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Review

Marine Actinobacteria Bioflocculant: A Storehouse of Unique Biotechnological Resources for Wastewater Treatment and Other Applications

Oluyemi Olatunji Awolusi [1], Adedeji Nelson Ademakinwa [2], Abidemi Ojo [3], Mariana Erasmus [3], Faizal Bux [1] and Mayowa Oladele Agunbiade [4,*]

1 Institute for Water and Wastewater Technology, Durban University of Technology, Durban 4000, South Africa; oluyemia@dut.ac.za (O.O.A.); faizalb@dut.ac.za (F.B.)
2 Department of Physical and Chemical Sciences, Elizade University, Ilara-Mokin 002, Nigeria; adedeji.ademakinwa@elizadeuniversity.edu.ng
3 UFS/TIA Saense Platform, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Bloemfontein 9301, South Africa; ojoao@ufs.ac.za (A.O.); ErasM@ufs.ac.za (M.E.)
4 Biocatalysis and Technical Biology Research Group, Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology, Cape Town 7535, South Africa
* Correspondence: Agunbiadem@cput.ac.za; Tel.: +27-717670208

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Abstract: The bioactive compounds produced by actinobacteria have played a major role in antimicrobials, bioremediation, biofuels, enzymes, and anti-cancer activities. Biodegradable microbial flocculants have been produced by bacteria, algae, and fungi. Microbial bioflocculants have also attracted biotechnology importance over chemical flocculants as a result of degradability and environmentally friendly attributes they possess. Though, freshwater actinobacteria flocculants have been explored in bioflocculation. Yet, there is a paucity of information on the application of actinobacteria flocculants isolated from the marine environment. Similarly, marine habitats that supported the biodiversity of actinobacteria strains in the field of biotechnology have been underexplored in bioflocculation. Hence, this review reiterates the need to optimize culture conditions and other parameters that affect bioflocculant production by using a response surface model or artificial neural network.

Keywords: marine environment; actinobacteria; flocculating activity; bioflocculants; application

1. Introduction

Actinobacteria have been isolated and screened from different ecosystems such as soil, freshwater, and marine environment and the bioactive compounds produced have been explored in different fields of biotechnology by different researchers [1,2]. The class *Actinobacteria* represents the group of microorganisms that harbor several important bioactive compounds that have been discovered including antimicrobials, antitumor agents, antiparasitics, anticancer agents, and enzymes [3,4]. Recently, bioactive compounds produced by actinobacteria have been explored in bioflocculation, which could serve as a replacement to chemical flocculants in wastewater treatments. Microbial flocculants are biopolymers which facilitates particles to particle flocculation through the process of forming bridges, thus resulting in the agglomeration of suspended particles (Figure 1). Extracellular polymeric substances including polysaccharides, glycoproteins or nucleic acids, proteins, and proteoglycans are the major components of bioflocculants [5,6]. Microbial flocculants potentiate huge remarkable applications in biotechnology, and this could be attributed to their biodegradability, unique flocculation performance and absence of toxicity [7,8]. They have been widely employed in

the treatment of different wastewaters, removal of steroid estrogen, precipitation of pharmaceutical proteins, adsorption of heavy metals, drinking water purification, food processing, and fermentation industries [9,10]. On the other hand, inorganic flocculants (polyaluminum chloride and ferric chloride) and organic flocculants (polyacrylamide and polyethylenimine) are also used in wastewater treatment and other biotechnological applications. However, their applications pose a threat to aquatic organisms and human beings [11]. For instance, applications of chemically synthesized compounds have been associated with various health diseases concerns including Alzheimer's, genotoxic disorders, and carcinogenicity. Another important factor is the cost of chemical flocculants which are not affordable for many developing countries. The setbacks attributed with chemical flocculants have necessitated the exploration of biodegradable flocculants which are environmentally friendly and can be used to replace the biotechnological applications of the synthesized flocculants. Biodegradable flocculants produced by microorganisms including actinomycetes are currently gaining traction due to their advantage of environmental friendliness. Some of these bioflocculant-producing microorganisms have been isolated from sludge, soil, wastewaters, rivers, dams, alkaline lake, and marine intertidal sludge [12,13]. Actinobacteria strains isolated from freshwater, dams, and sludge have been implicated in bioflocculation and their industrial applications in wastewaters treatment have been validated. However, there is paucity of information regarding marine actinobacteria bioflocculant and their role in flocculation. Hence, there is need to explore the diversity of marine actinobacteria for bioflocculation and validate their possible industrial applications in dye and heavy metals removal, wastewater treatment, and synthesis of nanoparticles. On this note, this review summarizes actinobacteria that have been isolated and screened from the freshwater environment and the need to explore marine actinobacteria bioflocculant and validate their potentials in biotechnology.

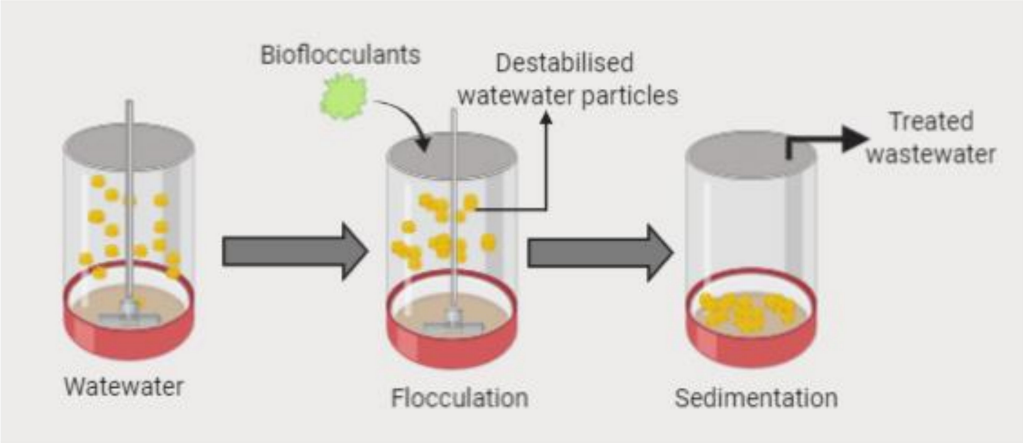

Figure 1. Diagrammatic description of the bioflocculation process (created with BioRender.com).

Flocculants can be classified into organic, inorganic and natural flocculants as itemized in Table 1 below.

Table 1. Classes of flocculants.

Inorganic Flocculants	Organic Flocculants	Natural Flocculants
Polyaluminum chloride	Polyacrylamide	Chitosan
Aluminum sulphate	Polyethylene amine	Cellulose
Aluminum chloride		Gum and mucilage
Ferric chloride		Sodium alginate
Alum		Tannin
Ferrous sulphate		Microbial flocculants

2. The Phylum *Actinobacteria*

Actinobacteria have played an important role in their associations with various higher organisms and the phylum includes species of *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Propionibacterium*, and *Tropheryma*, soil populations (*Micromonospora* and *Streptomyces* species), plant commensals (*Frankia* spp.), and gastrointestinal commensals (*Bifidobacterium* spp.). Interestingly, actinobacteria equally play an important role as both symbionts and pathogens in plant-associated microbial communities. According to Bergey's Manual of Systematic Bacteriology, phylum *Actinobacteria* is divided into 6 classes, including Actinobacteria, Acidimicrobiia, Coriobacteriia, Nitriliruptoria, Rubrobacteria, and Thermoleophilia. Among these, the class *Actinobacteria* is further subdivided into 16 different orders including Actinopolysporales, Actinomycetales, Bifidobacteriales, Catenulisporales, Corynebacteriales, Frankiales, Glycomycetales, Jiangellales, Kineosporiales, Micrococcales, Micromonosporales, Propionibacteriales, Pseudonocardiales, Streptomycetales, Streptosporangiales, and Incertae sedis [14]. The order Actinomycetales is now limited to the family members Actinomycetaceae, and the other suborders that were formerly part of this order are now known as distinct orders [15]. Thus, 43 of the 53 families that fall within the phylum *Actinobacteria* are designated to a single class *Actinobacteria*, whereas the other five classes represent only 10 families [16]. They are largely found in aquatic (freshwater and marine) and terrestrial environments. However, the greatest actinobacterial biodiversity lies in the oceans [14] and are considered the treasure house of secondary metabolites [17]. They are associated with the production of secondary metabolites and bioactive compounds which includes enzymes, antitumor and anti-parasitic agents, vitamins and antibiotics which are very active against pathogens [18]. It has been recently proposed that actinobacteria are distributed in marine environments and such includes mollusks, mangroves, fish and sponges, sediments, and seaweeds. Furthermore, *Dietza maris*, *Rhodococcus erythropolis,* and *Kocuria erythromyxa* as a representative of marine actinobacteria have been isolated from seafloor sediment [19]. According to Dharmaraj [20], the marine environment harbors varieties of actinobacteria which are not found in the terrestrial environment.

3. Bioactive Compounds from Marine Actinomycetes

Actinomycetes are Gram-positive aerobic bacteria with a high content of guanine and cytosine in their DNA and account for over 50% of all bioactive microbial compounds discovered as documented in Dictionary of Natural Products [21]. The genus *Streptomyces* is accountable for over 80% of the bioactive compounds produced among actinomycetes [22]. Furthermore, they are able to breakdown insoluble remains of other microorganisms and this includes lignocellulose and chitin [14]. In addition, they breakdown complex organic materials by providing different enzymes which include amylases, ureases, and cellulases. Thus, confirms the importance of *Streptomyces* in providing solutions for recycling chemicals that can pose problems to the biogeochemical process. Marine actinobacteria are prokaryotes and some of the actinobacteria genera include *Streptomycetes*, *Actinomycetes*, *Arthrobacter*, *Frankia*, *Micrococcus* and *Micromonospora*. It has been documented that microorganisms from marine sources have played a vital role in bioremediation and they are rich in biological macromolecules, thus affirming their importance in biotechnology [23]. Marine actinobacteria potentiate the capability of producing secondary metabolites as shown in Table 2 and the enzymes they produce have the capabilities of catalyzing different biochemical reactions [24]. It is noteworthy that marine actinobacteria could survive extreme climatic conditions such as high pressure, high salinity, and high temperature, thus modifying some physiological conditions to survive and enhance the production of novel bioactive compounds [25,26]. The presence of actinobacteria in different marine environments and habitats have been confirmed and validated by different researchers. The genus *Streptomyces* from marine and terrestrial environment have been found to be the highest producing strains of bioactive compounds.

Table 2. Some secondary metabolites produced by marine actinobacteria.

Compound	Species	Other Biological Activity	References
Antibacterial activity			
Abyssomicins	*Verrucosispora* sp.	–	[27]
Bonactin	*Streptomyces* sp.	Antifungal	[28]
Chloro-dihydroquino	*Streptomyces* sp.	Anticancer	[29]
Diazepinomicin	*Micromonospora* sp.	Anticancer; anti-inflammatory	[30]
Frigocyclinone	*Streptomyces griseus*	–	[31]
Essramycin	*Streptomyces* sp.	–	[32]
Lynamicins	*Marinispora* sp.	–	[33]
Marinopyrroles	*Streptomyces* sp.	Cytotoxic	[34]
Caboxamycin	*Streptomyces* sp.	Cytotoxic	[35]
Himalomycins	*Streptomyces* sp.	–	[36]
Chandrananimycin	*Actinomadura* sp.	Antialgal; antibacterial	[37]
N-(2-hydroxyphenyl)-2-phenazinamine(NHP)	*Nocardia dassonvillei*	Anticancer	[38]
Anticancer activity			
Salinosporamide A	*Salinispora tropica*	–	[39]
Caprolactones	*Streptomyces* sp.	–	[40]
3, 6-Disubstituted indoles	*Streptomyces* sp.	–	[41]
IB-00208	*Actinomadura* sp.	–	[42]
Antitumor activity			
Chinikomycins	*Streptomyces* sp.	–	[43]
Glyciapyrroles	*Streptomyces* sp.	–	[35]
Mechercharmycin A	*Thermoactinomyces* sp	–	[44]
Aureoverticillactam	*Streptomyces aureoverticillatus*	–	[45]
Arenicolides	*Salinispora arenicola*	–	[46]
Chalcomycin	*Streptomyces* sp.	–	[9]

3.1. Isolation and Maintenance of Cultivable Actinobacteria for Bioflocculant Production

Actinomycetes usually exist in mixed bacterial consortium in soil, water, marine sediments, and sponges [47,48]. For the sake of eliminating non-sporulating bacteria from samples, each sample is usually subjected to pre-heat treatment at 60 °C for 15 min. Afterwards, the samples are thoroughly shaken in sterile medium, vortexed for 2 to 5 min, and are serially diluted (10^{-4} and 10^{-6}) before plating on selective media for the isolation of actinobacteria. The selective isolation media generally employed includes: M1 Agar [49], ISP2 and NaST21Cx Agar [50], R2A Agar [51], and Marine Agar (MA) 2216 [52]. The media are usually supplemented with penicillin (100 μg mL^{-1}) or nalidixic acid (25 μg mL^{-1}), and cycloheximide (100 μg mL^{-1}) or potassium dichromate (50 μg mL^{-1}) to purposely inhibit the growth of other bacteria and fungi respectively. Bacterial colonies that show resemblance to actinobacteria under light microscope are then purified several times on respective media. For isolation and purification purposes, colonies are individually streaked out onto any isolation media of choice, prepared with sea water and eventually transferred on new plates until pure cultures

are obtained. Isolated bacteria are usually stored at −20 and −80 °C, in 20% glycerol and isolation medium for maintenance.

3.1.1. Plackett–Burman (PB) Design for the Screening of Bioflocculant Production

PB is a design tool developed for screening n factors specifically in $n + 1$ experimental studies. In comparison to the conventional full factorial design, PB design notably reduces the number of experiments required to reach the set goal, thus reducing the cost of resources in terms of labour and time. It involves using PB design to determine the variables that has great influence on bioflocculation activity and two levels of medium variable concentration, designated as +1 (high) and –1 (low) are usually explored. NCSS version 12 (NCSS, LLC, Kaysville, UT, USA) statistical software may be applied for designing and developing the PB experimental matrix according to the first-order regression equation as described in Equation (1):

$$Y = b_0 + \sum_{i=1}^{k} b_1 x_i$$

where Y is the response (bioflocculation activity), b_0 is the model intercept, b_i is the linear coefficient, x_i is the level of the independent variable and k is the number of variables involved. Although the model designed does not explain the synergy between the variables; however, it can be employed for screening in identifying variables that significantly influence the response [53]. Furthermore, variables that exhibit higher influence with respect to bioflocculation activity could be subjected to additional optimization studies.

3.1.2. Bioflocculation Process Optimization Using Central Composite Design (CCD)

The influence of the most significant process variables identified by the PB design could be further investigated using response surface methodology (RSM) coupled with CCD. In order to establish a relationship between the dependent variable and the independent variables, the bioflocculation activity are fit to a second-order regression model as shown in Equation (2):

$$Y = \delta_0 + \delta_1 A + \delta_2 B + \delta_3 C + \delta_{12} AB + \delta_{13} AC + \delta_{23} BC + \delta_{11} A^2 + \delta_{22} B^2 + \delta_{33} C^2 \quad (2)$$

where Y is the bioflocculation activity (response), δ_0 is the intercept term; δ_1, δ_2, and δ_3 are the coefficients of the linear terms; δ_{12}, δ_{13}, and δ_{23} are the coefficients of the interaction terms; δ_{11}, δ_{22}, and δ_{33}.

Each experimental condition is usually conducted in duplicate and the mean bioflocculation activity are recorded for the corresponding response. Various software including Design-Expert (Stat-Ease Inc., Minneapolis, MN, USA) [10], MODDE (Umetrics, Sweden) [54], Fusion Pro (S-Matix), and JMP (SAS Institute) [55] could be applied for the model and analyze the data statistically.

4. Factors Affecting Bioflocculant Production

The major factors that affect bioflocculant production include temperature, shaking speed, pH, inoculum size, cations, and carbon and nitrogen sources.

4.1. Effect of Inoculum Size

Inoculum size contributes immensely to the growth of cell and during the cause of secondary metabolites production. It has been documented that small inoculum size extends the stationary phase while larger inoculum size inhibits bioflocculant production by causing drastic overlapping in the niche of the organism [56]. In the study conducted by Agunbiade et al. [57], inoculum size of 1% (*v*/*v*) resulted in optimal flocculating activity of the bioflocculant produced by *Streptomyces platensis*.

In contrast, flocculating activity of 700 u/mL was attained when 4% (*v*/*v*) fermentation medium was used in the bioflocculant produced by *Bacillus licheniformis* [58].

4.2. Effect of Cations

Cations are very important in bioflocculant production and this has been proven in literature [59,60]. Cations facilitate the rate of flocculation by neutralizing the charge on the bioflocculant, thereby enhancing bridges formation between the particles and the bioflocculant [61]. It is worthy to note that the addition of cations to kaolin clay suspension enhances the size of floc formed, thereby increasing the rate of sedimentation processes [62]. For instance, optimum flocculating activity was attained with the bioflocculant produced by *Arthrobacter humicola* when Ca^{2+} and Mg^{2+} were used [12]. In addition, the bioflocculant produced by *Halomonas* sp V3a and *Bacillus aryabhattai* strain PSK 1 attained their optimum flocculating level when $CaCl_2$ as representatives of divalent cations was used [63]. Carboxylic functional groups of bioflocculant serve as adsorption sites for metal ions that promote complexes formation between bioflocculant and clay particles [64]. In another study, Fe^{3+} inhibited bioflocculant production while Mg^{2+}, Al^{3+}, Ca^{2+}, K^{+}, and Na^{+} enhanced better flocculating activity rate in the bioflocculant produced by *Bacillus* sp. [65].

4.3. Effect of pH

It has been noted that pH affects the microorganisms, their production of active compounds and activity [5]. The effect of pH on bioflocculant production has been reported by different authors. Alkaline medium support higher flocculating activity in protein bioflocculants while polysaccharide bioflocculants enhances better flocculating activity at a pH range of slightly acidic to alkaline medium [66]. The pH of the medium tends to have an impact on the surface charge and charged state of the colloidal particles, thereby affecting the flocculation effect [67]. The pH conditions for attaining optimum bioflocculant production varies with each organism. For instance, *Aspergillus flavus* bioflocculant attained its optimum production at pH 7 [5]. In another study, *Terrabacter* sp. thrives well at a pH range of 7–11, attaining its optimum flocculating activity at pH 8 [68]. Furthermore, bioflocculant produced by *Alteromonas* sp. was very active at neutral to moderately alkaline conditions and optimum flocculating activity of 600 U/mL was attained [69].

4.4. Effect of Carbon and Nitrogen Sources

Carbon and nitrogen sources play a vital role in the growth of microorganism, enzymes, proteins, and nucleic acid production. The requirement varies with different organisms. For instance, the optimum flocculating activity of 90.1% and the lowest activity of 29% was recorded when carbohydrate source and starch were used in the bioflocculant produced by *Bacillus salmalaya* [70]. On the other hand, when sucrose, glucose, lactose, and maltose were used as a single source, flocculating activity exceeding 60% after 72 h of cultivation were observed. Literature search has revealed that organic nitrogen sources are easily absorbed by microbial cells. Hence, they are more favourable for bioflocculant production as compared to inorganic nitrogen sources [71]. Interestingly, some microorganism can utilize either organic nitrogen sources or their combination to attain optimum flocculating activity [72,73]. A good example is observed in the bioflocculant produced by *Aspergillus parasiticus*, where sodium nitrate resulted in optimum bioflocculant production [74]. Contrastingly, when combined with ammonium sulfate, no bioflocculant was produced. In another study, yeast extract gave the best flocculating activity when used as an organic nitrogen source in the bioflocculant produced by *Cellulosimicrobium cellulans* L804 [75]

5. Characterization of Purified Bioflocculants

The phenol–sulphuric acid method, using glucose as a standard has been adopted in assaying for total sugar content of bioflocculant [76]. Protein content could be determined by the Bradford method using bovine serum albumin (BSA) as standard [77]. Additionally, the zeta potential and

charge density of bioflocculant could be measured according to the method of Pathak et al. [78]. Moreover, neutral sugar, amino sugar, and uronic acid content are usually quantified using standard methods [79]. The functional group of purified bioflocculant is analyzed with the aid of Fourier transformer infrared spectroscopy (FTIR). This is achieved by blending the purified bioflocculant homogeneously with KBr and the spectrum is monitored over the frequency range of 4000–400 cm^{-1} at ambient temperature using FTIR spectrophotometer. Furthermore, the molecular weight of purified fractions of polysaccharides could be analyzed using gel permeation chromatography system coupled with a specific refractive index. Scanning electron microscope (SEM) is usually employed to observe the surface morphology of the purified bioflocculant and Energy dispersive X-ray analysis is used to validate the different elemental compositions of the purified bioflocculant. The heat profile of the purified bioflocculant is monitored using thermogravimetric analyzer (weight loss versus temperature). This is usually achieved over a temperature range of 20 to 600 °C at a heating rate of 10 °C/min under a constant flow of nitrogen gas.

6. Applications of Actinobacteria in Biotechnology

They have been widely implicated in the production of many novel bioactive compounds like antitumor agents, antibiotics, enzymes, pigments, immune-suppressive agents, and bioflocculation materials [20]. They are good microbial transformations of organic compounds and some of the members of this genus can eventually participate in the bioconversion of wastes into high-value chemical products. Some of their application are briefly described below

6.1. Antimicrobials

Actinobacteria are best known as an agent that fight multi-drug resistant pathogenic organisms and antimicrobial compounds producers. The high increase in antibiotic resistance has urgently called for the screening and search for novel antibiotics with a new mechanism of action. Literature search has affirmed the genera *Streptomyces* and *Micromonospora* as the major genera of actinobacteria responsible for almost 80% of antibiotic production [80,81]. Some of the actinobacteria that possess antimicrobial abilities are *Streptomyces rimosus* [82] and *Streptomyces parvulus* [83]. Antibiotics produced from the bioactive compounds of actinobacteria can be classified into different classes. Example include aminoglycosides (kanamycin), anthracyclines (doxorubicin), macrolides (erythromycin), and ansamycins. Furthermore, some actinobacteria produce more than one antibiotic substance (*Streptomyces griesus*) and some antibiotic may be produced by different species of actinobacteria.

6.2. Enzymes

The bioactive compounds produced by genus *Actinomycetes* are responsible for the production of many chemical compounds, such as enzymes, antibiotics, nutraceuticals, antitumor agents, plant growth regulators, and vitamins [26,84]. Different genera of actinomycetes have been documented as the major sources of industrial enzymes that can be employed in biotechnological applications and biomedical fields [85]. Actinomycetes have been continuously studied and employed in the production of amylases, cellulases, proteases, chitinases, xylanases, tyrosinases, perosidaxes, laccasses, and pectinase [86]. It is worthy to note that actinobacteria enzymes are of great values in biotechnological applications such as food, industry, fermentation, textile, and paper industries. These enzymes secrets amylases on the outer part of the cells and it aids in extracellular digestion [87]. Furthermore, the bioactive compounds produced by *Streptomyces avermitilis* enhances the ortho-hydroxylation of resveratrol to piceatannol [88]. The applications of other actinobacteria enzymes are illustrated in Table 3.

6.3. Biofuels

Bioconversion of plant materials to sugars and their transformation into other compounds enhances the growth of a sustainable biofuel industry [89]. Literature search has validated that the genera *Streptomyces*, *Cellulomonas*, *Mycobacterium*, *Propionibacterium*, *Nocardia*, *Corynebacterium*,

and *Micromonospora* are very rich in carbohydrate degrading enzymes (glycoside hydrolase) [90]. Interestingly, the enzymes are important in the production of simple sugars which are subsequently converted into biofuels and other compounds of economic value. Actinobacteria also play an important role in detoxification of fuel associated toxic compounds.

Table 3. Selected Actinobacteria and their biotechnological applications.

Use	Enzyme	Applications
Detergent (laundry and dish wash)	Protease	Protein stain removal
	Amylase	Starch stain removal
	Lipase	Lipid stain removal
	Cellulase	Cleaning, color clarification, anti-redeposition (cotton)
	Mannanase	Mannanan stain removal (reappearing stains)
Starch and fuel	Amylase	Starch liquefaction and saccharification
	Amyloglucosidase	Saccharification
	Pullulanase	Saccharification
	Glucose isomerase	Glucose to fructose conversion
	Cyclodextrin-glycosyltransferase	Cyclodextrin production
Xylanase	Viscosity reduction (fuel and starch)	
Food (including dairy)	Protease	Milk clotting, infant formulas (low allergenic), flavor
	Lipase	Cheese flavor
	Lactase	Lactose removal (milk)
	Pectin methyl esterase	Firming fruit-based products
	Pectinase	Fruit-based products
	Transglutaminase	Modify visco-elastic properties
Baking	Amylase	Bread softness and volume, flour adjustment dough conditioning
	Xylanase	Dough stability and conditioning (in situ emulsifier)
	Lipase	Dough stability and conditioning (in situ emulsifier)
	Phospholipase	Dough strengthening
	Glucose oxidase	Dough strengthening
	Lipoxygenase	Bread whitening
	Protease	Biscuits, cookies
	Transglutaminase	Laminated dough strengths
Animal feed	Phytase	Phytate digestibility–phosphorus release
	Xylanase	Digestibility
	β-Glucanase	Digestibility
Beverage	Pectinase	De-pectinization, mashing
	Amylase	Juice treatment, low calorie beer
	β-Glucanase	Mashing
	Acetolactate decarboxylase	Maturation (beer)
	Laccase	Clarification (juice), flavor (beer), cork stopper treatment
Textile	Cellulase	Denim finishing, cotton softening
	Amylase	De-sizing
	Pectatelyase	Scouring
	Catalase	Bleach termination
	Laccase	Bleaching
	Peroxidase	Excess dye removal
Pulp and paper	Lipase Protease	Pitch control, contaminant control Biofilm removal
	Amylase	Starch-coating, de-inking, drainage improvement
	Xylanase	Bleach boosting

Table 3. *Cont.*

Use	Enzyme	Applications
	Cellulase	De-inking, drainage improvement, fiber modification
Fats and oils	Lipase	Transesterification
	Phospholipase	De-gumming, lyso-lecithin production
Organic synthesis	Lipase	Resolution of chiral alcohols and amides
	Acylase	Synthesis of semisynthetic penicillin
	Nitrilase	Synthesis of enantiopure carboxylic acids
Leather	Protease	Unhearing, bating
	Lipase	De-pickling
Personal care	Amyloglucosidase	Antimicrobial (combined with glucose oxidase)
	Glucose oxidase	Bleaching, antimicrobial
	Peroxidase	Antimicrobial
	L-Asparagine	Antitumor
	Neuraminidase	Antiviral agents
	Aminoacylase	Regulation of urea cycle

Source: [89].

6.4. Synthesis of Nanoparticles

Actinobacteria are major agents for producing nanoparticles, which exhibit different ranges of biological properties, namely antibacterial, antifungal, anticancer, anti-biofouling, antiparasitic and antioxidant. The genera, *Streptomyces* and *Arthrobacter,* are representative of actinobacteria that enhance the development of non-toxic methods of the formation of silver and gold nanoparticles. Literature search has revealed that 25 isolates of 49 synthesized silver nanoparticles are from the marine environment [91].

6.5. Bioremediation and Bioflocculation

The bioactive compounds produced by actinobacteria plays a pivotal role in organic carbon recycling and degradation of complex polymers. In addition, the capability of actinobacteria strains to produce cellulose and hemicellulose degrading enzymes enhances their potentials in degrading and solubilization of lignin and lignin related compounds [92]. Degradation of feather wastes was achieved by the ability of *Nocardiopsis* sp. SD5 to produce keratinase enzyme [93]. Bioflocculation involves the process of formation of flocs through extracellular polymeric substances (EPS) produced by the organisms [94]. This is achieved by secreting EPS, which may be adhesive or cohesive and aid in the agglomeration of suspended solids that are present in water or wastewaters [95]. Actinobacterial flocculants are harmless, biodegradable, and free of secondary pollutants; hence, they have been widely employed in biotechnology [96]. Some of the genera that have been implicated in flocculation and biotechnology include *Streptomyces* sp, *Arthrobacter* sp, *Brachybacterim* sp, *Streptomyces platensis*, and *Terrabacter* sp. [12,57]. Furthermore, an overview of microorganisms that have been screened for bioflocculation activity and validated in different field of industrial biotechnology are itemized in Table 4.

Table 4. Some of the microorganisms implicated in flocculation and their industrial applications.

Name	Source	Chemical Composition	Flocculating Activity (%)	Applications	Citations
Bacillus aryabhattai	Egyptian Agricultural soils	Glycoprotein	92.8% at 50 °C and 94.6% at pH 2.0	N/A	[97]
Ruditapes philippinarum	Aquaculture	Complex heteropolysaccharides	86.7% in deionized water and 91.8% in sea water	N/A	[98]
Sphingomonas Yabuuchiae	Chromotrophic acid waste water	Polysaccharides	0.4% (*w*/*w*) kaolin suspensions over pH 3.9 and 20–80 °C	Steroid estrogen removal	[10]
Alteromonas sp CGMCC 10612	Surface Sea water	Polysaccharides	2575.4 U/mL achieved in a 2-L fermenter	Dye decolorization	[69]
Bacillus cereus	Marine sponge	Polysaccharides	94% F/A in kaolin suspension	synthesis of Ag nanoparticles and bioremediation of wastewater	[99]
Bacillus Mucilaginous	Mixed activated Sludge	Extracellular polysaccharides	90% F/A in kaolin suspension	N/A	[100]
Bacillus Megaterium	Swine waste water treatment plant	Polysaccharides	90.2% in 4 L kaolin suspension	Arsenite removal	[101]
Streptomyces sp	Mangrove sediments	Polysaccharides	99.18% on *Nannochloropsis* culture medium	Recovery of microalgae	[102]
Bacillus cereus	Activated sludge Flocs	N/A	86.87%	Microalgae harvest	[103]
Rhodococcus erythropolis	Alkaline thermal pretreated sludge	N/A	N/A	Removal of Pb (II)	[104]
Scendesmus quadricauda	Algaetech International Sdn Bhd	Glycoprotein	flocculate 86.7% of *Scenedesmus quadricauda* cells in presence of $ZnCl_2$	Harvesting of biomass	[105]
Paenibacillus mucilaginosus	Soil sample	Polysaccharides	97% flocculation on kaolin clay suspension	Industrial waste water treatment	[106]

Table 4. *Cont.*

Name	Source	Chemical Composition	Flocculating Activity (%)	Applications	Citations
Bacillus sp. XF-56	Marine intertidal Sludge	N/A	Up to 93.5% hydrogen and bioflocculant produced in marine culture condition and 96.8% in fresh ones	N/A	[107]
Bacillus agaradhaerens C9	Alkaline lake sample	Polysaccharides Protein & nucleic Acids	95.29% kaolin suspension	Biofilms formation and harvesting of *Chlorella minuttssima*	[108]
Shinella albus Xn-1	Phycosphere of *Microcyctis aeruginosa* 7820	Non proteins & carbohydrate	86.65%	Harvest of *Chlorella vulgaris* biomass	[109]
Panebacillius polymyxa MBF-79	Recycled activated sludge samples	Glycoproteins	94.7% flocculation was achieved	Removal of arsenic acid	[110]
Klebsiella	Activated sludge	Polysaccharide	93.9% flocculation was achieved	N/A	[111]
Pseudomonas aeruginosa ZJU1	Water sample by a routine enrichment	Polysaccharide proteins & nucleic acids	N/A	Treatment of HABs caused by *Microcystis aeruginosa*	[112]
Klebsiella sp. TG-1	Waste water of a starchy factory	Polysaccharides	98% kaolin clay	Defecating Trona suspension	[113]
Bacillus firmus and *Bacillus licheniformis*	National collection of industrial microorganisms	Glycoproteins	N/A	Decolorization of dye and remediation of toxic metal solution	[114]

7. Conclusions and Recommendations

Microbial bioflocculants are extracellular polymeric substance comprising of polysaccharides, glycoproteins, and proteins that are usually produced by microorganisms during the process of secretion [5]. They have been proposed as a potential replacement to traditional and chemical flocculants (polyaluminum chloride, polyacrylamide and aluminum sulphate) as a result of the harmless and biodegradability potentials they exhibit. Interestingly, microbial flocculants isolated from different sources, such as freshwater environment and wastewater sludge have been reported in literature [68,105]. However, their applications on a large scale medium have been greatly hindered by weak flocculating activity, poor yield, and the exorbitant cost of production. Hence, there is an urgent need to isolate and screen potential actinobacteria producing bioflocculant strains from other sources and optimize their production cost before their application in biotechnology. It is worthy to note that *Nocardiopsis aegypta* sp. nov has been isolated from the marine environment and its potential flocculating ability has been established [115]. However, there is a dearth of information on validating the optimum culture conditions and confirming the potential application of the strain (*Norcadiopsis aegypta*) in biotechnology. Therefore, there is a need for the isolation of actinobacteria strains from different marine environments, screening for flocculating potentials and validating their application in different areas of bioflocculation. Furthermore, it would be important to optimize culture and fermentation conditions using different statistical package tool (response surface model and artificial neural network) prior to their production and applications on a large-scale medium. In addition, in vivo toxicological evaluation of marine actinobacteria purified bioflocculants need to be performed on wistar rats before implementing its industrial practical application on a large-scale medium

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