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Bioflocculants as Sustainable Alternatives in Water Treatment: Mechanisms, Applications, and Future Prospects

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ABSTRACT

Bioflocculants are emerging as promising alternatives to conventional chemical coagulants for the treatment of potable water and wastewater. Derived primarily from microbial sources and plant-based materials, these agents are biodegradable, non-toxic, and environmentally friendly. This study presents a comprehensive overview of their origins, chemical compositions, and mechanisms of action, with particular emphasis on microbial extracellular polymeric substances (EPS) and the roles of polysaccharides and proteins in flocculation. The work highlights the key parameters influencing bioflocculant production, and highlights the variety of microbial strains for their roles in wastewater treatment. Applications in high-turbidity water treatment have demonstrated removal efficiencies of up to 98% for suspended solids, with turbidity reductions generally ranging from 72% to 85% in microbial systems, and reaching 99% in specific biocoagulant treatments. Bioflocculants have also shown significant efficacy in removing heavy metals and dyes. Despite their advantages, challenges remain in large-scale implementation due to limited raw material availability, complex extraction processes, and reduced effectiveness in low-turbidity systems. The study also outlines future directions for improving microbial efficacy through nutrient optimization, pH and temperature control, and the use of microbial consortia. These findings highlight the potential of bioflocculants as sustainable solutions for water and wastewater treatment, especially in resource-limited or ecologically sensitive regions.

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1.0 INTRODUCTION

Water contamination by anthropogenic pollutants (including domestic, industrial, and agricultural waste) poses significant risks to public health (Okaiyeto *et al.,* 2016; Quina *et al.,* 2017). Waterborne diseases are increasingly prevalent due to the consumption of contaminated water (Pathak, 2014). Among the preferred methods for removing organic matter and pollutants from wastewater is flocculation, which facilitates the aggregation of fine particles into larger flocs that settle more readily (Bruno *et al.*, 2020).

Flocculants, categorized into inorganic, organic, and naturally occurring microbial bioflocculants, serve this function (Okaiyeto *et al.,* 2016; Nwodo *et al.,* 2012). Prior to discharge into aquatic environments, wastewater must undergo treatment using conventional and advanced technologies to ensure its safe conversion into potable water and treated effluent (Madaki *et al.,* 2013; Rajasulochana *et al.,* 2016). Flocculation and coagulation are indispensable phases in most water and wastewater treatment processes. Their primary goal is to remove suspended particles, thereby yielding clear effluent (Duan *et al.,* 2012). These processes often represent the initial stage in treatment frameworks (Edzwald, 1993; Ebeling *et al.,* 2003). Coagulants and flocculants, especially those with divalent and highly charged ions, are essential to initiating this process (Hosseini *et al.,* 2008). Additionally, negatively charged polymers with high molecular weight are extensively used (Lapointe *et al.,* 2020). Common chemicals used in this process include iron and aluminum salts, hydrated lime, magnesium carbonate, and various polymeric agents such as polyaluminum chloride and polyferric sulfate (Aragonés-Beltrán *et al.,* 2009; Moran, 2018; Weydts *et al.,* 2015).

Bioflocculants, which are also described as extracellular polymeric substances (EPS), offer a natural alternative to these chemical agents. They are derived from various microorganisms, including bacteria, algae, and fungi, through cell lysis and secretion processes. These bioflocculants consist of complex mixtures of biopolymers like nucleic acids, proteins, lipids, polysaccharides, glycoproteins, and cellulose. Because they are biodegradable and environmentally benign, they do not cause secondary pollution. Despite these advantages, broad commercial adoption of bioflocculants remains limited. This limitation is mainly due to relatively low flocculating efficiency, modest production yields, and the high cost of substrates (such as glucose, fructose, and glutamate) needed for their synthesis (Ajao *et al.,* 2018). Studies have shown that substrate expenses can make up a significant portion of the total production cost, reducing competitiveness against synthetic alternatives in specific industrial contexts. Moreover, downstream processes, particularly extraction and purification, can account for 30–50% of overall costs. Abu Bakar *et al.* (2021) emphasised the economic drawbacks of the large solvent volumes required for these recovery and refinement steps in large-scale systems.

1.2 Bioflocculant-Producing Microorganisms

Bioflocculant-producing microorganisms are capable of synthesizing and excreting extracellular polymeric substances (EPS) with flocculating activity as part of their metabolic processes. These organisms are ubiquitously distributed across diverse ecological niches such as soil, freshwater, marine environments, and wastewater systems. Under optimized cultivation conditions, these microbes can yield substantial quantities of bioflocculants as secondary metabolites.

The major groups of microorganisms known to produce bioflocculants include:

i. **Bacteria:** Numerous bacterial taxa have demonstrated strong bioflocculant-producing capabilities. Notable genera include *Bacillus*, *Pseudomonas*, *Proteus*, and *Rhodococcus*. These bacteria typically secrete high-molecular-weight biopolymers, such as polysaccharides and glycoproteins, which facilitate aggregation and sedimentation of suspended particles. For instance, *Bacillus velezensis* has shown removal efficiencies of 72.0% for turbidity, 62.0% for COD, and 53.6% for BOD in wastewater treatment (Agunbiade *et al.,* 2022). Other bacterial bioflocculants have achieved up to 99% removal of heavy metal ions.

ii. **Fungi:** Filamentous fungi have also been widely recognized for their potential in bioflocculant production. Genera such as *Aspergillus*, *Penicillium*, and *Trichoderma* produce extracellular biopolymers that exhibit strong bridging and adsorption properties, contributing to effective floc formation. For example, a fungal bioflocculant from *Aspergillus oryzae* demonstrated over 85% turbidity removal in a wide pH range (4.0–9.0) (Wang *et al.,* 2021). Another fungal bioflocculant from *Pichia* sp. showed removal efficiencies of 43% (COD), 64% (BOD), 73% (P), and 50% (N) in coal mine wastewater (Li *et al.,* 2022).

iii. **Algae:** Certain microalgae species, including *Chlorella*, *Spirulina*, and *Scenedesmus*, have been reported to produce flocculant-active substances. These algal-derived EPS possess unique physicochemical properties that make them suitable for use in eco-friendly flocculation systems. Microalgae cultivated in seafood wastewater achieved 92% harvesting efficiency through bioflocculation (Wang et al., 2023). Algae-based treatments are also noted for their lower energy consumption compared to traditional wastewater treatment methods due to reduced aeration and chemical dosing requirements.

Due to their diverse biochemical compositions and flocculating efficiencies, bioflocculants derived from these microbial sources have attracted significant interest in environmental biotechnology. They offer sustainable alternatives to synthetic flocculants in applications such as wastewater treatment, heavy metal removal, and bioremediation, thereby contributing to greener and more cost-effective environmental management strategies.

1.3 Characteristics of Bio-flocculants and Bio-coagulants

1.3.1 *Chemical Composition and Functional Mechanisms*

Biocoagulants can be extracted from plant, microbial, or animal sources (Amran *et al*., 2018; Muruganandam *et al.*, 2017). Their coagulative capabilities are largely attributed to the presence of biopolymeric compound**s** such as polysaccharides**,** proteins, and functionally active groups including hydroxyl (-OH) and carboxyl (-COOH**)** moieties (Manholer *et al.*, 2019; Choy et al., 2014). These chemical constituents facilitate floc formation via adsorption**,** polymer bridging**,** and charge neutralization mechanisms. In addition to their coagulative action, some biocoagulants exhibit antimicrobial and heavy metal removal properties, enhancing their utility in high-turbidity water systems (Gunaratna et al., 2007). Phytochemicals such as tannins and alkaloids, present in some plant-based biocoagulants, contribute to this antimicrobial activity (Choy *et al.,* 2014). However, conventional chemical coagulants may elevate the organic load in treated water, fostering microbial regrowth and affecting water color, taste, and odor (Amran *et al.,* 2018). Gunaratna *et al.* (2007) recommend removing such residuals via purification or filtration.

1.3.2 *Bioflocculant-producing microorganisms*

Bioflocculant-producing microbes represent a promising frontier in sustainable biotechnology, particularly in the field of water and wastewater treatment. These microorganisms—including species of *Bacillus*, *Aspergillus*, *Streptomyces*, and *Rhizobium*, synthesize extracellular polymeric substances (EPS) such as polysaccharides, glycoproteins, or nucleic acid complexes that facilitate flocculation by bridging or charge-neutralizing suspended particles. The diversity of microbial species capable of producing bioflocculants is enormous which allows for adaptability to various environmental conditions, enhancing their practical applicability in industrial, municipal, and agricultural wastewater systems. Advancements in microbial fermentation and metabolic engineering could continually support the need for improving the yield, efficiency, and functional properties of bioflocculants, reinforcing their potential as a green alternative to synthetic flocculants. Table 1 highlights some of the commonly reported bioflocculant producing microorganisms.

Table 1: Representative Bioflocculant-Producing Microbes and its Characteristics

Source Type	**Microorganism**	**Dominant Functional Groups/Compounds**	**References**
Bacteria	*Bacillus agaradhaerens* C9	Carboxyl, hydroxyl, amino, glycoproteins	Liu *et al*. (2015)
Bacteria	*Bacillus mucilaginosus*	Uronic acid, neutral sugars, amino sugars, carboxyl, hydroxyl	Deng *et al.* (2003)
Bacteria	*Bacillus salmalaya* 139SI-7	Carboxyl, hydroxyl, amino, polysaccharides, proteins	Tawila *et al.* (2018)
Bacteria	*Paenibacillus polymyxa*	Polysaccharides	Abdel Khalek *et al.* (2017)
Bacteria	*Bacillus licheniformis* strain W7	Polysaccharides, proteins, hydroxyl, carboxyl, amino groups	Wang *et al.* (2015)
Bacteria	*Bacillus velezensis*	Xylose, glucose	Agunbiade *et al*. (2022)

*(*Adopted from Setyo *et al.,* 2020*)*

1.3.3 *Safety and Environmental Considerations*

Residual traces of chemical coagulants may persist in treated water and contribute to issues such as pipe corrosion or the formation of toxic by-products like disinfection byproducts (DBPs) (Amran et al., 2018). For instance, studies have shown that aluminum-based coagulants can contribute to aluminum accumulation on pipe surfaces, potentially impacting lead release, and the use of chlorine-based disinfectants after chemical coagulation can lead to the formation of various DBPs, some of which are known carcinogens. In contrast, biocoagulants are non-corrosive**,** biocompatible, and non-toxic, making them safer alternatives in potable water treatment (Choy et al., 2014). Despite their proven efficacy, large-scale commercial adoption remains limited, primarily due to insufficient standardization and mechanistic understanding of their action (Choy *et al.,* 2014).

1.3.4 Properties of Bio-flocculants and Bio-coagulants

- Flocculating Efficiency: Capable of aggregating colloidal particles into larger flocs, facilitating their removal through sedimentation or filtration.
- Biodegradability: Readily decomposed by microbial action, contributing to a sustainable environmental footprint.
- Non-toxicity: Generally safe for human and ecological health, making them suitable for potable water applications.
- Cost-Effectiveness: Potential to be produced using inexpensive substrates, especially from agricultural or industrial waste streams.
- Environmental Versatility: Stable and effective across a wide range of pH, temperature, and salinity conditions.
- Selectivity: Some exhibit preferential binding or flocculation of specific contaminants (e.g., heavy metals, dyes, or microbes).

1.4 Advantages of Using Biocoagulants/Bioflocculants

Bioflocculants have a wide range of application and their application not only enhances the safety of treated water but also supports circular bioeconomy models by utilizing agricultural or microbial byproducts, thereby aligning with global sustainability goals in water and wastewater management.

i. Environmental friendliness

The application of biocoagulants and bioflocculants in drinking water and wastewater treatment has emerged as a promising environmentally sustainable alternative to conventional chemical-based coagulants. These biologically derived agents produce significantly fewer harmful by-products, thereby minimizing ecological risks and promoting safer aquatic environments (Abdelaal *et al.,* 2014). As reported by Dzionek *et al. (*2016), bio-based coagulation technologies exhibit minimal environmental toxicity compared to traditional methods. This highlights their potential applicability in potable water and wastewater treatment, particularly for effective turbidity reduction.

ii. Reliable Performance

Although bio-based coagulants may not yet be as extensively developed as synthetic counterparts, their efficacy in pollutant removal has been well established. Notably, *Azadirachta indica*-derived biocoagulants have demonstrated a remarkable capacity to eliminate up to 97.91% of microalgae from wastewater, surpassing the 91.62% removal rate achieved using conventional alum (Ali *et al.*, 2019). Furthermore, a chitosan-based coagulant extracted from mushrooms achieved 99% turbidity reduction in palm oil mill effluent at a low dosage of 10 mg/L, whereas alum required a significantly higher dosage of 1,200 mg/L to achieve only 93% reduction (Irhayyim, 2017). Bioflocculants have also exhibited strong antimicrobial performance, reducing Gram-positive and Gram-negative bacterial loads by 85.61% and 77.63%, respectively (Sha'arani *et al.*, 2019). Supporting these findings, Megersa *et al.* (2016) reported that plant-derived biocoagulants achieved 99% turbidity and 98% microbial removal from domestic wastewater, meeting established discharge standards. These results underscore the potential of bioflocculants and biocoagulants as effective and sustainable alternatives in potable and wastewater treatment, especially for turbidity control.

iii. Local Resource Utilization and Effluent Reduction

The use of locally sourced materials in the production of biocoagulants and bioflocculants presents a sustainable approach to minimize dependency on non-renewable raw materials. By leveraging indigenous biomass and microbial resources, particularly in remote or underdeveloped regions, production costs and logistical burdens can be significantly reduced (Aviso *et al.*, 2011). Many local materials, often treated as waste, have valuable properties that make them suitable for use as biocoagulants. Agricultural leftovers, food scraps, and industrial by-products are now being explored as practical sources for producing bioflocculants (Saranya *et al.*, 2019). Using these resources helps reduce waste, lowers effluent volumes, and promotes more sustainable approaches to water management that align with circular economy practices.

iv. Applicability in Remote Areas

The inherent adaptability of biocoagulants/bioflocculants makes them particularly advantageous for application in geographically isolated regions. Unlike chemical coagulants, which may be difficult and costly to transport, bio-based alternatives can be sourced and produced locally. Studies have reported successful deployment of these agents in rural and underserved regions, such as the use of Moringa oleifera seeds for water treatment in various parts of Africa and Asia, where conventional water treatment infrastructure is limited. For instance, communities in rural Bangladesh, Kenya, Southern Africa, Malaysia, India, and Indonesia have effectively utilized locally sourced bioflocculants to improve water quality, demonstrating their practical applicability and sustainability in resource-constrained environments. However, optimal utilization of local biomass for production remains a challenge requiring further research and policy support to enhance scalability and efficiency.

v. Reduction in Sludge Generation

Conventional coagulants such as alum are known to produce large volumes of sludge, which pose environmental and economic challenges for disposal. In the United Kingdom alone, potable and wastewater treatment processes generated approximately 270,000 tonnes of dry sludge in 2014 (Keeley *et al.,* 2014).Bioflocculants, in contrast, have been shown to reduce sludge generation by up to 30% compared to alum-based treatments (Kristianto *et al.,* 2019; Amran *et al.,* 2018). This reduction not only lowers operational costs associated with sludge handling and transport but also mitigates the environmental burden of sludge disposal.

vi. Valorization of Treatment Sludge

Sludge generated from bioflocculant-based treatment processes tends to be more biodegradable and nutrient-rich, thereby enhancing its potential for reuse. Anaerobic digestion of this sludge has been found to yield biogas as a valuable by-product, without adversely affecting microbial performance in the digester (Lichtfouse et al., 2019; Hamawand et al., 2017). Additionally, the organic-rich composition of sludge derived from wastewater treatment in sectors such as aquaculture, palm oil production, and coffee processing renders it suitable for use as a soil conditioner or organic fertilizer (You et al., 2019). With proper composting, such sludge can contribute to sustainable agricultural practices (Kirchmann et al., 2017), thus closing the loop between waste generation and resource recovery.

2. Biosynthesis of Microbial Flocculants

The synthesis of microbial flocculants is closely associated with microbial metabolism, particularly during the stationary or late exponential phase. Numerous studies have shown that the highest flocculating activity typically occurs during this stage of microbial growth, a phenomenon attributed to enhance metabolic by-products or autolysis mechanisms (Van Den Hende et al., 2017; Wu et al., 2020). Several environmental and operational parameters influence the biosynthesis of microbial flocculants, including temperature, pH, dissolved oxygen concentration, cultivation time, and the carbon-to-nitrogen ratio in the culture medium.

2.1 Impact of pH on Microbial Flocculant Production

The pH of the flocculation environment plays a crucial role in determining the electrostatic interactions between microbial flocculants and suspended particles. Since pH affects both the surface charge of colloidal particles and the ionic state of the flocculant, it directly influences the efficiency of floc formation through mechanisms such as charge neutralization (Yang *et al.,* 2018; Zouboulis *et al.,* 2004). Various microbial flocculants display different sensitivities to pH, and each has an optimal pH range within which flocculating activity is maximized (Zhang *et al.*, 2013; Wu *et al.,* 2018). For instance, a bioflocculant produced by *Bacillus mucilaginosus* has an optimal pH of 7.0, achieving over 90% flocculating activity, while another from *Bacillus licheniformis* showed optimal activity at pH 7.0 and maintained high efficiency across a broad range of pH 2-8 (Wang *et al.,* 2015). Conversely, some bioflocculants, like those from *Bacillus salmalaya*, exhibit reduced flocculation activity (<70%) under very acidic (pH < 5) or very alkaline (pH > 9) conditions (Tawila *et al.,* 2018). Outside this optimal range, the surface characteristics of suspended particles and the flocculant may be altered, reducing the efficacy of adsorption and charge interaction (Huang et al., 2015).

2.2 Impact of Temperature on Microbial Flocculant Production

Temperature influences microbial flocculant production through both direct and indirect mechanisms:

- **Indirect effects** involve the physiological response of the microbial cells to temperature. Each strain possesses an optimal growth temperature and a specific temperature range for maximum flocculant production (Li et al., 2019). For instance, many bacterial strains, including *Bacillus* species, show optimal bioflocculant production around 30-37°C, with flocculating activities often exceeding 90% at these temperatures (Tawila et al., 2018; Wang et al., 2015). Some bioflocculants, like those from *Bacillus aryabhattai* PSK1, have shown effective flocculating activity (≥80%) even at higher temperatures ranging from 60-95°C.
- **Direct effects** refer to the influence of temperature on the physicochemical behavior of the flocculant molecules themselves. As temperature increases, molecular motion is accelerated, potentially enhancing flocculation kinetics. However, in the case of protein-based flocculants, excessive heat may cause denaturation and structural collapse, leading to

decreased performance. In contrast, polysaccharide-based flocculants generally exhibit greater thermal stability and retain their adsorption capacity under elevated temperatures. For example, a purified bioflocculant from *Bacillus licheniformis* retained more than 78% of its flocculating activity after heating at 100°C for 25 minutes (Wang *et al.,* 2017). Consequently, the impact of temperature on flocculation efficiency is largely dependent on the biochemical nature of the flocculant.

2.3 Effect of Dosage on Flocculation Performance

The dosage of microbial flocculants is a critical factor that influences the effectiveness of the flocculation process. According to Qui et al. (2009), optimal dosing results in the saturation of available adsorption sites on suspended particles, thereby facilitating efficient bridging and aggregation. If the dosage falls below the optimal level, insufficient flocculant is available to neutralize particle charges or facilitate bonding, leading to incomplete flocculation. Conversely, an excess of flocculant can lead to inter-flocculant interactions or restabilization of particles, which can hinder aggregation efficiency (Ariffin et al., 2012; Wang et al., 2011; Blanco et al., 2008). For example, a bioflocculant produced by *Bacillus megaterium* showed peak flocculation (95%) at a dosage of 0.1 mg/mL, while another study found an optimal dosage of 0.7 mg/mL for a bioflocculant from *Bacillus velezensis* (Agunbiade *et al.,* 2022). These findings emphasize that the optimal flocculant dose varies with the type of suspended material and the properties of the flocculant, underscoring the importance of system-specific optimization.

3. Potential Applications of Microbial Flocculants in Wastewater Treatment

The primary objective of microbial flocculant research is to facilitate the removal of various pollutants from wastewater. Bioflocculants have demonstrated significant efficacy in treating real wastewater samples, including those contaminated with heavy metals, dyes, and suspended solids:

- **Heavy Metals:** Bioflocculants have shown promising results in removing heavy metals from industrial wastewater. For instance, a bioflocculant produced by *Bacillus subtilis* effectively removed heavy metals from lake water samples, achieving removal efficiencies of 92.9% for Aluminum, 94.3% for Zinc, 86.2% for Iron, and 68.1% for Copper (Agunbiade et al., 2019). Another study reported that the bioflocculant QZ-7 efficiently removed heavy metals from industrial wastewater, demonstrating its potential for real-world applications (Wang *et al.,* 2019).
- **Dyes:** Bioflocculants are highly effective in decolorizing textile industrial effluents. A bioflocculant produced by *Serratia ficaria* achieved a remarkable 99.9% color removal from pulp wastewater, along with 72.1% chemical oxygen demand (COD) reduction (Li *et al.*, 2013). Other bacterial isolates have also shown high decolorization rates, with removal efficiencies of up to 97.04% for textile industrial effluent (Ali *et al.,* 2019).
- **Suspended Solids:** Bioflocculants are widely recognized for their ability to remove suspended solids and turbidity. For example, a fungal bioflocculant isolated from food processing solid waste demonstrated significant turbidity removal from turbid water (Zhang *et al.,* 2020). In another study, a bioflocculant produced by *Bacillus velezensis* achieved 72.0% turbidity removal from wastewater (Agunbiade *et al.,* 2022). These examples highlight the practical applicability of bioflocculants in various wastewater treatment scenarios tailored treatment approaches. As such, the selection and performance of microbial flocculants must be matched to the specific type of wastewater and its constituent pollutants.

Various strains of microorganisms produce different types of flocculants, each with distinct biochemical compositions and flocculation mechanisms. Consequently, the effectiveness of microbial flocculants differs depending on the wastewater matrix being treated. Their performance is influenced by factors such as pH, temperature, the presence of heavy metals or organic matter, and the physicochemical interactions involved in floc formation.

Numerous studies have demonstrated the successful application of microbial flocculants in removing turbidity, suspended solids, heavy metals, and organic contaminants from different wastewater sources (Table 2). These applications underscore their versatility and efficiency in environmental remediation. A comprehensive summary of microbial strains and their respective flocculating performance in common wastewater types is presented in the following table, highlighting their promising role as eco-friendly alternatives in sustainable wastewater treatment.

Table 2: Microbial flocculants utilized in common wastewaters treatment

Wastewater Type	**Contaminant Category**	**Flocculating Mi croorganisms**	**References**
Coal Slurry Wastewater	Coal waste slurry	*Azotobacter chroococcum*	Zhang *et al. (*2012)
	Coal slurry	*Rh odopseudomonas spheroides*	Dong *et al. (*2015)
	Coal-water slurry	*N itrogen-fixing bacteria* *Mycobacterium tuberculosis* *Escherichia coli* *White rot fungi*	Vijayalakshmi *et al.* (2003)
	Fine coal	*Bacillus subtilis*	Sari *et al.*, (2019)
Food Wastewater	Bioethanol mill wastewater	*White-rot fungus Trametes*	
	Suspended Solids Metal Ions	*Bacillus subtilis R23* *B. lich enifomis* ATCC 9945A *B. lich enifomis* CCRC 12826 *B. lichenifor mis* CICC10099	Bajaj *et al.* (2011)
Poultry wastewater	Slaughterhouse	*Comamonas sp.*	Dlangamandla *et al.* (2016)
Starch wastewater		*Bacillus mucila ginosus* MBFA9 *Bacillus lich eniformis* X14	Deng et al. (2003)
Dairy wastewater	Fe, AL, Mn, Zn, COD	*Terrabacter*	Agunbiade *et al. (*2019)
Municipal wastewater	Harmful algae	*Klebsiella pneumoniae strain* NY1	Nie *et al.* (2011)
Landfill leachates	Humic acids	*Rhizomonas sp.*	Zouboulis *et al.* (2004)
Heavy Metal wastewater	Cu(II)	*Ultrasonic assisted Spirulina platensis*	Vardhan *et al.* (2019)
	Cu(II)	*Sulphuric acid modified Spirulina platensis*	Gunasundari, (2017)
	Cu(II)	*Paenibacillus sp. strain* A9	*(*Jiang *et al. (*2019)
	Pb(II)	*P. p olymyxa* CCTCC M206017	*(*Feng *et al.* (2013)
	Pb(II)	*Paenibacillus sp. strain* A9 (MBFA9)	Zhang *et al.* (2019)
	Pb(II) Zn(II)	*Paenibacillus polymyxa* GA1	*(*Zhou *et al.* (2017)
	Cr(II) Ni(II)	*H erbaspirillium sp. CH7* *Paenibacillus sp. CH11* *Bacillus sp. CH15* *Halomonas sp.*	Lin *et al.* (2011)
	Cu, Zn, Pb and Cd	*Paenibacillus polymyxa*	Huang *et al.* (2019)
	Fe (III) Cd(II)	*Aspergillus niger*	
	Minerals	*Rhodococcus opacus*	Czemierska *et al.* (2016)
Papermaking wastewater	CO D_{Cr} content absorbance	*Aspergillus niger*	Li *et al. (*2008*)*
	Detergents Cellulose	*Bacillus subtilis* *Rhodococcus* *Bacteroides succinicum*	Zhong, (2016)
	COD Colority	PSBF	Ren *et al.* (2015)
Textile wastewater	Cu(II), Pb(II) and Cd(II)	*Bacillus sp.* *Pseudomonas sp.*	Azzam *et al.* (2015)
	Biavin medium blue dye Cr(II) Ni(II)	*H erbaspirillium sp. CH7* *Paenibacillus sp. CH11* *Bacillus sp.CH15* *Halomonas sp*	Lin *et al.* (2011)
	Anthraquinone based Reactive Blue 19	*Enterobacter sp.* NCIM 5545	Holkar *et al. (*2014)
	Acid and reactive dyes	*Pleurotus osstreatus* *Aspergillus niger* *Penicillium spp.*	Ali *et al.* (2012)
Printing and dyeing wastewater	Congo red (CR)	*Klebsiella peneumoniae*	Wang *et al. (*2010)
	Methylene blue	*Paenibacillus polymyxa* GA1	

Adopted from Hiuru *et al.* (2020)

4. Mechanisms of Bioflocculation

From a biochemical perspective, microbial flocculants are primarily composed of polysaccharides and proteins produced during microbial metabolism. Some flocculants may also incorporate inorganic metal ions, which contribute to their structural or functional roles. Morphologically, microbial flocculants can exhibit either a fibrous structure or a compact spherical form. Their application in diverse wastewater treatment processes has been widely explored. Gasp and Adholeya (2007) showed that microbial systems made from bacteria, fungi, algae, and plant-based mixtures were effective at removing color from distillery wastewater.

Various microbial strains have been identified as effective flocculant producers, including *Enterobacter* spp. (Tang *et al.,* 2014; Wang *et al.,* 2009), *Halomonas* spp., *Citrobacter* spp. (Pant *et al.*, 2007), *Bacillus* spp. (Li *et al.,* 2009; Lee *et al.*, 2014; Ai *et al.,* 2018; Sathiyanarayanan *et al.,* 2013), and *Rhodococcus erythropolis* (Wen *et al.,* 2016; Kuyukina *et al.,* 2013; Lin *et al.,* 2019).

The general process of floc formation involves several steps:

iv. Dispersal of flocculant particles throughout the aqueous medium.

v. Adsorption of these particles onto suspended matter via collision.

vi. Surface binding and bridging on colloidal particles (Lin *et al.,* 2019; Wu *et al.,* 2019).

vii. Growth of microflocs into larger and more stable aggregates through further adsorption and inter-particle interaction (Pant *et al.,* 2007).

Several theoretical models have been proposed to explain microbial flocculation mechanisms, including Friedman's extracellular fiber theory, Crabtree's ester composite hypothesis, and Butterfield's adhesion model related to poly-β-hydroxybutyric acid (PHB) (Luvuyo *et al.,* 2013; Zhao *et al.,* 2012). While these models provide foundational concepts, explicit experimental validation or detailed practical applications directly linking to these specific historical models in recent bioflocculation literature are less commonly detailed. Currently, widely accepted mechanisms include adsorption bridging, charge neutralization, chemical interactions, and volume sweeping. In most systems, flocculation is the outcome of the synergistic action of these multiple mechanisms (Yang *et al.,* 2014), which vary depending on the specific microbial flocculant and environmental conditions.

4.1 *Adsorption Bridging*

According to the adsorption bridging theory, microbial flocculants act as high-molecular-weight macromolecules with multiple active sites and low charge density. These active sites bind colloidal particles, enabling floc formation through a three-dimensional network of large polymer chains, often stabilized by hydrogen bonding and van der Waals forces (He *et al.,* 2019; Xia *et al.,* 2018; Wang et al., 2010; Han *et al.,* 2019; Figdore *et al.,* 2018). For instance, a polysaccharide-based bioflocculant produced by *Bacillus* sp. was shown to effectively flocculate kaolin suspension primarily through adsorption and bridging mechanisms, with its long polymer chains extending to adsorb onto multiple particles (Wang et al., 2019). Effective bridging depends on adequate chain length and sufficient surface area to facilitate contact and adsorption across different particles (Hori *et al.,* 2010; Pant *et al.,* 2007).

4.2 Charge Neutralization

Most colloidal particles in water have a negative surface charge, which keeps them apart and prevents settling. Charge neutralization happens when positively charged parts of a microbial flocculant attach to these negatively charged surfaces. This reduces the repulsive forces between particles, making it easier for them to come together and form larger clusters, or flocs (Yang *et al.,* 2018; Zouboulis *et al*., 2004). For instance, a bioflocculant from Bacillus licheniformis contains many positively charged groups like amino and carboxyl groups that neutralize the negative charges on kaolin particles, resulting in effective flocculation (Wang *et al.,* 2015). Likewise, research on Paenibacillus species shows that the cationic parts of their bioflocculants are key to neutralizing charges and helping flocculate negatively charged contaminants (Lian *et al.,* 2016). The process often works even better in the presence of multivalent cations such as calcium and magnesium, which can further lower the zeta potential of the particles and support aggregation (He *et al*., 2019).

4.3 *Chemical Interactions*

Chemical reactions affected by pH changes also play a role in flocculation. PH changes can modify the ionization of flocculants and colloidal particles, influencing how strongly they interact (Obaja *et al.,* 2005; Renault *et al.,* 2009; Lee *et al.,* 2014). The most effective flocculation usually occurs at pH levels with the lowest zeta potential, promoting aggregation before any charge reversal occurs.

4.3.1 *Volume Sweeping*

Volume sweeping happens when microbial flocculants create clusters that trap smaller particles as they sink under gravity. This works like a sieve, with the dense floc structure providing enough connection points to catch suspended matter. Interestingly, the amount of flocculant needed depends on how polluted the water is. Water with more contaminants usually needs less flocculant because the particles are more likely to collide and stick together. In contrast, cleaner water often requires a higher dose to start the aggregation process.

5. Approaches to Improve Microbial Performance in Water and Wastewater Treatment

Several practical measures can help boost the effectiveness of microbial flocculants in treating water and wastewater:

i. Balancing Nutrients: Maintaining suitable carbon, nitrogen, and phosphorus levels supports healthy microbial growth and metabolism. This balance is essential for producing sufficient flocculants and achieving good treatment outcomes.
ii. Managing Aeration: Providing enough oxygen is key for aerobic microbes. Proper aeration encourages their growth and helps them generate flocculants more quickly.
iii. Controlling pH: Keeping pH within the right range creates conditions that favour microbial activity and stable floc formation.
iv. Regulating Temperature: Microbial processes depend heavily on temperature. Running treatment systems within the ideal temperature range for the specific microbes improves their performance and keeps the process steady.
v. Using Bioaugmentation: Adding selected high-performing microbes or commercial bioaugmentation products can strengthen the system's ability to break down harsh pollutants and increase overall reliability.
vi. Ongoing Monitoring: Regular checks on microbial populations and process conditions help spot problems early. This allows timely adjustments to keep the system running well.
vii. Limiting Toxic Inputs: Reducing harmful substances in incoming water protects the microbes, helping them stay active and effective.
viii. Optimizing Hydraulics: Designing tanks and flow systems with the correct retention times and flow patterns encourages microbes to settle and thrive, improving the treatment process.

6. Microbial Consortia as an Emerging Approaches in Microbial Engineering for Wastewater Treatment

Microbial consortia represent an evolving, green biotechnology approach that leverages the synergistic interactions of multiple microbial species. Employing a single microbial strain often yields suboptimal results, whereas consortia can degrade complex contaminants more efficiently. Various studies have endorsed their effectiveness (Hosseinzadeh et al., 2020; Ji et al., 2020). The advantages of using consortia include accelerated treatment rates, improved pollutant diversity tolerance, and ecological resilience. Among these, algal-bacterial consortia have gained attention due to their low energy requirements and ease of biomass recovery (Kang *et al.,* 2017). Liu *et al.* (2014) also reported their effectiveness in nutrient removal and biomass valorization. Malik *et al.* (2012) demonstrated that Ecobacter consortia successfully facilitated bioaugmentation for the biological removal of nitrogen compounds. The transformation of ammonium through microbial reduction reactions significantly decreased its concentration during treatment. Similarly, Qi *et al.* (2021) validated the performance of a well-established algal-bacterial consortium in the phycosphere, highlighting its promise for advanced wastewater treatment applications.

7. Challenges and Limitations in the Application of Bioflocculants

7.1 Limitations

Despite the increasing attention on bioflocculants and biocoagulants as environmentally friendly alternatives, several limitations hinder their full-scale adoption in water and wastewater treatment systems:

Limited Availability: Bioflocculants/biocoagulants are not readily available in commercial quantities. Active compounds require multistep extraction, particularly from microbial sources. The extraction process includes microbial cultivation, centrifugation, and the isolation of exopolysaccharides (EPS) using saline or EDTA solutions, followed by filtration and lyophilization (Castro et al., 2014; Aguilera et al., 2008; Jachlewski et al., 2015). Each step must be carefully executed to recover pure and functionally active compounds.

Raw Material Dependency: The sustainability of bioflocculant production is highly dependent on the consistent availability of suitable raw materials, which may vary by region and season.

Performance in Low-Turbidity Water: While effective in treating high-turbidity water, bioflocculants have shown limited efficiency in low-turbidity systems (Asrafuzzaman *et al.,* 2011). For instance, studies indicate that the flocculation efficiency of bioflocculants tends to decrease significantly when the initial turbidity of the water is below a certain threshold, often requiring the addition of co-flocculants or higher dosages to achieve satisfactory results (Wang *et al.,* 2019). This limitation is primarily due to the reduced collision frequency between particles in low-turbidity water, which hinders the bridging and charge neutralization mechanisms of bioflocculants (Li *et al.*, 2013).

Scaling and Equipment Compatibility: Industrial-scale application faces challenges such as ensuring continuous production, raw material sufficiency, and the adaptation of existing treatment infrastructure to meet the operational requirements of bio-based flocculation processes.

7.2 Key Challenges

The mass production of microbial bioflocculants demands a continuous supply of nutrient-rich media. Currently, conventional synthetic media are predominantly used, raising sustainability and cost concerns (Vaningelgem *et al.,* 2004; Moghannem *et al.,* 2018; Samer, 2015). In addition, translating laboratory results to industrial-scale, continuous-flow treatment systems remains underexplored, despite growing interest driven by the limitations of conventional coagulants. More advanced studies are required to evaluate and improve the capacity of bioflocculants to remove pathogenic microorganisms in real effluent. Most research remains at the bench-scale level, with few pilot or full-scale implementations available for validation.

7.3 Future Prospects

Agricultural and industrial wastes that contain ample fermentable sugars and nitrogen compounds are still largely untapped for producing bioflocculants on a commercial scale. Optimizing fermentation conditions and adjusting substrate levels in solid and liquid systems could help achieve higher production. Using mixed microbial cultures that include strains producing xylanase, cellulase, and bioflocculants may also remove the need for expensive pre-treatment steps and boost overall yields. In addition, studying the molecular and genetic basis of bioflocculant synthesis could open the door to engineering microbial pathways for better productivity and more reliable process control.

Future research should focus on addressing the identified limitations and expanding the practical applicability of bioflocculants. Specific directions include:

viii. **Cost-Effective Production and Raw Material Utilization:** Given the challenge of raw material dependency and high production costs, future studies should explore the utilization of readily available and low-cost agricultural residues, industrial by-products, and food waste as alternative nutrient sources for bioflocculant production. This includes optimizing fermentation processes using these waste streams to enhance yields and reduce overall costs.

ix. **Enhanced Performance in Low-Turbidity Water:** To overcome the limited efficiency of bioflocculants in low-turbidity systems, research should investigate strategies such as the development of novel bioflocculants with improved affinity for sparsely distributed particles, the synergistic application of bioflocculants with other treatment methods (e.g., membrane filtration, advanced oxidation processes), or the design of hybrid flocculation systems that combine the advantages of bioflocculants with minimal chemical aid.

x. **Genetic Engineering and Metabolic Pathway Optimization:** To improve bioflocculant yields and tailor their properties for specific applications, advanced genetic engineering techniques can be employed. This involves identifying and manipulating genes responsible for bioflocculant synthesis, optimizing metabolic pathways in producing microorganisms, and developing robust strains capable of high-level production under diverse environmental conditions.

xi. **Pilot- and Full-Scale Implementation and Validation:** A critical gap remains in the transition from laboratory-scale studies to pilot- and full-scale applications. Future research must prioritize conducting comprehensive pilot-scale trials using real wastewater samples to validate the efficacy, cost-effectiveness, and operational stability of bioflocculant-based treatment systems. This includes assessing long-term performance, sludge management, and overall environmental impact in practical settings.

xii. **Mechanistic Understanding and Standardization:** While mechanisms like adsorption bridging and charge neutralization are understood, further research is needed to fully elucidate the complex interactions between bioflocculants and diverse pollutants. This includes developing standardized characterization methods for bioflocculants and their performance, which will facilitate comparison across studies and accelerate their commercialization.

8. CONCLUSION

This review highlights the significant potential of bioflocculants as sustainable and eco-friendly alternatives to conventional chemical coagulants in water and wastewater treatment. The detailed analysis of their mechanisms, including adsorption bridging and charge neutralization, supported by experimental data from various microbial strains, underscores their efficacy in removing diverse pollutants. For instance, bioflocculants have demonstrated high removal efficiencies for heavy metals (e.g., 92.9% for Aluminum, 94.3% for Zinc), dyes (up to 99.9% color removal), and suspended solids (72.0% turbidity removal) in real wastewater samples. Their inherent advantages, such as biodegradability, reduced sludge generation (up to 30% less than alum), and applicability in remote areas, position them as crucial components of future sustainable water management strategies.

Despite these compelling benefits, the widespread industrial adoption of bioflocculants faces several challenges, including high production costs, raw material dependency, and limitations in treating low-turbidity water. Addressing these challenges necessitates focused future research, particularly in optimizing cost-effective production methods using agricultural waste, enhancing performance in low-turbidity environments, leveraging genetic engineering for improved yields, and conducting rigorous pilot- and full-scale validation studies. By bridging the gap between laboratory-scale promise and industrial-scale application, bioflocculants can fulfill their substantial potential as green technologies, contributing to cleaner water and a more sustainable environment.

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