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THE IMPORTANCE OF FLOCCULATING AGENTS IN BIOFLOC AQUACULTURE SYSTEM: NEW FLOCCULANTS AND THEIR PERFORMANCE ON WATER QUALITY, SLUDGE PRODUCTION, HEAVY METALS, AND MICROPLASTIC

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Abstract

Biofloc technology (BFT) is a promising new approach that has the potential to balance the needs of increased fish food production and sustainable aquaculture. Of late, there has been growing research on BFT and several reviews have been published that highlight the benefits of this technology. Studies focused on topics, including nitrogen recycling, carbon source selection, probiotics, species selection, stocking, and biofloc-based coupled models. However, the scope of research on BFT is expanding, and it is important to examine the latest findings to identify the future course of action. A recent literature search identified the use of novel flocculating agents in the BFT. The use of novel flocculating agents (such as chitosan) is a new approach to improving water quality in BFT systems. These advances include the development of new flocculants and the development of new biofloc-based feeds and supplements. The benefits of good flocculants in the BFT are as follows, i) improved floc formation, ii) improved water quality and reduced sludge production, iii) removal of heavy metals, iiii) microplastic degradation, and iiiii) improved fish and shrimp growth performance. In this review, the importance of flocculating compounds in the biofloc system are discussed.

Key words: biofloc technology, flocculating agents, chitosan, water quality, sludge production

In the year 2020, global hunger and the prevalence of undernourishment increased from 8.4% to 9.9% (FAO et al., 2021). This makes it even more challenging to achieve the Sustainable Development Goal (SDG) of zero hunger by 2030 (Allen et al., 2018). The growing population also puts a strain on food production systems, which are already facing challenges such as land and resource constraints. One way to meet the growing demand for food is to reduce food waste, but this is only a short-term solution (Van der Goot et al., 2016). In the long term, there is a need to find ways to produce more food in a sustainable way. Aquaculture is one of the most promising food production sectors that have the potential to alleviate global hunger. Out of total global fish production, 156 million tonnes were utilized for human dietary needs, and this will continue to grow to meet the expected global population of 9 billion by 2050 (Godfray et al., 2010). Fish is a good source of dietary protein, especially in developing countries (Nie et al., 2020). In fact, the global aquaculture sector is rapidly expanding at an annual growth rate of 5.2% per year, and it is expected to continue to grow in the coming years (FAO, 2024). One of the biggest concerns is the environmental impact of aquaculture, which can lead to water pollution and the spread of diseases. To address these challenges, there is a need to develop more sustainable aquaculture practices.

Biofloc technology (BFT) is a promising sustainable aquaculture practice that has the potential to address many of the challenges facing the aquaculture sector (Khanjani et al., 2024 a). BFT uses microorganisms to convert waste nutrients into food for fish and other aquatic organisms. This can help to improve water quality and reduce the environmental impact of aquaculture (Khanjani et al., 2025 b).

Biofloc is a matrix of bacteria, diatoms, phytoplankton, dead creatures, faeces, and polymeric compounds (Devi and Kurup, 2015). But because inappropriate floc development might occur, the traditional floc formation procedure can take many weeks or even crash. To address this difficulty and speed up floc formation, researchers use flocculating chemicals (Chutia et al., 2022). This is because flocculating agents can function as the surface area of microorganisms that produce flocs, as observed in several studies employing clay mineral (Xavier et al., 2022) and chitosan (Yunos et al., 2017; Chutia et al., 2022). Adding chitosan increased floc size by attaching microbes and other substances to its surface. However, optimal flocculating agent dosages prevent floc development crashes or no flocculation (Chutia et al., 2022). For instance, adding 30 ppm of chitosan (flocculating agent) increased floc volume, while adding more than 30 ppm decreased it due to its anti-microbial properties, which kill bacteria and other microbes. In biofloc systems, flocculating agents increase floc development and maintain water quality. The biofloc, on the other hand, uses very little flocculating chemicals, therefore there's room for research in this field. Thus, in this review, the importance of flocculating compounds in the biofloc system are discussed.

Biofloc technology: concept and advantages

BFT systems are typically operated in tanks or ponds that are enriched with organic matter, such as fish feed, plant extracts, or manure (Khanjani et al., 2022). This organic matter provides a food source for a variety of microorganisms, including bacteria, algae, and protozoa (Khanjani et al., 2024 a). These microorganisms form flocs, which are aggregates of cells that are suspended in the water. The flocs provide a habitat for the microorganisms and also serve as a food source for the fish (Emerenciano et al., 2025).

As a result, i) BFT improved water quality: The microorganisms in BFT systems help to consume waste nutrients, such as ammonia and nitrite, which can lead to water quality problems in traditional aquaculture systems. This can help to reduce the need for water exchange, which can save water and energy (Avnimelech, 1999; De Schryver et al., 2008; Khanjani and Alizadeh, 2024). ii) Reduced environmental impact: BFT systems can help to reduce the environmental impact of aquaculture by reducing the amount of waste that is produced. The waste nutrients that are consumed by the microorganisms in BFT systems are converted into biomass, which can be used as a food source for the fish or can be removed from the system and used as fertilizer (Khanjani and Sharifinia, 2024). iii) Increased production: BFT systems have been shown to improve the growth and survival rates of fish. This is likely due to the fact that the flocs provide a high-quality food source for the fish and also help to improve water quality (Khanjani et al., 2022). iiii) Reduced feed costs: The use of BFT systems can help to reduce feed costs. This is because the flocs provide a high-quality food source for the fish, which can reduce the amount of commercial feed that is needed (Khanjani et al., 2025 a). iiiii) Enhanced disease resistance: The microorganisms in BFT systems can help to enhance the disease resistance of fish. This is because the microorganisms produce antimicrobial compounds that can help to protect the fish from disease-causing bacteria (Khanjani et al., 2024 a, c).

Biofloc and flocculating agents

While the primary focus of BFT is on maintaining optimal water quality in a high-stocking, zero-water-exchange scenario, the formation of microbial flocs is essential for the system to function properly. There is a wealth of literature on various aspects of water quality in biofloc systems, but this section will focus on how the application of novel flocculating agents reported in the literature can influence floc formation.

In BFT systems, nutrients are recycled by maintaining a high C/N ratio. This promotes the growth of heterotrophic bacteria, which reduce the amount of ammonia in the culture system and generate new microbial biomass. The bacteria then aggregate with other natural microbial communities, particles floating in the water column, and themselves to form flocs. These flocs are made up of a variety of substances, *i.e.* polysaccharides (Avnimelech, 2009; Sears et al., 2006), proteins (Kuhn et al., 2010), humic substances (Basuvaraj et al., 2015), nucleic acids (Salehizadeh and Shojaosadati, 2001), and lipids (Wu et al., 2017).

Bioflocculation is a flocculation mechanism that occurs as a result of microorganisms synthesizing and secreting flocculants. A number of factors have an impact on flocculant formation and the bioflocculation process. Some of these include: the microorganism's genotype, physiological factors (Salehizadeh and Shojaosadati, 2001), and environmental factors (Wu et al., 2017). Microbial flocs are formed through a number of different mechanisms. One important mechanism is bacterial adhesion. Bacteria can produce extracellular polymeric substances (EPS) that allow them to adhere to each other and to other surfaces. These EPS can also act as a bridging agent, bringing together other particles and forming flocs (Mishra, 2016). Microbial flocs are aggregates of microorganisms that have formed through the process of flocculation (Li et al., 2020). They are typically composed of bacteria, algae, and protozoa, and they can be found in a variety of environments, including wastewater treatment plants, soil, and the human gut (Chahal et al., 2016). Under nitrogen limitation, flocs are mostly formed as slime or capsule layers. When the conditions are right, biofloc aggregates can range in size from microscopic to more than 1 mm, which is comparable to the majority of commercial fish pellets for small fish. The microbial biomass has typical densities of slightly more than 1.0 g wet weight per ml of floc aggregate, resulting in slow-sinking particles (1–3 m/hour) in the bioflocs.

Another important mechanism in the formation of microbial flocs is algae aggregation. Algae can also produce extracellular polymeric substances (EPS), which can help them to aggregate and form flocs (Zhang et al., 2019). In addition, algae can produce extracellular enzymes that can break down organic matter, releasing dissolved organic carbon (DOC). This DOC can then be used by bacteria as a source of energy, further promoting the formation of microbial flocs (Majhi et al., 2023).

Flocculation is the process of bringing together small particles to form larger aggregates, or flocs. This can be done naturally, through the action of microorganisms, or artificially, with the use of flocculating agents (Lee et al., 2014). Flocculants are typically synthetic polymers, but they can also be natural substances, such as microbial flocs (Lee et al., 2014). Flocculants work by bridging between two or more particles, creating a network that holds the particles together. This network can be physical, in the case of synthetic polymers, or it can be biological, in the case of microbial flocs. The type of flocculant that is used will depend on the specific application. For example, synthetic polymers are often used in wastewater treatment (Saravanan et al., 2022), while microbial flocs are often used in soil remediation (Ying and Wei, 2019).

Flocculation is a complex process that involves a number of different mechanisms. One important mechanism is electrostatic attraction. When two particles have opposite charges, they will be attracted to each other. This is the main mechanism that is responsible for the flocculation of suspended solids in water. Flocculants are typically made up of macromolecules like polysaccharides, proteins, or other polysaccharide-protein complexes. The carbohydrate components of polysaccharides are primarily glucose, galactose, and mannitose. As a result, they have excellent thermal stability. The benefits of suitable flocculants in the biofloc system include: i) improved water quality (Basuvaraj et al., 2015), ii) increased nutrient removal (Avnimelech, 2009), iii) reduced sludge production (Salehizadeh and Shojaosadati, 2001), iiii) improved growth performance of fish and shrimp (Kuhn et al., 2010), and iiiii) increased biosecurity (Khanjani et al., 2024 c).

Types of flocculants

Flocculation is a process by which colloidal particles come out of suspension to sediment in the form of floc or flake, either spontaneously or due to the addition of a clarifying agent. The action differs from precipitation in that, prior to flocculation, colloids are merely suspended, under the form of a stable dispersion (where the internal phase (solid) is dispersed throughout the external phase (fluid) through mechanical agitation) and are not truly dissolved in solution (Agunbiade et al., 2016). There are three main types of flocculants: inorganic, organic and specialty.

Inorganic flocculants are chemical compounds that are used to clump together (flocculate) small particles in water (Maćczak et al., 2020). This can be used to remove suspended solids, improve water clarity, and control algae growth. In biofloc technology, inorganic flocculants are used to help aggregate the biofloc, which is a mixture of living and non-living matter that is produced by the fish and other organisms in the system. The biofloc provides a food source for the fish and helps to improve water quality. According to the study by Wu et al. (2017) some of the most common inorganic flocculants used in BFT are:

a. Aluminum sulfate (alum) is a cheap and effective flocculant that is widely available. It is a metal salt that reacts with water to form positively charged ions. These ions bind to negatively charged particles in the water, causing them to clump together.
b. Ferric chloride is another metal salt that is commonly used as a flocculant. It is more expensive than alum, but it is also more effective. Ferric chloride reacts with water to form positively charged iron ions. These ions bind to negatively charged particles in the water, causing them to clump together.
c. Ferric sulphate is a less common flocculant that is made from iron and sulphur. It is similar to ferric chloride, but it is not as effective.

Inorganic flocculants can be used in biofloc systems to improve water quality and promote the growth of biofloc. However, they should be used with caution, as they can also kill beneficial bacteria and algae. The dosage of flocculant should be carefully controlled to avoid these problems. Some of the most common specialty flocculants are included in Table 1.

Key properties of the selected flocculants

a. Polyacrylamide (PAM): PAM is the most common organic flocculant used in biofloc technology. It is effective at removing a wide variety of pollutants, and it is relatively inexpensive.
b. Polyethyleneimine (PEI): PEI is a more effective flocculant than PAM at removing small particles and heavy metals. However, it is also more expensive.
c. Polyvinylamine (PVA): PVA is a non-ionic flocculant that is effective at removing both positively and negatively charged particles. It is relatively inexpensive and non-toxic.
d. Chitosan: Chitosan is a natural flocculant that is derived from chitin, which is a substance found in the shells of crabs and shrimp. Chitosan is effective at removing a variety of pollutants, and it is biodegradable and non-toxic (Pellis et al., 2022).

Table 1. A comparative study of potential flocculant types for use in BFT

Type	Example	Characteristics	Reference
Inorganic	Aluminium sulphate, ferric chloride, ferric sulphate	Effective, inexpensive, corrosive, can leave behind residual chemicals	Agunbiade et al. (2016)
Organic	Polyacrylamide, polyacrylic acid	Less corrosive than inorganic flocculants, do not leave behind residual chemicals, can be more expensive	Xia et al. (2017)
Specialty	Designed for specific applications, such as wastewater treatment or mining	Can be more effective or more cost-effective than general-purpose flocculants	Kurniawan et al. (2023)

e. Specialty flocculants: Specialty flocculants are a type of flocculant that is designed for specific applications. They are often more expensive than traditional flocculants, but they can provide better performance in certain situations.

There are three main mechanisms by which flocculants work:

a. Charge neutralization is the most common mechanism by which inorganic flocculants work. In this mechanism, the flocculant neutralizes the charge on colloidal particles, which causes them to aggregate (Cruz et al., 2020).
b. Electrostatic patch is a mechanism by which some organic flocculants work. In this mechanism, the flocculant attaches to the surface of colloidal particles, creating a patch of opposite charge. This patch attracts particles with the same charge, causing them to aggregate (Czemierska et al., 2015).
c. Polymer bridging is a mechanism by which most organic flocculants work. In this mechanism, the flocculant forms a network of polymer chains that traps colloidal particles. This network of polymer chains causes the particles to aggregate (Czemierska et al., 2015).

The water containing bioflocculant consists of a higher level of macromolecules, such as proteins, nucleic acids, polysaccharides, etc. (Wu et al., 2017). Chemical coagulants, such as metallic salts and natural biopolymers, have been extensively used for wastewater treatment due to their outstanding capabilities (Ebeling et al., 2003; Lee et al., 2014; Dauda et al., 2019). However, chemical coagulants are not biodegradable, generate toxic sludge, and significantly increase the retention of metallic ions in water, making their usage hazardous and potentially harmful (El Samrani et al., 2008).

Bioflocculants are a class of natural polymers that can be used as an efficient substitute for various synthetic flocculants. They are produced by microorganisms, such as bacteria and algae, and are therefore biodegradable and environmentally friendly. Bioflocculants are also more effective than chemical flocculants in removing a wide range of pollutants, including heavy metals, organic matter, and bacteria (Carneiro-Marra et al., 2019; Picos-Corrales et al., 2020) A comprehensive list of potential bioflocculants for flocculation is detailed in Tables 2 and 3.

Chitosan as novel flocculating agent

Chitosan-based bioflocculation is a process that uses chitosan, a natural polysaccharide, to bind insoluble particles and dissolved organic matter in wastewater (Lichtfouse et al., 2019). The resulting aggregates can then be separated using sedimentation and filtration.

Table 2. Bioflocculants used in wastewater treatment at various concentrations and having potential to be used in BFT

Source of flocculants	Effective concentration	References
Abelmoschus esculentus	80 mg/L	Carneiro-Marra et al. (2019)
Banana juice	90 mL	Gautam and Saini (2020)
Bitter gourd seed	400 mg/L	Babarao and Verma (2015)
Cactus	0.4 g/L	Deshmukh and Hedaoo (2018)
Castor oil cake	40 mL	Sheikh et al. (2016)
Cicer arietinum	0.1 g/L	Ferrari et al. (2016)
Cicer arietinum	68 mg/L	Laksmi et al. (2017)
Cotton seed oil cake	40 g/L	Narmatha et al. (2017)
Dolichos lablab	450 mg/L	Daverey et al. (2019); Laksmi et al. (2017)
Hibiscus rosa sinensi	500 mg/L	Awang and Aziz (2012)
Malva sylvestris (mallow)	12 mg/L	Anastasakis et al. (2009)
Moringa oleifera	1 g/L; 9.4 mL/ L	Chonde and Raut (2017); de Paula et al. (2018)
Ocimum basilicum	1.6 mg/L	Shamsnejati et al. (2015)
Opuntia mucilage	21.1 mg/L	Carpinteyro-Urban et al. (2012)
Plantago major L.	297.6 mg/L	Gautam and Saini (2020)
Plantago ovata	1.5 mg/L	Ramavandi and Farjadfard (2016); Mishra and Bajpai (2005)
Psyllium husk	0.4 g/L and 7.2 g/L	Gautam and Saini (2020)
Sechium edule (chayote)	15 mg/L	Almeida et al. (2017)
Strychno spotatorum	60 mL	Dehghani and Alizadeh (2016)
Surjana seed	30 g/L	Patel and Vashi (2013)
Tamarind indica	1250 mg/L	Laksmi et al. (2017)
Tanfloc SG	20 mg/L	Gautam and Saini (2020)
Trigonella foenum- graecus	0.1 g/L	Patil and Hugar (2015)
Zea mays	30 g/L	Patel and Vashi (2013)

Table 3. Chemical coagulants utilized for wastewater treatment

Coagulants	Chemical formula	Concentration	References
Aluminium sulphate	$Al_2(SO_4)_3$	7.2 mL/L and 40 mg/L	Akinwole et al. (2016); Dehghani and Alizadeh (2016); de Paula et al. (2018)
Ferric chloride	$FeCl_3$	3.6 mL/L	Sarparastzadeh et al. (2007); de Paula et al. (2018)
Ferric sulphate	$Fe_2(SO_4)_3$	400 mg/L	Babarao and Verma (2015)
Iron (III) chloride hexahydrate	$Cl_3FeH_{12}O_6$	500 mg/L	Gautam and Saini (2020)
Lime	CaO	600 mg/L	Lin et al. (2017)
Magnesium chloride	$MgCl_3$	120 mg/L	Verma et al. (2012)
Poly aluminium chloride (PAC)		500 mg/L	Gautam and Saini (2020)
Poly aluminium ferric chloride (PAFC)		3 mg/L	Gkotsis et al. (2017)
Poly aluminium silicate chloride (PASic)		9 mg/L	Liao and Zhang (2018)
Poly ferric acetate		24 mg/L	Wei et al. (2017)
Poly ferric sulphate		20 mg/L	Wei et al. (2017)
Potassium aluminium sulphate KAl	$(SO_4)_2{\times}12H_2O$	0.25 g/L	Malik (2018)

The bioflocculation process is similar to traditional flocculation, which uses metal salts or other chemicals to destabilize particles and cause them to clump together, however, chitosan-based bioflocculation is a more environmentally friendly process, as it does not use any harsh chemicals (Renault et al., 2009; Lichtfouse et al., 2019). Moreover, it is a versatile and effective method for removing pollutants from wastewater even with high concentrations of organic matter (Lichtfouse et al., 2019).

The bioflocculation process begins with the addition of chitosan to the wastewater. The chitosan chains then interact with the particles and dissolved organic matter in the wastewater, forming microflocs. These microflocs are then aggregated into larger flocs through agitation. The agitation causes the microflocs to collide with each other, and the chitosan chains to bridge between them and these larger flocs can be removed ultimately from the wastewater by sedimentation and filtration (Lichtfouse et al., 2019).

The bioflocculation process can vary significantly depending on the specific wastewater being treated and is influenced by a number of factors, including the type and concentration of chitosan, the pH of the wastewater, and the presence of other ions or molecules in the wastewater (Renault et al., 2009).

Mechanisms of chitosan-based bioflocculation:

- Charge neutralization: The chitosan chains have a positive charge, which can neutralize the negative charge of the particles in the wastewater. This causes the particles to lose their electrostatic repulsion, which allows them to come together and form microflocs (Liang et al., 2023).
- Bridging: The chitosan chains can also bridge between particles, forming physical links between them. This results in the formation of larger flocs (Eamrat et al., 2024).
- Entrapment: The chitosan chains can also entrap particles within their structure. This can further increase the size and stability of the flocs (Morin-Crini et al., 2019; Vidal and Moraes, 2019).
- Complexation: The chitosan chains can also form complexes with dissolved organic matter in the wastewater. This can help to remove the organic matter from the wastewater and improve the efficiency of the bioflocculation process (Hsu et al., 2024).

Overall, chitosan-based bioflocculation is a promising technology for the removal of pollutants from wastewater. It is a more environmentally friendly process than traditional flocculation, and it is effective for treating wastewater with high concentrations of organic matter.

Properties of chitosan as flocculant

The flocculating properties of chitosan are due to its cationic charge and its ability to form hydrogen bonds (Lichtfouse et al., 2019). In acidic media, the amino groups of chitosan are protonated, giving it a positive charge (Harugade et al., 2023). This positive charge allows chitosan to interact with negatively charged particles, such as clay, suspended solids, and colloidal particles. The hydrogen bonding between the amino groups of chitosan and the hydroxyl groups of other molecules also contributes to the flocculating ability of chitosan (Lichtfouse et al., 2019). The flocculating efficiency of chitosan is affected by a number of factors, including the molecular weight, the degree of deacetylation, the pH, and the ionic strength of the solution (Rahangdale et al., 2019). In general, chitosan with a higher molecular weight and a higher degree of deacetylation is more effective as a flocculant (Li et al., 2013). The pH of the solution also plays an important role, with chitosan being more effective at a pH below its isoelectric point (Roussy et al., 2005). The ionic strength of the solution can also affect the flocculating efficiency of chitosan, with higher ionic strengths reducing the efficiency of chitosan (Younis et al., 2019). Chitosan is a versatile biopolymer with a number of properties that make it an attractive flocculant for a variety of applications (Piekarska et al., 2023). Its cationic charge, its ability to form hydrogen bonds, and its high molecular weight and degree of deacetylation all contribute to its flocculating efficiency. Chitosan has been used successfully in a variety of applications, including water treatment, wastewater treatment, food processing, papermaking, and textiles (Zhang et al., 2019).

The formation of microbial flocs in biofloc systems is a complex process involving numerous physical, chemical, and biological events (Avnimelech, 2009). This can lead to a slow initial development of biofloc, and even once the biofloc is established, the system may still fail due to a lack of sustainable floc development (Figure 1).

However, many flocculating compounds have been shown to be effective in accelerating floc formation and improving floc quality. One such compound is chitosan, which is a naturally abundant polymer that is a deacetylated product of chitin. Chitosan has many beneficial properties, including biodegradability, biocompatibility, non-toxicity, and the ability to aggressively bind microbial cells. It also has a high hydrophobicity, which means it is soluble in acidic solutions but insoluble in water and most organic solvents.

In a study by Yunos et al. (2017), the efficacy of coagulation-flocculation utilizing chitosan as a natural biocoagulant to extract *Chlorella* sp. was investigated. The results showed that chitosan was effective in coagulating and flocculating *Chlorella* sp., with the highest flocculation activity being observed at a chitosan concentration of 30 mg/L.

Another study by Chutia et al. (2022) found that chitosan can also be used to improve the flocculation activity of bioflocs. In their study, they added different concentrations of chitosan (0, 10, 20, and 30 mg/L) to biofloc systems and found that the treatment with the highest concentration of chitosan (30 mg/L) had the maximum flocculation activity. They also found that chitosan was able to increase the size of bioflocs and reduce protozoan grazing, which can lead to improved floc utilization by fish.

Chitosan has been shown to be effective in the harvesting of microalgae, such as *Chlorella* sp. For example, in a study by Yunos et al. (2017), chitosan was able to achieve a high flocculation performance of 5 NTU of turbidity at a chitosan dosage of 30 mg/L. Other studies have also shown that chitosan can be used to effectively harvest microalgae from a variety of cultures, including wastewater, seawater, and freshwater (Rashid et al., 2013 a).

Overall, chitosan is a promising bio-coagulant for microalgae harvesting (Elcik et al., 2023). It is non-toxic, biodegradable, has a high flocculation ability, and can be used in small doses. These factors make chitosan a sustainable and environmentally friendly alternative to chemical coagulants for microalgae harvesting.

The benefits of good flocculants in the BFT

Improved floc formation

The flocculating processes in BFT systems can destabilize, flocculate, and precipitate colloidal particles. This allows for solid-liquid separation, which is essential for maintaining water quality and removing waste products from the system (Suresh et al., 2018). The flocculating process is initiated by the production of EPS by bacteria. EPS are sticky molecules that can bind to other particles, such as bacteria, algae, and detritus. This binding helps to form flocs, which are aggregates of particles that can be easily separated from the water.

Bioflocculants are a type of flocculant that is produced by microorganisms (Sheng et al., 2006). They are primarily recognized for their aggregating function and electronic neutralization (Padeniya et al., 2022). Aggregating function refers to the ability of bioflocculants to bind to particles and form flocs. Electronic neutralization refers to the ability of bioflocculants to neutralize the electrostatic charges on particles, which can help to promote floc formation. Bioflocculants can improve the system's ability to form floc by binding with biological and non-biological components. By acting as binding agents, bioflocculants also facilitate the floc harvesting process.

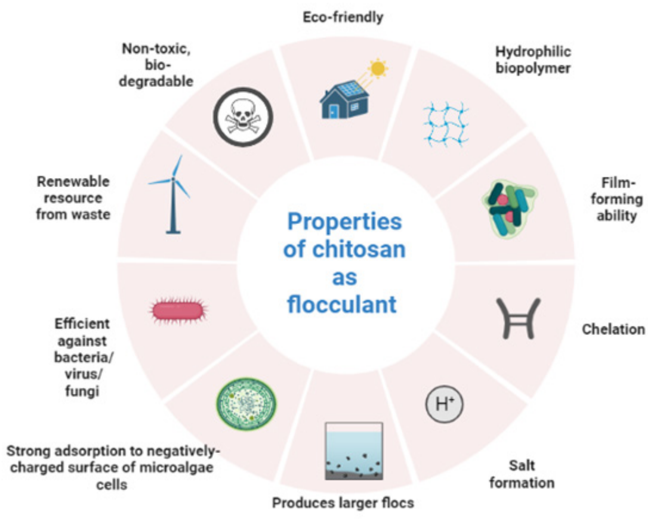

Figure 1. Properties of chitosan as flocculating agent

Improved water quality

Using BFT would enable ammonia toxicity issues to be resolved since increased nitrogen consumption by heterotrophic bacteria speeds up the nitrification process, which guarantees a decrease in the concentration of ammonium in culture systems (Hargreaves, 2006). By binding with uneaten feed and nitrogenous mass the flocculants play a critical role in maintaining the TSS and physical parameters of water.

Bioflocs play a role in maintaining water quality by absorbing suspended solids and nutrients, and they can also be used as a food source for fish. The improvement of water quality by BFT can be attributed to a number of factors, including:

i) The production of EPS: EPS are sticky molecules produced by bacteria. EPS bind to other particles, such as bacteria, algae, and detritus, and form flocs. Flocs are large aggregates of particles that can be easily removed from the water (Deng et al., 2023).

ii) The consumption of ammonia and other nitrogenous waste products: Bacteria in bioflocs can consume ammonia and other nitrogenous waste products. This helps to reduce the levels of these pollutants in the water, which can improve water quality (Jiménez-Ojeda et al., 2018).

iii) The production of oxygen: Photosynthetic algae in bioflocs can produce oxygen. This helps to improve the dissolved oxygen levels in the water, which is important for fish health (Ray et al., 2012).

iiii) The removal of suspended solids. Flocs in biofloc systems can trap suspended solids, such as algae, detritus, and fish faeces. This helps to improve water clarity and reduce the risk of algal blooms (Padeniya et al., 2022).

Furthermore, bioaugmentation via bioflocculants is one of those strategies to enhance the water quality. A study by Pekkoh et al. (2022) found that bioaugmentation with a combination of algae and bacteria could be effective in reducing ammonia toxicity in BFT systems. The study found that the dual-bioaugmentation treatment reduced ammonia concentrations by up to 80%, compared to a control treatment without bioaugmentation. The study also found that the dual-bioaugmentation treatment had no negative effects on fish growth or survival. Overall, BFT can be an effective way to improve water quality in aquaculture systems. By utilizing the natural processes of microbial communities, BFT can help to reduce waste production, improve dissolved oxygen levels, and remove suspended solids.

Potential for removing of heavy metals

The flocculants have the ability to absorb or remove the heavy metal from the system. BFT has been shown to be effective in removing heavy metals from aquaculture systems (Habib et al., 2023). For example, a study by Khanjani et al. (2024 b) found that BFT was effective in removing heavy metals from an aquaculture system. The study found that the concentration of heavy metals in the water of the BFT system was significantly lower than the concentration of heavy metals in the water of a traditional aquaculture system. Flocculants are substances that can cause small particles to clump together, or flocculate. This can be useful for removing heavy metals from water, as the flocculants can bind to the heavy metals and carry them away. One type of flocculant that is effective at removing heavy metals is *Cyanobacteria*. *Cyanobacteria* are a type of bacteria that can produce EPS. These EPS are sticky and can bind to heavy metals, forming flocs that can be easily removed from water. In a study by Bender et al. (1994, 1995), the cyanobacterium *Oscillatoria* sp. was shown to be effective at removing a variety of heavy metals from water, including Mn^{2+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+}, and Fe^{3+}.

Another type of flocculant that is effective at removing heavy metals is Zoogloea. Zoogloea is a type of slime mould that can produce EPS. Various studies have shown that zoogloeal species can remove heavy metals such as cadmium, lead, zinc and nickel (Yang et al., 2022; Nduka and Umeh, 2021), toxic compounds such as tetracycline (Yang et al., 2022; Chang et al., 2021), and nutrients such as nitrogen (Huang et al., 2015), nitrite (Yang et al., 2022; Chang et al., 2021) and phosphorous (Montoya et al., 2008) from the water. Flocculants can be a valuable tool for removing heavy metals from water. They are relatively inexpensive and easy to use, and they can be effective at removing a wide variety of heavy metals.

Microplastic degradation

It is clear that aquaculture has been negatively affected by microplastic (MP) contamination and bioaccumulation. The degradation of MPs is a complex process that can be affected by a number of factors, including the type of plastic, the environmental conditions, and the presence of microorganisms. Microorganisms, such as bacteria and fungi, can play a role in the degradation of MPs. These microorganisms can break down the plastic into smaller molecules, which can then be further degraded by other processes. For example, Shukri et al. (2022) isolated a bacterium, *Bacillus enclensis* having potential to degrade polyethylene, polypropylene, and polystyrene microplastics. The study found that the bioflocculants produced by *B. enclensis* had a flocculating activity of 93%, and they were able to reduce microplastic pollution by up to 80%.

Amini et al. (2022) conducted a review of studies on the biodegradation of MPs by marine bacteria. The study found that a variety of bacteria, including *Pseudomonas* sp., *Bacillus* sp., and *Vibrio* sp., have been shown to be able to degrade MPs. The study also found that the degradation rate of MPs by bacteria can be affected by a number of factors, such as the type of plastic, the concentration of bacteria, and the environmental conditions. Huang et al. (2022) studied the biodegradation of MPs by a marine bacterium, *Pseudomonas* sp. MB4. The study found that *Pseudomonas* sp. MB4 was able to degrade polyethylene MPs at a rate of 20% per day. The study also found that the degradation of MPs by *Pseudomonas* sp. MB4 was enhanced in the presence of oxygen and nutrients. Li et al. (2022) studied the biodegradation of MPs by a marine bacterium, *Bacillus cereus* B-1. The study found that *B. cereus* B-1 was able to degrade polystyrene MPs at a rate of 10% per day. The study also found that the degradation of MPs by *B. cereus* B-1 was enhanced in the presence of oxygen and nutrients. These studies provide evidence that microorganisms can degrade MPs, and they suggest that this is a promising area of research for developing new methods for MP remediation.

Floc harvesting

Floc harvesting has been carried out using a variety of methods, including centrifugation, coagulation-flocculation, filtration, and flotation. The coagulation-flocculation method is thought to be a promising technique for harvesting a large amount of microalgae biomass (Ahmad et al., 2011; Rashid et al., 2013 b).

Coagulation-flocculation is a process that involves the addition of a coagulant to a microalgae suspension, which causes the microalgae cells to flocculate. The flocculated microalgae cells can then be easily separated from the water by sedimentation or filtration. Inorganic salts and other chemical substances are commonly used as coagulants in the coagulation-flocculation process. However, concerns have been raised about the use of these chemical coagulants, as the residues may pose health risks to both humans and aquatic life.

There are a number of different methods for flocs harvesting, including:

i) Sedimentation: Flocs can be allowed to settle out of suspension under gravity. This is the simplest and most energy-efficient method of floc harvesting, but it is not very effective for harvesting small flocs or flocs that are suspended in a high concentration of solids (Asadi et al., 2021).

ii) Centrifugation: Flocs can be separated from water by spinning them in a centrifuge. This is a more effective method than sedimentation for harvesting small flocs, but it is also more energy-intensive (Matter et al., 2019).

iii) Filtration: Flocs can be filtered out of water using a variety of filters, including membrane filters, diatomaceous earth filters, and sand filters. This is a very effective method of floc harvesting, but it can be expensive to set up and operate (Cescon and Jiang, 2020).

iiii) Flocculation: Flocs can be formed by adding a flocculant to water. A flocculant is a substance that causes particles to aggregate. Once the flocs have formed, they can be harvested using any of the methods described above (Gregory, 2013). The choice of floc harvesting method will depend on a number of factors, including the size of the flocs, the concentration of solids in the water, the desired efficiency of the process, and the cost of the equipment. Flocs are also important in wastewater treatment. In the secondary stage of wastewater treatment, flocs are formed by bacteria and other microorganisms. These flocs help to remove suspended solids and other contaminants from wastewater. The flocs are then allowed to settle out of the wastewater and are eventually removed from the treatment plant either through.

• Electrocoagulation: This method uses an electric current to coagulate flocs. The electric current causes the particles to become negatively charged, which causes them to clump together, or

• Membrane separation: This method uses membranes to filter out flocs. The membranes have pores that are smaller than the flocs, so the flocs are trapped on the membrane while the water passes through.

Conclusion

Biofloc technology, as a new technology, still needs more research and development to be accepted by farmers. As a result of these advances, BFT is now a more reliable and sustainable aquaculture production system. Today, BFT is being used successfully by aquaculturists around the world. BFT systems work by creating a self-sustaining ecosystem in which beneficial bacteria, algae, and other microorganisms form flocs, or aggregates, in the water. These flocs provide a habitat for fish and other aquatic organisms, and they also help to improve water quality by removing waste products and nutrients. The use of flocculating agents in biofloc systems is a promising area of research. These agents have the potential to improve water quality, nutrient removal, and fish and shrimp growth performance. Further research is needed to optimize the use of these agents in biofloc systems. With the development of more efficient and stable flocculants, as well as a better understanding of the role of flocculants in disease prevention, it is possible to help reduce environmental effects and improve disease resistance in the biofloc system.

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Conflict of interest

Authors declare that no conflict of any kind exists with this submission.

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