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Review Article

Microbial Flocculants: Production, Mechanisms, Applications, and Future Perspectives

Drashti Jadav[1]*, Debabrata Sarkar[2] and Ronak Chhaya[2]

[1]R&D Department, Bioani India Pvt. Ltd., India

[2]Research and Development Division, Bioani India Pvt. Ltd., India

***Corresponding Author:** Drashti Jadav, R&D Department, Bioani India Pvt. Ltd., Lilapur, Ahmedabad, Gujarat, India.

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Abstract

Microbial flocculants are extracellular biopolymers produced by bacteria, fungi, yeasts, actinomycetes, microalgae, cyanobacteria, and mixed microbial communities. They have attracted considerable interest as sustainable alternatives to inorganic salts and synthetic organic polymers used in water and wastewater treatment. This review critically examines the production, composition, flocculation mechanisms, characterization, applications, limitations, and future development of microbial flocculants. Most reported products are polysaccharides, proteins, glycoproteins, or complex extracellular polymeric substances containing hydroxyl, carboxyl, amino, phosphate, and sulfate groups. Production is strongly affected by microbial strain, carbon and nitrogen sources, initial acidity or alkalinity, temperature, oxygen transfer, cultivation time, mineral ions, and fermentation strategy. Low-cost residues from agriculture, food processing, dairy operations, brewing, starch manufacture, and wastewater treatment can reduce medium costs while supporting circular resource use. Flocculation occurs through a combination of polymer bridging, charge neutralization, adsorption, ion-mediated cross-linking, hydrogen bonding, and hydrophobic interactions. The dominant mechanism depends on polymer charge, molecular size, functional groups, particle properties, water chemistry, and mixing conditions. Microbial flocculants have been investigated for turbidity removal, sludge settling and dewatering, dye decolorization, heavy metal capture, treatment of municipal and industrial effluents, aquaculture water improvement, and harvesting of microalgal biomass. Their principal advantages are biodegradability, low toxicity, renewable production, and broad functional diversity. However, variable composition, low or inconsistent yield, expensive recovery, storage instability, dependence on supporting ions, and limited pilot-scale evidence continue to restrict commercialization. Future progress will depend on standardized testing, economical feedstocks, robust downstream processing, engineered production strains, integrated omics, data-driven process control, life cycle assessment, techno-economic evaluation, and long-term validation in real wastewater systems.

Keywords: Microbial Flocculants; Bioflocculants; Extracellular Polymeric Substances; Wastewater Treatment; Flocculation Mechanisms; Biopolymer Production; Sustainable Water Treatment

Abbreviations

AFM: Atomic Force Microscopy; BOD: Biochemical Oxygen Demand; COD: Chemical Oxygen Demand; DLS: Dynamic Light Scattering; EPS: Extracellular Polymeric Substances; FTIR: Fourier Transform Infrared Spectroscopy; GC–MS: Gas Chromatography–Mass Spectrometry; GPC: Gel Permeation Chromatography; HPLC: High-Performance Liquid Chromatography; MBF: Microbial Bioflocculants; NMR: Nuclear Magnetic Resonance; RSM: Response Surface Methodology; SEM: Scanning Electron Microscopy; TEM: Transmission Electron Microscopy; TGA: Thermogravimetric Analysis; XPS: X-ray Photoelectron Spectroscopy.

Introduction

Coagulation and flocculation are essential solid-liquid separation operations in drinking water preparation, municipal wastewater treatment, industrial effluent clarification, mineral processing, food processing, and fermentation. Conventional treatment commonly uses aluminum or iron salts and synthetic polymers because they are effective, readily available, and relatively inexpensive. Nevertheless, chemical treatment may generate large quantities of sludge, introduce residual ions or polymer fragments, and increase the environmental burden of sludge handling. These concerns have stimulated research into biodegradable and biologically produced alternatives [22-24].

Microbial flocculants are high molecular weight products formed during microbial growth, cell surface development, biofilm formation, stress response, or cell lysis. They may be secreted into the culture medium or remain associated with the cell surface. Their active components include polysaccharides, polyomino acids, proteins, glycoproteins, uranic acids, lipids, nucleic acids, and composite extracellular polymeric substances. The abundance of hydroxyl, carboxyl, amino, phosphate, and sulfate groups enables interaction with colloids and dissolved contaminants through several physicochemical pathways [1-4].

Early work with *Corynebacterium glutamic*, *Bacillus subtilis*, and *Bacillus licheniformis* established that microorganisms could produce efficient flocculating polymers with defined chemical structures and high activity [1-3]. Later research expanded the microbial resource base to include marine bacteria, actinomycetes, yeasts, fungi, and mixed consortia [4,6-8,10,13]. Recent studies have demonstrated that individual bioflocculants can remove turbidity, dyes, metals, organic matter, and nutrients, while also improving sludge properties and aquaculture wastewater quality [11-21].

Despite significant laboratory progress, microbial flocculants remain less widely commercialized than conventional chemical products. Major obstacles include expensive culture media, low volumetric productivity, product variability, difficult purification, limited shelf life, and insufficient evidence from continuous pilot or full-scale operation. Comparisons among studies are also complicated by differences in kaolin concentration, test volume, mixing regime, settling time, dosage expression, cation addition, and calculation of flocculating activity [22-24].

This review integrates established and recent findings on microbial flocculant sources, production strategies, chemical composition, molecular mechanisms, analytical characterization, and environmental applications. It also evaluates current barriers to scale-up and identifies research priorities in strain engineering, low-cost feedstocks, standardized performance testing, process modelling, life cycle assessment, and hybrid treatment systems.

Materials and Methods

Review design and literature search

A narrative review approach was used because the literature includes heterogeneous microorganisms, cultivation conditions, purification procedures, flocculation assays, and wastewater matrices that are not suitable for direct quantitative pooling. Peer-reviewed literature was identified through PubMed, publisher databases, Crossruff linked records, and broad academic web searches. Searches combined the terms microbial flocculant, bioflocculant, extracellular polymeric substances, exopolysaccharide, flocculation mechanism, production optimization, wastewater treatment, dye removal, heavy metal removal, sludge dewatering, and microalgae harvesting. Foundational studies and recent articles available through July 2026 were considered.

Eligibility, extraction, and synthesis

Priority was given to original studies that reported the producing microorganism, culture conditions, product composition, flocculating activity, mechanism, characterization, or application. Review articles were used to identify historical developments, methodological limitations, and broader research trends. Studies with unclear microbial origin or without sufficient description of flocculation performance were used only for contextual discussion. Information was organized into microbial sources, production, downstream recovery, structural characteristics, mechanisms, applications, limitations, and future directions. Because experimental protocols varied substantially, reported performance values were interpreted as study-specific rather than as directly comparable rankings.

Results and Discussion

Definition, origin, and classification of microbial flocculants

Microbial flocculants are functional macromolecules produced by microorganisms that promote aggregation of suspended particles, cells, colloids, or dissolved contaminants into larger flocs that can be separated by sedimentation, flotation, centrifugation, or filtration. The term commonly overlaps with bioflocculant, microbial polymeric flocculant, exopolysaccharide flocculant, and EPS based flocculant. However, not every extracellular polymer is an effective flocculant. Useful products must combine adequate molecular size, accessible binding groups, favorable conformation, and compatibility with the treated water.

Classification can be based on biological source or chemical composition. Bacterial products are the most extensively studied because bacteria grow rapidly, are readily screened, and can be manipulated by fermentation and genetic methods. Fungi and yeasts can use complex substrates and often secrete robust polymeric materials. Actinomycetes provide chemically diverse polymers, while microalgae and cyanobacteria offer the added benefits of carbon fixation and nutrient recovery. Mixed cultures produce complex EPS matrices that can display broad pollutant affinity and operational resilience [4,7,8,10,13].

Based on composition, microbial flocculants may be predominantly polysaccharide, protein or polyomino acid, glycoprotein, or mixed EPS. Polysaccharide-rich products are usually thermally stable and negatively charged because of uranic acid, phosphate, or sulfate residues. Protein-rich products may provide amino and carboxyl groups and can show pH-dependent charge behavior. Composite products often provide several complementary interaction sites and may perform well against mixed pollutants.

Microbial source	Representative organisms	Typical components	Important features
Bacteria	*Bacillus, Paenibacillus, Pseudomonas, Rhodococcus, Corynebacterium*	Polysaccharides, proteins, polyglutamic acid, glyco-proteins	Rapid growth, broad diversity, convenient fermentation and strain improvement
Fungi	*Aspergillus, Penicillium, Rhizopus*	Polysaccharides, proteins, glycoproteins	Strong secretion capacity and toler-ance of complex substrates
Yeasts	*Pichia, Saccharomyces, Candida*	Mannans, glucans, proteins, glycoproteins	Established industrial cultivation and high biosafety potential
Actinomycetes	*Streptomyces, Nocardia*	EPS, glycoproteins, polysac-charides	Chemically diverse products and strong adsorption properties
Microalgae and cyanobacteria	*Chlorella, Scenedesmus, Arthrospira, Nostoc*	Sulfated polysaccharides, uranic acids, glycoproteins	Simultaneous nutrient recovery and carbon fixation
Mixed consortia	Activated sludge, biofilm, bacterial con-sortia	Complex EPS containing polysaccharides, proteins, lipids, and extracellular DNA	Broad functionality and environ-mental adaptability but difficult standardization

Table 1: Classification of microbial flocculants according to source and dominant composition.

Microbial producers and representative advances

Bacterial producers

Bacillus species are among the most frequently reported producers. *Bacillus subtilis* DYU1 produced a high molecular weight biopolymer containing polyglutamic acid and showed strong calcium-assisted flocculation [2]. *Bacillus licheniformis* produced a novel extracellular bioflocculant with high activity and established an early model for strain isolation, optimization, and structural characterization [3]. More recent *Bacillus* studies have expanded application from model kaolin suspensions to multi-pollutant treatment, including dyes, copper, chromium, and food processing wastewater [11,19].

Corynebacterium glutamic is notable for its widespread use in industrial biotechnology. The early REA series demonstrated production of polygalacturonic acid-based flocculants [1]. A more recent C. glutamic strain produced a polysaccharide and protein-containing bioflocculant active under strongly acidic conditions and removed several metals from real acid mine wastewater [17]. This example illustrates how the physiological properties of the producing microorganism can be matched to a difficult treatment environment.

Pseudomonas strains are attractive because of their metabolic flexibility and ability to form extracellular polymers. Pseudomonas sp. GO2 produced a bioflocculant that removed heavy metal ions [15]. Stenotrophomonas Pavani GXUN74707, a related Gram-negative environmental bacterium, showed efficient flocculation and improved chemical and biochemical oxygen demand in aquaculture wastewater [18]. Pseudomonas sp. GXUN74702 subsequently achieved high kaolin flocculation, good polymer yield, and measurable organic load removal from aquaculture wastewater [21].

Rhodococcus erythropolis can utilize sludge and livestock wastewater as inexpensive media while producing a functional bioflocculant [5]. This is important because medium cost is a major fraction of overall production cost. Marine bacteria also generate distinctive sulfated or salt-tolerant polymers. A marine isolate described by Chen and colleagues produced a bioflocculant suitable for dye wastewater treatment [6].

Fungi, yeasts, actinomycetes, and consortia

Fungal and yeast bioflocculants may be especially useful where inexpensive carbohydrate-rich wastes are available. Pichia kudriavzevii produced a carbohydrate-rich bioflocculant containing protein and uranic acid and demonstrated wastewater treatment potential [13]. Fungal systems can also combine polymer secretion with biosorption and biodegradation, although broth viscosity and separation of fungal biomass may complicate downstream processing.

Actinomycetes produce structurally diverse extracellular materials. Streptomyces platensis generated a bioflocculant that reduced chemical oxygen demand in river water and meat processing wastewater [7]. Their slower growth compared with many bacteria is a disadvantage, but actinomycetes may be valuable when unique adsorption or antimicrobial functions are required.

Mixed microbial consortia produce complex EPS in activated sludge, biofilms, and engineered co-cultures. A self-assembled consortium-derived bioflocculant showed useful pollutant remediation properties [8]. Consortium products may tolerate fluctuating water quality better than single-strain products, but changes in community composition can alter polymer yield and quality. Therefore, mixed culture production requires ecological monitoring and operating controls that preserve functional stability.

Production of microbial flocculants

Screening and strain selection

The development process normally begins with collection of environmental samples from activated sludge, soil, sediments, industrial effluents, fermented foods, or marine habitats. Isolates are screened by measuring clarification or optical density reduction in a standard suspension, commonly kaolin clay. A high apparent flocculation value is only an initial indicator. Promising strains should also be evaluated for polymer yield, reproducibility, biosafety, stability, dependence on cations, activity in real wastewater, and ease of product recovery [10,16,22].

Nutritional and environmental factors

Carbon source affects both biomass formation and the precursor pool for polymer synthesis. Glucose, sucrose, starch, molasses, glycerol, organic acids, and complex agro-industrial residues have been used. The optimal carbon source is strain-specific. Easily metabolized sugars may maximize yield, but low-cost residues can improve process economics even if productivity is moderately lower. Nitrogen source controls growth, enzyme synthesis, and the protein fraction of EPS. Organic nitrogen such as yeast extract or peptone often supports rapid production, whereas urea and ammonium salts may reduce cost [5,15,18-21].

Initial pH influences nutrient transport, enzyme activity, membrane function, polymer charge, and secretion. Most bacterial producers perform near neutral pH, but acid-tolerant strains can generate products useful for mine drainage or acidic industrial wastewater [17]. Temperature determines growth rate and biosynthetic activity, while aeration and agitation control dissolved oxygen and mass transfer. Excessive shear may damage cells or polymer chains; insufficient mixing can create nutrient and oxygen limitation.

Bioflocculant formation may be growth-associated, partially growth-associated, or prominent during the stationary phase. Cultivation time should therefore be optimized with simultaneous measurement of biomass, polymer concentration, and flocculating activity. An extended process does not always improve yield because secreted polymers may be degraded by microbial enzymes or altered by changes in broth pH.

Low-cost substrates and circular production

Fermentation medium is a major cost driver. Sludge hydrolysate, livestock wastewater, brewery wastewater, potato starch wastewater, dairy effluent, corn steep liquor, molasses, rice straw hydrolysate, soybean meal, and food processing residues have been investigated as alternative feedstocks. The ideal waste-based medium supplies carbon, nitrogen, minerals, and vitamins without inhibitory contaminants. Pretreatment may be required to improve hydrolysis, reduce toxicity, or sterilize the feedstock. Waste valorization can couple pollutant reduction with production of a useful water treatment agent, but variability in waste composition must be managed through feedstock characterization and blending [5,12,15,17,21].

Variable	Main influence	Practical consideration
Microbial strain	Polymer type, yield, biosafety, stress tolerance	Select for both production and application environment
Carbon source	Energy supply and precursor generation	Balance productivity with feedstock cost and consistency
Nitrogen source	Growth, enzyme synthesis, protein fraction	Use economical sources without reducing activity
Initial pH	Transport, enzyme activity, secretion, polymer charge	Control throughout fermentation when pH drift is large
Temperature	Growth rate and biosynthetic enzyme activity	Avoid conditions that favor biomass but reduce polymer yield
Aeration and agitation	Oxygen transfer, mixing, shear	Scale using oxygen transfer and power input rather than rpm alone
Cultivation time	Growth phase and polymer degradation	Harvest near maximum activity, not only maximum biomass
Trace ions	Enzyme cofactors and polymer assembly	Prevent inhibition or contamination from excessive metals
Fermentation mode	Productivity, contamination risk, scalability	Compare batch, fed batch, continuous, submerged, and solid state systems
Statistical optimization	Interaction among variables	Use response surface methods after preliminary factor screening

Table 2: Major variables affecting microbial flocculant production and practical optimization.

Fermentation scale up and downstream recovery

Most studies use shake flasks, but industrial translation requires stirred tank, airlift, or other controlled bioreactors. Scale up based only on vessel volume or agitation speed is unreliable. Oxygen transfer coefficient, power input, mixing time, gas flow, foam formation, viscosity, and heat removal should be evaluated. Highly viscous EPS broths can reduce oxygen transfer and create gradients that change product composition.

Extracellular products are generally easier to recover than cell-bound polymers. A typical sequence includes removal of cells by centrifugation or filtration, precipitation with ethanol or another solvent, dialysis or ultrafiltration, washing, and drying. Purification improves characterization but increases cost and solvent demand. For some wastewater applications, cell-free fermentation broth or partially purified concentrate may be sufficient. Product specification should therefore be linked to end use. Drinking water and food applications require stricter purity and safety than sludge thickening or industrial pretreatment.

Drying and formulation influence storage stability. Freeze drying preserve’s structure but is expensive. Spray drying is more scalable but may expose polymers to heat and shear. Encapsulation, carrier adsorption, blending with benign inorganic aids, and liquid concentrates are possible formulation options. Long-term data on activity retention, microbial contamination, moisture sensitivity, and reconstitution are still limited.

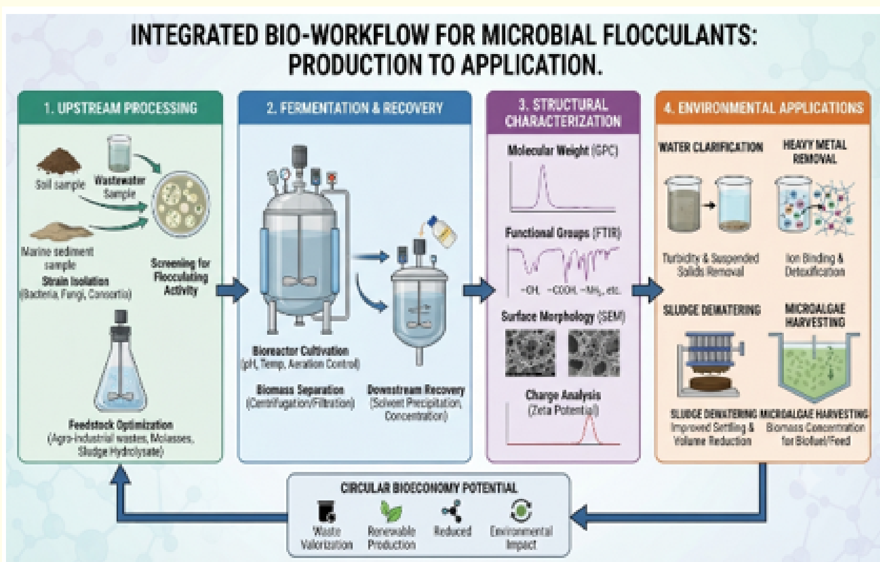

Figure 1: Integrated lifecycle workflow for microbial flocculants, tracking the process from upstream waste-based feedstock optimization and fermentation to multi-technique characterization and final environmental applications.

Mechanisms of flocculation

Chemical composition and structural characterization

The function of a microbial flocculant depends on its molecular architecture. Polysaccharide backbones provide long chains for bridging, while uranic acids, phosphate groups, sulfate groups, and amino sugars influence charge and binding affinity. Proteins contribute amino, carboxyl, aromatic, and sulfhydryl groups and may provide hydrophobic interaction sites. Glycoproteins combine chain extension with diverse binding chemistry. Extracellular DNA can contribute to network formation in complex EPS, although its direct role in purified bioflocculants is less frequently quantified.

Routine compositional analysis includes total carbohydrate, protein, uranic acid, and nucleic acid assays. Monosaccharide composition can be determined by HPLC, ion chromatography, or GC-MS after hydrolysis and derivatization. Molecular weight and distribution are measured using GPC or size exclusion chromatography. FTIR identifies broad functional group patterns, whereas NMR provides more detailed information on glycosidic linkages and polymer backbone structure. XPS examines surface elemental composition and chemical states.

SEM and TEM visualize particle surfaces and floc architecture before and after treatment. AFM can examine nanoscale topography and interaction forces. Zeta potential reveals changes in surface charge during addition of polymer and supporting ions. DLS provides particle size distributions, while TGA and differential scanning calorimetry assess thermal behavior. No single method establishes the mechanism. A convincing structure function interpretation normally combines composition, molecular size, spectroscopy, charge, and microscopic evidence [2,9,11,17-20].

Polymer bridging and adsorption

Polymer bridging is frequently the dominant mechanism for high molecular weight bioflocculants. Segments of a polymer chain adsorb onto one particle while unoccupied segments extend into the liquid and attach to other particles. Effective bridging requires sufficient chain length, partial rather than complete surface coverage, and adequate mixing to create collisions. Excess dosage may saturate particle surfaces, leaving no free segments for bridging and causing destabilization. Studies of *Bacillus, Pseudomonas*, and wastewater-derived biopolymers consistently support bridging through microscopy, molecular size, and dosage response [2,9,11,19-21].

Charge neutralization and ion-mediated cross-linking

Many particles and polysaccharide bioflocculants are negatively charged at neutral pH. Divalent or trivalent cations can compress the electrical double layer, neutralize negative sites, and form bridges between the polymer and particle surfaces. Calcium is widely used because it is effective and relatively benign, although magnesium, iron, and aluminum may also promote flocculation. Charge neutralization is indicated when zeta potential approaches zero as flocs form. However, high activity can occur without complete neutralization, demonstrating that charge effects often work together with bridging [17-21].

Hydrogen bonding, hydrophobic interaction, and complexation

Hydroxyl, carboxyl, amino, and phosphate groups can form hydrogen bonds and coordination complexes with contaminants. Carboxyl and phosphate groups bind metal ions, while amino and sulfhydryl groups can contribute selective metal affinity. Hydrophobic domains in proteins and lipids can associate with oils, dyes, and organic particles. In complex wastewater, several mechanisms act simultaneously. Therefore, mechanisms should be described as a dominant combination rather than assigned solely from a single FTIR spectrum or cation experiment.

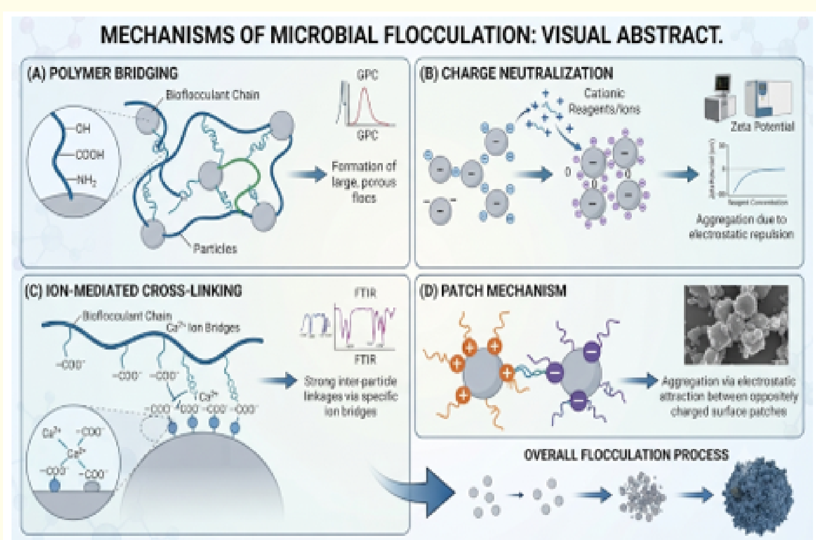

Figure 2: Schematic of the primary microbial flocculation mechanisms: (A) Polymer Bridging, (B) Charge Neutralization, (C) Ion-Mediated Cross-Linking, and (D) Patch Mechanism, along with key characterization methods.

Mechanism	Molecular basis	Typical evidence
Polymer bridging	Long chains adsorb on several particles and create a connected network	High molecular weight, optimum dosage, large irregular flocs, activity without full charge neutralization
Charge neutralization	Oppositely charged polymer or cation reduces electrostatic repulsion	Zeta potential moves toward zero and settling increases
Ion-mediated cross-linking	Divalent or trivalent ions connect anionic polymer groups and particle surfaces.	Strong dependence on calcium, magnesium, iron, or aluminum
Adsorption	Functional groups attach to mineral, organic, or biological surfaces	FTIR or XPS changes and rapid contaminant uptake
Complexation	Carboxyl, phosphate, amino, or sulfhydryl groups coordinate metal ions	Metal removal, spectroscopy, and pH-dependent binding
Hydrogen bonding	Polar groups interact with hydroxylated or oxygen-containing surfaces	pH and solvent effects together with abundant hydroxyl groups
Hydrophobic interaction	Nonpolar polymer domains associate with oils, dyes, and hydrophobic particles.	Improved removal of hydrocarbons or hydrophobic colorants
Sweep entrapment	Particles become trapped in a growing polymer or precipitate network	Dense floc matrix and strong dependence on coagulant aid

Table 3: Principal mechanisms involved in microbial flocculation.

Factors governing flocculation performance

Bioflocculant dosage has an optimum range. Insufficient dosage leaves particles unconnected, whereas overdosing may cause surface saturation, steric stabilization, charge reversal, or excessive viscosity. The optimum value must be established for each wastewater because particle concentration and composition influence polymer demand.

pH controls ionization of polymer functional groups and particle surfaces. It also affects metal hydrolysis and contaminant speciation. A product with broad pH tolerance is desirable for industrial use, but apparent tolerance in a kaolin test may not predict behavior in saline, oily, or metal-rich wastewater. Temperature influences polymer conformation, viscosity, and biological activity. Polysaccharide-rich products often retain activity after heating, while protein-rich products may be more sensitive.

Mixing is needed to distribute the flocculant and promote collisions, but excessive shear can break flocs or degrade long chains. Jar tests should report rapid mixing speed and duration, slow mixing conditions, settling time, vessel geometry, and sample depth for optical measurement. Water ionic strength, competing organic matter, surfactants, salts, and fine particles can enhance or inhibit performance. Standardized reporting would greatly improve comparison among studies [9,22].

Applications of microbial flocculants

Turbidity removal and water clarification

Kaolin clay remains the most common model for primary screening. Several *Bacillus* and *Pseudomonas* products have achieved high clarification under optimized conditions [2,3,11,18-21]. More meaningful application studies use river water, high-turbidity source water, brewery wastewater, or municipal effluent. Chen and colleagues combined production, performance testing, and identification of flocculation-related genes in a high-turbidity drinking water context [20]. For potable water application, pathogen safety, residual organic carbon, odour, color, endotoxin, and disinfection compatibility must be evaluated in addition to turbidity.

Municipal wastewater, activated sludge, and dewatering

Microbial polymers can improve aggregation, settling, and dewatering of activated sludge. EPS addition may influence the formation and mechanical stability of aerobic granular sludge [14]. Wastewater-derived bioflocculants may also create a resource recovery pathway in which organic carbon is converted into a functional polymer instead of being completely oxidized. However, the net environmental benefit depends on fermentation energy, chemicals used for extraction, polymer dosage, and final sludge properties.

Industrial wastewater and organic load reduction

Brewery, dairy, meat processing, starch, textile, pharmaceutical, mining, and aquaculture wastewater have been investigated. A *Bacillus velezensis* bioflocculant showed potential for brewery wastewater treatment [12]. Streptomyces platensis reduced chemical oxygen demand in meat processing wastewater [7]. The polysaccharide bioflocculant MBF 35A removed nutrients and organic load from meat product wastewater [19]. Pseudomonas and Stenotrophomonas strains improved chemical and biochemical oxygen demand in aquaculture wastewater [18,21]. In complex effluent, flocculation is most effective for particulate and colloid-associated pollutants and is often best used as pretreatment or polishing rather than as the only treatment step.

Producer or product	Key feature	Reported application	Reference
Corynebacterium glutamic	Polygalacturonase acid-based polymer	Foundational identification and characterization	[1]
Bacillus subtilis DYU1	High molecular weight polyglutamic acid containing biopolymer	Kaolin flocculation with divalent cations	[2]
Bacillus licheniformis	Extracellular bioflocculant produced after strain optimization	Model suspension clarification	[3]
Halomonas sp. and *Micrococcus* sp. *consortium*	Consortium-derived polymer	Broad pH and cation response	[4]
Rhodococcus erythropolis	Used sludge and livestock wastewater as medium	Low-cost production and wastewater treatment	[5]
Streptomyces platensis	Actinomycete-derived polymer	River and meat processing wastewater	[7]
Bacillus megaterium-derived wastewater polymer.	Size fractionation linked to adsorption and mixed particle flocculation	Mechanistic analysis	[9]
Bacillus subtilis ZHX3	Polysaccharide-rich product	Multiple pollutants including dyes and metals	[11]
Bacillus velezensis	Wastewater sludge isolate	Brewery wastewater treatment	[12]
Pichia kudriavzevii	Carbohydrate-rich yeast polymer	Wastewater clarification	[13]
Corynebacterium glutamic Cg1 P30	Acid-active, metal-binding product	Real acid mine wastewater	[17]
Stenotrophomonas Pavani GXUN74707	Optimized extracellular polymer production	Aquaculture wastewater	[18]
Bacillus subtilis 35A	High-yield polysaccharide-protein product	Dyes, metals, and meat wastewater	[19]
Pseudomonas sp. GXUN74702	Polysaccharide-rich polymer with practical yield	Aquaculture wastewater	[21]

Table 4: Representative studies illustrating microbial flocculant production and application.

Application	Main targets	Important evaluation criteria
Drinking water clarification	Turbidity, suspended solids, natural organic matter	Residual polymer, microbial safety, odour, color, disinfection compatibility
Municipal wastewater	Suspended solids, sludge, particulate organic matter, nutrients	Settling, dewatering, sludge volume, downstream biological effects
Textile wastewater	Dyes, colloids, chemical oxygen demand	Color removal, salt tolerance, pH range, sludge characteristics
Food and brewery wastewater	Proteins, fats, starch, biomass, organic load	Clarification, biochemical and chemical oxygen demand, biodegradability
Mining and metal wastewater	Iron, aluminum, copper, zinc, lead, chromium	Binding capacity, selectivity, acidic pH performance, metal recovery
Aquaculture wastewater	Suspended solids, feed residues, microbial biomass, organic load	Water quality improvement, toxicity to aquatic organisms, reuse potential
Sludge dewatering	Bound water and fine particles	Capillary suction time, specific resistance, cake solids, polymer dose
Microalgae harvesting	Microalgal cells	Harvest efficiency, biomass contamination, reuse of medium, product suitability
Emerging contaminants	Pharmaceuticals, microplastics, pathogens, complex mixtures	Realistic concentration, competing matter, regeneration, hybrid process performance

Table 5: Application areas, target pollutants, and main evaluation requirements.

Dyes, heavy metals, and emerging contaminants

Dyes can be removed by adsorption, charge interaction, and entrapment within flocs. A marine bacterial flocculant was applied to dye wastewater [6], and *Bacillus subtilis* products have shown high removal of cationic dyes [11,19]. Metal removal occurs through complexation, adsorption, ion exchange, and co-precipitation. *Bacillus firmus* polysaccharide, Pseudomonas GO2 polymer, and Corynebacterium glutamic bioflocculant illustrate the potential for binding metals in neutral or acidic water [15,17]. Evidence for pharmaceuticals, microplastics, and mixed emerging contaminants is growing, but many studies remain at batch laboratory scale and require realistic concentration ranges and competing matrices.

Microalgae harvesting and resource recovery

Harvesting microalgae is expensive because individual cells are small and remain suspended. Microbial flocculants or flocculant-producing bacteria can aggregate cells and reduce energy demand for centrifugation. The choice of product must consider whether the harvested biomass will be used for feed, food, fuel, or extraction of valuable compounds. A flocculant suitable for wastewater treatment may not be acceptable when product purity and toxicity are critical. Microalgal bacterial flocs also integrate nutrient removal, biomass production, and solid-liquid separation.

Advantages and limitations

The major advantages of microbial flocculants are renewable biological production, biodegradability, generally low toxicity, chemical diversity, and effectiveness at relatively low dosage in some systems. Their functional groups can support simultaneous particle aggregation and contaminant adsorption. Production from wastes can contribute to a circular bioeconomy by converting low-value organic streams into treatment materials.

These advantages should not be generalized without product-specific assessment. A crude fermentation product may contain cells, nutrients, metabolites, endotoxins, enzymes, or residual substrate. Biodegradability may reduce persistence but can also shorten shelf life or increase treated water organic carbon. Some products require calcium, iron, or aluminum aids, reducing the distinction from chemical treatment. Toxicity and ecotoxicity data are sparse for many new strains and formulations.

Economic limitations remain substantial. Yield is often reported as crude dry mass, purified polymer, or flocculating units, making comparison difficult. Solvent precipitation, dialysis, freeze drying, and chromatography are unsuitable for low-value, large-volume applications unless simplified. Process scale-up may change oxygen transfer and polymer structure. Real wastewater varies daily, while most studies use a single batch or synthetic suspension. These factors explain the gap between high laboratory activity and limited commercial deployment [22-24].

Future perspectives

Standardization and performance benchmarking

A standard minimum data set is urgently needed. Studies should report strain identity and deposit number, medium composition, polymer yield on a clear basis, purification method, composition, molecular weight distribution, dosage, cation concentration, pH, temperature, mixing regime, settling time, water composition, and statistical replication. Benchmarking against alum, ferric salts, and commercial polymers should use equal treatment objectives rather than only equal mass dosage. Standardized kaolin tests are useful for screening but must be supplemented with real wastewater and continuous operation.

Omics, metabolic engineering, and synthetic biology

Genome sequencing, transcriptomics, proteomics, metabolomics, and flux analysis can identify precursor pathways, glycosyltransferases, polymerization complexes, secretion systems, regulatory proteins, and stress responses associated with EPS production. Chen and colleagues linked high turbidity treatment performance with flocculation-related genes [20]. Future strain engineering may redirect carbon from biomass or by-products toward polymer synthesis, improve substrate utilisation, reduce product degradation, and tailor charge or functional group composition. Genetic modification must be balanced with containment, regulatory acceptance, and production stability.

Intelligent bioprocessing and robust scale-up

Data-driven models can integrate fermentation variables, online sensors, and polymer quality measurements. Machine learning may assist in strain ranking, medium optimization, prediction of activity, and control of batch variability. However, models require high-quality standardized datasets and external validation. Scale up should focus on oxygen transfer, rheology, mixing, foam, and downstream integration. Continuous or repeated batch production may improve productivity but increases contamination and genetic stability concerns.

Product formulation, hybrid systems, and reuse

Commercial products must be stable, easy to transport, rapidly soluble, and compatible with existing dosing equipment. Immobilization, encapsulation, spray-dried powders, carrier-based formulations, and composite bioflocculants may improve stability and reduce dosage. Hybrid systems combining microbial flocculants with membranes, adsorption, advanced oxidation, electrocoagulation, biological treatment, or constructed wetlands can address dissolved contaminants that flocculation alone cannot remove. Recovery and reuse of flocculant or captured metals could further improve economics.

Sustainability, regulation, and commercialization

Future claims of sustainability should be supported by life cycle assessment and techno-economic analysis. Important inputs include feedstock collection, sterilization, fermentation energy, aeration, solvent use, water demand, drying, transport, dosage, sludge production, and end of life. Pilot studies should measure long-term performance, process reliability, operator requirements, and compatibility with existing infrastructure. Product regulation will depend on intended use, purity, microbial safety, ecotoxicity, residual organic carbon, and consistency between batches.

Barrier	Consequence	Priority response
Low or inconsistent yield	High cost and variable product supply	High-producing strains, fed-batch strategies, pathway engineering, robust control
Expensive medium	Poor economic competitiveness	Validated agro-industrial wastes, feedstock blending, minimal nutrient supplementation
Complex recovery	High solvent, energy, and labor demand	Use crude or partially purified products where safe; membrane concentration and scalable drying.
Variable composition	Uncertain performance and difficult quality control	Define critical quality attributes and rapid analytical fingerprints

Cation dependence	Additional chemical use and operational complexity	Develop cation-independent or low-aid formulations and optimize water chemistry
Storage instability	Short shelf life and activity loss	Stabilizers, encapsulation, dry formulations, accelerated stability testing.
Nonstandard assays	Poor comparison among studies	International methods for yield, activity, dose, mixing, and wastewater characterization
Limited real wastewater evidence	Uncertain field reliability	Long-term pilot trials across seasons and variable loading
Incomplete safety data	Regulatory and public acceptance barriers	Toxicology, ecotoxicology, pathogen and endotoxin assessment
Uncertain sustainability	Green claims cannot be verified.	Life cycle assessment, techno-economic analysis, and comparison with incumbent products

Table 6: Current barriers and priority directions for commercialization.

Conclusion

Microbial flocculants are a diverse group of extracellular biopolymers with substantial potential for sustainable solid-liquid separation and pollutant removal. Bacteria remain the dominant production platform, but fungi, yeasts, actinomycetes, algae, cyanobacteria, and microbial consortia expand the range of structures and functions available. Product performance is governed by microbial genetics, feedstock composition, fermentation conditions, molecular weight, charge, functional groups, water chemistry, dosage, supporting ions, and mixing. Polymer bridging, adsorption, charge neutralization, ion-mediated cross-linking, hydrogen bonding, hydrophobic interactions, and metal complexation usually act together. Laboratory studies demonstrate promising removal of turbidity, dyes, metals, suspended solids, nutrients, and organic matter, as well as benefits for sludge treatment and microalgae harvesting. Nevertheless, broad commercial adoption requires more than high flocculating activity in kaolin suspension. Economical production, scalable recovery, stable formulation, standardized testing, safety evaluation, and long-term validation in real wastewater are essential. Integration of waste-based feedstocks, systems biology, metabolic engineering, advanced characterization, intelligent process control, and hybrid treatment offers a realistic path toward application-specific products. Future research should emphasize reproducible quality and verified environmental and economic benefit so that microbial flocculants can progress from promising laboratory materials to dependable components of modern water treatment.

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