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Microbial flocculants as an excellent alternative to synthetic flocculants for industrial application: A comprehensive review

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Abstract. Flocculation is used to effectively separate suspended colloids in domestic and industrial wastewater. Flocculants are classified into three categories as organic, inorganic and natural flocculants. Its flocculating properties, ease of use and commercial use have led to the widespread use of organic and inorganic flocculants. However, it can cause serious health problems due to its carcinogenicity and neurotoxicity. Therefore, natural bioflocculants are used to treat wastewater without harming humans and the environment. Natural flocculants are non-toxic, environmentally friendly and capable of flotation even at low concentrations. This article also discusses the classification, functions, mechanisms and applications of flocculants. Applications of natural flocculants and flocculation efficiency in the treatment of industrial wastes such as food, heavy metal and dyeing are discussed. Future studies will use methods to understand how agricultural and food wastes are used for cost-effective bioflocculant production. Bacterial consortia and new novel marine bacteria are indicated for large-scale industrial production.

Keywords: natural flocculants, chemical flocculants, microbial flocculant mechanism, industrial applications

INTRODUCTION

Today, the negative impact of water pollution and the limitation of water sources are irrefutable worldwide problems. In this way, water recycling and pollution are considered this century's most critical environmental problems (Shahedi *et al.,* 2020). Industrial wastewater is distinguished from home wastewater by its broad definition. The resulting effluent composition and characterization are entirely varied and quite complex due to the many types of industries and processes involved. Classification of industrial wastes based on their harmfulness to the environment are organic, solid, toxic, oily, acid-base, biological, nutrient, aerobic, thermal and sensory pollutants. A combination of various treatment procedures is usually necessary to thoroughly remove target pollutants, with each method having its benefits and drawbacks (Teh *et al.,* 2016).

The scattered particles in the water cannot be separated, which becomes one of the significant challenges in wastewater treatment. Separation of solid contaminants from wastewater is mandatory. Flocculation and sedimentation in this process are adopted from Porwal *et al.* (2015) research findings. The flocculants have been classified into three types, such as inorganic, organic and microbial flocculants. Organic flocculants are polyacrylamide component that significantly treats suspended solids and coarse particles. Ferric chloride and aluminium belong to inorganic flocculants that have a profound effect on treating suspended solids and colloidal substances. Microbial flocculants are categorized

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into extracellular metabolic follicles, intracellular extracellular follicles, and bacterial flocculants. Microbial flocculants have a broad range of industrial significance. However, certain flocculants obtained from microorganisms could have selective applications towards wastewater treatment (Li *et al.,* 2016; Aljuboori *et al.,* 2015). Both organic and inorganic sources of flocculants are used to treat wastewater (Ngo & Guo, 2009).

On the other hand, aluminium salt-based flocculant is used in wastewater treatment whereby sludge is produced and used as fertilizer for agricultural purposes, resulting in enhancing aluminium salt dosage in the soil, which creates soil contaminants and affects plants. By migration, infiltration, diffusion and deposition, free aluminium ions infiltrate groundwater, rivers, and lakes, polluting and endangering water supplies (Abbas *et al.,* 2017). Organic polymer flocculants have the advantage of low volume and rapid flocculation, but they do not leave readily biodegradable residues. The most widespread synthetic organic flocculant is polyacrylamide. However, polyacrylamide is not harmful, and its complex breakdown can lead to secondary pollution. Also, the polymer monomer acrylamide residue is a source of concern (Luvuyo *et al.,* 2013). Microbial flocculants are non-toxic polymer molecules produced by a bacterium or its metabolites, and the main components are polysaccharide, glycoprotein, protein, DNA and cellulose. The molecular chain length affects the flocculation activity of microbial flocculants, which are biological macromolecules. The effect of microbial flocculants improves with the length of the molecular chain.

Furthermore, the impact of flocculants is affected by the molecular structure. The bacterial flocculants settle the particles suspended in the water through the absorbing bridging vessel. Many hydrophilic molecules, including carboxyl and hydroxyl residues in the flocculants' molecular structure, are responsible for neutralising the chemical reaction and flocculation mechanism. This mechanism could facilitate the absorption of flocculants, which are bridged to the reaction group molecules and the aerosols (Li *et al.,* 2009; Zhao *et al.,* 2013; Guo *et al.,* 2014). Bioflocculants have been used for wastewater treatment for a long time, but concerns about their use have increased. This article addresses mainly the characteristics of microbial flocculants and their types, the flocculation mechanism, its advantages and finally, flocculants on further development and applications.

Classification of flocculants

In industries, flocculants enhance the settling of tiny particles - for example, in wastewater treatment, freshwater purification, and downstream processes (Salehizadeh & Shojaosadati, 2001). A classification of flocculants with subclasses is illustrated in Figure 1 (Okaiyeto *et al.,* 2015).

Inorganic flocculants

Inorganic flocculants like aluminium chloride, polyaluminium chloride, alum, ferric chloride, aluminium sulphate, and ferrous sulphate are widely used in wastewater treatment. When added to wastewater, suspended particles can bind with negatively charged metal ions to form cationic charges (Lee *et al.,* 2014). This interaction will form a micro floc and are aggregated to form macro flocs whereby settle down quickly from the solution (Suopajarvi *et al.,* 2013). Most of the inorganic flocculants have been used for the treatment of drinking and wastewater. Polyaluminium chlorides are widely used in water purification, but they are susceptible to pH, decreased activity at low temperatures, and massive amounts required for flocculation. It subsequently generates a large volume of secondary pollution, which challenges the wastewater treatment process (Wei *et al.,* 2003; Bratby, 2006; Sharma *et al.,* 2006). In this study, polyaluminium chloride contains aluminium, leading to serious health problems and unsafe drinking (Banks *et al.,* 2006). Recently, low molecular weight inorganic flocculants have been discovered, such as ferric polysilicates, which have high flocculation efficiency relative to other organic polymer flocculants (Shi & Tang, 2006; Moussas & Zouboulis, 2009). The composite of both inorganic-organic flocculants has higher flocculating activity than single inorganic-organic flocculants. For example, a composite of poly ferric chloride poly-dimethyl-diallyl-ammonium chloride showed increased flocculant efficacy than that of the single compound treating kaolin suspension (Shi & Tang, 2006; Wang *et al.,* 2007a; Gao *et al.,* 2008). Recently, the composite inorganic-organic coagulant such as poly-dimethyl-diallyl-ammonium chloride has been made by transplanting a cationic inorganic coagulant to organic polymers, deriving a complete flocculating activity from both molecules (Moussas & Zouboulis, 2009).

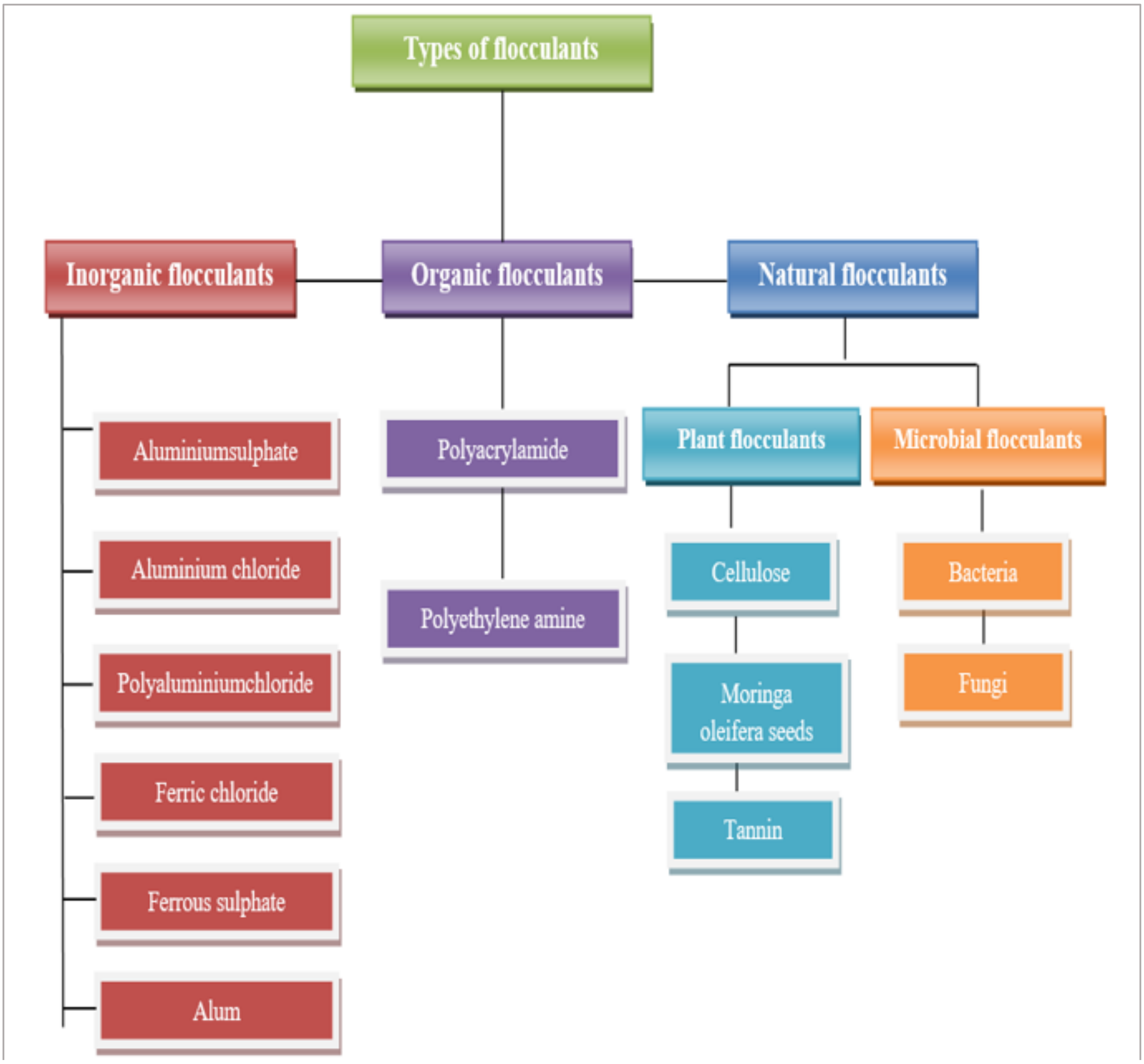

Figure 1. Broad classification of flocculants.

Organic flocculants

Nowadays, organic flocculants are used due to increased flocculation and coagulation efficiency when compared with inorganic flocculants (Kang *et al.,* 2007; Ahmad *et al.,* 2008). Organic synthetic polymers are classified into four types; positively charged cationic, negatively charged anionic, neutrally charged non-ionic and amphoteric (contains both cationic & anionic groups). These organic flocculant polymers differ in structure, charge type, composition and molecular weight. Organic flocculants are used for various industrial uses, including dredging fields, wastewater treatment etc. The high-polymer organic flocculants are universally used to obtain low-cost, low-dose effective flocculation activity (Kurane *et al.,* 1986; Salehizadeh & Shojaosadati, 2001). Polyacrylamide (PAA), polyethene amine and polyDADMAC (diallyl dimethylammonium chloride) are the organic flocculants (Singh *et al.,* 2000; Kang *et al.,* 2007; Lee *et al.,* 2014).

Natural flocculants based on plants and microorganisms

Natural flocculants are very important for water treatment because of their biodegradability, non-toxicity, lack of secondary pollutants, and environmentally friendly product. Natural flocculants are obtained in two ways: plant-derived flocculants and microbial-based flocculants. Flocculants for plants, fruit seeds, liquid juice and proteins or polysaccharides obtained from plant parts are used for flocculation. In microbial flocculants, many bacteria, fungi and yeast can be used to produce bioflocculant.

Plant flocculants

Cellulose

Recently, cellulose has become an essential component that implies industrial significance, including water, pharmaceutical, cosmetic, wood and paper industries (Das *et al.,* 2012). Cellulose bounded in polysaccharides is extracted from agricultural residues (Lee *et al.,* 2014) and contains a linear chain of β (1 → 4) D-glucose units. The annual production rate was estimated at 1011-1012 tons per year. Cellulose is derived from plants, microbes and animals (Kim *et al.,* 2006; Roy *et al.,* 2009). In 1838, Anselme Payen discovered cellulose from plant residues. Hermann Staudinger is the father of macromolecular chemistry and demonstrated the polymeric structure of cellulose in 1920. There are two types of cellulose, Iα and Iβ and the most stable structure. Anionic sodium carboxymethyl cellulose is prepared from cellulose, which is eco-friendly and complex with aluminium sulfate to remove turbidity in drinking water (Khiari *et al.,* 2010). Whereas municipal wastewater treated with ionized dicarboxylic acid nanocellulose was tested for flocculating activity using ferric sulfate as a coagulant (Subajarvi *et al.,* 2013).

Tannins

Tannins are biodegradable ionic substances derived from bark, fruits, leaves and other plants (Heredia & Martin, 2009). Its flocculating activity was checked to remove colloidal particles in drinking water treatment. Several research studies have experimentally proven that flocculating properties of tannins can eliminate the suspended colloidal particles in fresh water and remove dyes and pigments from synthetic raw water (Roussy *et al.,* 2005; Abdulsahib *et al.,* 2015; Zhou *et al.,* 2020). Recent researchers investigated the modified tannin (Tanfloc flocculant) to eliminate the impurities and flocculating capability in removing heavy metals from polluted water. Tanfloc is modified tannin by a physiochemical process and extracted from the bark of *Acacia mearnsii.* Tanfloc structure contains soluble salts and hydrocolloid gums with chemical modification, including quaternary nitrogen, to provide cationic behaviour for Tanfloc. They are used for flocculation without the aid of a coagulant and pH (Heredia, 2009).

Plant gums and mucilage

Gums and mucilages are derived from the plant leaves, barks, fruits and vegetables used in the water treatment. They are safe and replaceable with synthetic polymers for water treatment due to their non-toxicity and eco-friendliness. There are various plants used for the production of gums and mucilage, such as *Trigonella foenum-graecum* (fenugreek), *Tamarindus indica* (tamarind), *Plantago psyllium* (psyllium), Plantago *ovate* (isabgol), *Abelmoschus esculentus* (okra), and *Malva sylvestris* (mallow) (Lee *et al.,* 2014), *Opuntia ficus-indica* (cactus), *Cicer arietinum* (chickpea) (Lek *et al.,* 2018), *Aloe vera, Aloe arborescens, Cereus forbesii, Melocactus* sp., *Opuntia dilleni,* and *Stenocereus griseus* (Daza *et al.,* 2016).

The plant extracts/flocculants are obtained with aqueous extraction and precipitation method. It showed higher flocculating activities on textile water (Mishra & Bajpai, 2005), landfill leachate (Al-Hamadani *et al.,* 2011), tannery waste substance, sewage effluent (Mishra *et al.,* 2005), and biologically treated effluent (Anastasakis *et al.,* 2009). In these experiments, 85% of TSS (total suspended solids), 60% of COD (chemical oxygen demand), 70% of turbidity, and 90% of colour were removed. The efficiency of polymers obtained from desert plants reduces turbidity and colours, as demonstrated by *Opuntia dilleni* sp., 88.56% and 94.67%, respectively. In this study, *Stenocereus griseus* removed 88.58% of turbidity and 94.67% of colour, *Cereus forbesii* removed 88.31% of turbidity and 92.27% of colour, *Melocactus* sp. removed 97.15% of turbidity and 96.08% of colour, *Aloe arborescens* removed 92.74% of turbidity and 95.73% (Daza *et al.,* 2016).

According to Lek *et al.,* (2018), chickpea is a viable and low-cost alternative to synthetic inorganic coagulants and flocculants to treat palm oil mill effluent (POME). Studies have been conducted on *Cicerarietinum* (chickpea) that explores the effects of pH, dose, and quick mixing speed of turbidity, TSS, and COD elimination. The optimal conditions were 6.69 (pH), 2.6 g/l chickpea dose, and a quick mixing speed of 140 rpm. The BOD (biological oxygen demand), TSS and COD removal percentages at the optimum turbidity were 86%, 87%, and 56%, respectively. Fourier transform infrared spectroscopy (FTIR) is used in the detection of the presence of hydroxyl (OH), Carbon-Hydrogen (CH), hydrogen (NH), carbon-carbon (CC), carbonyl (CO), and carbon-nitrogen (CN) bonds in proteins and polysaccharides.

Moringa oleifera seeds

Moringa oleifera (MO) is a tropical plant categorized under the Moringaceae family, which contains water-soluble proteins that can be used for coagulation formation and reduce high turbidity in drinking water treatment. Fourteen species have been identified, and all species have coagulant properties to different degrees. MO is a widespread, fast-growing, drought-resistant tree that can grow in low humidity. MO seed reduced the oil quantity as a coagulant for the treatment of water to eliminate cyanobacteria from various natural water resources (Camacho *et al.,* 2017). According to Muyibi & Okuofu (1995), MO, as a primary coagulant, can remove turbidity around 80-99% for synthetic and raw water. Moringa seeds have soluble cationic proteins with a molecular weight of 13 kDa and an isoelectric pH range of 10 and 11 (Ndabigengesere *et al.,* 1995). Li & Pan (2013) developed a universal environmentally friendly method that turned sand into significant flocculants for eradicating Harmful Algal Blooms (HABs) in fresh and marine water bodies. The pH of the MO coagulant was utilized to change the isoelectric sand point was mainly increased from 4.5 to 10.5. However, normal sea sand changed alone by MO can remove 80 % and 20% of *Amphidinium carterae* (AC) and *Chlorella* sp. (CS), respectively, in salt water and 60% of *Microcystis aeruginosa* (MA) in freshwater. Chitosan was used to join and bridge the tiny MO-algae-sand flocs, which can remove 96 % of *Amphidinium carterae* (AC), and *Chlorella* sp. (CS) from saltwater and 90 % of *Microcystis aeruginosa* (MA). Biodegradable modifiers like MO and chitosan have achieved a higher removal rate of HAB by modifying the sand by the biocomponent mechanism. Figure 2 depicts the mechanism of action of MO and chitosan floc on modified sand with algal blooms in water.

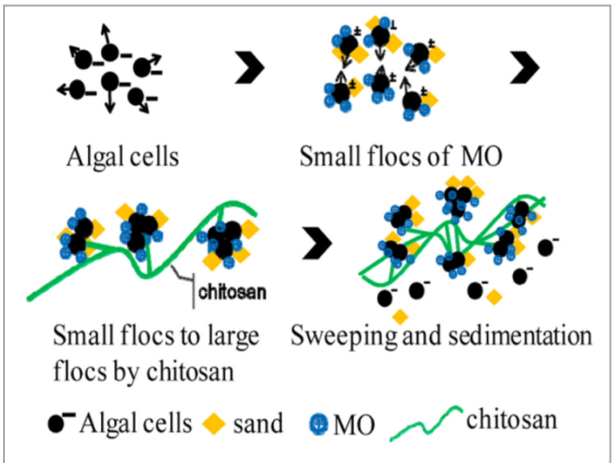

Figure 2. Removal of HAB by modified sand flocculant (*Moringa oleifera,* chitosan, sand) (Li & Pan 2013).

Microbial flocculants of bacteria and fungi

Bioflocculant is an eco-friendly, biodegradable and non-toxic macromolecular polymer synthesized from microorganisms (Liu *et al.,* 2010; Pathak *et al.,* 2017). Compared with organic and inorganic synthetic flocculants, bioflocculant has vast advantages and high flocculation efficiency. They are also harmless, biodegradable and non-toxic to biotic & abiotic ecosystems (Aljuboori *et al.,* 2015). Most chemical flocculants are susceptible to pH alterations in wastewater (Liu *et al.,* 2015). Bioflocculant has significant advantages in harvesting microalgae (Liu *et al.,* 2015b; Ndikubwimana *et al.,* 2016), dewatering of activated sludge (Guo *et al.,* 2017) and wastewater treatment, adsorption of heavy metal wastewater (Fan *et al.,* 2019), and settle down the solid suspended particles in ash flushing wastewater (Liu *et al.,* 2015).

Bacterial flocculants

Biological or flocculants have been applied in water, wastewater, and aquaculture industries. Water and wastewater treatment industries are significant users of flocculants since they are quick to apply and environmentally acceptable. Isolated bioflocculant-producing bacteria grown on yeast peptone glucose agar have a mucoid and ropy colony shape, according to Guo *et al.* (2017). *Bacillus infantis*, *Bacillus cereus*, *Bacillus safensis*, *Halomonas venusta*, *Nitroreductor aquimarinus,* and *Pseudoalteromonas*, seven bioflocculant-producing strains were performed in 64 consortiums. Among them, 19 consortia of bioflocculant exhibited a maximum flocculation rate of 80%. Two bioflocculant-forming bacterial species showed 80% flocculation activity when combined for flocculation activity. The marine bacteria have a high amount of exopolysaccharide and thus exhibit maximum flocculating activity when compared with other strains. For instance, *B infantis* and *N. aquimarinus* have the highest flocculation activity of 92.9 and 90.6%, respectively. In this investigation, the combined species of *B. infantis* and *B. cereus* flocs had maximum flocculation of 94.3%.

In a recent study, household kitchen wastes were employed to feed *Bacillus agaradhaerens* C9 to produce a bioflocculant to clean wastewater. Carbohydrates, polysaccharides, proteins, celluloses, inorganic salts, and pectin are all components of kitchen waste. These compounds can be used as essential nutrients for the growth of microorganisms and for synthesizing beneficial microbial metabolic products. *B. agaradhaerens* C9 can produce many degrading enzymes like pectinase, amylase, xylanase, protease, cellulase and lipase were utilized to convert household waste into flocculants. Consequently, 6.92 g/L of bioflocculant was produced using 40 g/L of kitchen waste. *B. agaradhaerens* C9 bioflocculant was used for 92.35% flocculation activity in 30 litres of iron heavy metal wastewater. (Liu *et al.,* 2019).

Fan *et al.* (2019) introduced *Klebsiella oxytoca* GS-4-08 as a new bioflocculant that relied solely on acetonitrile (ACN) for growth. Low-cost substrates for *K. oxytoca* GS-4-08 were used in a nitrile-containing industrial effluent, and the nitrile contents were utilized to make bioflocculants. It was initially used to remove heavy metals from water bodies, particularly Cd (II), Cr (VI), and Pb (II) metals that are hazardous to aquatic life. Complete degradation of ACN (1g/L) can take about 350 hours. In one litre of bioflocculant-producing *K. oxytoca* GS-4-08 in a synthetic medium, 4.6 g/L can be obtained. With the help of Fe^{3+}, these bioflocculants achieve flocculation efficiencies well above 90% in the kaolin clay solution method. Bioflocculants comprised polysaccharides (46.3%) and proteins (20.6%) adsorbed heavy metals in wastewater up to 112.2 mg^{-1} for Cu^{2+} and 439.2 mg^{-1} for Pb^{2+}, respectively. *Paenibacillus polymyxa* can also eradicate heavy metals like Cd, Pb, Cu, and Zn from wastewater via an adsorption process. Its ability to remove heavy metals achieved a maximum concentration of 233.3 mg/g, 551.1 mg/g, 250 mg/g, and 96.7 mg/g, respectively (Huang *et al.,* 2019).

Li *et al.* (2019) explored bioflocculants from *Streptomyces* sp. hsn06 with intense bioflocculation activity against *Chlorella vulgaris*. When the bioflocculant concentration was ten mg/L, the flocculation activity reached 68.7%. They used methylbenzene, butyl alcohol, dichloromethane, ethyl acetate, and trichloromethane as nutrients to synthesize bioflocculants. They are exposed to various temperatures and pH, and double and triple-double bonds regulate their interactions. The bioflocculant promotes cells of algae in the shape of a large floc. These bioflocculants were frequently utilized in the harvesting of *Chlorella vulgaris*. The different bacterial species that used carbon and nitrogen sources for their bioflocculant production are listed in Table 1.

Manivasagan *et al.* (2015) said that the production of polysaccharides-based bioflocculant to synthesize silver nanoparticles by *Streptomyces* sp. These biosynthesized silver nanoparticles have potent antibacterial activity against pathogenic bacteria like *Escherichia coli, Bacillus subtilis, Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The highest antibacterial activity was observed against *P. aeruginosa*, whereas the lowest was against *B. subtilis*. This study concluded that the excellent quality of bioflocculants could be synthesized from marine actinobacteria with antibacterial activity against waterborne pathogens.

The bacterium utilized n-hexadecane as a nutrient source to synthesize a novel polymeric bioflocculant and remove heavy metals from industrial wastewater. The 87.8% biofloculant activity removes heavy metals, including Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}, from the aqueous solutions with a concentration range of 1-50 mg/L^{-1} (Pathak *et al.,* 2017). *Bacillus salmalaya*

(139SI) was explored to develop an ideal bioflocculant QZ-7 for industrial wastewater treatment. The produced QZ-7 was tested for adsorption removal effectiveness for Zn^{2+} (81.3%), Pb^{2+} (77.9%), as (78.6%), Cd^{2+} (68.7%), and Cu^{2+} (76.1%) (Tawila *et al.,* 2019).

Table 1. Bacterial bioflocculants and their energy sources.

Bacterial strains	Carbon/nitrogen source	Reference
Agrobacterium sp. M-503	Urea, yeast extract, sucrose	Liu *et al.,* 2010
Arthrobacter sp. B4	Glucose, yeast extract	Yumei *et al.,* 2017
Bacillus agaradhaerens C9	Glucose, yeast extract	Liu *et al.,* 2015
Bacillus cereus and *pichia membranifaciens*	Alcohol, urea	Zhang *et al.,* 2015
Bacillus licheniformis X 14	Beef extract, peptone	Li *et al.,* 2009
Bacillus mojavensis 32A	L- glutamic acid	Elkady *et al.,* 2011
Bacillus pumilus	Urea, maltose, yeast extract	Makapela *et al.,* 2016
Bacillus subtilis F9	Sucrose, peptone	Giri *et al.,*2015
Chryseobacterium daeguense W6	Tryptone, glucose,	Zhang *et al.,* 2010
Halomonas sp. AAD6	Molasses	Sam *et al.,* 2011
Klebsiella sp. TG-1	Sucrose, yeast extract	Liu *et al.,* 2013
Klebsiella sp. ZZ-3	Glucose, urea	Yin *et al.,* 2014
Methylobacterium sp.	Peptone, glucose,	Ntsaluba *et al.,* 2011
Paenibacillus elgii B 69	Peptone, sucrose,	Li *et al.,* 2013
Proteus mirabilis	Glucose, tryptone	Zhang *et al.,* 2010
Pseudomonas aeruginosa	Petroleum hydrocarbon, peptone	Pathak *et al.,* 2017
Rhodococcus opacus	Glucose, yeast extract	Czemierska *et al.,* 2016
Solibacillus silvestrid W 01	Sorbitol, yeast extract	Wan *et al.,* 2013
Streptomyces sp. MBRC-91	Palm jiggery, yeast extract	Manivasagan *et al.,* 2015

Fungal flocculants

The fungal strains produce bioflocculants for various treatments such as water treatment, wastewater treatment, algal harvesting and several industrial purposes. According to Aljuboori *et al.* (2013), *Aspergillus flavus* was reported with 0.4 g of purified bioflocculant obtained from 1 L of the fermentation medium. The bioflocculants contained 28.5% proteins and 69.7% sugar, with 40% neutral sugars, 2.48% uronic acid, and 1.8% amino sugar. The components of neutral sugars are lactose, sucrose, xylose, glucose, mannose, galactose, and fructose in the molar ratio of 4.4:2.4:5.8:4.1:0.8:9.9:3.1. They exploit that the purified *A. flavus* IH-7 have amide, methoxyl, hydroxyl and carboxyl groups. The weight of the elements H, C, N, O, and S are 4.8%, 29.9%, 33%, 34.7%, and 2.0%, respectively.

Aljuboori *et al.* (2013) reported that the filamentous fungi *Aspergillus niger* is used as an effective bioflocculant for harvesting biomass that drastically reduce the production cost and economically viable renewable energy production (Prajapati *et al.,* 2016; Li *et al.,* 2017; Chen *et al.,* 2018). Oliveira *et al.* (2019) reported that the co-culture technique could be helpful for algal harvesting biogas production, reduce anaerobic digestion, and lower costs. They used *Aspergillus niger* co-cultured with *Spirulina maxima*, and *Synechococcus subsalsus* showed 94% bioflocculation efficiency. Selectively, *S. subsalsus* co-culture with fungal: cyanobacterial ratio 1:5 bioflocculation efficiencies up to 98% in 48 hours were acquired with carbon supplements. Despite the highest fungal: cyanobacterial ratio concentration, 1:5 shows lower efficiency in 54% obtained without carbon supplements. These results suggested a requirement for better co-culture conditions for efficient biomass densification.

Table 2. Fungal bioflocculants and their energy sources.

Fungal strains	Carbon/nitrogen source	Reference
Aspergillus flavus	Sucrose, peptone	Aljuboori *et al.,* 2013
Penicillum HHE-P7	Glucose, yeast extract	Liu & Cheng, 2010
Talaromyces trachysperms OU5	Glucose, urea	Fang & Shi, 2016
Aspergillus niger	Effluent from Palm oil mil	Aljuboori *et al.,* 2014
*Rhizopus*sp. M9 *Rhizopus* sp. M17	Potato starch wastewater, urea	Pu *et al.,*2014
Scenedesmus obliquus AS-6-1	Nitratre	Guo *et al.,* 2013
Talaromyces sp.	Glucose, urea	Fang & Shi, 2016
Filamentous fungal strain	Potato dextrose agar	Jebun *et al.,* 2015
Phanerochaete chrysoporium	Glucose potatoes	Zhang *et al.,* 2013

Mechanisms of the bioflocculant

Several flocculation mechanisms, such as Bridging, Charge neutralization, and adsorption, have been proposed (Lee *et al.,* 2014; Okaiyeto *et al.,* 2015). The actual mechanisms of bioflocculation reaction in the remediation of dissolved and suspended particulate matter are frequently aided by bridging and charge neutralization. Alternative processes primarily depend on the adsorption of flocculants on the particle surfaces (Bolto & Gregory, 2007).

Flocculation mechanism by bridging

The bridging mechanism can occur when the long-chain polymers of high molecular weight and low charge density particles are adsorbed in a long loop and tail (Figure 3a) (Caskey & Primus, 1986). It provides the possibility of attachment and interaction of polymer segments to other particles, and thereby it creates 'bridging' between particles, as shown in Figure 3b (Lee *et al.,* 2014). The effectiveness of the bridging mechanism relies on the molecular weight, the net charge of the flocculant and the molecules attract suspended particles and form aggregates (Yuan *et al.,* 2011). The polymer with high molecular weight and long chain is more effective than the particles with low molecular weight and short chain (Razali *et al.,* 2011).

For an effective bridge to be required, long-chain polymers must be sufficient to bind to the particle surface and extend the bridging formation from one particle to another. Surface particles are not used only for bonding long-chain polymer sections absorbed in other particles. Assume that the long-chain polymer should not be too long because the particles will be over-coated with the polymer with no base available to 'bridge' with the other particles. Here the particles are rearranged as shown in Figure 3. Low amounts of absorbed polymers are required, and excessive amounts will provide resorption. The amount absorbed should not be less; otherwise, adequate bridging contacts cannot be formed (Bolto & Gregory, 2007). The bridging flocculation mechanism can give essential flocs compared to other flocculation mechanisms.

Flocculation mechanism by charge neutralization

In the aqueous solution, tiny particles are moved continuously, similar to the Brownian movement. The repulsive electrostatic forces are more significant when charged particles are exposed than the Van der Waals keys. The bioflocculation absorption site is counter-charged, and charge neutralization is an essential mechanism behind the floc formation (Figure 4). In order to settle tiny particles present in the aqueous solution, counter charged mixture is required, which carries a positive charge that aids in neutralizing the particles, whereas negatively charged particles get stabilized. In many studies, optimal bioflocculation occurs at the levels of polyelectrolytes required to neutralize the particle charge. The particles accumulate under the Van der Waals force (Lachhwani, 2005).

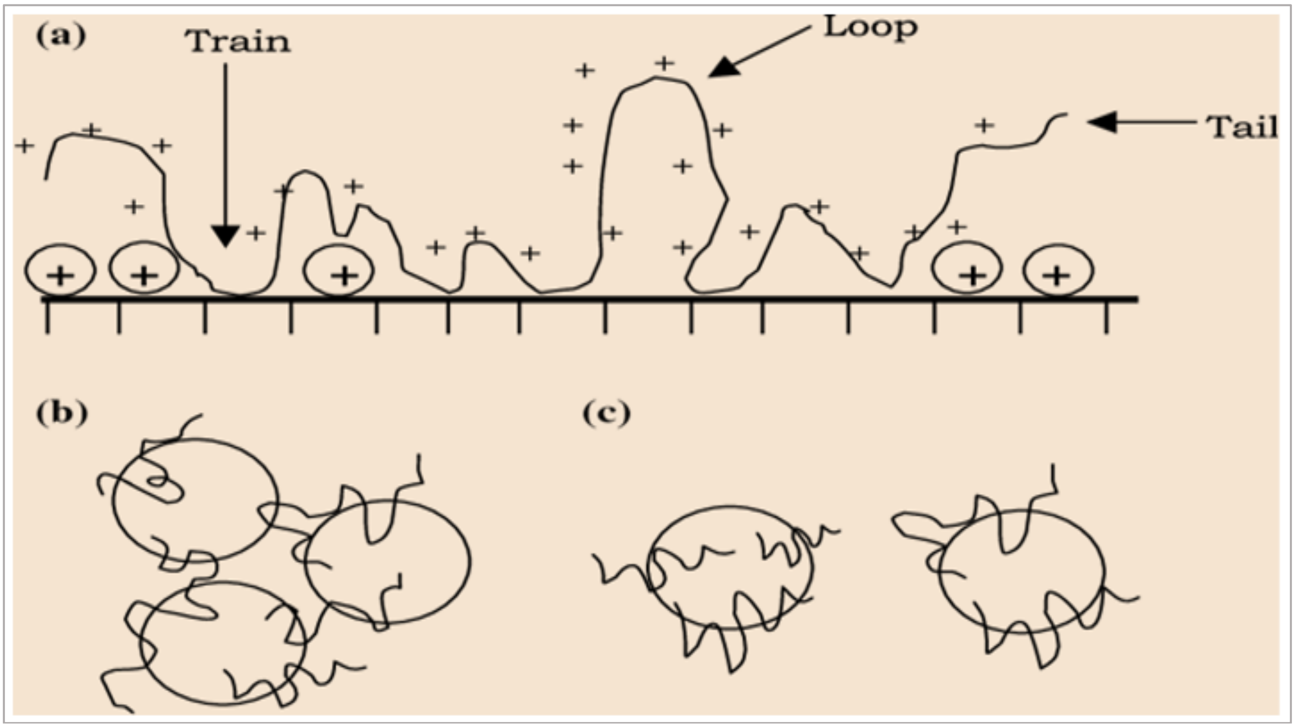

Figure 3. (a) Polymer adsorption and loop formation for binding, (b) aggregation of polymers bridging between particles (aggregation), (c) restabilisation of colloid particles (Sharma *et al.,* 2006).

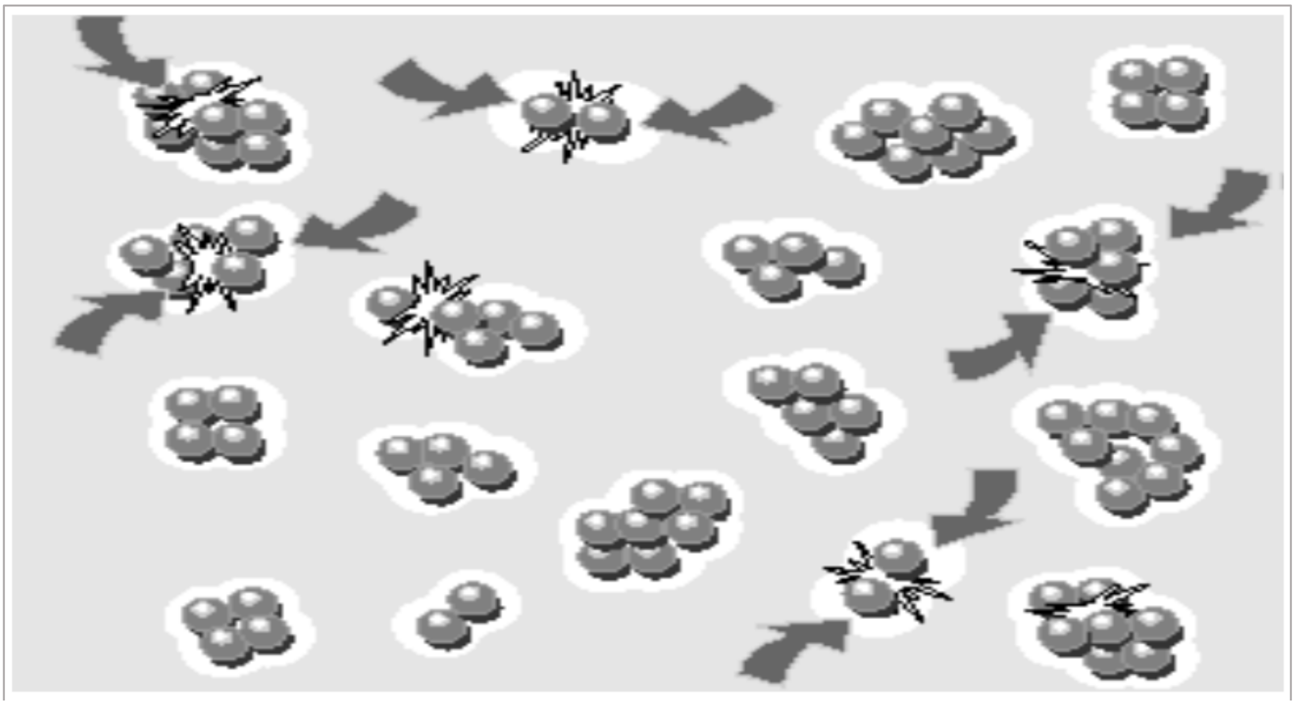

Figure 4. Flocculation of charged and uncharged particle (Lachhwani, 2005).

Flocculation mechanism by electrostatic patch

The electrostatic patch mechanism has a highly charged density of polyelectrolytes with low molecular weight adsorption on the opposite charge surface. The basic concept is that highly charged biopolymer adsorbs on a weak charged surface to neutralize the surface. The electrostatic attraction between the positive and negative particles approaches closely, giving agglomeration and flocculation (Blanco *et al.,* 2002). In this mechanism, the charge density of polyelectrolytes requirement should be higher for efficient flocculation. The bridging flocculation reduces when the charge density is reduced propositionally (Eriksson *et al.,* 1993).

Similarly, Zhang *et al.* (2010) described that the reduction in zeta potential is directly proportional to the repulsive force between the surface particles. Due to the surface charge density of the particles (Figure 5). Moreover, when the zeta potential value becomes zero, the repulsive force between the particles becomes more robust and gives better floc formation. In microbial flocculation, the Van der Waals force significantly destabilises the colloid formation into large particles. The pH and temperature of the integrated particles can affect the flocculation reactions due to environmental conditions. As the repulsive force changes, the integration of the phlox formation may disappear into the water (Lee *et al.,* 2014).

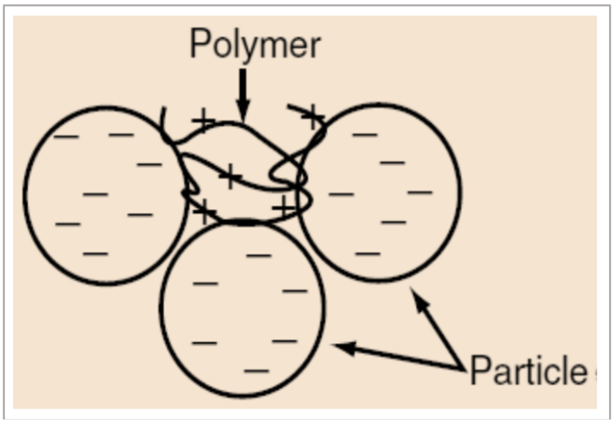

Figure 5. Flocculation by charge neutralization mechanism - a schematic view (Lee *et al.,* 2014).

Applications of microbial flocculants in treatment of industrial wastewaters

The use of microbial flocculation is essential in the water and wastewater industries. Various microbial flocculants are used in wastewater treatment. Microbial flocculants are often treated from diverse sources from industries' wastewater, wastewater with heavy metals, and textile dyes.

Food industry wastewater treatment

The bioflocculant-producing organism was obtained from the soil sample and identified as *Bacillus mucilaginosus* MBFA9 used in starch wastewater treatment. MBFA9 bioflocculant has a flocculating ability and can perform 99.6% bioflocculation activity against kaolin clay suspension with a dosage concentration of 0.1 ml/L. This MBFA9 strain treats stagnant wastewater inhabited by organic particles (Ca^{2+}). After stabilising the organic particles, the chemical oxygen requirement and the proportion of suspended solids were removed between 68.5% and 85.5%, respectively. *B. mucilaginosus* MBFA9 shows better flocculating activity compared to traditional chemical flocculants. MBFA9 contains the carboxyl and hydroxyl groups, consisting mainly of polysaccharide components such as 19.1% uronic acid, 47.4% neutral sugar and 2.7% amino sugars. *B. mucilaginosus* MBFA9 can be used in downstream processing due to the absence of toxins in the food and fermentation industry (Deng *et al.*, 2003). The bioflocculant produces microorganisms of *Rhizopus* sp. M9 and M17 were used to treat potato starch effluent water (PSW). PSW was used as a cheap growth substrate, and it was determined that culture conditions did not require adjusting for COD (1600 mg/L), potassium dihydrogen phosphate (KH_2PO_4) (0.04 g/L), urea (0.3 g/L) and pH. Optimal amounts of 0.1 m/L PSW and 10% $CaCl_2$ bioflocculant with five millilitres of Potato starch wastewater COD (54.09%), 1.1 g/L recycled PSW protein to help remove floc (Pu *et al.*, 2014).

According to a study by Qiao *et al.* (2019), *Paecilomyces* sp. (MBF2-1) bioflocculant synthesis flocculates *Trichosporon fermentans* and removes unwanted organic matter from the soybean oil refinery. The Soybean Oil Refinery (SOR) emits pollutants that pollute the environment and are high in fat and COD. MBF2-1 contains 75% polysaccharide, and it is floating. Compared to other bioflocculant, MBF2-1 only floats in high levels of *T. fermentans* and removes the organic components of SOR wastewater. Most importantly, MBF2-1 has 97% flocculating activity under alkaline conditions. Zeta identified potential measurement flocculate mechanisms and recommended net trap, bridging and sweeping mechanisms. MBF2-1 Bioflocculant SOR shows 95% flocculating activity of *T. fermentans* from wastewater. Furthermore, MBF2-1 removes the oil content and COD of SOR effluent within 30 minutes and reaches 55% and 53%, respectively. Bioflocculation is an effective process by which large–scale production in the food industry was replaced against centrifugation.

Heavy metals wastewater treatment

Due to industrial and urban development, heavy metal pollution has negatively impacted the environment. Industries, including mining and metallurgy, release vast quantities of heavy metal-containing waste into the environment. All these threats in living organisms worldwide because of this non-degradability and persistence. Even at lower concentrations, these heavy metals are toxic and which a deleterious effect on living organisms. Hence it is mandatory to remediate heavy metals from the environment. Conventional physical and chemical methods for environmental metal remediation are costly, non-specific, and have a high-energy requirement, which limits their use.

Furthermore, these processes are unsuccessful when the metal concentrations are less than 100 mg/L. Biosorption is a promising technology in heavy metal remediation at a low cost from sewage. Ayangbenro *et al.* (2019) used two bioflocculants that isolated bacteria from mining samples and tested them for their use in removing heavy metals from mine soil. Isolated *Pseudomonas aeruginosa* and *Pontoya* sp. have 71.3% and 51.7% flocculating activity against heavy metals. Bioflocculant production with an optimized pH of 7.5 and temperatures of 30°C with glucose and sucrose as a substrate for production. It can flocculate 51.2% cadmium, 52.5% chromium and 80.5% lead and 48.5% cadmium, 42.5% chromium and 73.7% b from *Pantoea* sp.

The production of bioflocculants should be more expensive than chemical flocculants. Nevertheless, use low-cost substrates, from residues of wastewater from livestock to the food industry, to reduce the cost of bioflocculant production. Previous research work done by Lei *et al.* (2018) focused on the decomposition of nitrile compounds, albeit small in recycling. Although bioflocculant are produced using low-cost substrates, nitrile–containing effluents as substrates for bioflocculant production are rarely reported. They discovered a new strain that could decompose acetonitrile into total nitrogen for their bioflocculant production. Novel strain *Klebsiella oxytoca* (A-GS408) decomposes 1g of acetonitrile within 15 days to produce 4.6 g/L^{-1} bioflocculant. The properties of the A-GS408 report revealed the carboxyl, hydroxyl, and amine groups in strains responsible for the absorption of heavy metals (Pd^{2+} and Cu^{2+}). It exhibits 90% fluorescence against kaolin and Fe^{3+} solution. The Freundlich adsorption isotherm equation determines the chemical bonding mechanism between heavy metals called chemisorption. Lin & Harichund (2011) preliminary showed that they treated three different industrial effluents with four bioflocculants by *Herbaspirillum* sp. CH7, *Paenibacillus* sp. CH11, *Bacillus* sp. CH15, and *Halomonas* sp., respectively. Every three industrial effluents (9 ml) were added to 1 ml of four bioflocculants to measure the flocculating activity by using ICP-OES (Inductively coupled plasma–optical emission spectrometry) at 550 nm wavelength. It removes several heavy metals in industrial effluents, Cr^{2+} 95%, Ni^{2+} 90%, and 50 to 80% turbidity in chemical effluents.

Table 3. Heavy metal degrading microbial flocculant and flocculation activity (%).

Microbial flocculants	**Heavy metals**	**Flocculation (%)**	**Reference**
Bacillus licheniformis KX657843	Cu (II): 58.82, Zn (II): 52.45	Cu (II): 86%, Zn (II): 81%	Biswas *et al.,* 2020.
Terrabacter sp. MUSC78T	Fe, Al, Mn, Zn	Fe: 77.7, Al: 74.8, Mn: 61.9, Zn: 57.6	Agunbiade *et al.,* 2019
Bacillus salmalaya 139SI	Zn^{2+}, As, Pb^{2+}, Cu^{2+}, Cd^{2+}	As: 78.6, Cd^{2+}: 68.7, Cu^{2+}: 76.1, Pb^{2+}: 77.9, Zn^{2+}: 81.3	Tawila *et al.,* 2019
Bacillus subtilis	Al, Zn, Fe, Cu	Al: 92.9, Zn: 94.3, Fe: 86.2, Cu: 68.1	Dih *et al.,* 2019
Halogeometricum borinquense A52	$Cr^{3+,}Ni^{2+,}Cd^{2+}$, Pb^{2+}	Cr^{3+}: 91.4%, Ni^{2+}: 89.6%, Cd^{2+}: 82.6%, Pb^{2+}: 64.6%	Chouchane *et al.,* 2018

Table 3 (continued). Heavy metal degrading microbial flocculant and flocculation activity (%).

Microbial flocculants	Heavy metals	Flocculation (%)	Reference
Pseudomonas aeruginosa IASST201s	Ni^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}	Ni^{2+}: 79.29 ± 0.12, Zn^{2+}: 73.7 ± 0.4, Cd^{2+}: 55.21 ± 0.24, Cu^{2+}: 52 ± 0.18, Pb^{2+}: 42.21 ± 0.18	Pathak *et al.,* 2017
Achromobacter xylosoxidans TERI L1	Ca^{2+}, Mg^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Ba^{2+}	Ca^{2+}: 76.8, Mg^{2+}: 55.1, Fe^{2+}: 50.6, Co^{2+}: 44.4, Cu^{2+}: 38.2, Ba^{2+}: 35.2	Subudhi *et al.,* 2016
Pseudomonas aeruginosa IASST201	Ni^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}	Ni^{2+}: 72.22 ± 0.22, Zn^{2+}: 55.42 ± 0.31, Cd^{2+}: 50.10 ± 0.13, Cu^{2+}: 36.19 ± 0.08, Pb^{2+}: 33.68 ± 0.16	Pathak *et al.,*2017
Thiobacillus thiooxidans	Zn, Cu	Zn: 95.24%, Cu: 39.84	Nagashetti *et al.,* 2013
Staphylococcus saprophyticus	Cr (VI), Pb, Cu	Cr (VI): 24.1%, Pb: 100%, Cu: 14.5%	Luptakova *et al.,* 2012
Paenibacillus sp.	Al^{3+} (0.2), As^{3+} (284), Cu^{2+} (2.0), Cr^{2+} (0.93), Cd^{2+} (0.1), Hg^{2+} (0.6), Fe^{2+} (0.94), Mn^{2+} (10.2), Ni^{2+} (0.1), Pb^{2+} (1.6), Zn^{2+} (252),	As^{3+}:21.7, Cu^{2+}: 0, Pb^{2+}: 75, Mn^{2+}: 27, Ni^{2+}: 0, Zn^{2+}: 33.4, Cr^{2+}: 0.10, Fe^{2+}: 1.30, Hg^{2+}: 27.8, Al^{3+}: 0, Cd^{2+}: 0	Lin & Harichund, 2011
Bacillus sp.	Al^{3+} (0.2), As^{3+} (284), Cd^{2+} (0.1), Cr^{2+} (0.93), Cu^{2+} (2.0), Fe^{2+} (0.94), Hg2+ (0.6), Mn^{2+} (10.2), Ni^{2+} (0.1), Pb^{2+} (1.6), Zn^{2+} (252)	As^{3+}:20.8, Cu^{2+}: 0, Pb^{2+}: 66.7, Mn^{2+}: 25.8, Ni^{2+}: 0, Al^{3+}: 0, Zn^{2+}: 31.9, Fe2+ (0.94), Hg2+ (0.6), Cr^{2+}: 0.10, Fe^{2+}: 1.00, Hg^{2+}: 16.7, Cd^{2+}: 0	Lin & Harichund, 2011
Halomonas sp.	Al^{3+} (0.2), As^{3+} (284), Cd^{2+} (0.1), Cu^{2+} (2.0), Cr^{2+} (0.93), Fe^{2+} (0.94), Hg^{2+} (0.6), Mn^{2+} (10.2), Ni^{2+} (0.1), Pb^{2+} (1.6), Zn^{2+} (252),	As^{3+}:19.3, Cr^{2+}: 0, Cu^{2+}: 0, Fe^{2+}: 0.80, Pb^{2+}: 27.1, Mn^{2+}: 24.2, Al^{3+}: 0, Ni^{2+}: 0, Zn^{2+}: 28.5, Cd^{2+}: 0, Hg^{2+}: 16.7	Lin & Harichund (2011)
Herbaspirillum sp.	Al^{3+} (0.2), As^{3+} (284), Cd^{2+} (0.1), Cu^{2+} (2.0), Fe^{2+} (0.94), Hg^{2+} (0.6), Mn^{2+} (10.2), Ni^{2+} (0.1), Pb^{2+} (1.6), Zn^{2+} (252), Cr^{2+} (0.93),	Al^{3+}: 0, As^{3+}:26.6, Cd^{2+}: 0, Cr^{2+}: 0.03, Cu^{2+}: 0, Fe^{2+}: 1.3, Hg^{2+}: 33.3, Mn^{2+}: 31.4, Ni^{2+}: 0, Pb^{2+}: 72.9, Zn^{2+}: 39.5	Lin & Harichund (2011)
Enterobacteriaccae	Pb, Cu, Cr (VI), Hg, Cd	Pb: 67.9%, Cu: 78.9%, Cr (VI): 55.8%, Hg: 43.23%, Cd: 58.9%	Rani *et al.,* 2010

Table 4. Literature work on bioflocculants and dye decolouration application.

Bioflocculants	**Source of isolate**	**Carbon/nitrogen source**	**Chemical analysis of bioflocculants**	**Functional groups**	**Flocculation (%) of dye removal**	**Reference**
Aliiglaciecola lipolytica	Seawater from Shandong, China	Glucose: 4 g/L	Intracellular laccase, azoreductase and lignin peroxidase	Protein-like and humic acid substances and tightly bound extra cellular polymeric substances (TB-EPS)	Congo Red (90%)	Wang *et al.,* 2020
Bacillus megaterium PL8	Leaves of *Camellia assamica*	Glucose: 20 g/L	Polysaccharide: 78.5%, Protein: 9.2% (w/w)	Methyl group and methylene groups	Congo Red (88.14%), Pb^{2+} ions (82.64%)	Pu *et al.,* 2020
Bacillus nitratireducens 4049	United Oil Palm Industries, Malaysia	Palm oil mill effluent	Polysaccharide: 80.2%, Protein: 20.24%	Hydroxyl, alkyl, carboxyl, and amino groups	EBT dye (90%)	Abbas *et al.,* 2020
Kocuria rosea BU22S	The sediments obtained from refinery harbour of Bizerte coast in Northern Tunisia contaminated with hydrocarbon compounds	Glucose: 15.61 g/L Peptone: 6.45 g/L	Polysaccharides: 71.6%, Uronic acid: 16.36%, Proteins: 2.83%	Hydroxyl, carboxyl, methoxyl, acetyl and amide	Reactive Blue 4 (76.4%), Acid Yellow (72.6%)	Chouchane *et al.,* 2018
Alteromonas sp. CGMCC 10612	Surface seawater South China sea (18°N, 116°E)	Glucose: 30 g/L Wheat flour: 1.5 g/L	Proteoglycan consisting of carbohydrate: 69.61%, Protein 21.56%	Hydroxyl, carboxyl and amino groups	Congo Red (98.5%), Direct Black (97.9%), Methylene Blue (72.3%)	Chen *et al.,* 2018
Enterobacter sp.	Anaerobic digester for biogas production from Institute of Chemical Technology, Mumbai, India	Glucose: 6 g/L	-	-	Anthraquinone Reactive Blue 19 (90%/24 h)	Holkar *et al.,* 2014

Brevibacillus laterosporus MTCC 2298 + *Galactomyces geotrichum* MTCC 1360	Obtained from Microbial Type Culture Collection, Chandigarh, India	Glucose: 6 g/L Tryptone: 2 g/L	Polysaccharides: 65%, Protein: 2.5%	Hydroxyl, carboxyl and amino groups	Scarlet RR (98%)	Waghmode *et al.,* 2012
Bacillus subtilis, *Exiguobacterium acetylicum*, *Klebsiella terrigena*	Northern wastewater treatment plant in Durban, South Africa.	2% of ethanol	Polysaccharides: 81.2%, Protein: 3.12%	Hydroxyl groups	Whale Azo dye (97.04%), Medi-blue Anthraquinone dye (80.61%), Fawn Azo dye (94.93%), Mixture of dyes (81.64%)	Buthelezi *et al.,* 2012

Dye decolourization treatment

Over the past few decades, industries have been considered significant contributors to environmental pollution, particularly in developing countries (Nourmoradi *et al.,* 2016). The industrial sectors such as textile, cosmetics, paper and pulp industries explicit huge pollutants, including dyes, into the water. Removing dyes from wastewater is a significant challenge, as more dye is entirely soluble in water-based solutions (Vimonses *et al.*, 2010). Wastewater treatment methods include activated carbon adsorption, oxidation, chemical coagulation/flocculation, electrochemical methods and membrane technology. A study by Bisht & Lal (2019) used a novel, environment-friendly bioflocculant obtained from *Bacillus* sp. BF-VB2 in the treatment of dye compounds present in wastewater. The novel bacterial strain *Bacillus* sp. (BF-VB2) was identified by 16S rRNA gene sequencing, and a new strain was submitted to the Gene Bank (MF362685). One milligram of BF-VB2 showed 99.0 ± 0.5% flocculation activity on 1980.0 mg of kaolin. It can reduce the textile wastewater colour (82.78% ± 3.03%), COD (92.54% ± 0.24%), TSS (73.59% ± 0.71%), and chlorine (81.90% ± 0.716%). BF-VB2 is a promising bioflocculant for potential water cleaners with high flocculation efficiency, thermal and pH stability, turbidity removal, and cation independence. The investigation provides experimental evidence of an actual scenario of bioflocculant application in wastewater treatment to remove dye components. The fungal strain *Penicillium xylanilyticus* BITSJ-11 produces bioflocculants and has profound usage in the discolouration of caustic and ionic dyes. It removes 99.56% of Congo red dye, rhodamine-B, biodegradable from dye effluent, highly stable in any condition and environmentally friendly for wastewater treatment (Saha *et al.,* 2020).

CONCLUSION AND FUTURE PROSPECTS

Chemical flocculants are widely used in different industries for low-cost removal of dissolved suspended solids and liquid separation. Based on the literature survey, it is unknown that those chemical flocculants are unhealthy for humans and the environment. Recently, bioflocculant has attracted industrialist and researchers due to their biodegradable and non-toxic properties. Most bacteria were used for the flocculation process because they are easy to cultivate and have less time for cultivation when compared with fungi. The bacterial bioflocculant contains intracellular and extracellular polymeric substances (EPS) with carbohydrates, protein and amino acids. The extracellular polymeric substance is involved in the aggregation and coagulation process. The mechanism of aggregation or coagulation may differ from each bioflocculant; it might be bridging, neutralization, or electrostatic path mechanisms. The zeta potential analyzer is commonly used to know the aggregation of the bioflocculant mechanisms.

Future studies will reveal the exact mechanism behind the flocculation with the help of advanced technology and cost-effective bioflocculant production. Research scientists proved that agricultural and food waste could be utilized for bioflocculant production. It will cut down the production cost instead of using commercially available chemicals. The new novel marine bacteria have excellent flocculating activity compared with isolates from soil and water. Large-scale industrial production is still restricted because of high production costs, low yield, and low flocculation capability. Optimizing conditions for flocculant production is the key point to improving bioflocculants yield and flocculation activity of bioflocculant. A bacterial consortium is another approach to enhance large-scale production and better flocculating activity than with single pure strains. The bioflocculants are selected according to their molecular weight and distribution of charge density of flocculants. The molecular weight and charge density are varied based on the microorganisms used. Bioflocculant production in industries is scarce, and most research studies focus on the laboratory level.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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