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Recent Approaches and Trends in Bio-flocculants for Environmental Sustainability: An Apodictic Review

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Abstract: Bio-flocculants are rich in polysaccharides that can flocculate colloidal and suspended particles by aggregating and forming flocs. They gained attention in wastewater treatment, the food industry, the dye industry, and downstream processing for their bio-degradability and eco-friendly activity. The current review focuses on bio-flocculant trends, involving information on microorganisms isolated and made to produce bio-flocculants and their applications in various fields. Furthermore, this review underscores bio-flocculants' significance in ecosystem preservation and their pivotal role in environmentally friendly pollution control strategies. By elucidating the intricate relationship between carbon and nitrogen sources, purification methods, characterization techniques, and the influence of metal ions on flocculation, this review contributes to a deeper understanding of bio-flocculant production and its potential applications in sustainable environmental management.

Keywords: bio-flocculant; production; environment-friendly; characterization; wastewater treatment.

1. Introduction

Flocculation is a process in which the suspended materials are aggregated using an aggregator and settle down the aggregates by means of gravity. Compounds required for flocculation are called flocculants and, based on the source it was categorized into inorganic flocculants (aluminum sulfate and polyaluminium chloride), Organic synthetic flocculants (Polyacrylamide derivatives, and Polyethylene amine) [1] and naturally occurring flocculants (Sodium alginate, chitosan, and microbial flocculants) [2]. Different types of bio-flocculants are available on the market, and their unique properties are based on their applications [3]. Among these polysaccharides, bio-flocculants have gained attention recently due to their better removal efficiency and eco-friendliness [3]. [4,5]Due to their cost-effectiveness and efficient flocculation, chemical flocculants are commonly used in drinking water processing and wastewater treatment industries, downstream processes in the food industry, and dye industries [4,5]. However, the use of these chemical flocculants can cause health and environmental problems. For example, residual aluminum from Polyaluminium chloride (PAC) and acrylamide monomers from polyacrylamide are known to be neurotoxic and carcinogenic to humans [6]. Flocculants are mostly preferred in wastewater treatment because of their dominant role in effective flocculation and cost-effectiveness. Water is an inevitable source for human beings. Many anthropogenic activities cause chemical contaminants in water resources, which pose potential risks to humans and the environment. There are various methods employed in wastewater treatment, among which flocculation is widely used in industries for its high efficiency and economic convenience [7]. Bio-flocculants have gained industry attention for their eco-friendliness, biodegradability, and safety for ecosystems, as well as for being substitutes for chemical flocculants. They are natural polymers produced by various microorganisms, including bacteria, fungi, algae, and even plants [8,9]. Microorganisms produce extracellular polymeric substances (EPS) and intracellular polysaccharides (IPS) through their metabolic pathways that are biodegradable and non-toxic, making them an environmentally friendly alternative to chemical flocculants [10]. The flocculation mechanism can be classified into cation-dependent bio-flocculation and cation-independent bio-flocculation [11]. Flocculation can be affected by various parameters such as concentration of bio-flocculants, pH, temperature, salinity, metal ions, etc. To optimize the parameters, Response surface methodology (RSM), a statistical tool, is used to offer reliable information on optimal performance [12]. Plackett-Burma design can be helpful for further optimization when there are more factors [13]. The production of bio-flocculant can be affected by carbon sources, nitrogen sources, the pH of the medium, etc. Agricultural waste such as rice stover and corn stover serve as inexpensive carbon sources [14]. Many different types of bio-flocculants are available in the market, with their unique properties and based on their application in culturing organisms producing bio-flocculants. Hence, bio-flocculants can be used as an alternate and efficient flocculation method. The advantages of the bio-flocculants are represented in Figure 1.

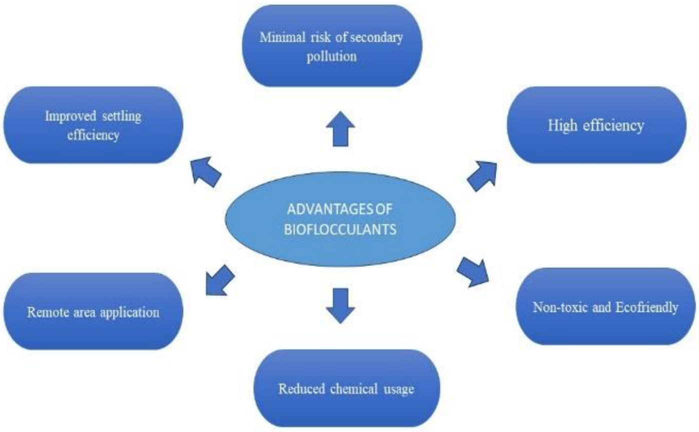

Figure 1. Advantages of bio-flocculants.

2. Materials and Methods

This review gives a comprehensive overview of Bio-flocculants, covering their various sources from where they are being isolated, growth media, compositions, production and purification techniques, factors affecting flocculating activity, advanced characterization techniques, and their vast applications in various sectors. This article incorporates insights from recent scientific research articles and reviews to enhance its breadth and depth. This review comprises 130 references, with the majority focusing on recent years' articles (around 44.7% from 2016-2023), highlighting the growing significance of Bio-flocculants and their applications in vast fields. The second most cited span between 2011 and 2015, accounting for around 28.79%. About 12.87% of the sources were cited from 2006 to 2010, and around 7.57% were cited from 2001 to 2005, with the remaining 5.3% predating 2000. These above-mentioned details are graphically represented in Figure 2.

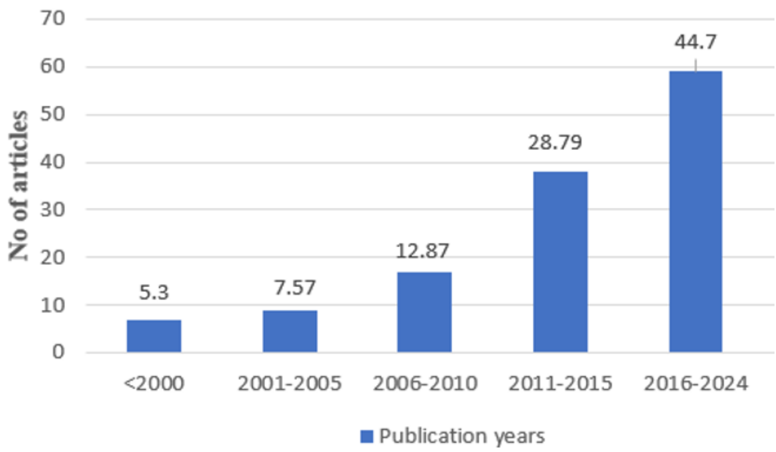

Figure 2. Year-wise reference detail.

3. Sources of Bio-flocculants

Bio-flocculants are being identified from various sources. The Exo polymeric substances (EPS) based bio-flocculants are produced by various organisms, including bacteria, fungi, algae, and yeast. Those polymeric substances can be polysaccharides, proteins, lipids, glycoproteins, glycolipids, etc., [15]. Researchers continue to explore and optimize bio-flocculant production from various sources to address environmental and industrial challenges. Table 3.1 displays the details of various microbes that produce effective bio-flocculants and the specific sources from which these microbes were isolated.

Table 1. Bio-flocculant-producing microbes and their source.

S. No	Name of the organism	Source	Reference(s)
1	*Proteus mirabilis TJ-1*	Mixed activated sludge	[16]
2	*Solibacillus silvestris W01*	Activated sludge	[17]
3	*Pichia kudriavzevii MH545928.1*	Kombucha tea, Greenheart Organics Pinetown in Durban KwaZulu-Natal Province, South Africa.	[18]
4	*Bacillus nitratireducens*	Palm oil mill effluent, wastewater pond of United Oil Palm Industries Sdn. Bhd., Malaysia	[19]
5	*Paenibacillus xylanilyticus.*	Water reservoirs and water treatment plants- Ranchi, India	[20]
6	*Aspergillus parasiticus*	China General Microbiological Culture Center	[21]
7	*Leuconostoc lactis KC117496*	Idly batter	[22]
8	*Bacillus subtilis and Pseudomonas sp*	Crude petroleum oil	[23]
9	*Enterobacter cloacae WD7*	recycled sludge from five seafood processing plants	[24]
10	*Bacillus sp, halobacillus sp, virgibacillus sp, cobetia sp.*	Algoa bay	[25–28]
11	*Klebsiella pneumoniae strain NY1 - MNXY1*	Municipal wastewater treatment plant	[29]
12	*Bacillus pumilus*	Thyume River in Alice, Eastern Cape, South Africa.	[30]
13	*Bacillus megaterium SP1*	Pond water	[31]
14	*Chryseobacterium daeguense W6*	Backwashing sludge of biologically aerated filter	[32]
15	*Klebsiella sp. - OS-1*	Brewery wastewater	[33]
16	*Bacillus circulans*	Soil samples from Sun Yat-sen University (Guangzhou, China)	[34]
17	*Corynebacterium glutamicum CCTCC M201005*	China Center for Type Culture Collection (CCTCC, Wuhan, China)	[35]
18	*Scenedesmus sp.*	Korean Collection for Type Cultures	[36]
19	*Vagococcus sp. W31*	Wastewater samples - Little Moon River -Beijing	[37]
20	*Myxobacterium nannocystis sp. NU-2*	Salt soil sample from Huanghai Sea, China.	[38]

S. No	Name of the organism	Source	Reference(s)
21	*Streptomyces platensis*	Strekfontein dam	[39]
22	*Bacillus velezensis*	Sludge wastewater sample, KwaZulu Natal Province, South Africa	[40]
23	Actinobacterial strain AUABF-*Streptomyces sp.*	Mangrove sediments of Vellar estuary, Southeast Coast, India	[41]
24	*Corynebacterium glutamicum* Cg1-P30	China General Microbiological Culture Collection Centre	[42]
25	*Aspergillus oryzae*	China General Microbiological Culture Collection Centre	[43]
26	*Citrobacter youngae* GTC 01314	Pathogenic Bacterial Genetic Resource Stock Center, School of Medicine, Gifu University, Japan	[44]
27	*Klebsiella oxytoca* GS-4-08	A laboratory strain cultured in a lab in China	[45]
28	*Leuconostoc citreum* SJ-57	Sweet Potato sour liquid	[46]
29	*Chromobacterium violaceum* LT-5, *Citrobacter Koseri* LT-6	Tapioca wastewater at Sukaraja region, Indonesia	[47]
30	*Klebsiella pneumoniae*-UKD24	Domestic Sewage Treatment plant	[48]
31	*Bacillus mojavensis* strain 32A	Egyptian salt production pond	[49]
32	*Vibrio navarensis* Strain 34, *Pseudoalteromonas ganghwensis* Strain WAB2121, *Staphylococcus gallinarum* Strain-E2, *Cytobacillus kochii* Strain FJAT-46231	Pangandaran coastal shrimp ponds	[50]
33	*Arthobacter sp.* 5J12A	Tyume River, South Africa	[51]
34	*Bacillus arybhattai*	Soil and Cassava root samples	[52]
36	*Phyla proteobacteria*	Felda Maokil palm oil mill, Labis, Johar	[53]
37	*Bacillus subtilis* F9	Wastewater sludge of Periyar Maniammai University, India	[54]
38	*Cellulosimicrobium cellulans L804*	Corn farmland samples	[55]
39	*Turicibacterium sanguinis ZCY83*	Methanol wastewater treatment plant, China	[56]
40	*Bacillus licheniformis, Bacillus firmus*	National Collection of Industrial Microorganisms, Pune, India	[57]
41	*Achromobacter xylosoxidans* Strain TERI L1	Activated sludge sample, Bongaigaon oil refinery	[58]
42	*Bacillus marisflavi* NA8	Palm oil mill, Labu, Malaysia	[59]
43	*Bacillus agaradhaerens* C9	Alkaline lake sample	[60]
44	*Pseudomonas aeruginosa* Strain IASST201	Numaligarh refinery limited, Bongaigaon refinery limited, Noonmati refinery of Indian oil corporation, Assam, India.	[61]
45	*Bacillus aryabhattai* Strain PSK1	Egyptian agricultural soil	[62]
46	*Pseudomonas aeruginosa* ZJU1	Institute of Hydrobiology, Wuhan, China.	[63]
47	*Pseudomonas veronii* L918	Nehe City, China	[14,64]
48	*Bacillus amyloliquefaciens* ABL19	Soil and activated sludge samples, Adeti stream, Nigeria	[65]
49	*Bacillus subtilis*	Intertidal one of Bohai, China	[66]
50	*Pseudomonas sp* HP2	Forest soil, Ningbo, China	[67]

4. Nutritional Requirements for Bio-flocculant Production

Bio-flocculants are biopolymers produced by microorganisms during their growth for various metabolic activities. They are biodegradable and produce no secondary pollution; their intermediates do not pose any risk to the ecosystem [18]. An optimized production medium should include sufficient carbon and nitrogen sources, sulfur, phosphorous, vitamins, and other minor nutrients for a better bio-flocculant yield. The carbon source is essential for cell growth, and nitrogen is important for synthesizing amino acids, nucleic acids, and enzyme synthesis. Sulfur and phosphorous are needed to synthesize proteins and co-enzymes from some vitamins [68]. The growth of the microorganism is also directly influenced by various environmental parameters, including pH, temperature, aeration, and agitation. The temperature of the culture greatly influences the flocculant yield in the microorganism as various genetic factors and enzymes responsible for the flocculant activity get triggered at specific temperatures [69]. Detailed information on the optimized growth medium, growth condition, and respective bio-flocculant yield for various bio-flocculant-producing microorganisms is provided in Table 4.1.

Table 2. Details of various medium compositions and growth conditions used for bio-flocculant production.

S. No	Bio-flocculant producing microbe	Medium composition (Per Liter)	Growth conditions (pH, temp, agitation)	Yield	Reference
1	*Proteus mirabilis*	Glucose - 10g; Peptone - 1g; $MgSO_4$ .7 H_2O - 0.3 g; K_2HPO_4 - 5g; KH_2PO_4 - 2g.	pH 7.0 25°C 130 rpm	1.33 g/L	[16]
2	*Paenibacillus xylanilyticus*	Dextrose - 10g; Peptone - 5g; Casein hydrolysate - 5g; $MgSO_4$ .7 H_2O, - 0.2 g; K_2HPO_4 - 5g; KH_2PO_4 - 2g; NaCl - 0.1g	pH 6.8±0.2 30°C 180rpm	2.3 g/L	[20]
3	*Streptomyces sp*	Palm jaggery - 20g; Yeast extract - 2.5g; NH_4NO_2 - 1.0g; $MgCl_2$ - 0.2g; K_2HPO_4 - 5g; NaCl - 0.5g.	pH 7.0 30°C 180rpm	3.5 g/L	[70]
4	*Bacillus subtilis*	Glucose - 30g; Yeast extract - 8g; K_2HPO_4 - 2g; $MgSO_4$ - 0.25g; $C_5H_8NO_4Na$ - 10g.	pH 7.5 35°C 180rpm	0.134 g/L	[71]
5	*Klebsiella sp*	Glucose - 20 g; $(NH_4)_2SO_4$ - 0.2g; KH_2PO_4 - 2 g; K_2HPO_4 - 5 g; NaCl - 0.1 g; Urea - 0.5 g; yeast extract - 0.5 g	pH 7.0 35°C	94.7%	[33]
6	*Pichia kudriavzevil*	Glucose - 20g; KH_2PO_4 - 2g; K_2HPO_4 - 5g; NaCl - 0.1g; CH_4N_2O - 0.5g; $MgSO_4$ - 0.2g; Peptone - 0.5g.	pH 7 35°C 140rpm	2.836 g/L	[18]
7	*Bacillus licheniformis*	Sucrose - 10g; Yeast extract -1g; Urea - 1g; KH_2PO_4 - 0.1g; K_2HPO_4 - 0.1g; NaCl - 0.1g; $MgSO_4.7H_2O$ - 0.2g.	pH 7.2 - 7.5 37°C 200rpm	2.93 g/L	[72]
8	*Klebsiella pneumoniae*	Glucose - 20g; Yeast extract - 0.5g; Urea - 0.5g; $(NH_4)_2SO_4$ - 0.2g; KH_2PO_4 - 0.2g; K_2HPO_4 - 0.5g; $MgSO_4.7H_2O$ - 0.2g; NaCl -0.1g.	pH 7 30°C 200rpm	1.67 g/L	[29]
9	*Chryseobacterium daeguense*	Glucose - 1 g Tryptone - 2 g Mg $(NO_3)_2$ - 0.2 g	pH 5-7 30°C 180 rpm	1.2 g/L	[32]
10	*Bacillus circulans*	Glucose - 10g; $CO(NH_2)_2$ - 0.44g; K_2HPO_4 - 2.5g;	pH 8 37°C 160rpm	0.78 g/L	[34]

S. No	Bio-flocculant producing microbe	Medium composition (Per Liter)	Growth conditions (pH, temp, agitation)	Yield	Reference
		KH_2PO_4 - 2g.			
11	*Aspergillus parasiticus*	Sucrose - 30g; $NaNO_3$ - 3g; $MgSO_4.7H_2O$ - 0.5g; KCl - 0.5g; $FeSO_4$ - 0.01g; K_2HPO_4 - 1g.	pH 6.0 30°C 140 rpm	0.56 g/L	[21]
12	*Bacillus pumilus*	Maltose - 20g; K_2HPO_4 -5 KH_2PO_4 - 2g; Urea - 0.5g; Yeast extract - 0.5g; $(NH_4) SO_4$ - 0.2g; $MgSO_4.7H_2O$ - 0.2g.	pH 7 28°C 160rpm	2.5 g/L	[30]
13	*Bacillus megaterium*	Glucose - 20g; K_2HPO_4 - 5g; KH_2PO_4 - 2g; $MgSO_4.7H_2O$ - 0.5g; $(NH_2)_2SO_4$ - 0.2g; NaCl - 0.1g; Urea - 0.5g; Yeast extract - 0.5g.	pH 7 30°C 160rpm	-	[31]
14	*Bacillus sp F19*	Sucrose - 20g; Yeast extract - 2.5g; KH_2PO_4 - 2g; K_2HPO_4 - 5g; NaCl - 0.1g.	pH 7 30°C 200rpm	1.47 g/L	[73]
15	*Bacillus sp. HXG-C1*	Glucose - 10g; Peptone - 1g; $MgSO_4.7H_2O$ - 0.3g; KH_2PO_4 - 2g; K_2HPO_4 - 5g.	pH 7 28°C 160rpm	72.4%	[25]
16	*Bacillus salmalaya* 139SI-7	Sucrose - 10g; Glucose - 5g; Yeast extract - 1.5g; Urea - 1.5g; K_2HPO_4- 0.1g; KH_2PO_4 - 0.1g; NaCl - 0.1g; $MgSO_4.7H_2O$ - 0.2g.	pH 7.0 ± 0.2 35.5°C 150rpm	2.75 g/L	[74]
17	*Bacillus sp*	Glucose- 10g; Yeast extract - 2.5g; $MgSO_4$ - 0.8g; K_2HPO_4 - 5g; KH_2PO_4 - 2g; Urea - 0.5g; NaCl - 0.1g.	pH 7 37°C 200rpm	0.16 g/L	[75]
18	*Bacillus subtilis and Pseudomonas sp.*	Glucose - 10g; NH_4Cl - 7g; K_2HPO_4 - 0.5g; $MgSO_4$ - 0.5g; $FeCl_3$ - 0.04g; $CaCl_2$ - 0.15g; $MnSO_4$ - 0.14g.	pH 7.2 to 7.5 30°C 200rpm	-	Click or tap here to enter text.[23]
19	*Virgibacillus species*	Glucose - 20g; Urea - 0.5g; Yeast extract - 0.5g; $(NH_4)_2SO_4$ - 0.2g; K_2HPO_4 - 5g; KH_2PO_4 - 2g; NaCl - 0.1g; $MgSO_4.7H_2O$ - 0.2g.	- 30°C 160rpm	2.43 g/L	[27]
20	*Alcaligenes faecalis* HCB2	Glucose - 20g; Yeast extract - 0.5g; KH_2PO_4 - 2g; K_2HPO_4 - 5g; $(NH_4)_2SO_4$ - 0.2g;	- 30°C 165rpm	4 g/L	Click or tap here to enter text.[6]

S. No	Bio-flocculant producing microbe	Medium composition (Per Liter)	Growth conditions (pH, temp, agitation)	Yield	Reference
		NaCl - 0.1g; $MgSO_4$ - 0.2g; CH_4NO_2 - 0.5g.			
21	*Bacillus mucilaginosus*	Soluble Starch - 1.5g; K_2HPO_4 - 0.6g; Yeast extract - 0.3g; $MgSO_4.7H_2O$ - 0.02g; NaCl - 0.01g.	pH 8 30°C 140rpm	0.7 g/L	[78]
22	*Corynebacterium glutamicum*	Sucrose - 10g; KH_2PO_4 - 0.1g; Corn Steep Liquor - 0.5ml; Urea - 0.5g; NaCl - 0.1g; $MgSO_4.7H_2O$ - 0.2g.	pH 7.8 28°C 120rpm	-	[35]
23	*Paenibacillus polymyxa* AM49	Glucose - 40g; NH_4NO_3 - 1g; KH_2PO_4 - 0.3g; K_2HPO_4 - 0.3g; $MgSO_4.7H_20$ - 0.1g; $MnSO_4.4H_2O$ - 0.1g. NaCl - 0.05g; $CaCO_3$ - 0.4g. Yeast extract - 0.1g; Soytone - 0.1g; Tryptone - 0.1g.	pH 7 30°C 200rpm	2.35 g/L	[36]
24	*Serratia ficaria*	Glucose - 10g; Yeast extract - 0.5g; Urea - 0.5g; NaCl - 0.1g; KH_2PO_4 - 5g.	pH 7.0 to 7.2 120rpm	95.4%	[79]
25	*Vagococcus sp.* W31	Glucose - 20g; KH_2PO_4 - 2g; K_2HPO_4 - 5g; $(NH_4)_2SO_4$ - 0.2g; NaCl - 0.1g; Urea - 0.5g; Yeast extract - 0.5g.	pH 6.5 25°C 120rpm	2.3 g/L	[37]
26	*Actinobacterium Streptomyces sp.*	Yeast extract - 2.5 g; Palm jaggery - 20g; NH_4NO_2 - 1g; $MgCl_2$ - 0.2g; K_2HPO_4- 5g; NaCl - 0.1g.	pH 7.0 30°C 200rpm	99.18%	[41]
27	*Chromobacterium violaceum*	Glucose - 10g; Sucrose - 10g; Peptone -0.5g; Yeast extract - 1.5g; Urea - 1g; $(NH_4)_2SO_4$ - 0.5g; KH_2PO_4 - 1.5g; K_2HPO_4 - 4.5g; $MgSO_4$ - 0.2g; NaCl - 0.1g.	pH 7.3 27°C 120rpm	68.92%	[47]
28	*Chryseobacterium daeguense W6*	Glucose - 1g; Tryptone - 2g; $Mg(NO_3)_2$ - 0.2g.	pH 6 30°C 180rpm	92.43%	[80]
29	*Aspergillus flavus*	Sucrose - 12g; Peptone - 7.52g; $MgSO_4.7H_2O$ - 0.5g; KCl - 0.5g; FeSO4- 0.01g; KH_2PO_4 - 1.0g.	pH 7.5 28°C 150 rpm	-	[81]
30	*Bacillus cereus* and *Bacillus thuringiensis*	Starch - 20g; Yeast extract - 3g; CaCO3 - 5g; KH_2PO_4 - 0.5g; MgSO4.7H2O - 0.2g;	pH 7.2 37°C	-	[82]

S. No	Bio-flocculant producing microbe	Medium composition (Per Liter)	Growth conditions (pH, temp, agitation)	Yield	Reference
		NaCl - 0.2g; CaSO4 - 0.1g; FeSO4.7H2O - 0.01g; Agar - 15g; MnSO4.7H2O - traces; Na2MoCu.2H2O - traces.			
31	*Actinomycete Streptomyces sp hsn06*	Glucose - 10g; NaCl - 24g; NH_4Cl - 1g; $MgSO_4.7H_2O$ - 0.5g; Yeast extract - 0.6g; KH_2PO_4 - 2g; $K_2HPO_4.3H_2O$ - 6.5g	28°C 120rpm	92.7%	[83]
32	*Kocuria rosea BU22S*	Glucose - 10g; NaCl - 2g; Peptone - 5g; KH_2PO_4 - 0.25g; $MgSO_4$ - 0.2g.	pH 8.0 30°C	4.72±0.02 g/L	[84]
33	*Proteus mirabilis*	Glucose - 20g; KH_2PO_4 - 5g; K_2PO_4 - 2g; Urea - 0.5g; Yeast extract - 0.5g; $MgSO_4.7H_2O$ - 0.2g; $(NH_4)_2SO_4$ - 0.2g; NaCl - 0.1g.	30°C 160rpm	70%	[85]

5. Composition and Characterization of Bio-flocculants

Bio-flocculants are high molecular weight polymers produced by microbes in their life cycle. These polymers comprise various compounds and functional groups that provide unique physiochemical properties. The composition of bio-flocculants may vary depending on the microorganisms, substrate, and environmental conditions. Generally, bio-flocculants are composed of polysaccharides, proteins, glycoproteins, lipids, glycolipids, and organic and inorganic components. In addition to that, it also contains organic acids like pyruvic acid, acetic acid, inorganic ions, and neutral sugar [86]. Generally, bio-flocculants have higher sugar content than proteins, but the bio-flocculants produced by *Chryseobacterium daeguense* have higher protein content than sugar [87]. Microbial exopolysaccharides (EPS) are the main chemical components of bio-flocculants. These are polymers secreted by microorganisms with diverse chemical compositions, resulting in various properties that cannot be seen in plant-derived polysaccharides. EPS is widely used in the food industry as viscosifying, stabilizing, gelling, or emulsifying agents. The composition of bio-flocculants is studied and estimated using various assays to analyze their properties. These assays aid in detecting the presence of specific functional groups and biomolecules responsible for the unique properties of the bio-flocculants. Phenol sulfuric acid assay [88] is widely used for quantitatively estimating total carbohydrates in bio-flocculants. Bradford [89] and Lowry's assay are used to quantify protein [90]. Anthrone assay for neutral sugar, carbazole sulfate assay for uronic acid, and Elson morgan assay for amino sugar are various biochemical assays used for characterizing the bio-flocculants. Nucleic acids were measured by using a diphenylamine colorimetric assay [91]. High-Performance Gel Permeation Chromatography (HPGPC) is used to measure the molecular weight of bio-flocculants. XPS analyses atomic composition and mass fraction. The thermal stability of bio-flocculants is determined by TGA analysis. Further, the complete structural characterization of EPS can be determined by using techniques such as 2D NMR analysis, which involves Correlated spectroscopy (COSY), Total Correlated spectroscopy

(TOCSY), Nuclear Overhauser Effect Spectroscopy (NOESY), Heteronuclear Multiple Quantum Correlation (HMQC) and Heteronuclear Multiple bond Correlation (HMBC) experiments [92]. The average molecular weight of the bio-flocculant is 4.79 × 10^5 Da [93], and most of the bio-flocculants are in the range of 2.5 x 10^6 Da [4]. Also, the functional group analysis is carried out by FTIR spectroscopy [94], X-ray photoelectron spectroscopy (XPS), and NMR spectroscopy [95]. Infrared spectrophotometry analysis reveals the presence of carboxyl, hydroxyl, and methoxy groups in the bio-flocculants [3]. These hydroxyl, amide, and carboxyl derivatives further contribute to their flocculation activity [97]. To determine the secondary structure of bio-flocculants, IR spectroscopy was performed with Bruker FTIR spectrometer and Deuterated triglycine sulfate (DTGS) as detector [91]. The integrated information about the microbial source, its bio-flocculants, the chemical composition of the bio-flocculant, and relevant analytical techniques used in bio-flocculant characterization are listed in Table 5.1.

Table 3. Composition of bio-flocculants from different microbial and plant sources.

Bio-flocculant producing organism	Bio-Flocculant	Chemical composition	Analytical method used	Reference (s)
Enterobacter sp.	ETH - 2	Total sugar - 91.7% Total protein -1.8%	SEM, FTIR, GPC, XPS.	[97]
Bacillus sp.	ISTVK1	Carbohydrate - 72.4% (Eb)	Phenol sulfuric acid assay	[98]
		Protein - 2.12% (Eb)	Bradford assay	
		Eb- carbon (40.92%), oxygen (58.09%), sodium (0.59%), sulfur (0.35%) and phosphorus (0.06%).	EDX	
Bacillus sp.	ISTVK1	Carbohydrate - 54.8% (Em)	Phenol sulfuric acid assay	[98]
		Protein - 3.0% (Em)	Bradford assay	
		Oxygen (76.7%), sodium (12.47%), phosphorous (6.38%), carbon (3.49%), magnesium (0.8%)	EDX	
Rhizobium radiobacterF2 and Bacillus sphaeicusF6	CBF F-26	Carbohydrate - 85.82%	Phenol sulfuric acid assay	[93]
		Proteins - 1.86 %	Bradford assay	
Bacillus licheniformis X-14	ZS-7	Carbohydrate -91.5%	Phenol sulfuric acid assay	[95]
		Protein - 8.4%	Bradford assay	
		Uronic acid - 16.4 %	Carbazole sulfuric acid assay	
		Pyruvic acid -7.1% Acetic acid - 0.5%	Friedman method	
		Carbon - 32.1% Hydrogen - 6.2% Oxygen - 55.1% Nitrogen - 5.3% Sulfur - 1.3%	Elemental analysis	
Proteus mirabilis	TJ-F1	Total protein: 63.1%	Bradford assay	Click or tap here to enter text.[16]
		Total sugar: 30.9%	Phenol sulfuric acid assay	
		Neutral sugar: Uronic acid: Amino sugar = 8.2: 5.3: 1	Anthrone assay: Carbazole sulfuric acid assay: Elson-morgan assay	
Bacillus subtilis	MSBF17	Total sugar: 58.6%	Phenol sulfuric acid assay	[12]
		Total protein: 17.8%	Bradford assay	
		Neutral sugar: 4.2% Uronic acid: 41.3% Amino sugar: 1.2%	Trifluoracetic acid	
		C: H: O: N: S= 42.35: 7.62: 39.51: 7.98: 1.56	Elemental analysis	
Bacillus sp.	MBFF19	Total sugar: 66.4%	Phenol sulfuric acid assay	Click or tap here to
		Total protein 16.4%	Lowery assay	
		Amino sugar: 0.5%	Elsom-Morgan method	
		Neutral sugar: 3.6% Uronic acid: 37%	Carbazole-sulfuric acid assay	

Bio-flocculant producing organism	Bio-Flocculant	Chemical composition	Analytical method used	Reference (s)
		C: H: O: N: S = 36.9: 5.9: 51.7: 4.4: 1.1	Elemental analysis	enter text.[24]
Aspergillus parasiticus	Bio-flocculant from Aspergillus parasiticus	Total sugar: 76.3%	Phenol sulfuric acid assay	[4]
		Total protein: 21.6%	Lowery-Folin assay	
Alcalignes faecalis	MBF-16	Total sugar: 90.9%	Phenol-sulfuric acid assay	[101]
		Total protein: 7.1%	Bradford assay	
		Neutral sugar: 20.11%	Anthrone reaction	
		Uronic acid: 30.23%	Carbazole-sulfate reaction	
		Amino sugar: 0.88%	Elson-morgan assay	
		C: O: H: N = 39.61: 33.42: 7.43: 7.22: 0.92	Elemental analysis	
Klebsiella pneumoniae YZ-6	MBF-6	Total carbohydrates:95.1%	Phenol sulfuric acid assay	[102]
		Total protein: 3.4%	Bradford assay	
		Neutral sugar: 50%	Anthrone method	
		Uronic acid: 8.4%	Carbazole sulfate acid reaction	
		Amino sugar: 5.8%	Elson-Morgan method	
		C: H: O: N: S =40.29: 6.52: 44.45: 7.52: 1.01	Elemental analysis	
Cellulosimicrobium cellulans L804	MBF-L804	Total sugar: 68.6%	-	[103]
		Total protein: 20%	-	
Colocasia antiquorum	CYBF (Plant bio-flocculant)	Total sugar: 65%	Phenol-sulfuric acid assay	[8]
		Total protein: 9%	Bradford assay	
		Uronic acid: 13%	Carbazole assay	
Paenibacillus elgii B69	MVF	Total protein - Nil	Bradford assay	Click or tap here to enter text.[79]
		Nucleic acid - Nil	UV spectrophotometer	
		Glucose: glucuronic acid: Xylose: Mannose = 1:0.5: 1.15: 0.46	Trifluoracetic acid assay	

5.1. Bio-flocculant efficacy studies

A study should be conducted on the effectiveness of purified bio-flocculants. The Trona suspension method, activated carbon method, soil, cellulose powder, and Kaolin suspension method are some methods for determining the flocculating efficiency of bio-flocculants [105]. Kaolin clay suspension is one of the widely preferred methods to determine the flocculating activity of bio-flocculants. For this, the Kaolin solution mixed with the purified bio-flocculant is subjected to stirring and left for a while to settle the Kaolin particles at the bottom. The supernatant of the solution is then taken for further optical analysis.

The following equation is used to determine the percentage of flocculating activity of the bio-flocculant.

$$\text{Flocculating activity} = \frac{(A-B)}{A}\times 100 \qquad (1)$$

where A is the Optical Density (OD) of kaolin clay suspension at 550 nm, and B is the OD of the treated solution at 550 nm.

6. Bio-flocculant Production Strategies

The transition from laboratory-scale bio-flocculant production to large-scale implementation is a pivotal process that demands careful consideration and execution. Suitable microbial strains must be chosen for bio-flocculant production to achieve the highest and desired production levels in large-scale prospects.

Various cultural conditions influence the growth of the microorganism. The temperature during the growth of microorganisms, pH of the growth medium, initial inoculum size, mode of fermentation, agitation, and the carbon-nitrogen ratio all directly influence the large-scale production of bio-flocculants.[69,106]. The optimum temperature for the growth of these microorganisms is 37±2°C [68].

The initial pH of the culture medium also plays an important role in producing bio-flocculants. Based on the pH requirement, the microorganisms are classified into three categories: acidophilic, alkaliphilic, and neutrophilic [69]. However, most bio-flocculant-producing microbes are efficient at neutral pH [21]. pH also influences the nutrient absorption capability of the microorganism. At neutral pH, the nutrient absorption capability is high due to the neutral electric charge of the growth medium, indicating higher bio-flocculant production [74].

The volume of the microbial inoculum also has a significant effect on the overall growth parameters. The lower the volume of the microbial inoculum, the more the microbe will have an extended lag phase, while the greater the volume of the microbial inoculum will have an elevated growth potential, leading to nutrient depletion before product production. It is found that the optimal 1% (v/v) of the inoculum is better for the maximum production of the bio-flocculants [34,79].

Carbon sources are considered the essential factors for the growth and metabolism of an organism, resulting in the production of bio-flocculant. There are various sources of carbon like glucose, fructose, galactose, lactose, maltose, sucrose, etc. [26,28]. Nitrogen sources play an important role in the production of the enzymes, amino acids, and nucleic acids required for the growth and metabolism of organisms. Urea, peptone, yeast extract, ammonium chloride, and ammonium sulfate are some of the nitrogen sources used. The organic nitrogen source yeast extract showed the best bio-flocculant yield, and in the case of using inorganic nitrogen sources, ammonium nitrate showed a good yield [73].

Also, the agitation of the growth medium inoculated with the culture regulates the amount of oxygen in the medium, nutrient absorption, and enzyme activities [107]. At the initial stage, that is, during the lag phase, the cell mass and the production of bio-flocculant are low, and the biomass concentration is low, so the oxygen requirement is very low for the microbe to adapt itself to the culture conditions. However, once the microbe starts growing, there will be a drastic increase in biomass concentration, and the production of bio-flocculants and the oxygen demand of the microbes will be higher [34]. The overall bio-flocculant production cycle is represented in Figure 6.1.

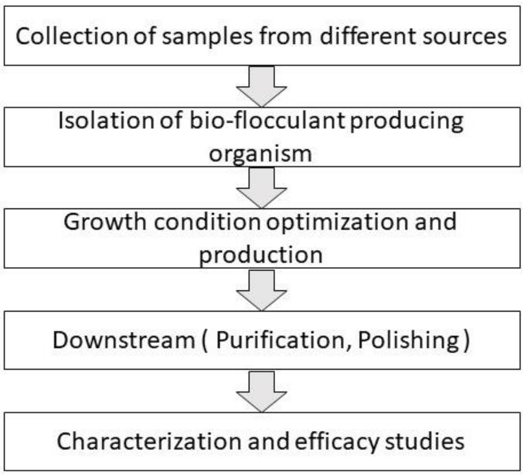

Figure 3. Flowchart representing the processes in the production of bio-flocculants.

7. Bio-flocculant Purification: Recent Approaches

The purification process is an essential part of the bio-flocculant production process. Once the bio-flocculants are synthesized, they should get rid of the residual matter, and the purity of the compound significantly affects the activity.

After fermentation of the culture broth, the general procedure is centrifuging the culture medium immediately, followed by adding distilled water to remove the insoluble substances and centrifuging again at a specific rpm for an optimized time. The preliminary purification step is to add two volumes of ice-cold ethanol to the supernatant, and the precipitate is vacuum-dried to get a purified bio-flocculant [108].

There are various methods available for further purification of bio-flocculants, which are detailed below.

The cold ethanol and cetylpyridinium chloride method involves centrifuging the culture broth at 4000 rpm for 30 minutes and adding two volumes of cold ethanol to the culture broth. This leads to the formation of a precipitate, and the precipitate is redissolved in the deionized water. 2% cetylpyridinium chloride solution is added with continuous stirring and allowed for a few hours. The precipitate is then collected and dissolved in 0.5 M NaCl solution. The crude bio-flocculant is then treated with two volumes of cold ethanol. After overnight dialysis at 4°C, the remaining precipitate is isolated using a vacuum drying technique[16,78].

The cold ethanol method deals with centrifugation of the fermented broth at 5000rpm for 10 minutes. The supernatant is poured into 4 volumes of cold ethanol to precipitate the bio-flocculant. Again, centrifugation is done to collect the resulting precipitate at 10,000rpm for 15 minutes and then redissolve in water. The crude bio-flocculant obtained is purified by dialyzing overnight at 4°C in distilled water and lyophilized[76,79].

Accordingly, some researchers have mentioned the method of chloroform and n-butanol in that the cell suspension will be heated at 100°C for 10 mins and cooled to room temperature (for the inactivation of enzymes) followed by centrifugation. The crude solution is treated with the sevage reagent (Chloroform: n-butanol at 5:1 v/v) to remove the proteinaceous compounds. The EPS- Exopolysaccharides were precipitated with cold ethanol (three times the volume) and left overnight. The precipitate is again centrifuged and dissolved in mill Q water. The crude EPS was dialyzed at 4°C with Milli Q water for 48 hours and can be further taken for various applications [22].

In the chloroform-methanol precipitation method, the culture broth will be initially centrifuged at 8000rpm at 4°C for 30 minutes. Two volumes of ethanol are added to the supernatant. The solution is left agitated for 12 hours at 4°C. The precipitate is then freeze-dried to obtain crude bio-flocculant. The crude product is then dissolved in distilled water to yield a solution of 1%w/v. A mixture of chloroform and methanol (2:1 v/v) was added and again agitated for about 12 hours. The supernatant was centrifuged for 30 minutes at 4°C and lyophilized to obtain a purified bio-flocculant [77]. The information on various purification methods is listed in Table 7.1

Table 4. Details of various purification methods.

S.No	Bio-flocculant producing organism	Nature of bio-flocculant	Purification method	Reference
1	*Bacillus subtilis and Pseudomonas*	Glycoprotein	Cold Ethanol	[76]
2	*Bacillus licheniformis*	Proteoglycan	Ice cold ethanol	[72]
3	*Leuconostoc lactis* KC117496	Glucan	Chloroform: n-butanol at 5:1, v/v and cold ethanol	[22]

S.No	Bio-flocculant producing organism	Nature of bio-flocculant	Purification method	Reference
4	*Virgibacillus species*	Uronic acid	Ethanol	[27]
5	*Proteus mirabilis* TJ-1	Carboxy 1, amino group	Cold ethanol and cetylpyridinium chloride	[16]
6	*Alcaligenes faecalis* HCB2	Polysaccharides	Ethanol precipitation, Chloroform and Methanol (2:1 v/v)	[6]
7	*Bacillus mucilaginosus*	Polysaccharides	Cold ethanol and cetylpyridinium chloride	[78]
8	*Bacillus thuringiensis* S13	Saturated fatty acids, brominated compounds	Cold ethanol	[109]
9	*Paenibacillus polymyxa* AM49	_	Cold ethanol	[36]
10	*Serratia ficaria*	Polysaccharides	Cold ethanol	[79]

8. Mechanism of Action

The mechanism of action of bio-flocculants involves the bridging of suspended particles in water, which leads to the formation of larger flocs with polymers that can settle more easily. Here, the polymer, especially neutral, acts as a bridge between the colloidal particles via adsorption[110]. Bio-flocculants can also modify the surface properties of suspended particles, making them more hydrophobic or hydrophilic, enhancing their aggregation and settling. Additionally, bio-flocculants can act as coagulants, destabilizing the suspended particles and promoting their aggregation. The specific mechanism of action of bio-flocculants can vary depending on their chemical composition and the properties of the suspended particles in the water [92]. It attracts and binds suspended particles in a liquid, forming larger clumps that can be easily removed through sedimentation or filtration. The functional groups in the bio-flocculant structure, such as carboxyl, hydroxyl, amino, and phosphate groups, contribute to the flocculation process. It can flocculate in two mechanisms, namely charge neutralization and bridging [111]. Bridging occurs when the bio-flocculant molecules form a bridge between two or more particles, causing them to aggregate. Charge neutralization occurs when the bio-flocculant and the suspended particles are oppositely charged molecules that neutralize the surface charge of the particles, allowing them to aggregate together. Adding K^+ ions decreases the negative charge between bio-flocculant and suspended particles. This aids in compressing the double-layer kaolin particles, weakens the static repulsive force, and aggregates each other, forming flocs [6]. Adsorption occurs when the bio-flocculant molecules adsorb onto the surface of the particles, causing them to clump together [106].

Furthermore, there are three theories that explain the flocculating property of bio-flocculants: The Alginate theory, The Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory, and the Divalent Cation Bridging (DCB) theory. The alginate theory proposes that bio-flocculants can act as glue by producing a gel-like substance that traps suspended particles together, promoting aggregation. DLVO theory is named after the scientists who developed it: Derjaguin, Landau, Verwey, and Overbeek. It explains that two opposing forces act on particles in a suspension: attractive Vander Waals forces and repulsive electrostatic forces. Flocculation occurs when the attractive forces between the particles exceed the repulsive force. This can be achieved by adding the flocculant, which neutralizes the electrostatic repulsion between particles and promotes the formation of large aggregates. Ca^{2+} increases the effect of bridging and absorption by inducing charge neutralization. However, it is not the main mechanism; it plays a minor role in the flocculating activity, but trivalent cations such as Fe^{3+} can inhibit ion exchange due to its higher charge density [7]. DCB theory explains the role of divalent cations, such as calcium and magnesium ions, in promoting the aggregation of colloidal particles in suspension. It proposes that divalent cations act as a bridge between particles, promoting their aggregation by reducing the repulsive forces between them. In a suspension, the suspended particles and bio-flocculants carry a net negative charge due to the ionization of their surface functional groups, resulting in repulsive electrostatic force between them. Here, the divalent cations such as Ca^{2+} and Mg^{2+} possess positive charge and act as a bridge between bio-flocculant and suspended particles, promoting aggregation. However, the effectiveness of the bio-flocculant depends upon its ability to interact with the particles in the suspension, and it is influenced by various factors, including charges, size, pH, and concentration of the flocculants used. The bridging effect is due to the strong binding affinity of the divalent cations for the negatively charged surface groups on the particles. Recent research suggests that the DCB theory is the most accurate in describing the role of cations in bio-flocculation [106].

9. Factors Affecting Flocculation Activity

Flocculation can be affected by several parameters such as pH, temperature, salinity, metal ions, bio-flocculant concentration, mixing speed, mixing time, and suspended solids.

9.1. Effect of metal ions.

Adding monovalent cations such as Na^+, K^+, and Mn^+ greatly reduces the flocculating activity by increasing the effluent concentration in suspended solids. Few studies suggest that potassium ions had little impact on floc strength [2]. Divalent cations such as Ca^{2+} and Mg^{2+} promote flocculating activity by decreasing the electrostatic interaction between the bio-flocculant molecules and Kaolin particles that could flocculate other compounds such as oil, dyes, and algae[4]. Magnesium ions improve the flocculating properties; however, it was reported that Mg^{2+} efficiency increases with lower concentrations of sodium ions. Some bio-flocculant requires Zn^{2+} for effective flocculating activity [3]. However, due to their higher surface charge density, trivalent cations such as Fe3+ inhibit flocculation at higher concentrations. A low concentration of Mn $^{2+}$ improves the production of enzymes such as dehydrogenase and phosphatase, which was beneficial in improving sludge flocculation and hydrophobicity and in removing chemical oxygen demand (COD) and total phosphorus (TP). However, a high concentration of Mn^{2+} (>10mg/ml) produced negative effects on the removal of COD and TP by reduced production of EPS [98]. However, certain microorganisms, such as Aspergillus flavus and Klebsiella pneumoniae,ave no effect on the concentration of metal ions for flocculation [101,102].

9.2. Effect of pH.

pH is one of the deterministic factors that influences growth and bio-flocculant production [76]. Also, the pH requirement varies for different organisms. Some organisms need an acidic environment for their survival and bio-flocculant production. For example, *Aspergillus paraciticus* shows that the acidic environment is best suited for growth and bio-flocculant production [106]; similarly, some organisms need alkaline or neutral pH. The pH requirement for *Citrobacter sp*. is between 7.2 to 10. Bio-flocculant production initiates at low pH and gets maximum at mild pH [103], while in algae, flocculation is mediated in alkaline conditions.

9.3. Effect of temperature.

The temperature significantly impacts microbes' growth, metabolism, and overall activity. Different microbes have specific temperatures at which they thrive, and variations outside these ranges can affect their biological processes. Generally, bio-flocculants with peptide or protein backbone are thermolabile, i.e., decomposition or structural and functional modifications occur due to abnormalities in temperature. However, the flocculants possessing carbohydrate backbone are thermally stable [30]. Production of bio-flocculants occurs in a wide range of temperatures, and the maximum efficiency varies from species to species. The bio-flocculant produced by *Aspergillus flavus* has maximum efficiency at 25°C, while the bio-flocculant produced by *Streptomyces platensis* has maximum efficiency at 50°C [101,2].

Table 5. Factors affecting the flocculating activity of bio-flocculants.

Bio-flocculant	Sources of bio-flocculant	pH range for effective bio-flocculant activity	Temperature range for effective bio-flocculant activity	Influence of metal ions in bio-flocculants activity	Optimal concentration for effective bio-flocculant activity	Reference
IH-7	*Aspergillus flavus*	Flocculating pH range: 4-8 Optimum pH: 4	Temperature range: 5-45°C Optimum temperature: 25°C Efficiency: 88.6%	Addition of monovalent or divalent cations doesn't influence the flocculating rate	Concentration: 0.5-20 mg/L Optimal concentration: 5 mg/L	[101]
MBF-6	*Klebsiella pneumoniae*	Flocculating pH range: 2-12 Optimum pH: 4	Temperature range: 0-70°C Optimum temperature: 30°C	Addition of metal ions doesn't influence the flocculating rate	Concentration: 10-100 mg/L Optimal concentration: 50 mg/L	[102]
MBF-L804	*Cellulosimicrobium cellulans*	Flocculating pH range: 2-13 Optimum pH: 5	Temperature range: 1-70°C Optimum temperature:35°C Efficiency: 92.68%	Addition of metal ions (Ca^{2+}, Mg^{2+}) increses the flocculation activity above 91.67%	Concentration: 2-22 mg/L Optimal concentration: 12 mg/L	[103]
Bio-flocculant from *Streptomyces platensis*	*Streptomyces platensis*	Flocculating pH range: 3-12 Optimum pH: 7	Temperature range: 50-100°C Optimum temperature:50°C	The addition of divalent cations such as $MgCl_2$ increases the flocculating activity	Concentration: 0.1-1 mg/mL Optimal concentration: 0.2 mg/mL	[2]
MBFGA1	*P. polymyxa*	Flocculating pH range: 5-9 Optimum pH: 9	-	Addition of $cacl_2$ enhances the flocculating activity	Concentration: 0.001-0.007 w/w Optimal concentration: 0.004 w/w	[111]
TKF04 (Bio-flocculant producing strain)	*Citrobacter sp*	Flocculating pH range: 2-11 Optimum pH: 7	Temperature range: 3-95°C Optimum temperature:50°C	Addition of cations doesn't influence the flocculating rate	Concentration: 0.1-1000 mg/mL Optimal concentration: 1 mg/mL	[44]
MBFF19	*Bacillus sp*	Flocculating pH range: 2-9 Optimum pH: 4	-	-	Concentration: 0-80 mg/L Optimal concentration: 20 mg/L	[100]

10. Applications of Bio-flocculants

Bio-flocculants find applications across different industries for their eco-friendly nature. It has a wide range of applications in wastewater treatment, biodiesel production, bioremediation, the food industry, the downstream dye industry, etc. Different strains of bacteria involved in different applications are listed below.

Table 6. Applications of bio-flocculants in various streams.

Sources of bio-flocculants	Applications	Reference(s)
Alcaligenes faecalis HCB2, *Pichia kudriavzevii*	It has a wide range of applications for removing BOD COD in coal mining wastewater treatment.	[6]
Rhodobacter sphaeroides	This species has the ability to flocculate coal slurry particles	[12]
Acaligenes faecalis subsp. Phenolicus ZY-16	MBF -16 bio-flocculant extract from the species was applied to remove toxic Microcystin-LR (MCLR) synthesized by cyanobacteria from water.	[101]
Aspergillus parasiticus	At acidic conditions, they were effective in the dye removal process.	[4]
P.polymyxa GA1	Lead ion Pb (II) was effectively removed by GA1 strain, which finds application in metal ion treatment.	[111]
Bacillus megaterium PL-8, *Chryseomonas luteola*	It has a wide range of applications in wastewater treatment and dye removal.	[7]
Pseudomonas veronii L918	It is used in coal-fired power plants to treat the ash-flushing wastewater generated from coal burning.	[14]
Streptomyces platensis SFD- 20, *Halobacillus, Virgibacillus, Oceanobacillus, Serratia ficaria*	It has a high flocculating activity and could be applied in river and wastewater treatment.	[2,99]
Bacillus agaradhaerens C9	It has the potential to harvest microalgae *Chlorella minutissima* UTEX2351 and be used in biodiesel production.	[60]
Acinetobacter baumannii YNWH 226	In aerobic conditions, it has the potential to degrade Congo red and can be used in dye removal treatment.	[32]
Paenbacillus elgii B69	It has applications in heavy metal adsorption, such as nickel (16%), cadmium (16%), chromium (15%), cobalt (9%), and lead (7.5%).	[104]
Rhizomonas sp	Removal of humic acid and treatment of landfill leachates	[10]
Citrobacter youngae GTC 01314	Sludge conditioning and dewatering	[44]

11. Conclusion

Bio-flocculants represent a sustainable alternative to synthetic counterparts, offering environmentally friendly and non-toxic solutions across various applications such as bioprocessing, dye decolorization [20], drinking water treatment [30], and wastewater management [39]. While production costs may be higher and some production challenges persist, their benefits are increasingly recognized. Notably, bio-flocculants derived from species like Vagococcus sp. find applications in the food and fermentation industry, underscoring their safety for human consumption. Additionally, Isaria cicadae 1 shows promising efficacy in coal-treated wastewater flocculation, presenting an economically viable and eco-friendly solution without secondary pollution risks. The rising interest in biodegradable bio-flocculants for wastewater treatment reflects growing scientific and technological attention in this field [108]. While numerous microorganisms exhibit bio-flocculation capabilities, exploration of untapped oceanic microorganisms can enhance productivity. Overall, bio-flocculants offer a compelling avenue for sustainable and effective wastewater treatment, with ongoing research poised to unlock further innovations.

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Conflicts of Interest

The authors declare no conflict of interest.

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