



POTENTIAL OF BIOFLOC TECHNOLOGY IN AQUACULTURE WASTEWATER TREATMENT

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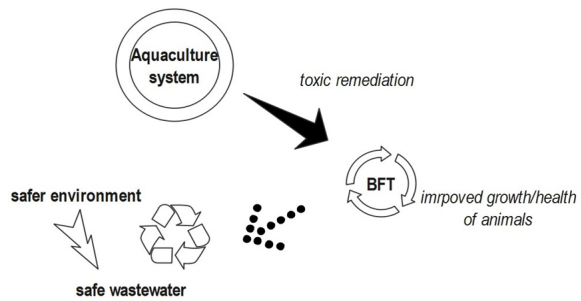
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HIGHLIGHTS

- Biofloc Technology (BFT) can serve as an eco-friendly solution for treating aquaculture wastewater.
- BFT emphasises the role of microbial communities in nutrient absorption and water quality enhancement.
- BFT mitigates toxins through bioleaching and microbial decomposition, contributing to a balanced environment in aquaculture systems.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article History:

Submitted final draft: 19 December 2023

Accepted: 21 December 2023

Published: 15 January 2024

Keywords:

Sustainable practices, water quality, microbial communities, environmental impact, microbial biomass, toxic remediation.

ABSTRACT

The increasing global demand for aquatic products and decline in wild fisheries pose a challenge in achieving the United Nations’ Sustainable Development Goal 14, which is to conserve and sustainably use marine resources. The depletion of fish populations due to overfishing, destruction of aquatic habitats as well as climate change has adversely affected aquatic ecosystems, which leads to further pressure in establishing food security. To meet the rising demand for fish products, countries have turned to aquaculture, but the industry itself faces many environmental challenges, particularly in wastewater management. This review explores the potential of using biofloc technology (BFT) to treat wastewater. BFT utilises microbial ecosystem processes to remove excess nutrients and acts as a natural “cleaning” mechanism. It transforms organic waste into valuable microbial biomass, which enhances water quality and minimises the ecological footprint of aquaculture. In this way, BFT reduces the amount of solid waste generated, increases the level of dissolved oxygen and creates an environment that is less conducive for the growth of harmful bacteria, thus reducing the need for chemical treatments. This paper also discusses the role of BFT in toxic remediation by analysing the nature and composition of aquaculture wastewater. This study provides a comprehensive overview of the mean values for various water quality parameters in aquaculture and biofloc water, and compares them with aquaculture standards.

Introduction

Global efforts are currently dedicated to the conservation and sustainable utilisation of aquatic resources in line with the United Nations' Sustainable Development Goal 14. This goal focuses on the significance of oceans, seas and marine ecosystems in fostering life, biodiversity and economic wellbeing (Duarte *et al.*, 2020; Huck, 2023). However, this goal is threatened by the convergence of factors that lead to a decline in wild catch. The primary impediment to achieving this objective is the escalating global demand for aquatic products, resulting in extensive overfishing. Unregulated and excessive fishing practices have put a strain on fish populations and disrupted the balance of aquatic ecosystems (Bulat *et al.*, 2023; de Moraes *et al.*, 2023). The practices adopted by these fishing activities often result in habitat destruction, further exacerbating the potential of wild fisheries to meet global demand. According to Arostegui *et al.* (2021), unchecked and excessive fishing techniques have depleted fish populations, leading to imbalances in aquatic ecosystems. In addition, climate change has also altered natural systems, causing an increase in sea temperatures, ocean acidification and changing currents. These changes have disrupted the distribution and behaviour of wild stocks and, consequently, their abundance and availability to fisheries.

Aquaculture is the fastest growing food-producing sector worldwide, expanding and intensifying across various regions. Its development is driven by the need to sustain fish supplies in the face of levelling off capture fisheries production (Dong & Dong, 2023). Over the past four decades, aquaculture has significantly increased global fish production, providing a substantial portion of the world's fish for human consumption. Aquaculture accounts for nearly half (45%) of the global supply of fish (Stieglitz *et al.*, 2023). With continued growth, it is expected to surpass wild fisheries in the production of fish for direct human consumption. It is anticipated that the global population will reach approximately nine billion

individuals by 2050; concurrently, there is an increasing demand for protein-rich sustenance. Aquaculture has proven to be a reliable substitute for wild fisheries, as evidenced in recent studies (Engin & Koyuncu, 2023; Khanjani *et al.*, 2023). Concern over declining wild catches has economic implications, focusing on the interconnectedness of aquatic ecosystems and the need for responsible resource management. The aquaculture subsector serves not only as a means to satisfy the increasing demand for aquatic products, but also as a platform for promoting sustainable practices, minimising strain on wild populations, and upholding environmental integrity, as described by Noor and Harun (2022).

Aquaculture production, driven by the increasing demand for food security, has become a major global industry (Verdegem *et al.*, 2023). While it addresses the need for output to meet growing population needs, the growth in aquaculture activities has created concerns about environmental sustainability, particularly in wastewater management. During the culture of fish and other aquatic species, waste accumulates, including unutilised feed, faeces and other organic matter, posing a significant environmental challenge (Elvines *et al.*, 2023). Discharging untreated or poorly treated wastewater into surrounding ecosystems will cause water pollution, habitat degradation and proliferation of harmful algal blooms. Therefore, effective wastewater management is required to mitigate these environmental impacts and ensure long-term sustainability of the industry (Yavuzcan Yıldız & Pulatsü, 2022; Alfeus & Gabriel, 2023). Untreated or poorly treated wastewater contains a variety of pollutants, including excess nutrients, organic matter and potentially harmful chemicals. Furthermore, the discharge of untreated aquaculture wastewater into the environment can introduce pathogens and diseases, posing a risk to wild and farmed aquatic species. This action has led to the spread of diseases, negatively impacting biodiversity and potentially leading

to economic losses in the aquaculture sub-sector (Priyadarshini *et al.*, 2023). The long-term sustainability of the aquaculture industry relies on maintaining a balance between meeting the increasing global demand for aquatic products and safeguarding aquatic ecosystem health.

Biofloctechonology (BFT) is a sustainable and eco-friendly solution for aquaculture wastewater management based on the activities of microbial communities in biofloc. These microorganisms absorb and assimilate excess nutrients, such as nitrogen and phosphorus, acting as a natural filtration system that prevents nutrient build-up, water quality issues and environmental degradation (Anawar & Chowdhury, 2020; Athukorala, 2021). BFT also promotes the conversion of organic wastes, such as uneaten feed and faeces, into valuable microbial biomass. This waste conversion minimises solid waste accumulation in water and produces a protein-rich source useful for cultured aquatic animals as supplementary feed or directly consumed by cultured species (Omitoyin *et al.*, 2009). The presence of bioflocs contributes to improved water quality by reducing suspended solids and maintaining optimal levels of dissolved oxygen, which are crucial for the health and growth of aquatic species, thereby minimising the risk of diseases and stress among cultured organisms. Furthermore, BFT establishes an environment in which beneficial microorganisms outcompete potential pathogens, aid in disease prevention and control, and reduce the need for chemical interventions. By effectively managing wastewater within aquaculture systems, BFT can significantly reduce the environmental impact of aquaculture operations, mitigate the discharge of nutrient-rich water into surrounding ecosystems, and minimise the potential release of untreated wastewater into natural water bodies. This study examines the operational dynamics of BFT in the context of aquaculture wastewater management, while simultaneously considering the feasibility of implementing this technology as a means of promoting sustainable aquaculture production practices.

Water Quality of Aquaculture Wastewater Versus Biofloc Water

The aquaculture industry has rapidly grown since 2000, where the impact of its wastewater has become significant and can adversely affect surrounding ecosystems (Ahmad *et al.*, 2022). The generation of wastewater is predicted at 20.15 m³/kg production/year, based on the average of intensive ponds, extensive ponds and flow-through systems (Tom *et al.*, 2021; Oktaviyani *et al.*, 2023). Wastewater generation also significantly increased in 2010 owing to the expansion of the inland culture system. The volume of wastewater produced by the aquaculture industry is expected to increase in tandem with the expansion of inland aquaculture systems. This substantial wastewater output highlights the urgent need for effective wastewater management practices in the aquaculture sector to mitigate the environmental impact on surface water resources. Over 80% of the global wastewater is produced primarily in low- and lower middle-income countries with minimal treatment before discharge into waterways (Nag *et al.*, 2023).

Table 1 presents the concentrations recorded by some reports on aquaculture wastewater. The wide range of turbidity levels, from 0.85 to 404.00 NTU with a mean of 197.93, indicates varying content of suspended particles, which can affect light penetration in culture systems (Li *et al.*, 2021). The large variations in reported physicochemical parameters show the complex composition of aquaculture effluents (Cheng *et al.*, 2020; Iber *et al.*, 2023). The reported variation in biochemical oxygen demand (BOD) and dissolved oxygen (DO) levels ranged from 1.06 to 317.00 mg/l and 1.32 to 9.60 mg/l, respectively. This portends oxygen depletion as reported by Liu *et al.* (2022) and Han *et al.* (2019). Similarly, there was a wide variation in ammonia concentrations (0.05 to 7.06 mg/l). Kurniawan *et al.* (2020) stated the need for efficient wastewater treatment to prevent toxicity to aquatic organisms and reduce its potential to cause eutrophication in rivers and lakes.

Table 1: Water quality parameters of aquaculture wastewater

Parameters	Scientific Reports									
	(Igwegbe & Onukwuli, 2019)	(Delrue <i>et al.</i> , 2021)	(Omotade <i>et al.</i> , 2019)	(Eddiwan <i>et al.</i> , 2020)	(Nuwansi <i>et al.</i> , 2021)	(Nizam <i>et al.</i> , 2020)	(Jusoh <i>et al.</i> , 2020)	(Nuwansi <i>et al.</i> , 2019)	(Lukwambe <i>et al.</i> , 2018)	(Bennett <i>et al.</i> , 2019)
Turbidity (NTU)	404.00			0.85	91.20	205.00			83.50	
pH	7.90	5.75	6.91	8.51	8.88	8.29	7.80	8.78		6.30
EC ($\mu\text{S}/\text{cm}$)	1963.00		400.00		2630.13	95.73		2883.35		
TDS (mg/l)	695.00				657.80	59.49		641.39		0.41
Chlorides (mg/l)		291.00								
Total hardness (mg/l)					290.15			310.35		
Calcium hardness (mg/l)					102.21			112.31		
Temperature ($^{\circ}\text{C}$)	30.20		27.80	30.31	24.50	27.77	30.00	22.50		
BOD (mg/l)	317.00		46.80			1.06				
DO (mg/l)	9.60		4.17	6.87	1.32	4.63	6.70	1.44		
Nitrate (mg/l)		235.00	8.50	0.12	55.12		0.39	1.33		
Phosphate (mg/l)		96.90	2.50	0.03	12.91	0.35		13.15	0.78	4.50
Ammonia (mg/l)			0.08		1.46	4.20	1.07	1.59	0.78	0.05

Table 2 presents a summary of scientific reports on biofloc water. BFT has been recognised as a promising approach for enhancing water quality, waste treatment and disease prevention in intensive aquaculture systems (Chen *et al.*, 2022). The parameters reported perform a crucial role in determining the effectiveness of BFT in maintaining favourable water conditions for aquaculture. Turbidity and total dissolved solids (TDS) serve as important indicators of water quality, and the reported values fall within the acceptable range for aquaculture systems (Su *et al.*, 2020). The pH levels, ranging from 6.58 to 8.50, are also suitable for aquaculture, as they align with the optimal range for most aquatic organisms (Nisar *et al.*, 2022). The electrical conductivity of 1210.00 $\mu\text{S}/\text{cm}$ indicates the presence of dissolved salts and minerals, which are vital for aquatic life and within the acceptable range for aquaculture (Jamal *et al.*, 2020). The variation in total “hardness” from 47.80 to 1850.00 mg/l may reflect different water sources or treatment methods. While the upper limit is high, it is important to consider the specific requirements of the cultured species, as some species can tolerate higher hardness levels (Sadi *et al.*, 2022). The temperature range of 24.70°C to 30.20°C is suitable for many aquaculture species, falling within the optimal range for growth and metabolism in a biofloc system (Kasan *et al.*, 2021). BOD levels, ranging from 18.40 to 77.75 mg/l, are critical for assessing organic pollution. The lower BOD values indicate good water quality, while the higher values may necessitate further monitoring and management to prevent oxygen depletion (Putra *et al.*, 2020). The dissolved oxygen levels, reported between 4.71 mg/l and 8.60 mg/l, are within the recommended range for most aquaculture species, ensuring adequate oxygen for fish and other organisms (Reinoso *et al.*, 2019). The nitrate concentrations, varying from 0.29 mg/l to 82.40 mg/l, and the range of ammonia levels from 0.01 mg/l to 1.90 mg/l, are important for assessing nutrient levels and potential toxicity. While the reported values cover a wide range, they provide insights into the nutrient dynamics

within the aquaculture system and the efficiency of BFT in nutrient management (Kurniawati *et al.*, 2021).

Table 3 presents a mean comparison of some quality parameters from aquaculture wastewater and biofloc water, as well as the recommended standards. The turbidity of both aquaculture wastewater and biofloc water exceeded the acceptable range, while the pH levels were suitable. The electrical conductivity also exceeded the limits for both sources. The total dissolved solids fell within the recommended ranges and the temperatures were within the recommended range. The BOD was higher in aquaculture wastewater, and nitrate levels exceeded standards in wastewater, but were within limits in biofloc aquaculture. Phosphate levels in biofloc exceeded the lower limit, but were below the upper limit, and ammonia levels exceeded their respective recommended standards in both wastewater and biofloc water.

Nature and Composition of Aquaculture Wastewater

Aquaculture waste consists of suspended solids, organic matter, nitrogen and phosphorus compounds, whose concentrations vary based on the type of system, feed quality and water exchange rates (Jaganathan *et al.*, 2022; Nagaraju *et al.*, 2023). The suspended solids arise from unconsumed feed, faeces of cultured organisms and remains of deceased organisms. The quantity of these waste components is indicated by the water’s BOD or chemical oxygen demand (COD). The degradation of organic waste typically results in the production of nitrogenous compounds, including ammonia, nitrite, nitrate and organic nitrogen. These compounds can cause water toxicity, eutrophication and acidification, as noted in a study by Ji *et al.* (2023). Phosphorus compounds, on the other hand, come from phospholipids and nucleic acids. Apart from influencing the breakdown of these organic materials, the respiration of aquatic organisms generates carbon dioxide, which influences water pH, alkalinity and dissolved oxygen in the aquaculture system. Several authors have reported that growth hormones,

Table 2: Water quality parameters of biofloc water

Parameters	Scientific Reports									
	(Ray & Lotz, 2017)	(Valenzuela-Jiménez <i>et al.</i> , 2021)	(Kamena <i>et al.</i> , 2022)	(AftabUddin <i>et al.</i> , 2020)	(Fischer <i>et al.</i> , 2020)	(Hosain <i>et al.</i> , 2021)	(Mansour <i>et al.</i> , 2022)	(Tom <i>et al.</i> , 2021)	(Lima <i>et al.</i> , 2021)	(Kavitha <i>et al.</i> , 2017)
Turbidity (NTU)	90.10									
pH	7.70	7.40	8.50	7.59	7.95	7.28	7.65	7.60	7.86	6.58
EC ($\mu\text{S}/\text{cm}$)							1210.00			
TDS (mg/l)		451.20								
Chlorides (mg/l)										
Total hardness (mg/l)			1850.00					47.80		
Calcium hardness (mg/l)										
Temperature ($^{\circ}\text{C}$)	29.10	26.50	29.00	27.60	24.70	27.88	28.24		30.20	27.50
BOD (mg/l)									18.40	77.75
DO (mg/l)	6.40	7.10	8.60	5.78	7.60	6.15		6.70	4.71	6.98
Nitrate (mg/l)	39.30	82.40	50.00	1.89		42.33	0.29		10.80	
Phosphate (mg/l)								2.08		
Ammonia (mg/l)	0.10	0.40	0.05	0.43	0.69		0.09	0.01	1.90	

Table 3: Values of aquaculture wastewater and biofloc water and standards for aquaculture

Parameters	Wastewater		Aquaculture Standards	
	Aquaculture Effluent	Biofloc Water	Lower Limit	Upper Limit
Turbidity (NTU)	197.93	90.10		25.00
pH	7.68	7.61	6.50	9.00
EC (μ S/cm)	1594.44	1210.00		50.00
TDS (mg/l)	424.35	451.20	500.00	1000.00
Chlorides (mg/l)	291.00			
Total hardness (mg/l)	300.25	948.90		
Calcium hardness (mg/l)	107.26			
Temperature ($^{\circ}$ C)	27.58	27.86	27.00	31.00
BOD (mg/l)	121.62	48.08		
DO (mg/l)	4.96	6.67	6.00	
Nitrate (mg/l)	50.08	28.38		7.00
Phosphate (mg/l)	13.43	2.08	0.10	0.20
Ammonia (mg/l)	7.06	0.46		0.30

antibiotics and feed additives are deleterious substances that are harmful to the environment when discharged as aquaculture waste.

Feed is the primary source of waste in aquaculture. The amount of supplemental feed proportionally affects waste production (Sharma *et al.*, 2022). Various factors influence waste generation in aquaculture water, including nutrient composition, flotation, feed size relative to fish size, feed quantity per unit time, feeding method and storage duration. The feeding method can differ according to the type of culture used. In a comprehensive system, fish primarily depend on naturally occurring organisms for nourishment, which is complemented by fertilisation of the pond. However, this outdoor cultivation method has failed to meet the current demand for aquaculture products. Semi-intensive culture, which involves moderate to high stocking densities and utilises a combination of natural food production and supplemental feed, relies on partial feeding. Modern intensive aquaculture systems rely on high-quality artificial feed optimised for rapid growth.

Despite the stringent regulation of chemical usage in contemporary aquaculture, certain substances, including medications, disinfectants and antifoulants continue to be used. Medication, including antibiotics, anaesthetics, ectoparasiticides, endoparasiticides and vaccines are administered to treat and control parasites (internal and external) and microbial infections (Jima & Megersa, 2018). Salts are also used to reduce stress in fish, whereas lime is applied to treat pond bottoms for acidity during pond preparation. However, pathogens represent a less frequently considered form of waste in aquaculture systems, particularly when present at concentrations below those detrimental to cultured fish (Dauda *et al.*, 2019; Ojha *et al.*, 2022). Nevertheless, the discharge of pathogens from wastewater can negatively affect aquatic organisms in natural water bodies. These water bodies harbour pathogen loads and additional inputs from fish culture systems may induce stress or mortality in fish stocks.

Solid waste primarily arises from uneaten feed and faecal matter of cultivated fish. At times, the catch may include fish that died

during the rearing process (Chiquito-Contreras *et al.*, 2022). Solid waste can be subdivided into suspended and settled solids. Suspended solids comprise fine particles that remain buoyant in water and pose the most significant challenge in terms of removal from culture systems unless coagulation or sedimentation techniques are employed (Boyd, 2020). Settled solids consist of larger particles that descend to the bottom within a short period and can be readily eliminated from the culture column. Solid waste has been identified as the most hazardous in fish culture systems, and it warrants prompt and effective removal (Markande *et al.*, 2016). Under optimal management practices, including proper feed storage, efficient feeding and utilisation of appropriate feed sizes, approximately 30% of feed is transformed into solid waste (Henriksson *et al.*, 2019; Asiri & Chu, 2020). This proportion depends on the type of culture system because solid waste removal is more readily accomplished in recirculating aquaculture systems than in flow-through systems. Solid waste poses a significant threat as it can obstruct fish gills which leads to mortality, particularly in the case of large settled particles (Saha & Azam, 2021). Prolonged retention and decomposition of these wastes will contribute to increased total suspended and dissolved solids. Furthermore, they may increase nitrogenous compounds and induce stress in cultivated fish. When solid waste accumulates within an aquaculture system, aerobic bacterial activity intensifies, augmenting COD, BOD and depletion of oxygen in the culture column.

Mechanism of Toxic Remediation Using Biofloc Technology

The remediation of toxins in aquaculture systems through the application of BFT is achieved by harnessing the inherent capabilities of microbial communities to neutralise and detoxify harmful substances (Abakari *et al.*, 2022; Da Silva *et al.*, 2023). BFT employs the metabolic processes of microorganisms to effectively detoxify a broad spectrum of pollutants such as ammonia, nitrites, heavy metals and organic contaminants. Figure 1 presents a schematic illustration of the remediation processes enhanced by BFT.

The use of BFT in aquaculture has gained significant attention because of its potential to promote sustainable and efficient fish farming. One crucial aspect of biofloc systems is water quality management and several processes have contributed to its improvement. Water quality management in a biofloc system is accomplished by bioleaching, microbial decomposition, bioflocculation and biomass formation. These processes collectively contribute to nutrient cycling, suspended solid removal, and the creation of a balanced and productive environment for aquatic organisms.

Bioleaching is a process in which microorganisms release nutrients bound to organic and inorganic matter (Jones & Santini, 2023; Wu *et al.*, 2023). Heterotrophic bacteria in biofloc systems act as efficient bioleachers by secreting enzymes that break down complex compounds into simpler forms. Heterotrophic bacteria such as *Bacillus* spp., *Pseudomonas* spp., *Vibrio* spp. and *Aeromonas* spp. play a critical role in facilitating the degradation of complex organic and inorganic compounds. These bacteria secrete enzymes like proteases, lipases, amylases and cellulases that break down these compounds into simpler forms (Wang, *et al.*, 2022). Through specialised enzymes, *Bacillus* species can degrade uneaten feed, faeces and detritus, releasing essential nutrients, such as nitrogen and phosphorus, into the water (Zheng *et al.*, 2020). Similarly, *Pseudomonas*, *Vibrio* and *Aeromonas* enhance nutrient cycling by breaking down proteins, lipids, carbohydrates and cellulose, thereby promoting water quality improvement in biofloc systems. The integrated enzymatic activities of these bacteria release essential nutrients, such as nitrogen and phosphorus, making them readily available for uptake by cultured organisms.

Microbial decomposition is important to maintain the nitrogen cycle in biofloc systems. The nitrogen cycle in biofloc systems involves a diverse array of bacteria that are responsible for ammonification, nitrification and denitrification. Ammonification, which involves the conversion of organic nitrogen compounds into ammonia

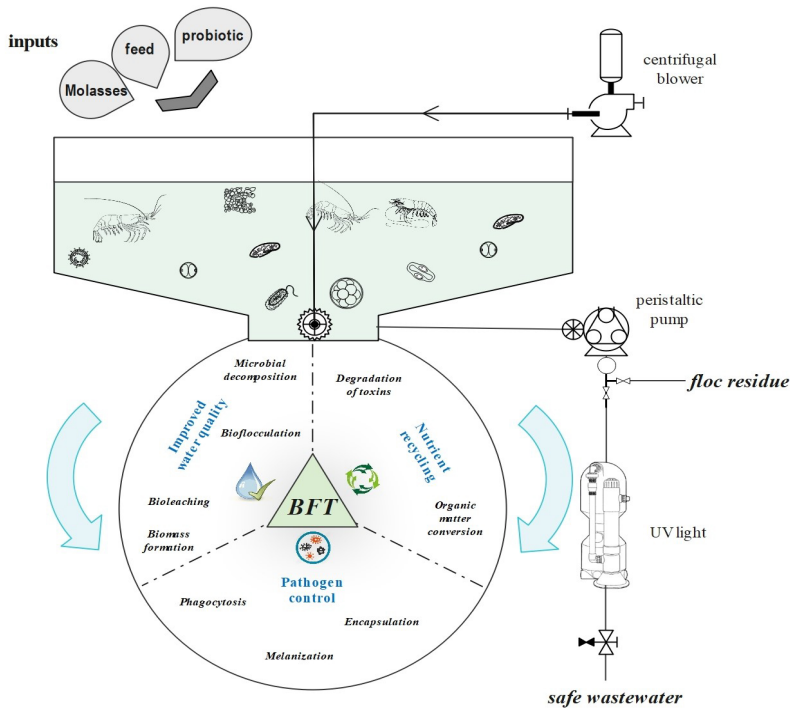


Figure 1: Schematic illustration of the toxic remediation processes in BFT

or ammonium ions, is aided by certain bacteria, such as *Bacillus*, *Clostridium* and *Actinomyces* (Shen *et al.*, 2022). Nitrification is controlled by ammonia-oxidising bacteria (AOB) (*Nitrosomonas* and *Nitrosococcus*), which convert ammonia to nitrite, while nitrite-oxidising bacteria (NOB) (*Nitrobacter* and *Nitrospira*) further oxidise nitrite into nitrate. Denitrification, which occurs under anaerobic conditions, involves *Pseudomonas*, *Paracoccus* and *Bacillus* bacteria, which convert nitrate into nitrogen gas or nitrous oxide. Ammonifying bacteria convert organic nitrogenous compounds into ammonia, which is subsequently oxidised to nitrites and nitrates by nitrifying bacteria. Furthermore, denitrifying bacteria convert nitrates back into nitrogen gas, thereby completing the cycle.

Pseudomonas spp. reduces nitrates to nitrites and ultimately to nitrogen gas, whereas *Paracoccus* bacteria aids the conversion of nitrate to nitrogen gas. Additionally, denitrifying strains of the genus *Bacillus* play a significant role

in completing the nitrogen cycle by transforming nitrates back into nitrogen gas (Font Nájera *et al.*, 2021). These diverse denitrifying bacteria collectively ensure the effective removal of nitrates, preventing their accumulation, and promoting a balanced nutrient environment in biofloc systems. This microbially driven nitrogen cycle prevents the accumulation of harmful ammonia and nitrite levels, ensuring a stable and safe aquatic environment for cultured organisms.

Biofloculation is a phenomenon in which microorganisms (primarily bacteria) form aggregates or flocs. These flocs condense suspended particles, including uneaten feed, organic matter and pathogens, thereby facilitating their removal from the water column. Biofloc formation is attributed to the production of extracellular polymeric substances (EPS) by certain bacteria (An *et al.*, 2023). EPS acts as a binding agent, causing particles to clump together and settle at the bottom of the system or be removed through mechanical filtration.

Biofloculation not only improves water clarity but also enhances the general water quality by reducing suspended solids and organic load (Boehm *et al.*, 2020). Biofloculation results in the cultivation of diverse microbial communities, along with suspended particles. Microbial biomass comprises bacteria, microalgae and other microorganisms, which contribute to nutrient cycling and water quality improvement. The presence of robust microbial biomass serves multiple purposes, such as providing a supplementary food source for cultured organisms, enhancing water quality through nutrient uptake, and acting as a biological filter by removing excess organic matter.

The reliance of BFT on microbial communities creates innate mechanisms for fighting pathogens. This defence mechanism is mediated by phagocytosis, melanisation and encapsulation. Phagocytosis is a fundamental immune response mechanism in biofloc systems undertaken by heterotrophic bacteria, microalgae and protozoa (Kumar, 2020; Iunes *et al.*, 2021). In this process, specialised cells, often referred to as phagocytes, engulf and digest foreign particles, such as bacteria, viruses and other pathogens. Some of the protozoa involved in phagocytosis are ciliates (*Tetrahymena* and *Paramecium*), amoebae (*Acanthamoeba* and *Hartmannella*) and flagellates (*Euglena*). Ciliates employ cilia to create a feeding current, capturing particles and microorganisms, which are then engulfed into specialised feeding grooves (Abraham *et al.*, 2019). On the other hand, amoebae use pseudopodia, extensions of the cell membrane, to surround and engulf particles or microorganisms, enclosing them in phagosomes for digestion with lysosomal enzymes. Flagellates also exhibit phagocytic activity by forming feeding grooves and transporting engulfed particles to their food vacuoles. While bacteria in biofloc systems contribute to nutrient cycling and organic matter decomposition, the primary phagocytic role is attributed to these protozoa, forming a dynamic microbial community that enhances the overall health and success of the biofloc system in aquaculture settings.

Phagocytosis is initiated around recognised pathogens (phagocytes) in a complex defence mechanism peculiar to the type of immune response, in which the pathogen is attached to the surface receptors of the phagocyte (Lee *et al.*, 2020). The phagocyte experiences structural modifications, extending pseudopodia to completely envelop the pathogen and create a membrane-bound vesicle known as a phagosome. This phagosome then fuses with lysosomes and intracellular organelles that contain an array of digestive enzymes. The release of these enzymes into the phagosome facilitates the breakdown of the structural components of the pathogen (proteins, lipids and nucleic acids) (McEwan, 2017). The resulting degradation yields smaller fragments and releases nutrients that can be utilised by phagocytes or other cells. The remnants of the digested pathogen are either discharged through exocytosis or, in the case of specialised immune cells, are transferred to the cell surface for antigen presentation.

Melanisation is a defence mechanism employed by biofloc systems, involving the synthesis and deposition of melanin, a dark pigment, in response to pathogenic threats. This process is particularly crucial in combating external parasites, fungi and certain bacterial infections. Melanisation is often associated with the activity of cells known as hemocytes, which are found in various aquatic organisms within the biofloc system (Zhang *et al.*, 2022; Lumaquin-Yin *et al.*, 2023). Hemocytes are known to release enzymes that convert phenols into melanin, which is deposited around the pathogen to form a dark capsule to prevent it from spreading in the biofloc system. Melanisation response not only serves as a physical barrier against pathogens, but also activates other immune responses within the system, contributing to a complete defence strategy in BFT (Koike & Yamasaki, 2020). Encapsulation, on the other hand, is a complex defence mechanism in biofloc systems, where foreign particles or pathogens are encapsulated by layers of host cells. This process involves concerted efforts by immune cells, including hemocytes and specialised cells called coelomocytes (Mohajeri *et al.*, 2022).

Capsules were created to surround the detected pathogens to isolate and neutralise the threat. The encapsulation process has proven to be particularly effective in immobilising larger parasites, thereby preventing them from causing harm to the cultured organisms. Additionally, encapsulated pathogens can be targeted by phagocytes for further elimination.

Conclusion

This study illustrates the promising role of BFT as an eco-friendly solution for aquaculture wastewater treatment. BFT harnesses the activities of microbial communities to absorb and assimilate excess nutrients and to convert organic waste into valuable microbial biomass. A comprehensive overview of aquaculture wastewater composition emphasises the diverse nature of waste generated, including suspended solids, organic matter, nitrogen compounds, phosphorus compounds, and potential deleterious substances. The discussion of the mechanism of toxic remediation by BFT elucidates how microbial communities in biofloc systems contribute to nutrient cycling, suspended solid removal, and the creation of a balanced environment. The processes of bioleaching, microbial decomposition, bioflocculation, and innate defence mechanisms against pathogens collectively contribute to the success of BFT in promoting sustainable and efficient fish farming.

Acknowledgements

This work is funded by the Ministry of Higher Education (MOHE), Malaysia, under the Higher Institution Centre of Excellence (HiCoE), Institute of Tropical Aquaculture and Fisheries (AKUATROP) programme [Vot. No. 63933, JPT.S(BPKI) 2000/016/018/015 Jld.3 (23) and Vot. No. 56050, UMT/PPPI/2- 2/5 Jld.2 (24)].

Conflict of Interest

All authors declare that they have no conflicts of interest.

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