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Review

Microbial Flocculants as an Alternative to Synthetic Polymers for Wastewater Treatment: A Review

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Received: 29 September 2018; Accepted: 24 October 2018; Published: 1 November 2018

Abstract: Microorganisms such as bacteria, fungi, and microalgae have been used to produce bioflocculants with various structures. These polymers are active substances that are biodegradable, environmentally harmless, and have flocculation characteristics. Most of the developed microbial bioflocculants displayed significant flocculating activity (FA > 70–90%) depending on the strain used and on the operating parameters. These biopolymers have been investigated and successfully used for wastewater depollution in the laboratory. In various cases, selected efficient microbial flocculants could reduce significantly suspended solids (SS), turbidity, chemical oxygen demand (COD), total nitrogen (Nt), dye, and heavy metals, with removal percentages exceeding 90% depending on the bioflocculating materials and on the wastewater characteristics. Moreover, bioflocculants showed acceptable results for sludge conditioning (accepted levels of dry solids, specific resistance to filtration, moisture, etc.) compared to chemicals. This paper explores various bioflocculants produced by numerous microbial strains. Their production procedures and flocculating performance will be included. Furthermore, their efficiency in the depollution of wastewater will be discussed.

Keywords: microbial flocculants; wastewater treatment; growth media; coagulation-flocculation

1. Introduction

The conventional coagulation-flocculation technique applied for wastewater treatment is widely used, showing significant treatment efficiency regarding the removal of organic material, suspended solids, and heavy metals [1]. In addition, it provides benefits for the wastewater treatment system, such as higher resistance to toxic loadings and massive amounts of organics, conducting simplicity, energy savings, etc. [2]. The chemical flocculants universally used in this process included inorganic (polyaluminum chloride, ferric chloride, etc.) and organic flocculants (such as polyacrylamide and its derivatives) [3]. These chemicals stay in wastewater after treatment and sludge and may cause health and ecological complications [4]. Consequently, the discarding of treated wastewater in the environment may cause serious disadvantages for human health, since the used chemicals are reported to be related to various health effects (Alzheimer's disease, neurotoxicity, carcinogenic, genotoxic

properties, etc.) [5]. Residual chemicals destroy aquatic life and make the water inappropriate for human consumption. Both synthetic organic and inorganic flocculants were reported to be responsible for neurotoxicity and carcinogenicity. It was also demonstrated that Alzheimer's disease is linked to aluminum remaining in treated water [6]. A recent study showed the toxicity of both anionic polyacrylamide and cationic polymers for aquatic invertebrates and fish [7]. In this context, it was reported that cationic polymers tend to accumulate in fish gills, interfering with gill function and ion regulation, causing fish death and consequently reducing the supply of healthy fish for human consumption [8]. Moreover, monomers resulting from the degradation of polyacrylamide under specific environmental conditions are considered to be a likely human carcinogen and neurotoxin. Therefore, many authorities have restricted the use of chemical polymers in various industrial applications [9]. In addition, these products are costly and may not be available locally. Hence, there is a need to consider other flocculants offering a new sustainable strategy. This sustainable approach is based on the use of bioflocculants in the coagulation-flocculation treatment for the removal of pollutants from wastewater [5]. Natural biological origin materials, such as beans, moringa, maize, cactus, etc., were investigated [5]. Recently, more attention has been diverted to microbial flocculants produced by various microorganisms (actinomycetes, fungi, bacteria, and algae) widely distributed in soil and water [10–12]. Microbial flocculants that are produced during the microorganism growth varied in composition (polysaccharides, proteins, DNA, cellulose, sugar, protein, polyamino acids, etc.). They are active biocompounds, biodegradable, without degraded intermediate pollutants, environmentally harmless, and have flocculation properties. For these reasons, examinations were carried out to determine their efficiency for wastewater treatment from various origins. This review aims to explore the production of microbial flocculants and their applications in wastewater treatments.

2. Bioflocculant-Producing Microorganisms

Bacteria, fungi, and microalgae were showed to produce bioflocculants. Microorganisms are selected based on various factors (morphology, the presence of slimy extracellular polysaccharides, etc.) using different methods and reagents (Congo red, crystal violet and $CuSO_4$ solution, chelating agents, colorimetric methods, etc.). The flocculating activity (FA) was commonly evaluated using a kaolin suspension. The produced bioflocculant was also subject to qualitative analyses. Fourier transfer infrared radiation was used to analyze the structure of the bioflocculant. Interestingly, many sources (sludge, soil, sediments, river, seawater, etc.) were investigated to isolate microorganisms that yield flocculating substances (e.g., polysaccharides, proteins, and glycoproteins).

2.1. Bacteria

Several bacterial strains belonging to various classes (Actinobacteria, Alphaproteobacteria, Bacilli, Deltaproteobacteria, Gammaproteobacteria, Proteobacteria, etc.) have been reported to produce flocculants (Table 1) [13–49]. For example, salt production pond *Bacillus mojavensis* strain 32A was found to produce proteoglycan flocculant (98.4% polysaccharide and 1.6% protein) with an interesting FA of 96.11% recorded at pH 10 [14]. In the presence of specific growth conditions (L-glutamic acid and NH_4Cl as nutrient sources), this strain yields 5.2 g/L of the extracted biopolymer. Another Bacillus strain isolated from freshwater (*Bacillus pumilus* ZAP 028) produced a thermostable and wide pH range flocculating agent (FA = 69.8%) [18]. In this case, results were obtained in the presence of maltose and several nitrogen sources (e.g., yeast extract, urea and ammonium sulfate) with 4% (*v*/*v*) of inoculum and pH 7. The bioflocculant content was 75.4% polysaccharide, 5.3% protein, and 15.4% uronic acid [18].

Table 1. Examples of bacteria investigated for flocculant production.

Strain (Source)	Carbon/Nitrogen Sources	FA [1]/Flocculant Composition (PS [2], P [3])	Reference
Bacillus agaradhaerens C9 (alkaline lake)	glucose, yeast extract	FA: 80.63% PS: 65.42%, P: 4.70%	[13]
Bacillus mojavensis 32A (salt production pond)	L-glutamic acid, NH_4Cl	FA: 96.11% PS: 98.4%, P: 1.60%	[14]
Bacillus sp. XF-56 (marine intertidal sludge)	glucose, yeast extract	FA: 93.5%	[13]
Bacillus subtilis F9 (wastewater sludge)	sucrose, peptone	FA: 90% PS: 88.3%, P: 10.10%	[15]
Bacillus licheniformis X14 (soil)	beef extract, peptone	FA: 98% PS: 91.5%, P: 8.4%	[16]
Bacillus licheniformis	glucose, NH_4Cl	FA: 96% PS: 91%, P: 9%	[17]
Bacillus firmus	glucose, NH_4Cl	FA: 89%	[17]
Bacillus pumilus (fresh water)	maltose, yeast extract, urea, ammonium sulfate	FA: 69.8%	[18]
Bacillus CPO8, *Bacillus* CPO13 and *Pseudomonas* CPO14 (contaminated crude petroleum oil)	glucose, NH_4Cl	FA: 92.17–97.59% PS: 91%, P: 9%	[19]
Bacillus velezensis 40B (brackish water)	glucose, yeast extract	FA > 98% PS: 98%, P: 2%	[20]
Bacillus cereus (cultivated soil)	starch, yeast extract	FA: 75% PS: 16.99%, P: 3.01%	[21]
Bacillus cereus (soil)	glucose, sucrose, fructose, lactose, starch, urea, peptone, yeast extract	FA: 75% PS: 91%, P: 9%	[21]
Bacilli subtilis CZ1003	glucose, beef extract	FA: 36.2%	[22]
Bacillus subtilis	glycerol, NH_4Cl	FA: 23.77% Poly-γ-glutamic acid	[23]
Bacillus thuringiensis (cultivated soil)	starch, yeast extract	FA: 76.3% PS: 15.23%, P: 84.73%	[21]
Solibacillus silvestris W01 (activated sludge)	sorbitol or starch, yeast extract	FA: 90% P: 75.1%, P: 24.9%	[24]
Paenibacillus elgii B69 (soil)	sucrose, peptone, yeast extract	FA: 90% P: 100%	[25]
Paenibacillus mucilaginosus GIM1.16 (soil)	sucrose, yeast extract	FA: 89.7% P: 100%	[26]
Pantoea agglomerans BH18 (mutant strain 2-103)	glucose, tryptone, yeast extract, beef extract	FA: 87.5%	[27]
Chryseobacterium daeguense W6 (biological aerated filter sludge)	glucose, tryptone	FA: 96.9% PS: 13.1%, P: 32.4%	[28]
Proteus mirabilis (activated sludge)	glucose, peptone	FA: 92.8%	[29]
Proteus mirabilis (activated sludge)	glucose, peptone	FA: 95.6% PS and P	[29]
Klebsiella sp. PB12 (river water)	nutrient poor medium (with glucose or lactose or mannose)	FA: 80% PS: 72.32%, P: 14.12%	[30]
Klebsiella sp. ZZ-3 (activated sludge)	glucose, $NaNO_3$, NH_4Cl, urea	FA: 94.5% PS: 84.6%, P: 6.1%	[31]
Klebsiella sp. TG-1 (starch factory wastewater)	sucrose, yeast extracts, beef extract (using trona suspension)	FA: 86.9% PS: 84.6%, P: 11.1%	[32]
Pseudomonas Aeruginosa	petroleum hydrocarbons, peptone	FA: 87.8% PS and P	[33]
Turicibacter sanguinis (wastewater sludge)	methanol wastewater $(NH_4)_2SO_4$, yeast extract	FA: ND [4] PS: 74.1%, P: 24.2%	[34]

Table 1. *Cont.*

Strain (Source)	Carbon/Nitrogen Sources	FA [1]/Flocculant Composition (PS [2], P [3])	Reference
Arthrobacter sp. B4	glucose, yeast extract	FA: 99% PS: 100%	[35]
Streptomyces sp. MBRC-91 (sourse NI)	palm jiggery, yeast extract, NH_4NO_2	FA: 96.3%	[36]
Achromobacter sp TL-3 (activate sludge)	ethanol, glycerol, peptone, yeast extract	FA: 95%	[37]
Rhodococcus opacus (sourse NI [5])	glucose, yeast extract	FA: 82% PS: 64.6%, P: 9.44%	[38]
Rhodococcus erythropolis (activated sludge)	swine wastewater	FA: 94.5% P: 99.2%	[39]
Rhodococcus rhodochrous (sourse NI)	glucose, NH_4Cl	FA: 22.5% PS: 62.86%, P: 10.36%	[40]
Halomonas stenophila HK30(saline-wetland)	MY medium	FA: 72.06% sulphated heteropolysaccharide	[41]
Halomonas sp. AAD6 (camaltı saltern area)	pretreated molasses	FA: ND PS: 90%, P: 0.5%	[42]
Agrobacterium sp. M-503 (propylene epoxide wastewater sludge)	sucrose, yeast extract, urea	FA: 74.5% PS: 85% P: 3%	[43]
Rothia sp. (Ruditapes philippinarum conglutination mud)	saccharose, $(NH_4)_2SO_4$, peptone	FA: 86.22% P	[44]
Chryseobacterium daeguense W6 (source NI)	glucose, tryptone, Mg $(NO_3)_2$	FA: 90% PS and P	[45]
Strain B31 (source NI)	glucose, urea	FA: 90.21% PS and P	[46]
Various bacterial isolates (tapioca wastewater)	glucose, sucrose, peptone, yeast extract, urea	FA: 13.54–71.38%	[47]
Methylobacterium sp. Obi (river water)	glucose, peptone	FA: 72% PS	[48]
Bacillus cereus and *Pichia membranifaciens* (activated sludge)	alcohol, urea	FA: 40–90%	[49]

[1] flocculating activity; [2] polysaccharide, [3] protein, [4] not determined, [5] not indicated.

More recently, two isolates from Egyptian soil (*Bacillus cereus* and *Bacillus thuringiensis*) were reported to produce a significant amount of bioflocculants [21]. The total carbohydrate bioflocculant contents were 16.99% and 15.27%, while the total protein content were 83.01% and 84.73%, respectively, for *Bacillus cereus* and *Bacillus thuringiensis*. The maximum FA (75% to 76.3%, respectively, for *Bacillus cereus* and *Bacillus thuringiensis*) were obtained at pH 7–8 and temperature 30–40 °C during the growth period from 72 to 96 h and in the presence of starch and yeast extract [21]. Different carbon sources were used for the growth of sludge-isolated strain (*Bacillus* sp. XF-56). However, glucose was most favorable for bioflocculant production (FA = 93.5%). Interestingly, at the initial pH (5–10), this strain yields hydrogen (optimum yield at pH = 7) and bioflocculants (optimum yield at pH = 8). This strain resists higher salt concentration, offering broad application potential for fresh and marine wastewater [13].

Likewise, several strains belonging to the Gamma proteobacteria class were isolated from various sources. River water [30], activated sludge [31] and starch wastewater [32] were used to select *Klebsiella* sp. strains. For example, *Klebsiella* sp. ZZ-3, isolated from activated sludge yields an effective, pH-tolerant, and thermostable bioflocculant [31]. In this case, the bioflocculant composition was found to be 84.6% polysaccharides (containing, specifically, rhamnose, mannose, and galactose) and 6.1% protein. Starch wastewater was also used to isolate another strain (*Klebsiella* sp. TG-1). The purified microbial flocculant consisting of polysaccharides and proteins (84.6% and 11.1%, respectively) showed a FA of 86.9% obtained with trona suspension [32].

Bacterial strains belonging to the Actinobacteria class (*Arthrobacter*, *Rhodococcus* sp. *Streptomyces*, etc.) were investigated by many researchers. *Rhodococcus erythropolis* isolated from activated sludge was cultivated in swine wastewater as a carbon and nitrogen source. The produced flocculant consist mainly of protein (99.2%) with higher FA of 99.2% [39]. However, another strain (*Rhodococcus rhodochrous*) growing in the presence of glucose and NH_4Cl produced proteoglycan flocculant (62.86% polysaccharide and 10.3% protein) with a lower FA (22.5%)) [40].

Nevertheless, many strains belonging to the Proteobacteria class possess the ability to synthesize bioflocculants. For example, a strain identified as *Agrobacterium* sp. M-503 (from propylene epoxide wastewater sludge) produced a biopolymer (85%p polysaccharide and 3% protein) with an acceptable level of FA (75%) [43]. Amjres et al. (2015) [41] isolated *Halomonas stenophila* HK30 from saline wetland that is able to produce sulphated heteropolysaccharide with an efficient FA (72.06%).

Based on the collected information, the majority of research activities has mainly been oriented to the isolation of new strains and the production of bioflocculant using pure culture. However, the possibility was reported recently of combining microbial strains to produce bioflocculants with better FA than pure strains. Table 2 [50–58] presents several consortia evaluated for their bioflocculant production.

Table 2. Examples of microbial consortia investigated for bioflocculant production.

Consortium (Source)	Carbon/Nitrogen Sources	FA [1] and Flocculant Composition (PS [2], P [3])	Reference
Cobetia sp. OAUIFE, *Bacillus* sp. MAYA (sediment)	glucose, urea, yeast extract, $(NH_4)_2SO_4$	FA: 90% uronic acid: 66%, P: 31%	[50]
Cobetia sp. OAUIFE, *Bacillus* sp. MAYA, and *Bacillus* sp. Gilbert (sediment)	glucose, urea, yeast extract, $(NH_4)_2SO_4$	FA: 87.4% (kaolin) F A: 96.4% (river water) FA: 93.7% (brewery wastewater) FA: 82.2% (dairy wastewater) PS and P	[51]
Staphylococcus sp. BAFRT4, *Pseudomonas* sp. CYGS1 (source NI [4])	Brewery wastewater	FA: 96.8% PS and P	[52]
Different stains (pharmaceutical, sugar and hoggery waste water)	sucrose, yeast extract, urea	FA: 76%	[53]
Halomonas sp. Okoh and *Micrococcus* sp. Leo (sediment)	glucose, yeast extract	FA: 86% Polycoprotein	[54]
Halobacillus sp. Mvuyo and *Oceanobacillus* sp. Pinky (sediment)	sodium carbonate, urea, yeast extract, $(NH_4)_2SO4$	FA: 98.3% PS and P	[55]
Streptomyces sp. Gansen and *Cellulomonas* sp. Okoh (river)	sucrose, peptone	FA: 98.9% PS and P	[56]
Biological sludge (municipal sewage)	NI	FA: 98.5% amino-polysaccharide	[57]
Biological sludge (municipal sewage)	NI	FA: 99.5% PS	[58]

[1] flocculating activity; [2] polysaccharide, [3] protein, [4] not indicated.

A culture mixture of *Cobetia* sp. OAUIFE and Bacillus sp. MAYA produces a bioflocculant containing 66% uronic acid and 31% protein. At a flocculant dose of 0.8 mg/mL, at pH 8 and in the presence of Ca^{2+}, optimum FA (90%) was obtained [50]. Similarly, a produced bioflocculant by growing both *Halomonas* sp. Okoh and *Micrococcus* sp. Leo was shown to be controlled by Ca^{2+}, Mn^{2+} and Al^{3+}, thermostable and active at pH (2–10), with an optimum FA of 86% at pH 8. Consequently, the bioflocculant may be used to replace the synthetic flocculants widely used in wastewater treatment [54]. An interesting bioflocculant (FA = 98.9%) was also produced by a mixture of *Streptomyces* sp. *Gansen* and *Cellulomonas* sp. Okoh [56] grown in an optimized medium (containing sucrose, peptone, and magnesium chloride). It has been reported that the extracted bioflocculant contains polysaccharides (neutral sugar, amino sugar, and uronic acids) and proteins [56]. Because bioflocculation represents a dynamic process occurring in an aerobic activated sludge system, the sludge may contain large numbers of bioflocculant-producing microbial strains. Microorganism aggregate of the biological sludge secretes mainly flocculating materials (e.g., polysaccharides, proteins, glycoproteins, etc.) at different concentrations. Biological sludge

from municipal wastewater was used for bioflocculant production by Zhang et al. (2013) [57] and Sun et al. (2012) [58]. In these studies, hydrochloric acid treatments were used to extract flocculating active ingredients. The highest flocculating rate fraction can be purified from the crude bioflocculant. The conducted experiment allowed the purification of an amino-polysaccharide bioflocculant with an optimum FA of 98.5% at pH 10.5 and 3.0% (v/v) as a dose [57]. Likewise, FA of the purified polysaccharide reached 99.5% at the same conditions (3.0% v/v and pH 10.5) [58].

2.2. Fungi and Microalgae

A limited number of studies on fungal bioflocculant have been reported (Table 3) [3,59–68]. Among the isolated fungal strains, *Aspergillus flavus* was shown to produce a bioflocculant composed mainly of polysaccharide (69.7%) and protein (28.5%), with an excellent FA (<90%) without cation addition. Interestingly, the use of sucrose and peptone allowed optimal bioflocculant production [3].

Table 3. Examples of fungi and algae investigated for bioflocculant production.

Strain (Source)	Carbon/Nitrogen Sources	FA [1] and Flocculant Composition (PS [2], P [3])	Reference
Aspergillus flavus (source NI [3])	sucrose, peptone	FA > 90% PS: 69.7%, P: 28.5%	[3]
Penicillum strain HHE-P7 (source NI)	glucose, yeast extract	FA: 96%	[59]
Talaromyces trachyspermus OU5 (source NI)	glucose, urea	FA > 92.5% PS: 84.6%, P: 15.2%	[60]
Fungal strain (soil)	glucose, NH_4Cl_2	FA: 80	[61]
Phanerochaete chrysosporium (source NI)	glucose potatoes	FA: 93.5 (coal slurry) Acidic polysaccharide	[62]
Aspergillus flavus (source NI)	sucrose, peptone	FA: 97.4%	[63]
Aspergillus niger (source NI)	palm oil mill effluent, glutamic acid	FA: 81% PS: 66.8%, P: 31.4%	[64]
Filamentous fungal strain (river water)	potato dextrose agar	FA: 59.34–99.18%	[65]
Penicillum strain HHE-P7 (source NI)	glucose, yeast extract	FA: 93%	[59]
Rhizopus sp. M9 *Rhizopus* sp. M17 (soil)	potato starch wastewater, urea	FA: 90.2%	[66]
Scenedesmus quadricauda (source NI)	nitrate	FA: 86.7% PS: 56.7%, P: 41%	[67]
Scenedesmus obliquus AS-6-1 (source NI)	nitrate	FA: 80–85% (for microalga) PS: 100%	[68]
Talaromyces sp. (soil)	glucose, urea	FA: 92.5% PS: 84.6%, P: 15.2%	[60]

[1] flocculating activity; [2] polysaccharide, [3] protein, [4] not indicated.

Another strain of *Aspergillus niger* was reported to produce bioflocculant (composed of 66.8% polysaccharide and 31.4% protein) while growing in palm oil mill effluent supplemented with glutamic acid [64]. The produced bioflocculant was thermostable and able to flocculate industrial wastewater, especially with salinity up to 10% and in cold regions [63]. A fungal strain, *Phanerochaete chrysosporium*, produce an acidic polysaccharide having a higher FA of coal slurry (93.5%) [62]. More recently and for the first time, the production of a proteoglycan (84.6% polysaccharide and 15.2% proteins.) bioflocculant by *Talaromyces* sp. was reported. Interestingly, 20 mg/L of the proteoglycan allowed more than 92.5% FA [60].

Regarding the flocculating capability of microalgae, various species have been reported to produce flocculants during the stationary phase in batch cultures. However, fewer studies on microalgal flocculant properties have been recorded. For the first time, Guo et al. (2013) studied an extracellular biopolymer from *Scenedesmus obliquus* AS-6-1. The produced bioflocculant is a 127.9 kDa polysaccharide that flocculates

freely-suspended microalgal cells. However, another strain, *Scenedesmus quadricauda*, was shown to produce significant amounts of bioflocculant composed of sugar (56.7%) and protein (41%) [68]. The self-flocculation efficiency reached 96.8% at pH 7 with biomass concentration 0.21–39 g/L [67].

3. Microorganism Growth Conditions for Bioflocculant Production

Microbial growth bioflocculant production has been reported to be influenced by various factors such as carbon sources, nitrogen sources, oligoelements, and operating parameters (temperature, pH, inoculum size, aeration rate, etc.). In order to increase the bioflocculant production, growth factors were optimized using statistical analyses. As reported before, a wide variety of microorganisms isolated from various sources, utilizing various nutrient sources and growing under various conditions, are able to produce bioflocculants with different characteristics.

At the beginning of the microbial bioflocculant investigation, media containing simple carbon and nitrogen were utilized for culturing new isolates. Carbon sources included sugar alcohols and organic acid. However, nitrogen sources included peptone, urea, yeast extract, NH_4Cl, etc. Tables 1–3 show the favorable nutrient sources allowing the obtention of flocculanting polymers with significant FA. Glucose and yeast extract were efficient for several microbial strains such as *Bacillus velezensis* 40B [20] and *Rhodococcus opacus* [40], allowing the production of bioflocculants having different rates of FA (98% for *Bacillus velezensis* 40B and 22.5% for *Rhodococcus opacus*). L-glutamic acid has been reported to have an effective role in the culture of *Bacillus mojavensis* 32A and flocculant productivity [14]. Interestingly, using glucose for *Penicillium* sp. HHE-P7 allowed the production of bioflocculant having a FA of 95% [59]. For *Solibacillus silvestris* W01, an optimal flocculant amount was reached with maltose [24]. Similarly, Zhang et al. (2010) [29] concluded that glucose and peptone were favorable for *Proteus mirabilis* to synthesize bioflocculant [29]. Compared with various nitrogen sources, peptone was highly appropriate for *Paenibacillus elgii* B69 to produce bioflocculant [25]. In the case of *Aspergillus flavus*, sucrose allowed the obtention of the maximum flocculant amount. However, this amount was negatively affected by fructose and glycerol. Interestingly, growth media supplemented by yeast extract and urea significantly enhanced the bioflocculant production [3]. Similarly, nitrogen sources such as $NaNO_3$, NH_4Cl, and urea stimulate the growth of Klebsiella sp. ZZ-3, allowing the production of glycoprotein with FA ranging from 90.4% to 94.5% [31]. In addition, glycerol and ammonium enhance significantly the growth of *Bacillus*. However, the produced flocculant showed a lower FA of 23% [23].

Byproducts and wastes generated by the agroindustrial sector contain a considerable amount of nutrients (carbon, nitrogen, oligoelements, etc.) useful for microbial growth. In this context, multiple studies demonstrated that agroindustrial residues (sugarcane, starch molasses, corn-steep liquor, soybean juice, etc.), which are mainly composed of polysaccharides, could be used as substrates for microbial growth and bioflocculant production. Similarly, wastewater and sludge, which are abundant raw materials containing enough carbon, nitrogen, phosphorus, and micronutrients, could sustain microbial growth for bioflocculant production. According to Zhao et al. (2017), *Rhizobium radiobacter* and *Bacillus sphaericus* were able to synthesize flocculating materials while growing in wastewater supernatant of anaerobic co-digestion (corn straw and molasses wastewater) [22]. Methanol wastewater was used as a growth medium to produce bioflocculant useful for arsenite removal [34]. Potato starch wastewater was used for the culture of two strains of *Rhizopus*, allowing the production of an efficient bioflocculant MBF917 for wastewater treatment [66]. Moreover, Guo et al. (2014) reported the use of wastewater sludge to prepare biopolymers with flocculating activity exceeding 92% [39].

The pH is also an important factor in the microbial culture. It was reported to affect the growth, bioflocculant production, and FA. Each microbial strain has an optimum pH for growth and bioflocculant production. The impact of pH can be illustrated by some examples reported for various microbial strains. For example, the *A. flavus* bioflocculant was produced at pH values ranging between 5 and 9 and the highest FA was obtained at neutral pH [3]. In the case of *S. silvestris*, the pH of growth was in the range pH 7–9 with an optimum FA at pH 8 [24]. For the *C. daeguense* strain,

the maximum amount of flocculating material was reached at pH 6 [29]. For *Penicillium purpurogenum*, pH 5.5 allowed the highest bioflocculant production [59].

Based on this reported information, it is vital to point out the role of pH during the flocculation procedure. In the presence of protein as a bioflocculant, an alkaline pH is required to ensure the flocculation [68]. However, polysaccharidic bioflocculants tolerate a pH ranging from slightly acidic to slightly alkaline conditions.

Similar to pH, the temperature plays an essential role in microbial culture. Depending on the microbial stain, the growth temperature significantly affects the growth, the bioflocculant production, and FA. As reported by many authors, a high temperature may change the protein or peptide structure of the bioflocculant, causing polymer damage and reducing the FA. In addition, the inoculum size and the time course of bioflocculant production are considered two critical factors. Both the bioflocculant production characteristics and the FA varied during the growth depending on the microbial strain and the inoculum size. For many strains such as *S. silvestris* [24] and *Streptomyces* sp. [56], the bioflocculant was produced during the logarithmic growth phase. For others strains such as *A. flavus*, the bioflocculant was produced at the same time as the cell growth with a maximum at the stationary phase [3].

Therefore, it is apparent that every microorganism has its specific operating parameters to maximize bioflocculant yield and FA. In order to determine the precise time at which the microbial culture should be stopped, the growth medium composition (carbon, nitrogen, and growth factors) and culture operating parameters (pH, temperature, aeration, inoculum size, etc.) should be optimized for each microbial strain.

4. Applications of Microbial Bioflocculants for Wastewater Treatment

Microbial bioflocculants are eco-friendly materials, harmless and biodegradable. They are composed of polysaccharides, proteins, and glycoproteins. Their degraded intermediates are safe for humans and the environment. Moreover, microbial enzymes responsible for bioflocculant degradation are present in the environment (wastewater, sludge, soil, sea, etc.). Because of the increasing requirement for environmental quality, bioflocculant performance has been investigated for wastewater treatment to remove solids, organic pollutants, and heavy metals. Sludge conditioning was also studied using microbial bioflocculants.

4.1. Microbial Flocculants for Turbidity and Organic Pollutant Removal

The use of microbial flocculants as flocculating substances in various municipal and industrial wastewater was proven to cause a significant reduction in levels of SS, turbidity, and COD, as indicated in Table 4 [25,27,57,60,69–73].

Table 4. Effluent treated by microbial flocculants for turbidity and organic pollutant removal.

Effluents	Strains/Operating Conditions	Removal Efficiencies (%)	Reference
Swine wastewater COD [1]: 1372–3025 mg/L pH: 7.5 Turbidity: 230-800 NTU	*Bacillus* xn12 and *Streptomyces* xn17 Flocculant: 9% v/v + $CaCl_2$ (1 wt %) at pH 11	COD: 42% (xn12) 46% (xn17) Turbidity: 82% (xn12) 87% (xn17)	[57]
Swine wastewater COD: 1372–3025 mg/L pH: 7.5 Turbidity: 230–800 NTU	*Bacillus* xn12 + *Streptomyces* xn17 Flocculant: 9% v/v + $CaCl_2$ (1 wt %), pH 8	COD: 42% Turbidity: 91%	[57]
Swine wastewater COD: 6746 mg/L NH_4^+-N: 785 mg/L TKN[2]: 1158 mg/L TP[3]: 153 mg/L Turbidity: 35,742 NTU	*Talaromyces trachyspermus* OU5 Flocculant: 5% v/v, 15 min	COD: 52.1% TKN: 39.7% NH_4^+-N: 18.6% TP: 21.5% Turbidity: 75%	[60]

Table 4. *Cont.*

Effluents	Strains/Operating Conditions	Removal Efficiencies (%)	Reference
Municipal wastewater TS[4]: 340 mg/L COD: 193 mg/L Turbidity: 58 NTU pH: 6.7	Mixture of strains isolated from secondary municipal sludge and cultivated in sterilized sludge. Flocculant: 2 mg broth exopolysaccharide/L + (20 mg/L Fe^{3+}; 20 mg/L Al^{3+}, 200 mg/L Ca^{2+}, 200 mg/L Mg^{2+})	COD: 80.4% (20 mg/LFe^{3+}) 78.1% (20 mg/LAl^{3+}) 74.5% (200 mg/LCa^{2+}) 74.6% (200 mg/LMg^{2+})	[69]
Brewery wastewater pH: 4.5 TS: 2362 mg/L Turbidity (NTU): 4063 COD: 1985 mg/L	Mixture of strains isolated from secondary municipal sludge and cultivated in sterilized sludge. Flocculant: 12.4 mg B-EPS/L + 40 mg/L Fe^{3+}; 40 mg/L Al^{3+}, 250 mg/L Ca^{2+}, 250 mg/L Mg^{2+})	COD: 87.4% (40 mg/L Fe^{3+}) 86.2% (40 mg/L Al^{3+}) 88.4% (250 mg/L Ca^{2+}) 85.7% (250 mg/LMg^{2+})	[69]
Aquaculture wastewater COD: 35.6 mg/L NH_4^+-N: 6.43 mg/L SS [5]: 27.1 mg/L	*Bacillus megaterium* SP1 Inoculation: 1×10^4 CFU/mL, 30 °C, pH 7	COD: 64% NH_4^+-N: 63% SS: 83.8%,	[70]
Potato starch wastewater COD: 7965 mg/L Turbidity: 712 NTU pH: 6	*Rhizopus* sp. M9 + *Rhizopus* sp. M17 Flocculant: 0.1 mL/L (0.5 mL M17: 0.5 mL M9) + 5 mL/L 10% $CaCl_2$	COD: 54.09% Turbidity: 92.11%	[66]
Municipal wastewater	*Klebsiella pneumoniae* NY1 Flocculant: 44 mg/L	SS: 72%, BOD 89% COD: 84%,	[71]
Landfill leachate wastewater COD: 1944 mg/L Turbidity: 1440 NTU, Chromaticity: 512 times, SS: 11.04 g/L, pH: 6.5.	*Pichia membranifaciens*: Flocculant: 2% v/v + $CaCl_2$ pH 7	COD: 42% Turbidity: 44% Chromaticity: 41% SS: 51%	[49]
Landfill leachate wastewater COD: 1944 mg/L Turbidity: 1440 NTU Chromaticity: 512 times, SS: 11.04 g/L, pH: 6.5	*Bacillus cereus* Flocculant: 2% v/v + $CaCl_2$ pH 7	COD: 45% Turbidity: 48% Chromaticity: 58% SS: 59%	[49]
Landfill leachate wastewater COD: 1944 mg/L Turbidity: 1440 NTU Chromaticity: 512 times, SS: 11.04 g/L pH: 6.5	*Bacillus cereus* and *Pichia membranifaciens* Flocculant: 2% v/v + $CaCl_2$, pH 7	COD: 73% Turbidity: 50% Chromaticity: 70% SS: 74%	[49]
Starch wastewater COD: 9660 mg/L Turbidity: 2098 NTU Chromaticity: 320 times SS: 1.094g/L, pH: 2.3	*Pichia membranifaciens*: Flocculant dose: 2% v/v + $CaCl_2$, pH 7	COD: 58% Turbidity: 54% Chromaticity: 57% SS: 34%	[49]
Starch wastewater COD: 9660 mg/L Turbidity: 2098 NTU Chromaticity: 320 times SS: 1.094 g/L, pH: 2.3	*Bacillus cereus* Flocculant dose: 2% v/v + $CaCl_2$, pH 7	COD: 81% Turbidity: 59% Chromaticity: 69% SS: 36%	[49]
Starch wastewater: COD: 9660 mg/L turbidity: 2098 NTU Chromaticity: 320 times SS: 1.094 g/L, pH: 2.3	*Bacillus cereus* and *Pichia membranifaciens* Flocculant: 2% v/v + $CaCl_2$, pH 7	COD: 86% Turbidity: 66% Chromaticity: 89% SS: 41%	[49]
Paper mill wastewater pH 8.57	Crude and purified bioflocculant from *Paenibacillus mucilaginosus* GIM1.16: Flocculant: 0.5–4 mg/L	COD: 70–75.2% SS: 81.5–88%	[72]
Biological product factory wastewater pH 7.11	*Paenibacillus mucilaginosus* GIM1.16: Flocculant: 0.5–4 mg/L	COD: 83.2-86.9% SS: 88.8–92%	[72]
Garbage incineration Plant wastewater pH 6.08	Crude and purified bioflocculant from *Paenibacillus mucilaginosus* GIM1.16: 0.5–4 mg/L	COD: 59.7–60.7 SS: 68–69.8	[72]
Municipal wastewater	*Paenibacillus elgii* B69 Culture broth: 3% v/v + 1% v/v $CaCl_2$ (1 wt %) solution Jar tester: 10 min 200 rpm, then 5 min at 40 rpm, 10 min standing	COD: 68% Turbidity: 83% Color: 88%	[25]

Table 4. *Cont.*

Effluents	Strains/Operating Conditions	Removal Efficiencies (%)	Reference
Tannery wastewater COD: 1082.2 mg/L Chrominance: 2410.8 mg/L Nt: 452.83 mg/L	*Bacillus cereus* CZ1001, *B. subtilis* CZ1002, and *B. fusiformis* CZ1003 Flocculant: 0.2 g/L for COD, 0.11 g/L for Chrominance, 0.11 g/L for Nt	COD: 22.71–97% Chrominance: 2.74–70.97% Nt: 22.71–38.43%	[73]
Ash-flushing wastewater SS: 18.33 g/L pH: 9.88	*Pseudomonas veronii* L918 Flocculant: 2.83 mg/L, Jar tester: rapid mixing for 2 min, followed by slow mixing for 1 min	FA: 92.51%	[27]

[1] Chemical oxygen demand, [2] Total Kjeldahl nitrogen, [3] Total phosphorus, [4] total solids, [5] Suspended solids, [6] total nitrogen.

Wastewater from various origins such as swine, municipal use, breweries, aquaculture, potato starch, landfill leachate, and tannery wastewater was subject to microbial flocculation. Studies were related to the optimization of culture conditions (pH, temperature, inoculum size, bioflocculant dosage, etc.) in order to maximize bioflocculant yields, FA, and pollutant removal. Obtained results varied depending on the used strain for bioflocculant production and on the wastewater characteristics (pH, COD, SS, P_t, N_t, etc.). For example, Tang et al. (2014) reported a flocculant from *Paenibacillus mucilaginosus* with removal efficiencies of 70–75.2% and 81.5–88%, respectively, for COD and SS from paper mill wastewater. The same bioflocculant allowed the removal of 88.8–92% of SS from biological factory wastewater, with 83.2-86.9% reduction of COD [73]. Similar work was conducted with garbage incineration plant wastewater (pH 6.08), giving a COD ranging from 59.7% to 60.7% and SS removal in the range of 68–69.8%. However, it is important to point out that the used polymer doses were between 0.5 and 4 mg/L [72].

Starch wastewater, characterized by high COD (COD: 9660 mg/L), suspended solids (1.094 g/L), turbidity (2098 NTU), and low pH (2.3), was also subject to flocculation treatment by *Bacillus cereus* bioflocculant (2% v/v in the presence of $CaCl_2$ at pH 7). This treatment allowed 81%, 59%, and 36% COD, turbidity, and SS removal, respectively. These values were increased while using the mixture of bioflocculant of *Bacillus cereus* and *Pichia membranifaciens* bioflocculants in the same conditions, with maximum COD, turbidity, and SS reduction of 86%, 66%, and 41%, respectively. However, lower removal rates (COD, turbidity, and SS reduction of 58%, 54%, and 34%, respectively) were obtained using only *Pichia membranifaciens* bioflocculant [49]. The same combined flocculant mixture of *Bacillus cereus* and *Pichia membranifaciens* allowed the highest levels of COD (73%), turbidity (50%), and SS (74%) removal for landfill leachate wastewater [49].

An interesting opportunity based on the direct addition of microbial strains to wastewater was performed by other authors. In this context, it was demonstrated that adding *Bacillus megaterium* SP1 (inoculation: 1×10^4 CFU/mL, 30 °C, pH 7) to aquaculture wastewater could efficiently reduce the COD and SS levels and accelerate the bioflocculation process [70]. Therefore, a microbial polymer could substitute chemicals (e.g., $Fe_2(SO_4)_3$, $AlCl_3$, etc.), during the treatment of industrial wastewater. A heterogeneous biopolymer prepared by a consortium (*Rhizopus* sp. M9 and M17) allowed many advantages (working at lower dose, without pH control, cheap cost of preparation, and significant elimination rates of turbidity and COD) while treating potato starch wastewater [66].

4.2. Microbial Flocculants for Heavy Metal Removal

In addition to organic pollutant removal, microbial bioflocculants were shown to be able to remove metals from an ion solution and real wastewater, as represented in Table 5 [17,22,34,44,74–79].

Table 5. Heavy metal removal by microbial flocculants.

Effluents	Strains/Operating Conditions	Removal Efficiencies (%)	Reference
Electroplating wastewater Cr (VI): 280 mg/L	Bacterial strains xn11 + xn7 culture broth: 2% *v*/*v*, pH 7.5, 100 rpm,1 min	Cr (VI): 28%	[74]
Chemical industry effluent As^{3+}: 284 mg/L; Cu^{2+}: 2 mg/L; Pb^{2+}: 1.6 mg/L; Mn^{2+}: 10.2 mg/L; Ni^{2+}: 0.1 mg/L; Al^{3+}: 0.2 mg/L; Zn^{2+}: 252 mg/L; Cr^{2+}: 0.93 mg/L; Cd^{2+}: 0.1 mg/L; Fe^{2+}: 0.94 mg/L; Hg^{2+}: 0.6 mg/L	*Herbaspirillium* sp. flocculant: 1000 mg/L, agitation: 30 s.	As^{3+}: 26.6%; Cu^{2+}: 0%; Pb^{2+}: 72.9%; Mn^{2+}: 31.4%; Ni^{2+}: 0%; Al^{3+}: 0%; Zn^{2+}: 39.5%; Cr^{2+}: 0.03%; Cd^{2+}: 0%; Fe^{2+}: 1.3%; Hg^{2+}: 33.3%.	[75]
Biavin 109 medium blue dye As^{3+}: 0 mg/L; Cu^{2+}: 0.2 mg/L; Pb^{2+}: 0.02 mg/L; Mn^{2+}: 39.2 mg/L; Ni^{2+}: 0.09 mg/L; Al^{3+}: 0.3 mg/L; Zn^{2+}: 1.21 mg/L; Cr^{2+}: 0.15 mg/L; Cd^{2+}: 0.96 mg/L; Fe^{2+}: 3.01 mg/L; Hg^{2+}: 0 mg/L	*Herbaspirillium* sp. Flocculant: 1000 mg/L agitation: 30 s.	As^{3+}: 0%; Cu^{2+}: 27.9%; Pb^{2+}: 25%; Mn^{2+}: 71.1%; Ni^{2+}: 89.2%; Al^{3+}: 22.1%; Zn^{2+}: 8%; Cr^{2+}: 94.9%; Cd^{2+}: 0%; Fe^{2+}: 65.3%; Hg^{2+}: 0%.	[75]
Whale dye As^{3+}: 0 mg/L; Cu^{2+}: 0.18 mg/L; Pb^{2+}: 0.38 mg/L; Mn^{2+}: 35 mg/L; Ni^{2+}: 5.81 mg/L; Al^{3+}: 0.39 mg/L; Zn^{2+}: 1.25 mg/L; Cr^{2+}: 0.03 mg/L; Cd^{2+}: 0.96 mg/L; Fe^{2+}: 1.3 mg/L; Hg^{2+}: 0 mg/L	*Herbaspirillium* sp. Flocculant: 1000 mg/L agitation: 30 s.	As^{3+}: 0%; Cu^{2+}: 13.1%; Pb^{2+}: 5.5%; Mn^{2+}: 16%; Ni^{2+}: 17.4%; Al^{3+}: 11.5%; Zn^{2+}: 16.8%; Cr^{2+}: 54.9%; Cd^{2+}: 0%; Fe^{2+}: 11.2%; Hg^{2+}: 0%.	[75]
Simulated electroplating wastewater	*Rhizobium radiobacter* and *Bacillus sphaericus* Flocculant: 374 mg/L pH 6, contact time: 40 min	Zn^{2+}: 90% Cu^{2+}: 90% Cr^{6+}: 30% Ni^{2+}: 65%	[22]
Metal ion solution $Cr_2O_7^{2-}$: 1 mg/L Ni^{2+}: 20 mg/L	*Ruditapes philippinarum* ZHT4-13 Flocculant: 2 g/L, 1 min	Ni^{2+}: 19.2% $Cr_2O_7^{2-}$: 69.3%,	[44]
Arsenite solution $NaAsO_2$: 1.0 ppm	*Turicibacter sanguinis* ZCY8 Culture broth: 1 g/L, 20 °C. Jar tester: 2 min at 200 rpm followed by 40 rpm for 30 min, settlement period: 1–6 min	Arsenite: 86.1%	[34]
Primary treated wastewater Ni: 48 mg/L; Al: 26.9 mg/L Fe: 14.2 mg/L; Zn: 17.4 mg/L Cu: 76 mg/L	*Cloacibacterium normanense* NK6 Broth-EPS: 35 or 50 mg/L, 250 rpm and 30 °C, 0–12 h.	Ni: 85%; Al: 73% Fe: 71%; Zn: 65% Cu: 36%	[76]
Synthetic wastewater Fe^{3+}: 171-999 mg/L Pb^{2+}: 88-917 mg/L	*Bacillus mucilaginosus*. Flocculant: 40% *v*/*v*, 29 °C, 150 r/min, 15 min.	Fe^{3+}: 15–27% Pb^{2+}: 30–78%	[77]
Synthetic wastewater Arsenate: 0.5 mg/L Arsenite: 0.5 mg/L	*Paenibacillus polymyxa* ZCY-79 Flocculant: 120 mg/L, pH 7, 60 min.	Arsenate: 98.9% Arsenite: 84.6%	[78]
Chromium solution Cr (VI): 10–100 mg/L pH: 4–8	*Bacillus licheniformis* Flocculant: 2 g/L, 150 rpm, 1–2 h.	Cr (VI): 88% (at pH 7)	[17]
Chromium solution Cr (VI): 10–100 mg/L pH: 4-8	*Bacillus firmus* Flocculant: 2 g/L, 150 rpm, 1–2 h.	Cr (VI): 77% (at pH 7)	[17]
Aqueous solution $Pb(NO_3)_2$: 1 g/L	*Paenibacillu polymyxa* CCTCC M206017 Flocculant: 4×10^{-3}% (*w*/*w*) Jar tester: 1 min at 1500 rpm followed by 40 rpm for 2 min, settlement period: 1–6 min pH: 8–9.	Pb: 99.85%	[79]

Among the works using real wastewater, a bacterial culture broth of two strains (xn11 + xn7) was used to flocculate electroplating wastewater (Cr (VI) initial concentration of 280 mg/L). A 2% (*v*/*v*) inoculum acting for 1 min at pH 7.5 and under agitation (100 rpm) achieved 28% Cr (VI) elimination. This points to the adsorption properties of the bacterial bioflocculant for Cr (VI) [74].

More recently, it was demonstrated that *Rhizobium radiobacter* and *Bacillus sphaericus* were effective for the elimination of Zn^{2+}, Cu^{2+}, Cr^{6+}, and Ni^{2+} from simulated electroplating wastewater. A bioflocculant dose of 374 mg/L (acting at pH 6 for 40 min) allowed 90% removal for both Zn^{2+} and Cu^{2+}, 65% for Ni^{2+}, and only 30% for Cr^{6+} [22]. In addition, Yao et al. (2013) [77] reported significant removal of

Fe^{3+} and Pb^{2+} from wastewater using *B. mucilaginosus* bioflocculant. Interestingly, heavy metals were removed by adsorption and by the formation of carbonate minerals in the presence of CO_2, which can cause waste disposal problems [77]. Another microbial bioflocculant showed maximum removal efficiency of arsenic (84.6%) and arsenite (98.9%) from synthetic wastewater [78]. Also, a maximum Pb (II) ion removal efficiency of 99.85% was reported by Feng et al. (2013) [79]. This removal level could be related to the charge neutralization and adsorption bridging.

4.3. Microbial Flocculants for Dye Decolorization

Various microbial bioflocculants showed their decolorization ability for different textile dyes, as summarized in Table 6 [17,44,49,73,74,80–83]. However, the majority of studies were performed using a solution of mixed dyes (basic fuchsin, reactive black, terasil yellow, orange G, methylene blue, crystal violet, malachite green, etc.).

Table 6. Dye decolonization by microbial flocculants.

Effluents	Strains/Operating Conditions	Removal Efficiencies	Reference
Dye solution Basic fuchsin: 100 mg/L Reactive black: 50 mg/L	Bacterial strains: xn11 + xn7 Culture broth: 3.3% v/v + $CaCl_2$ (1 wt %)	Basic fuchsin: 93% Reactive Black: 35%,	[74]
Dye solution Terasil yellow: 0.2 g/L	*Chryseomonas Luteola* Culture broth: 0.13% v/v + $CaCl_2$ (1 wt %), pH 7	COD [1]: 33.25%, Turbidity 38.22%	[80]
Dye solution (10 mg/L) Methylene blue Crystal violet Malachite green	*Ruditapes philippinarum* ZHT4-13 Flocculant: 2 g/L, 1 min	Methylene blue: 86.11% crystal violet: 97.84% Malachite green: 99.45%	[44]
Dye solution Methylene blue: 50 mg/L	*Ruditapes philippinarum* Flocculant: 50 mg/L, sodium dodecyl sulphate SDS: 8 mM, Ca^{2+}: 5 mM Jar tester: 5 min at 200 rpm, followed by 30 min at 40 rpm, 1 h settlement period	Methylene blue: 98.63%	[81]
Dye solution (10 mg/L) orange G, methylene blue, crystal violet and malachite green	*Bacillus firmus* Flocculant: 2 g/L, 30 min	Orange G: 58% Methylene blue: 72% Crystal violet: 84% Malachite green: 90%	[17]
Mixed dye from textile industrial effluents (Dianix yellow S-6G, Dianix navy CC, etc.)	flocculant produced by mixture of various strains	Whale: >97.04%, Mediblue: 80.61% Fawn: 94.93% Mixed dye: 81.64%	[82]
Dye wastewater Cibacron yellow: 20–150 mg/L COD: 40–190 mg/L	*Sphingomonas paucimobilis* Culture broth: 0.13% v/v + $CaCl_2$ (1 wt %), pH 7	COD > 80%	[83]
Printing and dyeing wastewater COD: 760 mg/L Turbidity: 165 NTU Chromaticity: 1200 times SS [2]: 0.348 g/L, pH: 8.7	*Pichia membranifaciens*: Culture broth: 2% v/v + $CaCl_2$ (1 wt %), pH 7	COD: 45% Turbidity: 49 % Chromaticity: 46% SS: 58%	[49]
Printing and dyeing wastewater COD: 760 mg/L Turbidity: 165 NTU Chromaticity: 1200 times SS: 0.348 g/L, pH: 8.7	*Bacillus cereus* Culture broth: 2% v/v + $CaCl_2$ (1 wt %), pH 7	COD: 49% Turbidity: 73% Chromaticity: 70% SS: 58%	[49]
Printing and dyeing wastewater COD: 760 mg/L Turbidity: 165 NTU Chromaticity: 1200 times SS: 0.348 g/L, pH: 8.7	*Bacillus cereus* and *Pichia membranifaciens* Culture broth: 2% v/v + $CaCl_2$ (1 wt %), pH 7	COD: 57% Turbidity: 78% Chromaticity: 78% SS: 63%	[49]
Tannery wastewater COD: 1082.2 mg/L Chrominance: 2410.8 mg/L Nt[3]: 452.83 mg/L	*Bacillus cereus* CZ1001, *B. subtilis* CZ1002, and *B. fusiformis* CZ1003 Flocculant: 0.2 g/L for COD, 0.11 g/L for Chrominance, 0. 11 g/L for total nitrogen	COD: 22.71–25.97% Chrominance: 12.74–70.97% Nt: 22.71–38.43%	[73]

[1] Chemical oxygen demand, [2] Suspended solids, [3] Total nitrogen.

Among these works, the removal of methylene blue, crystal violet and malachite green (at 10 mg/L) was conducted using a flocculant from *Ruditapes philippinarum* ZHT4-13. A polymer dose of 2 g/L applied for 1 min allowed removal percentages of 86.11% (for methylene blue), 97.84% (for malachite green), and 9.49% (for crystal violet) [44]. The use of *Ruditapes philippinarum* flocculant for methylene blue (at 50 mg/L) removal was optimized using a jar test Interestingly, higher removal (98.63%) was obtained under optimal operating conditions of 50 mg/L of bioflocculant in the presence of sodium dodecyl sulphate (8 mM) and Ca^{2+} (5 mM). The jar test operating conditions were rapid agitation (200 rpm for 5 min), followed by slow agitation (40 rpm for 30 min) and settlement (for 1 h) [81]. Similarly, *Bacillus firmus* bioflocculant was also tested for a dye solution containing orange G, methylene blue, crystal violet, and malachite green (at 10 mg/L). In this experiment, optimal conditions (bioflocculant dose = 2 g/L, 30 min agitation) allowed the removal of 90% for malachite green, 58% for orange G, 72% for methylene blue, and 84% for crystal violet [17].

Experiments with real wastewater were also conducted using a microbial culture broth or extracted bioflocculant. Among these experiments, a culture broth of microbial consortium (*Bacillus cereus* and *Pichia membranifaciens*) was used to treat printing and dyeing wastewater. A culture broth of 2% *v*/*v*, pH 7 and in the presence of 1% (*v*/*v*) of $CaCl_2$ allowed the removal of 57% of COD, 63% of SS, and 78% of the turbidity [49]. In the same operating conditions, the use of each strain alone showed different performance. *Bacillus cereus* allowed the removal of 49% of COD, 73% of SS, and 70% of the turbidity; however, *Pichia membranifaciens* removed 45% of COD, 58% of SS, and 49% of the turbidity [49]. The variability in performance could be explained by the variability of functional groups (hydroxyl, amino, phosphate, and carboxyl groups) present in the polymer molecules produced by each strain. Interestingly, the combination of the two strains may allow most substances present in wastewater to bond and, consequently, enhanced the removal efficiency.

In another study, tannery wastewater (initial COD: 1082.2 mg/L) treatment was assessed using the bioflocculant that resulted from the growth of three bacterial strains (*Bacillus cereus* CZ1001, *B. subtilis* CZ1002, and *B. fusiformis* CZ1003). The study showed the variability of the optimum bioflocculant doses for the removal of COD, chrominance, and total nitrogen. It showed that 0.2 g/L of the flocculant allowed COD removal percentages in the range 22.71–25.97%, while a 0.11 g/L dose allowed nitrogen removal percentages in the range 22.71–38.43%. At the same concentration, the chrominance removal reached 12.74–70.97% [81]. Thus, microbial bioflocculant can be considered as a potential agent to treat industrial wastewater containing dyes in high concentrations. However, various parameters (pH, temperature, flocculant doses, etc.) should be optimized depending on the characteristics of the wastewater to be treated.

4.4. Microbial Flocculants for Sludge Dewatering

In order to prepare sludge for dewatering processes, a conditioning process using chemical polymers should be applied. Sludge dewatering allowed the obtention of a product that was dry enough, thereby reducing the storage volume and limiting the energy used during the process of sludge incineration. As indicated above, the use of chemical polymers presents various disadvantages. Interestingly, using biomaterials for wastewater sludge conditioning represents a new sustainable technology. To the best of our knowledge, very few works have described the possibility of using microbial bioflocculants for sludge conditioning. Sludge dewatering was evaluated regarding dry solids (DS) and specific resistance to filtration (SRF). As indicated in Table 7 [29,84–88], bioflocculants showed significant results similar to those obtained with chemical polymers such as polyacrylamide (PAM), polyaluminum chloride (PAC), $FeCl_3$, and $Al_2(SO_4)_3$. For example, *Acidithiobacillus ferrooxidans* flocculant improved the dewaterability of anaerobically digested sludge compared to PAM. The microbial polymer significantly reduced capillary suction time (CST) and the SRF of sludge by 74% and 89%, respectively, and these values are higher than with PAM. Interestingly, the *Acidithiobacillus ferrooxidans* biopolymer reduces the moisture content of sludge to 70% and improves the clarity of the filtrate in terms of removal of total suspended solids and total dissolved solids [84]. Moreover, microbial bioflocculants offered the lowest optimum dosage,

as reported by Guo and Ma (2015) [85]. An optimal dosage of 1.6 g/L showed better performance (DS and SRF values) than $FeCl_3$ and $Al_2(SO_4)_3$, which are added at an optimal dose of 8 g/L [85].

Table 7. The use of microbial flocculants for sludge dewatering and comparison with synthetic polymer.

Sludge	Treatment Conditions with Flocculant	Sludge Characteristics after Treatment	Reference
Anaerobically digested sludge (municipal wastewater plant)	*Acidithiobacillus ferrooxidans* bioflocculant	SRF = 0.36 × 10^{13} m/kg	[84]
SRF [1] = 3.29 × 10^{13} m/kg	(inoculation with 10^8 cells/mL, 30 min, 180 rpm)	CST = 10.1 s	
CST [2] = 38.7 s		Moisture: 70.3%	
		Organic matter: 74.5%	
		Calorific value: 4013 cal/g	
Anaerobically digested sludge (municipal wastewater plant)	Polyacrylamide (PAM) 67% and 89%	SRF = 1.08 × 10^{13} m/kg	[84]
SRF [1] = 3.29 × 10^{13} m/kg		CST = 16.25 s	
CST [2] = 38.7s		Moisture: 71.2%	
		Organic matter: 66%	
		Calorific value: 3815 cal/g	
Secondary sludge (municipal)	Bioflocculant from pre-treated sludge (1.5 g/L)	DS = 22.5%	[85]
DS [3] = 13.2%		SRF = 3.4 × 10^{12} m/kg	
SRF = 11.3 × 10^{12} m/kg			
	$Al_2(SO_4)_3$ (8 g/L, pH 6.5)	DS = 15.9%;	
		SRF = 4.7 × 10^{12} m/kg	
	PAM (0.15 g/L, pH 7.5)	DS = 24.2%;	
		SRF = 3.2 × 10^{12} m/kg	
	PAC (4 g/L, pH 7.5)	DS = 20.6%;	
		SRF = 3.8 × 10^{12} m/kg	
	$FeCl_3$ (8 g/L. pH 6.5)	DS = 16.4%;	
		SRF = 4.5 × 10^{12} m/kg	
Secondary sludge (municipal)	Bioflocculant of *Paenibacillus polymyxa*	DS = 21.7%;	[86]
DS = 13.2%	(1.5 g/L, pH 7.5)	SRF = 3.6 × 10^{12} m/kg	
SRF = 11.3 × 10^{12} m/kg			
Secondary sludge	Bioflocculant of *Paenibacillus polymyxa*	DS = 20.8%;	[87]
DS = 13.2%;	(1.5 g/L, pH 7.5)	SRF = 3.9 × 10^{12} m/kg	
SRF = 11.3 × 10^{12} m/kg			
Secondary sludge	Bioflocculant of *Klebsiella pneumoniae* (0.1%/wt/v)	DS = 59.97%.	[88]
SRF = 11.64 × 10^{12} m/kg	$Al_2(SO_4)_3$	SRF = 4.66 × 10^{12} m/kg	
	PAC		
		SRF = 6.25 × 10^{12} m/kg	
		SRF = 5 × 10^{12} m/kg	
Secondary sludge	Bioflocculant of *P. mirabilis* TJ-1	SFR = 9 × 10^5 mL/kg	[29]
SRF = 29 × 10^5 mL/kg	Poly (acrylamide P(AM-DMC): TJ-F1 + CaCl		
pH 6.23		SFR = 15 × 10^5 mL/kg	
Moisture: 96.81%		SFR = 2.5 × 10^5 mL/kg	
VSS [4]/TSS [5]: 55%			

[1] Specific resistance to filtration, [2] Capillary suction time, [3] Dry solids, [4] Volatile suspended solids, [5] Total suspended solids.

5. Conclusions

This review has considered the potential employment of microbial flocculants as a substitute for conventional chemical agents in wastewater treatment. Efforts have focused on the isolation, identification, and characterization of effective bioflocculant-producing microorganisms. Interestingly, microorganisms isolated from several sources, utilizing various nutrient sources and growing under

various conditions, are able to produce bioflocculants with different characteristics. Microbial growth conditions (strain, inoculum, nutrient sources, operating parameters, etc.) are studied in order to determine a typical procedure to maximize both bioflocculant production and flocculating activity. Bioflocculant chemical characteristics such as polymer content are related to the microbial strain and substrates used. Hence, data related to the conditions of microbial flocculant production are required to establish a strategy for scientific research and the commercial application of biopolymers in wastewater treatment. As discussed above, the potential use of microbial flocculants for wastewater treatment has been verified. They have shown significant results in removing pollutants from wastewater such as suspended solids, turbidity, COD, total nitrogen, dye, and heavy metals. At a laboratory scale, many examples of bioflocculants displayed significant flocculating activity, where the efficiency of pollutant removal exceeded 90% depending on the microbial strain used to produce the flocculant and on the wastewater characteristics. Therefore, extensive research is required to determine the optimal bioflocculation procedures for each type of wastewater. Also, in order to understand the bioflocculation mechanism, more experiments needed to be conducted taking into account the modifications in different treatment systems. Finally, the efficacy of the bioflocculation should be examined at a large scale, in real conditions and for a variety of wastewater systems, followed by a techno-economic assessment.

Author Contributions: Conceptualization, F.B.R.; S.M.S. and W.M.; investigation, F.B.R.; data curation, F.B.R. and S.M.S.; writing—original draft preparation, F.B.R. and S.M.S.; writing—review and editing, W.M.; supervision, W.M.; project administration, F.B.R.; S.M.S. and W.M.

Funding: Not applicable.

Acknowledgments: The authors would like to express their gratitude to King Khalid University, Saudi Arabia for providing administrative and technical support. The authors would also like to express their gratitude to the Research Center of Advanced Materials, King Khalid University, Saudi Arabia for support. Likewise the authors thanks University of Bisha, Kingdom of Saudi Arabia.

Conflicts of Interest: There are no conflicts of interest to declare.

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