.

To investigate whether bioaugmentation improves the performance of algal-bacterial biofloc, in this study, a probiotic *Bacillus* consortium (BC) as a bioaugmented bacterial seed and a microalgal consortium (MC) as a bioaugmented microalgal seed were used. Four different strategies, including i) non-bioaugmentation, which was unsterilized aquaculture wastewater (AW) without the addition of BC and MC; ii) bacterial bioaugmentation, which was AW with the addition of BC; iii)

microalgal bioaugmentation, which was AW with the addition of MC, and iv) dual bioaugmentation, which was AW with the addition of both BC and MC, were compared under aeration conditions using AW and fed-batch supplementation with molasses and rice bran as alternative nutrient sources and biomass support materials. The biofloc formation performance using indicators such as biofloc yield, the growth of bacteria and microalgae, and flocculating activity were measured to select the most effective strategy for bioaugmentation. Furthermore, the influence of bioaugmentation on EPS generation, morphological structure, microbial population, and water quality was determined, which through detailed exploration better grasps the bioaugmentation process and its implications. Specifically, these findings provide some fundamentals that allow for the exploration of novel algal-bacterial biofloc formations for advanced wastewater utilization and further practical applications of algal-bacterial biofloc, and describe the role of microbes in nutrient removal and symbiosis in the system.

2. Materials and methods

2.1. Materials

The culture medium used in this study contained 0.4 g/L of molasses, 0.2 g/L of rice bran, and 1 L of aquaculture wastewater. Aquaculture wastewater (AW) was obtained from a Nile tilapia (*Oreochromis niloticus*) farm in Chang Mai, Thailand. Molasses and rice bran were locally purchased from farmers. Rice bran was sieved through a 1 mm mesh to eliminate larger fibers and other particles before use. Molasses, which was employed in this research, had a total carbon content of 40.17 %w/w and a total nitrogen content of 0.44 %w/w, while rice bran had a total carbon content of 85.77 %w/v and a total nitrogen content of 0.38 %w/v. The native microorganisms present in the AW were used as the initial inoculum (5 log CFU/mL). The dried culture of *Bacillus* syntrophic consortia containing *Bacillus* spp. and *Bacillus aryabhattai* (>40,000 CFU/mL) obtained from Nilebiofish Co., Ltd. (Chiang Mai, Thailand) was used as the *Bacillus* consortium (BC). Mixed microalgae consortia, including *Scenedesmus dimorphus* (50%) and *Chlorella* sp. (50%), obtained from the Algal and Cyanobacterial Research Laboratory, Chiang Mai University, Thailand, were used as the microalgal consortium (MC).

2.2. Experimental setting

For the formation of algal-bacterial biofloc, the experiment was performed in duplicate 15 L batch photobioreactors with an operational volume of 10 L culture medium at 25 ± 2 °C, with continuous light feeding of 36 μmole/m^2/s, and an aeration rate of 0.2 L/min. Molasses (0.4 g/L) and rice bran (0.2 g/L) were continuously supplied to the photobioreactor every 3 days. The initial dried BC was set at 0.1 g/L. The initial MC was set at 0.02 OD$_{660}$. Four treatments of culture systems were tested:

1. Control biofloc system: AW system without the addition of BC or MC (AW; non-bioaugmentation);
2. Biofloc system with MC addition (AW + MC; microalgal bioaugmentation);
3. Biofloc system with BC addition and without MC addition (AW + BC; bacterial bioaugmentation);
4. Biofloc system with BC and MC addition (AW + BC + MC; dual bioaugmentation).

The cultivations of all treatments were performed for 15 days. The total number of bacteria, chlorophyll *a* (Chl a) concentration of microalgae, and biofloc yields were measured every 3 days. The pH, turbidity, chemical oxygen demand (COD), ammonium-nitrogen (NH$_4^+$–N), nitrate–nitrogen (NO$_3^-$–N), and phosphate–phosphorus (PO$_4^{3-}$–P) of the culture system were detected at days 0, 7, and 15. At the end of the experiment, the flocculation efficiency, the concentration of extracellular polymeric substances (EPSs), the morphology of the biofloc, and the microbial community were characterized.

2.3. Analytical methods

The total number of bacteria was monitored by dilution plate counting on plate count agar and colony-forming units was calculated as the number of bacteria cells (CFU/mL) after 24 h of incubation at 37 °C. Microalgal growth was measured by determining Chl a concentration following the process described by Lomakool et al. (2021). Briefly, a 10 mL of sample suspension was filtrated through 4.7 cm Whatman GF/C glass fiber filters. The filtered sample was mixed with 10 mL of 90% methanol, boiled at 70 °C for 20 min, and then centrifuged at 6000 rpm for 15 min. The supernatant was collected and the above steps were repeated until the extraction liquid was colorless. The optical density (OD) of the supernatant was measured at 630, 645, 665 and 750 nm with a UV–vis spectrophotometer. The Chl a concentration (mg/L) was calculated using the following equations (Eq. (1)):

$$Chl\ a = (11.6A - 1.31B - 0.14C) \times [(V/V_f) \times (1/L)] \tag{1}$$

where A is OD$_{665}$ − OD$_{750}$, B is OD$_{645}$ − OD$_{750}$, C is OD$_{630}$ − OD$_{750}$, V is the total volume of extract (mL), V$_f$ is the volume of the sample (mL), and L is the light path length of the width of the cuvette (cm).

The flocculation activity was calculated using the following equations (Eq. (2)):

$$Flocculation\ activity = [(A - B) / B] \times 100 \tag{2}$$

where A and B are the absorbency of the microalgal culture at 680 nm before and after resting for 30 min, respectively.

The biofloc yield was calculated in terms of dried biomass (g/L). The suspension sample (10 mL) was filtrated through 4.7 cm Whatman GF/C glass fiber filters and washed it twice with distilled water. The filtered biomass was then dried at 60 °C until it reached a consistent weight. Total biomass was gravimetrically determined. The biofloc yield (g/L) and productivity (mg/L/day) were calculated using the following equations (Eq. (3) and Eq. (4)), respectively:

$$Biofloc\ yield = [(A - B) / C] \times 100 \tag{3}$$

$$Biofloc\ productivity = (B_2 - B_1) / (t_2 - t_1) \tag{4}$$

where A and B represent the total weight of dried filter paper after and before sample filtration, respectively, C represents the total volume of suspension sample (mL), and B$_1$ and B$_2$ represent the biofloc yield (mg/L) at time t$_1$ (start of cultivation) and t$_2$ (end of cultivation), respectively.

The pH value was measured using a pH meter (Starter 31000, OHAUST, USA). The COD was analyzed using standard methods (APHA, 1995). The turbidity value and the concentrations of NH$_4^+$–N, NO$_3$–N, and PO$_4^{3-}$–P were determined using a DR2100 spectrophotometer (HACH, USA).

The EPSs of algal-bacterial biofloc was extracted using formaldehyde under a mild alkali condition (Wang et al., 2019). Briefly, the suspension sample (5 mL) was mixed with 60 μL formaldehyde for 30 min after washing with 0.01 mol/L phosphate-buffered saline (PBS, pH 7.4). Then, 5 mL of 1 mol/L NaOH was added, and the mixture was incubated at 200 rpm and 4 °C for 90 min to dissolve polysaccharides and proteins. The supernatant was collected by centrifugation at 10,000 rpm for 20 min. The polysaccharides were measured as glucose equivalents through a phenol-sulfuric acid assay (Ruangrit et al., 2021). The proteins were measured as BSA equivalents using a Bradford protein assay (Ruangrit et al., 2021). The morphology and size of the algal-bacterial biofloc was characterized using light microscopic analysis. For the calculation of average biofloc size, a biofloc size of 20 granular was measured through an ECLIPSE Ni-E upright microscope (Nikon, China) and then calculated using NIS-elements D software.

The algal community of biofloc was analyzed using microscopic observation and recorded the top three algal species using three levels of scores: well-observed (+++), moderately observed (++), and less-observed (+).The bacterial community in the biofloc was analyzed using high-throughput 16S rRNA gene amplicon sequencing analysis. The samples were taken for DNA extraction using a DNA kit (Tiangen Biotech). The genomic DNA were purified using a DNA Clean and Concentrator Kit (Zymo Research). The 16S rRNA genes of distinct regions (16S V3-V4) were amplified using a specific primer (341F; CCTAYGGGRBGCASCAG and 806R; GGACTACNNGGGTATCTAAT). After purification using a Qiagen Gel Extraction Kit (Qiagen, Germany), the PCR products were used for high-throughput sequencing via a HiSeq2500 PE250 Illumina sequencing instrument (IIlumina Inc, San Diego, USA). The sequencing data were analyzed using QIIME software (version 1.7.0) and USEARCH software (version 7.0.1001). After quality filtration, the remaining sequences were grouped into operational taxonomic units (OTUs) based on the SILVA database with a 97% similarity identify cutoff, which provides genus- and species-level categorization.

2.4. Statistical analysis

All experiments were performed in the triplicate. An analysis of variance was conducted to measure the significance of results, and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Algal-bacterial biofloc formation

Fig. 1 compares the formation of algal-bacterial symbiosis biofloc for the different bioaugmentation strategies: including i) the control biofloc system, which was an unsterilized AW system without the addition of BC or MC (AW; non-bioaugmentation); ii) biofloc system with BC addition and without MC addition (AW + BC; bacterial bioaugmentation); iii) biofloc system with BC and MC addition (AW + BC + MC; dual bioaugmentation), and iv) biofloc system with MC addition (AW + MC; microalgal bioaugmentation), cultivated in a 10 L photobioreactor with fed-batch supplementation of molasses and rice straw as a low-cost nutrient. The algal-bacterial biofloc yield for all bioaugmentations was higher than that of the non-bioaugmented control (Fig. 1a). At the end of the experiment, the yield of AW + BC + MC was the highest, at 0.56 g/L, followed by that of AW + MC (0.48 g/L), AW + BC (0.43 g/L), and AW (0.12 g/L). This indicated that BC and MC addition led to a higher biofloc yield. Compared with the non-bioaugmented control, the biofloc productivity with BC and/or MC addition increased in the range of 2.98- to 3.84-fold (Fig. 1b), proving that bioaugmentation promoted biofloc formation. Similarly, Ekasari et al. (2021) discovered that mixing biofloc seed with microalga *Chlorella* sp. increased biofloc production by up to 1.75-fold compared to the production of a monoculture biofloc. However, biofloc yields not only contain the weight of the algal-bacterial biofloc but also the undegradable particles from the fed-batch supplementation of rice bran, which provided biomass support particles for both algae and bacteria, hence promoting the formation of the biofloc. This finding is consistent with the results of Romano et al. (2018) and Addo et al. (2021), who found that the addition of rice straw and bran to biofloc systems not only provided an alternative nutrition supply but also served as a biomass support material for the production of bioflocs. Notably, the bioflocs produced in this study were both algal-bacterial symbiosis bioflocs and microbe-rice bran complexes.

During algal-bacterial biofloc formation in this study, bacteria growth rapidly increased in all treatments (Fig. 2a), with a comparable growth rate of 0.33–0.36 generations per hour, indicating that the presence of sufficient oxygen (O_2) in the aeration process promotes bacterial activity in bioflocs. Although microalgae can produce more O_2, the growth rate of bacteria was not enhanced in the AW + MS or AW +
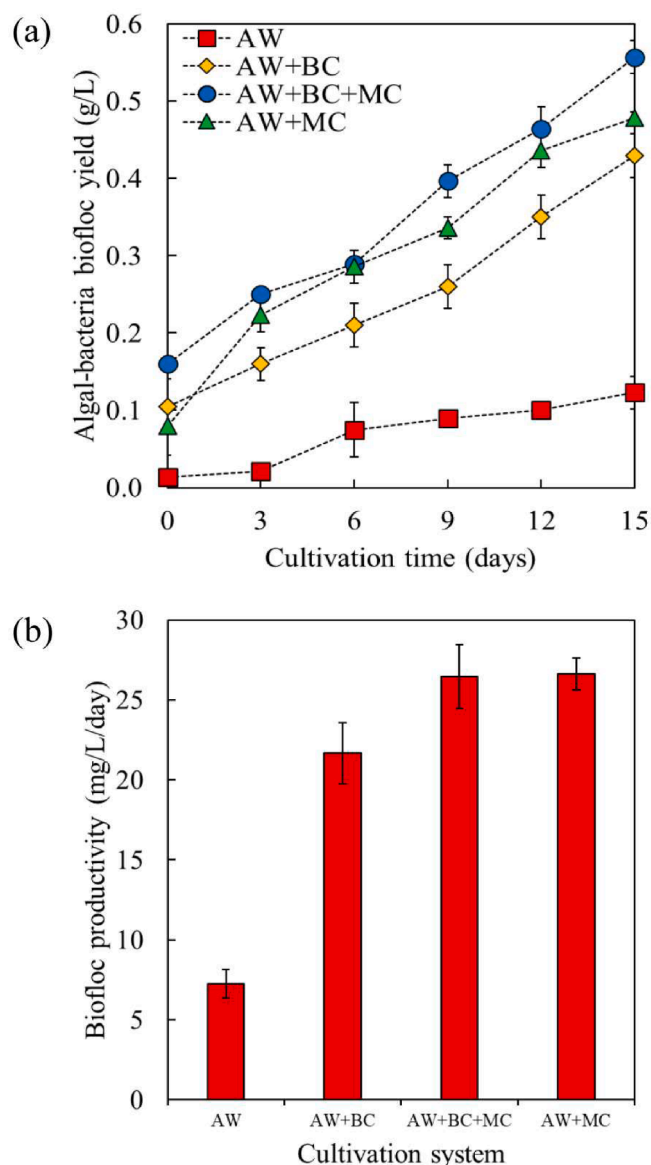

Fig. 1. The yield (a) and productivity (b) of algal-bacteria biofloc formed in batch photobioreactor under different cultivation system (AW: non-bioaugmentation; AW + BC: bacterial bioaugmentation; AW + MC: micro-algal bioaugmentation; AW + BC + MC: dual bioaugmentation).

BC + MS systems (Fig. 2a). The imbalanced development of the two microorganisms likely resulted in the lack of improvement in bacterial growth. However, the bacterial growth rate with BC addition in the AW + BC system was lower than in the AW system. The insignificant improvement in bacterial growth rate with all BC and/or MC additions might be attributed to the competition between the algae and bacteria and/or bacteria and bacteria for space and nutrients (Huo et al., 2020). Zhao et al. (2019) found that in operation over the long term, excessive O_2 in the aeration process and the O_2 supply provided by microalgal cells are limiting factors, which, in turn, restrict the proliferation of bacterial cells.

According to Fig. 2b, the microalgae quickly developed and achieved their maximum growth after 12 days of cultivation, producing chlorophyll at 1253.17 µg/L (AW + MC system), 833.69 µg/L (AW + BC + MC system), 664.31 µg/L (AW system), and 663.37 µg/L (AW + BC system). The substantial increase in chlorophyll content resulted from the increased production of algal biomass. This finding showed that the algae can use the CO_2 produced by bacteria as well as the CO_2 created
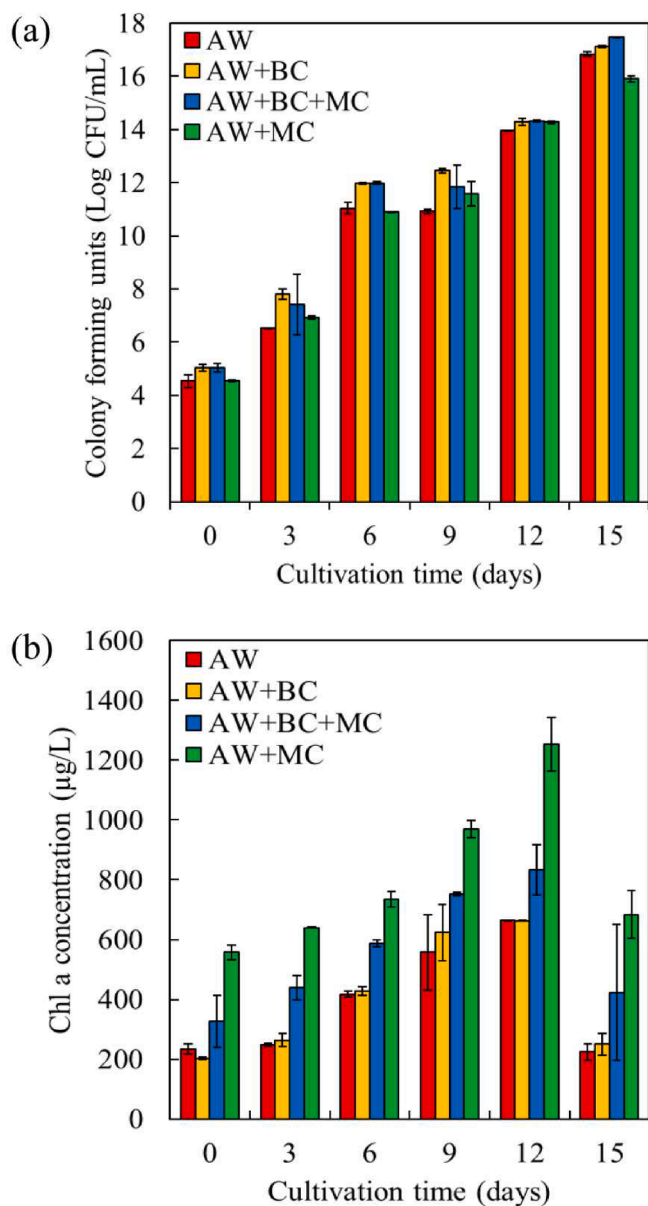
Fig. 2. The growth of bacteria (a) and algae (b) during algal-bacteria biofloc formation under different cultivation system (AW: non-bioaugmentation; AW + BC: bacterial bioaugmentation; AW + MC: microalgal bioaugmentation; AW + BC + MC: dual bioaugmentation).

via aeration feeding. The detectability of chlorophyll in the systems without microalgae addition (AW + BC and AW systems) was possibly due to the growth in native microalgae and diatoms contaminated with originally unsterilized aquaculture wastewater. Notably, the formation of symbiotic algal-bacterial biofloc without BC addition in the AW + MC system might have increased the microalgal biomass and resulted in the maximum chlorophyll content. The low chlorophyll content of the AW + BC + MC system occurred due to the shading effect of the algal population or the competition for resources with fast-growing bacteria, both of which were present. While the AW + MC had a lower biofloc yield, which meant a lower number of solid particles. This allowed a greater amount of light to enter the system, leading to higher chlorophyll content. Unfortunately, after 15 days of cultivation, the chlorophyll concentration in all treatments was lower than at 12 days (Fig. 2b). A similar finding was reported by Nguyen et al. (2019), who found that the light restriction and self-shading caused by the cocultivation of microalgae and bacteria detrimentally impacted microalgal

photosynthesis, resulting in lower chlorophyll synthesis.

The interactions between microalgae and bacteria, as well as nutrient balance and competition for nutrient intake, contribute to the establishment of algal-bacterial biofloc symbiosis, involving sophisticated and poorly understood processes (Wang et al., 2019). Phytohormones produced by bacteria may increase the photosynthesis activity by stimulating the pigment production pathway (Kumsiri et al., 2021). According to Gangwar et al. (2014), bacteria not only create phytohormones but also release other molecules such as enzymes, vitamins, and other micronutrients. These compounds have the potential to increase the synthesis of microalgal biomass and the accumulation of biochemicals in their cells, such as chlorophyll. Certain conditions in the culture system benefit the development of microalgae and are advantageous for algal-bacterial biofloc formation. However, other conditions may be deleterious to bacterial growth. Hazardous metabolites released by algae, such as volatile halogenated chemicals and fatty acids, may restrict bacterial colonization and prevent them from spreading (Berthold et al., 2019). Similarly, Dalvi et al. (2021) discovered that a combination of factors, including pH, DO, sunlight, and the metabolic activities of algae, might inactivate bacteria found in wastewater through multiple mechanisms. According to Dao et al. (2018) and Kumsiri et al. (2021), the chemical signals generated by the microalgae may be used for cell–cell communication, but because they are not nutrients, they may decrease gene expression and/or physiological activity, ultimately leading to reduced bacterial growth rates. In conclusion, the use of bioaugmentation strategies may result in both positive and negative interactions.

Sedimentation evaluation indices like flocculation activity are often used in the biofloc system, which measures the settleable algal-bacterial biofloc and/or microbe-rice bran complexes from a height of two-thirds from the bottom in an aliquot of the culture after resting for 30 min. An increase in flocculation activity indicates a more efficient and economical biomass harvesting process (Wang et al., 2022). The flocculation activities of the AW, AW + BC, AW + BC + MC, and AW + MC systems were 78.79%, 88.04%, 71.91%, and 61.81%, respectively (Fig. 3a), suggesting that BC addition increased settleability whereas MC addition decreased sedimentation. The findings may be explained by referring to the cell surface characteristics of MC biomass. According to Bhattacharya et al. (2017), microalgal cell surfaces are negatively charged owing to the presence of carboxylic (–COOH), amine (–NH$_2$), and phosphate (–PO$_4$) groups. When the culture pH is greater than 5, the –COOH groups start to dissociate, which results in a net negative charge. The charge creates a repulsive force between the microalgal cells, which prevents the cells from settling to the bottom of the liquid in which they are floating, resulting in lower sedimentation efficiency. In all systems, the free algae and bacteria clustered together with or without undegradable rice bran particles to generate the bioflocs using the EPSs that they produced. An optimum ratio of microalgae to bacteria offers a more suitable precondition for the production of algal-bacterial biofloc (Nguyen et al., 2019), resulting in higher flocculation activity. Lower flocculation activity might have occurred due to the remaining non-settleable algae-bacteria and/or free algae with the high cell concentration of MC present in the AW + BC + MC and AW + MC systems, whereas the AW and AW + BC systems had higher flocculation activity with a low MC concentration. There was a statistically significant variation in the flocculation activity of algal-bacterial biofloc for the different levels of initial MC concentration. Wang et al. (2019) found that using a low inoculation concentration resulted in higher flocculation activity. At the same inoculation level, the flocculation activity of various algae varied considerably. Therefore, the adoption of self-flocculating algal strains may increase settleability in both the AW + BC + MC and AW + MC systems, which requires further investigation.
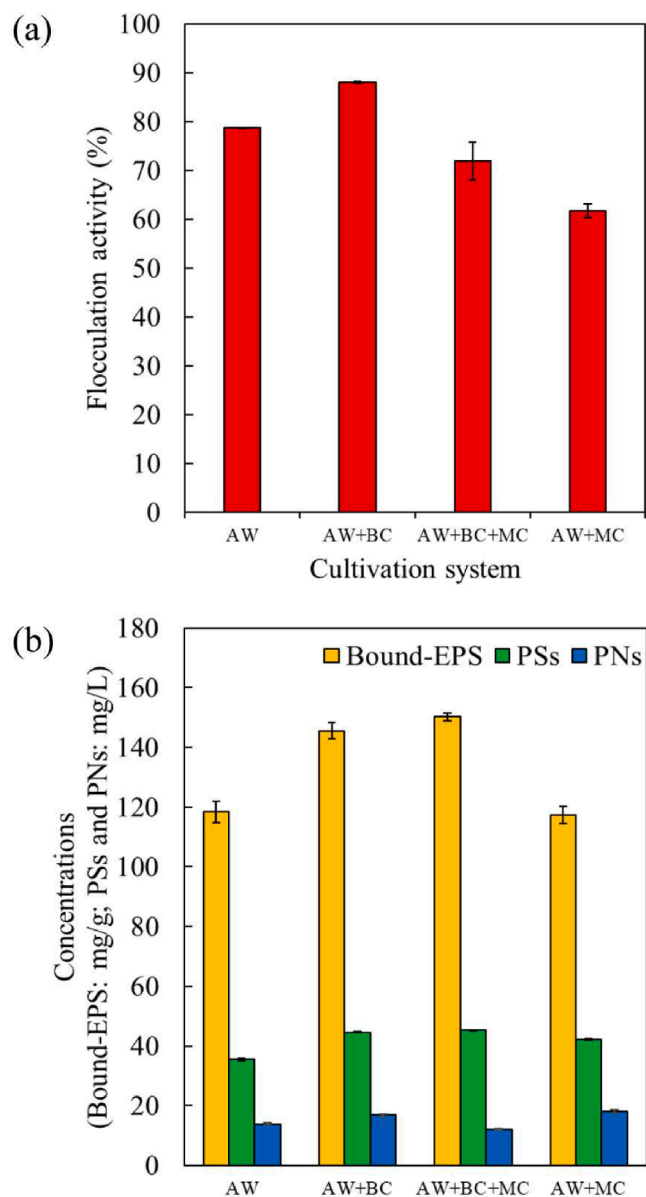
Fig. 3. Flocculating activity (a) and EPS production (b) of algal-bacteria biofloc derived from different cultivation system (AW: non-bioaugmentation; AW + BC: bacterial bioaugmentation; AW + MC: microalgal bioaugmentation; AW + BC + MC: dual bioaugmentation).

3.2. Characterization of algal-bacterial biofloc

3.2.1. EPS production

In this study, the algal-bacterial biofloc formation demonstrated that the EPS generated by algae and bacteria is a critical factor in its formation (Fallahi et al., 2021). EPSs are found in the form of microbial secretions and is mostly composed of proteins (PNs) and polysaccharides (PSs), which represent 50–72% of the total amount of EPSs found (Tang et al., 2021). EPSs are divided into two types: soluble and cell-bound EPS. Soluble or free EPS refers to EPS that is not tightly bound to cells or that is available in solution, whereas bound EPS is strongly attached to cells (Fallahi et al., 2021). However, free EPS is often incapable of forming algal-bacterial biofloc. Tightly bound EPSs (TB-EPSs) and loosely bound EPSs (LB-EPSs) are two types of bound EPSs. Wang et al. (2022) found that a layer of bioflocs can be formed by TB-EPSs, which firmly attached to the cell surface and formed the inner layer. An outer layer of bioflocs was formed by LB-EPSs in a dispersed slime.

In this study, the amounts of bound EPSs, PNs, and PSs in algal-bacterial bioflocs were determined, as well as the ratio of PNs to PSs. As shown in Fig. 3b, at the end of cultivation, the EPS content in all systems was significantly higher than at the beginning of cultivation. The content of bound EPSs of AW + BC + MC (150.22 mg/g bioflocs) was higher than that of AW + BC (145.61 mg/g bioflocs), AW (118.50 mg/g bioflocs), and AW + MC (117.31 mg/g bioflocs) systems. This demonstrated that the addition of BC to the system increased the amounts of bound EPSs, leading to an increase in flocculation efficiency and the generation of biofloc. However, there was no improvement in the EPS content with the addition of MC to the system, indicating that bacteria were the primary producers of EPSs and that the increased microalgal growth resulted in higher biofloc yields but less secretion of EPSs. Moreover, the trend in flocculation activity did not follow the trend in bound EPSs. At the end of cultivation, there was a significant rise in the number of EPSs produced, but there was no corresponding increase in the formation of bioflocs. This indicates that not only does the function of EPSs play a role in the formation of algal-bacterial bioflocs, but cell surface charge interaction also plays a role in this process. A similar observation was reported by Wang et al. (2022).

The concentration of PSs and PNs in EPS were not significantly different between the non-bioaugmented and bioaugmented treatments (Fig. 3b). This demonstrated that both PSs and PNs in the EPSs were functional in the formation process of both algal-bacteria symbiosis bioflocs and microbe-rice bran complexes. According to Wang et al. (2022), PSs can promote bioflocculation and further increase biofloc formation and expansion depending on their viscosity, rheological behavior, and adherence; PNs can induce microbial flocculation through hydrophobic interactions. In addition, biofloc formation occurs between EPSs and microbes, with or without rice bran as a biomass support material, through complicated interactions such as London forces, electrostatic interactions, and hydrogen bonding (Fallahi et al., 2021), which result in the formation of bioflocs.

3.2.2. Morphological structure

The morphological structure of the algal-bacterial biofloc was determined by light microscopic analysis. The algal-bacterial biofloc changed color to a brownish-green, indicating that the algae appeared in the biofloc consortia in a different proportion (see supplementary material). The microscopic images (see supplementary material) and SEM photograph (see supplementary material) also proved that the biofloc obtained in this work contains both algal-bacterial symbiosis bioflocs and microbe-rice bran complexes. From microscopic images at 10x magnification (see supplementary material), a denser interior was found in the algal-bacterial biofloc in the AW + BC, AW + MC, and AW + BC + MC systems compared to the AW system, suggesting that bioaugmentation strategies improve the morphology of algal-bacterial bioflocs. The 10x microscopy images also showed that even though the addition of BC increased the granularity of the biofloc, the high density of free algal cells or algae that were not attached filled more accessible spaces in the systems with MC addition. Wang et al. (2022) proposed that adding EPS-producing algae as MC can increase the volume of algal-bacterial bioflocs while improving the settleability of the system.

The 40x microscopy images (see supplementary material) show that algae (e.g., diatoms, cyanobacteria, and microalgae) appeared in both the exterior and interior sections of the algal-bacterial biofloc. Without the addition of MC (see supplementary material), the algae grew and attached to the biofloc. This verified that some native algae were present in the initially unsterilized aquaculture wastewater, resulting in the presence of measurable chlorophyll in both the AW and AW + BC systems. Notably, although algal-bacterial biofloc formed without the addition of MC, the formation efficiency was lower than that obtained with MC addition. A similar observation was reported by Ekasari et al. (2021). The algal-bacterial biofloc sizes ranged from 50 to 700 μm with average sizes of 327, 343, 408, and 425 μm for AW, AW + BC, AW + MC,

and AW + BC + MC systems, respectively, indicating that MC addition enhanced the size of algal-bacterial biofloc. Despite the presence of free algae, the addition of MC and BC in the dual-bioaugmentation system led to the largest algal-bacterial biofloc. Similarly, Liu et al. (2018) discovered that the addition of algae improved the morphology of algal-bacterial consortia. Notably, the dual-bioaugmentation strategy has considerable potential for the creation of both algal-bacterial symbiotic biofloc and microbe-rice bran complexes.

3.2.3. Microbial community

The dominant composition of the microbial communities in the algal-bacterial biofloc was analyzed using microscopic observations for determining the algal community, while high-throughput 16S rRNA gene amplicon sequencing analysis was used to characterize the bacteria community. Through microscopic analysis (see supplementary material), it was found that the top three algal strains in the biofloc of the non-bioaugmented control (AW system) were green microalgae, namely *Tetradesmus lagerheimii* (46.01%), *Chlorella* sp. (25.77%), and *Closterium* sp. (12.88%); *Chlorella* sp. (40.87%) was the most abundant algae in the biofloc in the AW + BC system, followed by T. lagerheimii (24.35%) and Closterium sp. (16.52%). With MC addition to both AW + MC and AW + BC + MC systems, the top three algal strains were similar and mainly consisted of microalga *Scenedesmus dimorphus* (43.54–48.39%), diatom *Nitzschia* sp. (17.42–21.77%), and *Chlorella* sp. (12.24–13.55%) (Fig. 4a). The relative abundance of microalgae was higher than that of diatoms in all systems (Fig. 4a). Compared with diatoms, microalgae may have a stronger ability to compete for nutrients or grow at a faster rate, which may have been the primary cause of this finding. Considering the relationship between the size of algal-bacterial biofloc and the observed strains of algae, it was discovered that there was no connection between the algal strains and the formation of algal-bacterial biofloc. This could be because EPSs and cell surface charges were involved in the formation of both the algal-bacterial symbiosis biofloc and the microbe-rice bran complexes (Gaignard et al., 2019; Chindanonda et al., 2021; Wang et al., 2022). Additionally, the results indicated that the top three algae strains observed in the system had a stronger affinity for the bacteria in biofloc than the other algal strains. These top three algae strains are capable of producing EPSs (Gaignard et al., 2019; Chindanonda et al., 2021), so may play a role in the interaction between algae and bacteria during algal-bacterial biofloc formation. However, these data cannot be used to demonstrate the existence of an interaction between microalgae and bacteria in biofloc because of the difficulties in observing all algae under a microscope. Therefore, the whole profile of the algae in the algal-bacterial biofloc must be investigated because this information may be useful in a better understanding of the algal-bacterial consortium.

To investigate the impact of bioaugmentation on bacteria community composition, the 16S rRNA gene sequencing technique was used to analyze the composition of the bacteria community at the phylum, class, and genus levels. The results are given in Fig. 4. At the phylum level (Fig. 4b), AW, AW + BC, AW + MC, and AW + BC + MC mostly consisted of *Proteobacteria* (24.53%, 41.22%, 38.85%, and 43.38%), *Bacteroidota* (14.79%, 10.45%, 14.28%, and 10.18%), *Firmicutes* (17.94%, 10.13%, 10.84%, and 20.54%), *Verrucomicrobiota* (17.82%, 4.77%, 4.33%, and 2.28%), *Chloroflexi* (9.39%, 13.21%, 14.05%, and 4.60%), and *Actinobacteriota* (2.97%, 9.40%, 3.94%, and 3.84%, respectively). *Proteobacteria* were the most numerous species in all of the systems studied, implying that the aeration conditions used in this study were more conducive to the growth of *Proteobacteria*, which may play an important role during algal-bacterial biofloc formation. Zhao et al. (2018) identified a correlation between *Proteobacteria* and green algae, which both increased in this study. *Proteobacteria* increased by 37–44% when BC and/or MC were added compared with the non-bioaugmentation strategy (Fig. 4b). Although *Bacillus* consortiums belonging to the phylum *Firmicutes* were added in both the AW + BC and AW + BC + MC systems, *Firmicutes* were not the most numerous bacterial species in either system
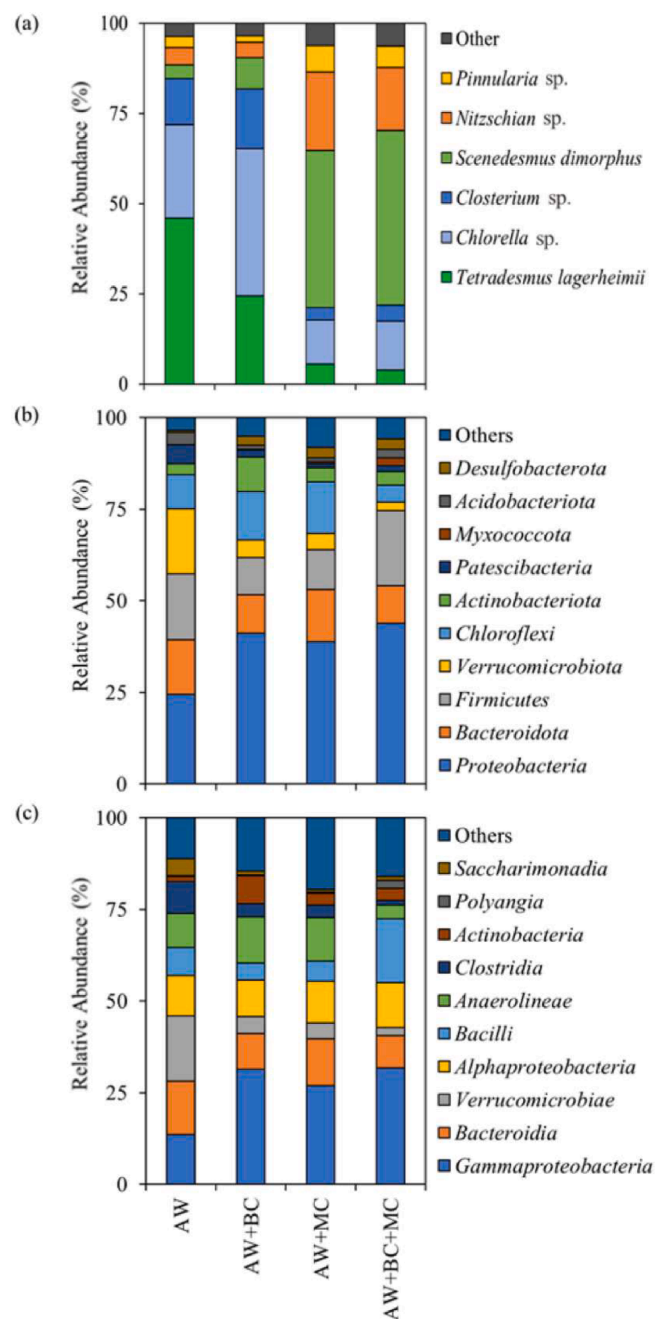

Fig. 4. Composition and relative abundance of algal (a) and bacterial community at (b) phylum level and (c) class level of algal-bacteria biofloc after 15 days of cultivation under different cultivation system (AW: non-bioaugmentation; AW + BC: bacterial bioaugmentation; AW + MC: micro-algal bioaugmentation; AW + BC + MC: dual bioaugmentation).

(Fig. 4b). *Verrucomicrobiota* levels substantially reduced after bioaugmentation (Fig. 4b). It is thought that competition occurred between the algae and bacteria and/or bacteria and bacteria for space and nutrients (Huo et al., 2020). This caused a shift in the bacteria community after bioaugmentation. The phyla *Proteobacteria*, *Bacteroidota*, *Firmicutes*, *Verrucomicrobiota*, *Chloroflexi*, and *Actinobacteriota* were the most often observed in biofloc samples, with variable proportions of each phylum based on the environmental conditions (Addo et al., 2021; Shen et al., 2021).

At the class level (Fig. 4c), the *Gammaproteobacteria*, which are members of the phylum *Proteobacteria*, were the most abundant class in all bioaugmentation systems (AW + BC, AW + MC, and AW + BC + MC),

accounting for 26.97–31.68% of the total, whereas *Verrucomicrobiae* was the dominant class in the non-bioaugmentation system (AW), accounting for 17.80%. The classes *Gammaproteobacteria* and *Alphaproteobacteria* dominated the *Proteobacteria* phylum in this study. *Alphaproteobacteria* (9.90–12.20%) were also present in all the systems (Fig. 4c). Both *Gammaproteobacteria* and *Alphaproteobacteria* are aerobic bacteria (Addo et al., 2021) that were able to grow well in all of the systems used in this study with the aeration used. The higher abundances of *Gammaproteobacteria* and *Alphaproteobacteria* were connected with the EPS content owing to their secretion of EPSs (Shen et al., 2021), which could be inferred from the slightly larger EPS content in bioaugmented than non-bioaugmented systems (Fig. 3b). All bioaugmentation systems showed significant reductions in the levels of *Verrucomicrobiae* belonging to *Verrucomicrobiota* and *Clostridia* belonging to *Firmicutes*, with reductions ranging from 75% to 88% and 60% to 86%, respectively, compared with the non-bioaugmented system (Fig. 4c). The *Bacillus* and/or microalgal consortiums that were added to the systems may have unfavorably affected the growth of *Verrucomicrobiae* and *Clostridia*. Both *Bacillus* and microalgae can release possibly harmful metabolites (Andrić et al., 2020; Kumsiri et al., 2021), which may particularly restrict the colonization of *Verrucomicrobiae* and *Clostridia*. In addition, class *Bacteroidia* (8.93–14.67%), belonging to phylum *Bacteroidota*; *Anaerolineae* (3.88–12.51%), belonging to phylum *Chloroflexi*; and *Actinobacteria* (1.45–7.73%), belonging to phylum *Actinobacteriota* were identified in all systems (Fig. 4c). Some of these bacteria were previously identified as including species producing algal growth agents (Kumsiri et al., 2021), which may have allowed them to proliferate in the systems alongside the algae to create an algal-bacterial biofloc. The presence of the *Anaerolineae* in all systems revealed that the algal-bacterial biofloc developed in this study comprised two layers, with the first layer being aerobic and the second being anaerobic in composition.

3.3. Changes in water quality

The changes in the water quality parameters such as pH, conductivity, turbidity, COD, NH_4^+–N, NO_3^-–N, and PO_4^{3-}–P in the bioaugmented and non-bioaugmented systems with fed-batch supplementation of rice bran and molasses are shown in Table 1. Results demonstrated that aquaculture wastewater and agricultural waste could be potentially used as an alternative nutrient source to promote the growth of both bacteria and algae and to enhance the formation of algal-bacterial biofloc. All systems had a pH that was slightly alkaline. On days 7 and 15, the pH of bioaugmented systems tended to stabilize in the range of 7.4–7.8 and 7.9–8.0, respectively; the pH in the non-bioaugmented system tended to stabilize in the range of 8.2–8.5 on days 7 and 15. A stable pH is beneficial for algal-bacterial biofloc formation (Wang et al., 2019). According to Pacheco-Vega et al. (2018), pH

levels beyond the range of 7.2 to 8.8 may affect the proliferation of bacteria and microalgae. Additionally, pH levels between 6.5 and 8.0 are adequate for algal-bacterial biofloc formation, whereas values less than 7.0 are detrimental to the nitrification process, according to Emerenciano et al. (2017). In terms of turbidity, the values in all systems dramatically increased after 7 and 15 days of biofloc formation, with the ranges of 158–414 and 243–585 FAU, respectively; in all bioaugmented systems, the levels were higher than those in the non-bioaugmented treatment (Table 1). Turbidity is a measurement of the degree to which water loses its clarity as a result of the presence of suspended particles in the water. The rise in turbidity was attributable to algae and bacteria clumping together to create an algal-bacterial biofloc, which led to a remarkable increase in the biofloc yields (Fig. 1 and Table 1). Although turbidity rises as biofloc yields increase, it also decreases the depth to which light can permeate the system. This may restrict the amount of light that can be used for algal pigment production (Kumsiri et al., 2021). As shown in Fig. 2a and Table 1, it was found that the chlorophyll content decreased when the turbidity increased. This might have occurred due to light-limited conditions where algae become entangled in bioflocs. Similarly, Nguyen et al. (2019) found that lower light penetration might cause algal synthesis of pigments, especially chlorophyll, in algae-bacterial biofloc. Light penetration may also be a factor limiting the amount of *Chloroflexi* because these bacteria require light to create energy for their growth. With AW + BC + MC dual bioaugmentation (Fig. 4 and Table 1), the amount of *Chloroflexi* substantially decreased with increasing turbidity. However, comparing the AW + BC and AW + MS systems with the AW (non-bioaugmented) system, these bacterial levels were higher. One explanation for this finding is related to competition for nutrient uptake between *Chloroflexi* and *Bacteroidota*. They generally use the same form of substrate for their proliferation (Ferreira et al., 2021), which implies that the distribution of *Chloroflexi* and *Bacteroidetes* in the algal-bacterial biofloc might be related to water turbidity, light penetration, and nutritional balance.

The electrical conductivity (EC) of water is a characteristic that shows its ability to transfer an electrical current and, as a result, can be used to approximate the concentration of dissolved particles or ions in the water (Kaewthong and Wattanachant, 2018). Comparing day 0 with days 7 and 15, the EC values in all bioaugmentation systems increased up to 665–714 and 744–782 μs/cm, respectively, while the EC values in the non-bioaugmented system on days 0, 7, and 15 were similar and ranged between 445 and 464 μs/cm (Table 1), indicating the impact of bioaugmentation. An increase in the EC values of all bioaugmentation systems revealed more frequent ammonification through the action of the bacteria. Ammonification is sometimes called mineralization, which refers to any chemical process in which organic matter presenting amine or amide groups (R-NH$_2$) is transformed into ammonia or its ionic form, ammonium (NH_4^+), as the end result (Romillac, 2019). The rice bran that was batch-fed as the nutrient supplement in this study may have R-NH$_2$

Table 1
Water quality parameters in bioaugmentations (AW + BC, AW + MC, and AW + BC + MC) and non-bioaugmentation (AW) systems at day 0, day 7, and day 15.

Parameters	Day 0	Day 7				Day 15			
		AW	AW + BC	AW + MC	AW + BC + MC	AW	AW + BC	AW + MC	AW + BC + MC
pH	7.06 ± 0.05[a]	8.20 ± 0.23[b]	7.75 ± 0.11[c]	7.68 ± 0.08[c]	7.46 ± 0.01[c]	8.46 ± 0.04[b]	7.94 ± 0.06[b]	7.99 ± 0.01[b]	7.99 ± 0.01[b]
Conductivity (μs/cm)	454.5 ± 2.1[a]	445.0 ± 7.1[a]	676.5 ± 6.4[b]	713.5 ± 5.0[b]	665.5 ± 7.8[b]	463.5 ± 0.7[a]	744.0 ± 5.7[b]	782.0 ± 2.8[b]	753.0 ± 14.4[b]
Turbidity (FAU)	151.0 ± 36.8[a]	158.0 ± 5.7[a]	406.5 ± 77.5[b]	374.0 ± 65.5[b]	414.5 ± 50.2[b]	243.5 ± 34.7[c]	520.0 ± 34.0[d]	523.0 ± 45.3[d]	584.5 ± 88.4[d]
COD (mg/L)	448.0 ± 73.9[ab]	560.0 ± 22.6[a]	532.0 ± 84.9[a]	512.0 ± 90.5[a]	384.0 ± 45.3[a]	516.0 ± 39.6[a]	704.0 ± 79.2[c]	656.0 ± 56.6[c]	704.0 ± 45.3[c]
Ammonium (mg/L)	0.26 ± 0.01[a]	0.22 ± 0.01[a]	8.24 ± 0.68[b]	11.60 ± 0.34[b]	12.28 ± 1.98[b]	1.28 ± 0.00[a]	14.56 ± 0.91[bc]	14.32 ± 2.94[bc]	8.64 ± 1.47[b]
Nitrate (mg/L)	0.90 ± 0.14[a]	0.90 ± 0.14[a]	1.30 ± 0.14[a]	0.50 ± 0.14[a]	2.70 ± 0.20[b]	1.50 ± 0.71[a]	1.00 ± 0.00[a]	1.50 ± 0.71[a]	2.00 ± 0.00[a]
Phosphate (mg/L)	9.80 ± 1.30[a]	4.79 ± 0.83[b]	8.57 ± 3.42[a]	11.92 ± 0.34[c]	11.12 ± 0.79[c]	7.78 ± 2.83[ab]	11.76 ± 1.81[c]	16.64 ± 0.45[d]	13.36 ± 1.92[cd]

groups that were converted into NH_4^+ by ammonification bacteria, causing both NH_4^+ and EC concentrations to rise. Ferreira et al. (2021) found that *Bacteroidota* are responsible for the ammonification of organic matter. One of the other possible reasons for this finding is that Gram-negative bacteria and some bacteria in the phylum *Proteobacteria* are capable of fixing nitrogen in the air (Ayre et al., 2021; Ferreira et al., 2021). They were able to turn N_2 from the system's continuous air supply in this study into NH_4^+, which led to a rise in the EC. These results imply that *Proteobacteria* and *Bacteroidota* were responsible for the elevation in EC values in all of the bioaugmentation systems through ammonification and nitrogen fixation. These bacteria were the top two bacteria in the algal-bacterial biofloc, accounting for more than half of the species richness, but the abundance of these bacteria was lower by approximately 40% in the non-bioaugmented system (Fig. 4). Additionally, the presence of more NH_4^+ in the systems may have been associated with an increase in EC (Table 1). More recently, Vishwakarma et al. (2022) highlighted that the wastewater from aquaculture sources also contains nitrogenous sources that contribute to NH_4^+ formation. This might be one of the variables that led to an increase in the EC values.

The changes in COD values on days 0, 7, and 15 are shown in Table 1. During algal-bacterial biofloc formation, both algae and bacteria have the ability to absorb oxidizable organic matters found in the systems, functioning as nutrition sources for their growth and resulting in a decrease in COD (Kumsiri et al., 2021). However, it could not assess the capability of the systems to eliminate COD in this study because of the fed-batch supplementation of rice bran and molasses every three days. According to Table 1, the COD value recorded on day 7 were 560, 532, 512, and 384 mg/L in the AW, AW + BC, AW + MC, and AW + BC + MC, respectively; on day 15, they were 516, 704, 656, and 704 mg/L, respectively. An explanation for the lower COD values in the AW + BC + MC system (dual bioaugmentation) on day 7 is that the system had a high organic compound removal capacity. Additionally, the higher COD values in all bioaugmentation systems on day 15 could have occurred due to the excess organic compounds in the systems. Both algae and bacteria have the potential to produce soluble organic compounds such as free EPSs (Wang et al., 2022), which contribute to an increase in COD levels. In addition, aerobic conditions promote the production of a variety of bacterial hydrolytic enzymes, such as amylase and protease, which can lower the fiber content of complex carbohydrates in rice bran and, as a result, increase their organic solubility (Romano et al., 2018). This may have been the primary cause of this increase in COD values. In a microalgal-bacterial system, Wang et al. (2022) found that the dominant bacteria were *Proteobacteria*, *Bacteroidota*, and *Firmicutes*, all of which have a high capacity to use organic compounds. Moreover, the presence of *Bacillus*, belonging to the *Firmicutes*, in the biofloc can promote COD removal (Li et al., 2018). These bacteria are found in the top three species in this study (Fig. 4), which indicated that they were the first bacterium groups to appear in the shifting COD levels, so were responsible for this finding.

During algal-bacterial biofloc formation, changes in the composition of nitrogen compounds affect the diversity of microbes and the synergistic interaction between microalgae and bacteria in the biofloc. In this study, changes in the levels of NH_4^+–N and NO_3^-–N were observed on days 0, 7, and 15, which showed variation between the non- and bioaugmented systems (Table 1). On days 7 and 15, NH_4^+–N and NO_3^-–N concentrations were higher than on day 0. Both algae and bacteria can eliminate NH_4^+–N through three main mechanisms: oxidation to NO_3^-–N and NO_2^-–N, assimilation into biomass (algae and heterotrophic bacteria), and volatilization as NH_3 (Fallahi et al., 2021). Sepehri and Sarrafzadeh (2018) reported that autotrophic bacteria (also known as nitrifying bacteria) conduct nitrification in aerobic conditions, resulting in the production of nitrite and nitrate, whereas heterotrophic bacteria and algae have a strong tendency to absorb ammonium. This indicates that the increase in NH_4^+–N during algal-bacterial biofloc production may not only have been related to ammonification and nitrogen fixation,

but also to an imbalance in the nitrification process. NO_3^-–N is directly absorbed by algae and bacteria via the assimilation route or indirectly through the dissimilation pathway, in which nitrate is transformed to nitrogen gas through the denitrification process (Fallahi et al., 2021). According to Addo et al. (2021) and Wang et al. (2022), the presence of a few groups of *Proteobacteria*, such as *Nitrobacter*, promotes the oxidation of nitrite to nitrate. This might have been the major reason for the increase in NO_3^-–N levels that was observed (Table 1). This proved that there was a wide variation in the nitrogen conversion patterns between the non- and bioaugmented systems, which was most likely caused by changes in the microbial populations that are involved in the nitrogen cycle.

According to Han et al. (2019) and Vishwakarma et al. (2022), in algal-bacterial culture, the C/N ratio might be altered to improve carbon assimilation, which would then promote nitrogen assimilation even more. They recommended maintaining a ratio of C/N in the culture medium or wastewater of around 6.1:1, which is much higher than the ratio of C/N in aquaculture wastewater (Han et al., 2019). Including some nutrients in the wastewater is the simplest way to get a balanced nutritional profile. In a real-world application, solid organics derived from a variety of sources have a better-balanced nutritional profile and become ideal for the development of algal-bacterial biofloc (Romano et al., 2018; Addo et al., 2021).

According to Table 1, PO_4^{3-}–P concentrations ranged from 4.79 to 11.92 and 7.78 to 16.64 mg/L on days 7 and 15, respectively, indicating differences between the bioaugmented and non-bioaugmented systems. In the literature, algal-bacterial consortia were reported to remove PO_4^{3-}–P by assimilation, which includes adsorption and precipitation (Fallahi et al., 2021). At the beginning of the culture phase, PO_4^{3-}–P forms on the cellular surface and then assimilates with the proliferation of the microorganisms (Delgadillo-Mirquez et al., 2016; Fallahi et al., 2021). PO_4^{3-}–P precipitation appears most often at pH 9–11 (Bunce et al., 2018), which is relatively uncommon in algal cultivation (Fallahi et al., 2021). Adsorption was the most important mechanism affecting variations in PO_4^{3-}–P concentrations in this study due to the observed pH level being below 9. In addition, the higher PO_4^{3-}–P concentrations might have occurred because heterotrophic bacteria, known as phosphate-accumulating bacteria, can release and uptake PO_4^{3-}–P, resulting in variability in PO_4^{3-}–P concentrations (Ji et al., 2020).

According to these findings, the establishment of algal-bacterial biofloc and alterations in water quality are both influenced by native microorganisms as well as bioaugmented microbes. Algae may support bacterial nutrient transformation (nitrification, denitrification, and phosphate accumulation), whereas bacteria can stimulate algal nutrient uptake via growth acceleration. The relationship between the microbial population and environmental conditions plays a key role in the nutrient cycle and the consumption of nutrients.

4. Conclusions

Bioaugmentation has the potential to be a strategy to successfully form symbiotic algal-bacterial bioflocs in aquaculture wastewater consisting of native microorganisms compared to non-bioaugmentation. Fed-batch supplementation of molasses and rice bran as alternative nutrient sources and biomass support materials not only helps form symbiotic algal-bacterial bioflocs but also continuously generates microbe-rice bran complexes. Dual bioaugmentation through the consortium addition of probiotic bacteria and microalgae maximized biofloc yields within a well-established microbial community. Microbial community analysis revealed that bioaugmentation altered the configuration of the microbial community as well as the nutrient cycling processes.

CRediT authorship contribution statement

Jeeraporn Pekkoh: Conceptualization, Methodology, Investigation,

Resources, Writing – review & editing, Supervision, Funding acquisition. **Chatchawan Chaichana:** Conceptualization, Investigation, Resources, Writing – review & editing, Supervision, Funding acquisition. **Theera Thurakit:** Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization, Project administration. **Kittiya Phinyo:** Methodology, Software, Validation, Formal analysis, Investigation, Writing – review & editing. **Sureeporn Lomakool:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Khomsan Ruangrit:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Kritsana Duangjan:** Investigation, Writing – review & editing. **Nakarin Suwannarach:** Investigation, Writing – review & editing. **Jaturong Kumla:** Investigation, Writing – review & editing. **Benjamas Cheirsilp:** Investigation, Writing – review & editing, Supervision. **Sirasit Srinuanpan:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2022.127469.

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