# RESEARCH

**Open Access** 

# Molecular detection of bacteria isolated from polluted environment and screening their ability to produce extracellular biopolymer flocculants

Hawraa Qays Al-assdy<sup>1\*</sup>, Wijdan Hussein Al-Tamimi<sup>1</sup> and Asia Fadhile Almansoory<sup>2</sup>

# Abstract

**Background** Microorganism bioflocculants are the large molecules released by microbes during growth and lysis. Bioflocculants are used in remediation wastewater and are thought to be more environmentally friendly. In the present study, 16 bacteria were isolated from hydrocarbons contaminated soil, sludge, and wastewater from different locations (Washing and lubrication stations of Zubair, Qurna, and Jazira, Beach of Shatt Al -Arab, and Al-Shuaiba Refinery) in Basrah city, south of Iraq. The isolates were identified by *16S rDNA* gene sequencing analysis. All isolated bacteria were subjected to a flocculants production test using a mineral salt medium. Bioflocculant activity was determined using kaolin clay and enhanced by addition cation (CaCl<sub>2</sub>).

**Result** The results showed that bacterial isolates were under 10 genera (*Alishewanella, Stutzerimonas, Pseudomonas, Bacillus, Pantoe, Acinetobacter, Escherichia, Exiguobacterium, Franconibacter, Lysinibacillus*), and nine isolates were recorded as new strains. Besides, the Phylogenetic tree was constructed to evaluate their close relationship and evolution between them. *Alishewanella* sp. was the most diverse and dominant genus among sixteen isolated bacteria. The isolates *Shewanella chilikensis, Exiguobacterium profundum*, and *Alishewanella jeotgali* were the most effective producing bioflocculant, where the flocculation activity recorded at 92.40%, 92.25%, and 91.65%, respectively. The ion Ca<sup>2+</sup> removes most large molecules and reduces solution absorption from 1.918 (kaolin clay) to 1.258.

**Conclusion** The contaminated environments harbor a diverse bioflocculant producing bacteria. The capacity of bacterial genera to produce bioflocculants varies, requiring the selection of optimal bacteria for bioflocculant production and their application in water treatment as effective alternatives to synthetic flocculants. The considerable flocculation activity seen suggests a potential for industrial applications. Moreover, more research on the process parameters is required to determine the possibility of large-scale production and to identify a compound responsible for flocculation activity.

Keywords Bacteria isolation, Bioflocculants, Ca<sup>2+</sup> ion, Flocculation activity, Turbidity removal

\*Correspondence:

Hawraa Qays Al-assdy

<sup>1</sup> Department of Biology College of Science, University of Basrah, Basrah, Iraq

<sup>2</sup> Department of Ecology, College of Science, University of Basrah, Basrah, Iraq

# 1 Background

Freshwater resources are widely regarded as the most significant reservoirs worldwide. All living organisms need access to clean drinking water to survive [34]. Long-term droughts, growing industries, and population growth reduce the amount of water. The accelerating growth of population, rapid urbanization, agricultural activity, and industrialization have raised the need for clean



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

hawraa.qais@uobasrah.edu.iq

water and the production of an enormous quantity of waste [4]. Every individual in developing nations produces between 30 and 70 mm<sup>3</sup> of wastewater annually [17]. The range of pollutant concentrations in wastewater is about 250–1000 mg/l of chemical oxygen demand (COD), higher than 10<sup>9</sup> microbial cells/ml and 350–1200 mg/l of total solids (including suspended and dissolved particles), in addition to heavy metals, and nutrients [46]. The discharge of effluent into waterbodies without sufficient treatment may result in detrimental effects on both human and environmental health [5].

Techniques for treating wastewater include filtration, absorption, ion exchange, flotation, sedimentation, and flocculation [5]. Flocculation is a straightforward and inexpensive wastewater treatment technique that includes the agglomeration, separation, and elimination of suspended matter, particles, and color [16]. Flocculants may be divided into three categories: inorganic flocculants, such as aluminum sulfate  $Al_2(SO_4)_3$  and poly aluminum chloride; synthetic organic flocculants, like polyethyleneimine and polyacrylamide byproducts; and bioflocculants, like chitosan sodium alginate [23]. The wastewater treatment process, low-cost microalgae harvest, dewatering of activated sludge, and downstream processes of the fermentation industry can be applied using flocculant [28, 44].

Microbial bioflocculants are macromolecules released by microbes, sometimes producing them at high rates. The extracellular bioflocculants are produced from broth fermentation. In recent years, microbial bioflocculants have received much interest as a possible substitute for chemical flocculants [39]. Extracellular polymeric substances (EPS) bioflocculants are divided into exopolysaccharides (EPSs), internal polysaccharides, and cell wall polysaccharides. They comprise polysaccharides, proteins, lipids, glycoproteins, and nucleic acid. EPSs have gained substantial consideration in recent years due to their outstanding oxidation resistance, emulsification capabilities, and protective ability [13, 18, 24]. It has been observed that various microorganisms, such as actinomycetes, fungi, bacteria, and microalgae, produced bioflocculants. Bacillus sp. is a common bacterium genus used in the production of bioflocculants [9].

Bioflocculant-producing bacteria (BPB) are selected for their morphology and capacity to generate slimy extracellular polysaccharides with high molecular weight [2]. *Pacilomyces* sp., *Nocardia marae* YK1, *and Rhodococcus erythropolis* S-I produce protein flocculants, *Alcaligenes latus* KT201 and *Bacillus subtilis* IFO3335 produce polysaccharide flocculants while *Arcuadendron* sp. TS-4, *Arathrobacter* sp., and *Bacillus salmalaya* 139SI-7 produce glycoprotein bioflocculants [3, 47]. In wastewater treatment, many species of bacteria that produce bioflocculants were used, including *Bacillus nitratireducens* [1], *Bacillus licheniformis* NJ3 [25], and *Bacillus sp.* DAS10-1 [10].

The treatment of industrial wastewater is a popular area of study worldwide, and flocculation is regarded as an excellent technique for pollutant removal [42]. Screening for microorganisms that produce bioflocculants, and developing programs for fermentation condition optimization, may improve the yield of bioflocculants [33, 48]. This study aims to identify the biopolymers generated by microorganisms through metabolic processes, elucidate their interaction mechanisms with environmental contaminants in water, and apply them as alternative techniques to synthetic polymers in water treatment.

# 2 Methods

### 2.1 Collection of samples

Soil, wastewater, and sludge samples were collected from seven sites during the three months from November 2022 to January 2023 Table 1. Soil and sludge samples were taken from five sites at a depth of 5 cm using a sterile shovel and deposited in sterile containers, and 500 ml of wastewater samples were collected. All samples were collected and sent to the laboratory for examination.

### 2.2 Isolation of bacteria

Using conventional culture-based techniques, the microbial content of the samples was detected (colony counts and numbers of isolates of various colonies). One ml of wastewater sample was added to 9 ml of sterile distilled water, then serially diluted to  $10^{-5}$  and cultured by spreading on Nutrient (Himedia, India) and MacConkey agar (Merck, Germany) plates, which incubated at 37 °C for 24 h. One gram of soil and sludge samples were activated by 100 ml of Nutrient broth (Himedia, India) and incubated in a shaker for one h. This was then diluted to  $10^{-7}$  and cultured, as mentioned previously. The different isolates were subcultured using streaking techniques on nutrient agar to get pure isolates. Nutrient agar slants

Table 1	sites of sample collection	۱
lable i	sites of sample conection	ł

Code of location	Name of location	Type of sample	
HA	Remediation basins of Al-Shuaiba Refinery	Wastewater	
HB	Al-Shuaiba Refinery	Soil	
HC	Al-Shuaiba Refinery	Sludge	
HD	Washing and lubrication station of Zubair	Soil	
HE	The beach of Shatt Al -Arab	Soil	
HF	Washing and lubrication station of Qurna	Soil	
HR	Washing and lubrication station of Jazira	Soil	

were used for storing pure bacteria for a moderately long term at 4 °C.

# 2.3 Molecular characterization of bacterial isolates 2.3.1 DNA extraction

The bacterial isolates underwent characterization through *16S rDNA* gene analysis. Bacterial DNA isolation using the Presto TM Mini gDNA Bacteria Kit by Geneaid. Subsequently, the *16S rDNA* gene was amplified via polymerase chain reaction (PCR) using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The PCR protocol entailed an initial denaturation step at 96 °C for 3 min, followed by 27 cycles comprising denaturation at 96 °C for 30 s, annealing at 56 °C for 25 s, and elongation at 72 °C for 15 s, with a final elongation step at 72 °C for 10 min [30].

# 2.4 Identification of bacterial species and analysis of the sequence data

The Macrogen company (South Korea) performed the purification and sequencing of PCR products. The obtained *16S rDNA gene* sequences underwent proofreading using Chroma's services. Bacterial species identification was achieved through the Basic Local Alignment Search Tool (BLAST) provided by the National Center for Biotechnology Information (NCBI) [27]. The sequences were input into the BLAST platform, enabling comparison with others to determine bacterial species. Furthermore, a phylogenetic tree was constructed using MEGA X [26].

### 2.5 Determination of bioflocculant activity

Media of bioflocculant production was prepared by dissolving glucose  $C_6H_{12}O_6$  20g(Himedia, India), potassium dihydrogen phosphate KH<sub>2</sub>PO<sub>4</sub> 2 g(Himedia, India), dipotassium phosphate K<sub>2</sub>HPO<sub>4</sub> 5 g (Thomas Baker, India), ammonium sulfate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.2 g (Merck, Germany), sodium chloride NaCl 0.1 g (Himedia, India), urea CH<sub>4</sub>N<sub>2</sub>O 0.5 g (Himedia, India), magnesium sulfate MgSO<sub>4</sub> 0.2 g (Hopkin and Williams, England), and yeast extract 0.5 g (Himedia, India), in 1 L of distilled water. The medium was autoclaved at 121 °C for 15 min. Biofloc production media 100 ml was inoculated with bacterial isolate 5% and incubated in a shaker at 150 rpm, 37 °C for 72 h. The production media of 10 ml was centrifuged at 8000 rpm for 10 min to separate bioflocculant from bacteria. Two ml of supernatant, 3 ml of CaCl<sub>2</sub> 1% (Riedel De Haen Ag Seelze Hannover, Germany), and 100 ml of kaolin clay 4 g/l (Himedia, India) were mixed at 200 rpm for 2 min, then at 100 rpm for 5min. The best bioflocculant performance was obtained from the highest flocculation activity using a spectrophotometer [50]. The following equation was used to compute the flocculating activity:  $A\% = \frac{A-B}{A} \times 100\%$ , where (*A*) the control and (*B*) sample optical density measured at 550 nm.

# 2.6 Effects of cation on the flocculating activity of bioflocculant

The influence of cation on the flocculation activity of bioflocculant was studied by adding  $CaCl_2$  at a concentration of 1%. Flocculating efficiency was also measured by spectrophotometer in the same manner as described in the section above [45].

### **3 Results**

# 3.1 Isolation and identification of bacterial isolates

In the present study, 16 bacterial isolates were isolated from an environment contaminated with hydrocarbons from Al-Shuaiba Refinery, Washing and Lubrication Station, and the beach of Shatt Al -Arab in Basrah City. The Gram staining results showed that Gram-negative bacteria were the predominant 75% compared to Gram-positive 25%, Fig. 1.

The particular amplification of 16S rDNA gene sequences was examined in all isolates. When using a set of universal primers, 27 F and 1492R, PCR amplification of 16S rDNA produced a single amplification of around 1500 bp for each strain. The strain's whole DNA was amplified to create a 16S rDNA fragment, partially sequenced later, the isolates' sequences were verified and edited using BioEdit, and the modified sequences were examined using BLAST online (In line with NCBI's alignment). After alignment with other 16S rDNA sequences, the degree of similarity of isolates was 98.5–100% to the reference strain. The results showed that bacterial isolates were under 10 genera (Alishewanella, Stutzerimonas, Pseudomonas, Bacillus, Pantoe, Acinetobacter, Escherichia, Exiguobacterium, Franconibacter, Lysinibacillus). Three out of these isolates were under the genus Alishewanella



**Fig. 1** The percentage of bacteria isolated from contaminated sites. Most of the bacteria isolated from environments contaminated with oil hydrocarbons were gram-negative

(Alishewanella agri, Alishewanella aestuarii, Alishewanella jeotgali) also the genus Stutzerimonas (Stutzerimonas stutzeri, Stutzerimonas balearica strain IMB16-137, Stutzerimonas balearica strain IMB16-093). Two species of Bacillus paramycoides were also identified (Table 2). Figure 2 depicts the phylogenetic tree illustrating the relationships between the selected 16 different strains of contaminated environment.

Of these 16 strains, nine novel strains were added to the GenBank database and assigned the accession numbers, Table 3.

Samples	Isolates code	Closet specie	Identity %	Accession no
HA	HA1	Stutzerimonas stutzeri strain IRQNWYF2	100	MT261835
	HA2	Alishewanella agri strain ZJY-583	99.42	KP282808
	HA3	Alishewanella aestuarii B11	98.5	NR_044344
	HA4	Bacillus paramycoides strain 3664	100	MT538528
HB	HB1	Exiguobacterium profundum strain SSPZ15	100	MT353657
	HB2	Stutzerimonas balearica strain IMB16-093	100	MG190658
	HB3	Pantoea alhagi	99.93	MW296119
HC	HC1	Shewanella chilikensis strain YZF-10	99.93	ON365757
	HC2	Acinetobacter oryzae strain WC13	100	MW296119
	HC3	Alishewanella jeotgali KCTC 22429	99.35	NR_116459
	HC4	Pseudomonas oleovorans subsp. oleovorans JCM 5968	99.72	LC462171
HD	HD1	Franconibacter daqui strain L17	99.80	OK509193
	HD2	Lysinibacillus boronitolerans strain Mix24	100	MH385002
HE	HE1	Stutzerimonas balearica strain IMB16-137	99.86	MG190736
HF	HF1	Bacillus paramycoides strain 3664	99.72	MT538528
HR	HR1	Escherichia coli strain EcSa193	100	OR261008





Fig. 2 The NJ phylogenetic tree construction method illustrated the evolutionary relationships among bacterial isolates

Code of samples	Isolates code	New bacterial strains	Identity %	Accession no. of new strain
HA	HA2	Alishewanella agri strain HAQ15	99.42	OR885475
	HA3	Alishewanella aestuarii strain HAQ17	98.5	OR890432
HB	HB3	Pantoea alhagi strain HAQ11	99.93	OR885473
HC	HC1	Shewanella chilikensis strain HAQ	99.93	OR885468
	HC3	Alishewanella jeotgali strain HAQ8	99.35	OR885471
	HC4	Pseudomonas oleovorans strain HAQ9	99.72	OR885472
HD	HD1	Franconibacter daqui strain HAQ26	99.80	OR890434
HE	HE1	Stutzerimonas balearica strain HAQ25	99.86	OR890433
HF	HF1	Bacillus paramycoides strain HAQ36	99.72	OR890435

Table 3 The bacterial isolates were identified as novel bacterial strains

The 16 BPB isolates were screened in the present study using kaolin clay suspension. All isolates' flocculating activity (FA) ranged from (15.4–92.4) %, Fig. 3. The effect of CaCl<sub>2</sub> on kaolin clay in water was recorded. The absorption measurement shows the reduction in solution absorption from 1.918 (kaolin clay) to 1.258 after adding 3 ml of CaCl<sub>2</sub> to kaolin clay, Fig. 4. The ion Ca<sup>2+</sup> removes most large molecules.

Most BPB isolates showed a high flocculating activity, Fig. 5. The most effective isolate was *Shewanella chilikensis* strain YZF-10 (92.4%), followed by *Exiguobacterium profundum* strain SSPZ15, *Alishewanella jeotgali* KCTC 22429, *Stutzerimonas balearica* strain IMB16-093 and *Stutzerimonas balearica* strain IMB16-137 (92.25, 91.65, 68.84 and 60.1)%, respectively.

# **4** Discussion

Microorganism resources are available in nature and include many advantageous biological features. Microbes develop compounds or processes to live in various environmental conditions, including severe salinity, high pressures, and very low temperatures [8]. Enzymes, peptides, and polysaccharides are metabolites that contribute to microbial adaptation and defensive mechanisms [35]. Alyousif [7], refers to bacteria as one of the most extensively studied bacteria in industrial biotechnology because of their enzymes. The results showed that Gramnegative bacteria were the predominant (75) % compared to Gram-positive (25) %. The result agreed with previous studies. Obi et al. [31] refer to Gram-negative bacteria outnumbered Gram-positive bacteria among the isolated genera, which included Klebsiella, Pseudomonas, Mycobacterium, Bordetella, Pusillimonas, Brucella, Bacillus, Achromobacter, Ochrobactrum, Advenella, Mesorhizobium, and Raoultella. Among the 21 bacterial isolates,



Fig. 3 Flocculation activity of bacterial isolates. In this experiment, the bacterial supernatant of sixteen bacteria was used with an additive CaCl<sub>2</sub> to remove kaolin clay from water. Most of the bacteria could flocculate kaolin clay. Three genera of isolates achieved flocculation activity and reached more than 90%



Fig. 4 A kaolin clay B kaolin clay and CaCl<sub>2</sub>. The figure shows the effect of CaCl<sub>2</sub> on removing large clay particles from water and roles on flocculation activity

7 (33.33%) were identified as Gram-positive bacteria. In contrast, 14 (66.66%) were identified as Gram-negative bacteria [6].

The results showed that bacterial isolates were under 10 genera (Alishewanella, Stutzerimonas, Pseudomonas, Bacillus, Pantoe, Acinetobacter, Escherichia, Exiguobacterium, Franconibacter, Lysinibacillus). Three out of these isolates were under the genus Alishewanella (Alishewanella agri, Alishewanella aestuarii, Alishewanella jeotgali) also the genus Stutzerimonas (Stutzerimonas stutzeri, Stutzerimonas balearica strain IMB16-137, Stutzerimonas balearica strain IMB16-093). Two species of Bacillus paramycoides.

The research results are compatible with many previous studies, such as Basheer et al. [11], who isolated *Acinetobacter* sp., *Moraxella* sp., *Bacillus* sp., *Vibrio* sp., and *Alcaligenes* sp. from soil contaminated with diesel. Another study was done by Cheng et al. [14], who isolated *Alishewanella* sp. from seawall muddy water. The *Exiguobacterium profundum* that tolerated the stress of heavy metals was isolated from industrial cities in a study made by [37]. Moreover, *Shewanella seohaensis* was isolated from hydrocarbon contaminated soil in a study by Ram et al. [36]. Furthermore, Biswas et al. [12] studied the *Acinetobacter* and *Exiguobacterium* isolated from Petrochemical wastewater. Last but not least, another study by Ogunlaja et al. [32] investigated the bioflocculant producing *Lysinibacillus fusiformis* isolated from oil-polluted soil. Identifying novel microorganisms with high bioflocculant production capabilities from unconventional environments, such as crude oil refineries, is crucial for improving the efficiency of existing bioflocculants, predominantly in these environments [49].

Flocculation is a cheap and simple method for treating wastewater [16]. The polymerization of many particles is called the flocculation process [21], which uses flocculants to aggregate, separate, and remove color, particles, and suspended solids [19].

Kaolin clay manufacture is a significant mineral industry. It is utilized in several sectors, including coating pigments, cement, chemicals, paint, rubber, paper filler, and ceramics. In addition, it acts as an extender in waterbased substances such as paint and ink and a useful addition that can enhance the properties of the substance, for which it creates a constant dispersion. Kaolin clay is a common yet difficult material used in hydrometallurgical, mineral processing operations, and waste tailings treatment [43].

Kaolin's colloidal size, permanently negatively charged, and anisotropic structure of basal sides make it difficult to settle and consolidate. When diffused in water, it may form a muddy suspension [43]. Kaolin's floc structures, generated by flocculant-assisted gravity thickening, can retain substantial water due to interfacial chemistry and particle interaction, making dewatering challenging [29]. The clay processing sector has difficulty removing clay



Fig. 5 A Kaolin clay, B kaolin clay, CaCl<sub>2</sub>, and supernatant of bacterial isolates. The beaker marked with (c) is a control containing kaolin clay and CaCl<sub>2</sub>

particles from liquid waste before they are released into the ecosystem [15].

Most BPB isolates showed a high flocculating activity. The current results agreed with the study of Al khafaji et al. [6], which isolated *Exiguobacterium profundum* with flocculant activity at 88%. Shinde and Jadhava, [41] isolated *S. stutzeri* with high flocculant activity. Bioflocculants contain negatively charged groups like amines, uronic acids, and carboxylic groups. Ca<sup>2+</sup> neutralizes groups within the bioflocculant, making it more positive and facilitating the flocculation of negatively charged kaolin clay particles [45].

The bioflocculant's amino and carboxylate groups promote its adsorption on clay surfaces, forming floc. This demonstrates that  $Ca^{2+}$  stimulates the bioflocculant. Flocculation is generated by adding cations, which reduces charge density and causes inter-particle bridging between kaolin particles. The capacity of  $Ca^{2+}$  to compress the charged dual layer of kaolin clay particles reduces electrostatic repulsion, leading to a partial loss of particle stability. It is reasonable to believe that cations support flocculation by stabilizing and neutralizing the remaining negative charges of the carboxyl groups of acetic, pyruvic, and uronic acids in an acidic polysaccharide that forms a bridge between kaolin particles [38].

 $Ca^{2+}$  is thought to be a stimulating factor for most extracellular polysaccharide bioflocculants. Bridging mediated by  $Ca^{2+}$  ions was regarded as one of the key flocculating processes [22]. Han et al. [20] showed that adsorbed active sites on the molecules of flocculants allow colloidal particles to be adsorbed on them. Charge attraction results from the attraction of hydrogen and electronegative atoms when the charges carried by the molecules of flocculants and contaminants are opposite. The adsorption happens at effective attachment sites rather than across the whole molecule chain [40].

# **5** Conclusions

The current study came up with the following conclusions: First, the bacteria were isolated from different contaminated locations in Basrah city. Second, the predominant bacteria were Gram-negative, and the genetic identification showed 10 genera with nine recorded new strains. Third, the genera of Alishewanella and Stutzerimonas were superior to other genera in the sense that most bacterial isolates can produce bioflocculant. Fourth, Shewanella chilikensis, Exiguobacterium profundum, and Alishewanella jeotgali recorded high levels of flocculant activity of kaolin clay removal from water 92.40%, 92.25%, and 91.65%, respectively. However, the considerable flocculation activity seen suggests a potential for industrial applications. Moreover, more research on the parameters of the process is required for the possibility of large-scale production.

#### Abbreviations

- EPS Extracellular polymeric substances
- EPSs Exopolysaccharides
- BPB Bioflocculant-producing bacteria
- FA Flocculating activity

#### Acknowledgements

Not applicable.

#### Author contributions

HQA collected samples, isolated and diagnosed bacteria, in addition to testing flocculation activity and contributed to writing the research. WHA and AFA analyze results and contributed to writing the research. All authors confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with its submission to Beni-Suef University Journal of Basic and Applied Sciences.

#### Funding

No funding was received for conducting this study.

### Availability of data and materials

Not applicable.

# Declarations

**Ethics approval and consent to participate** Not applicable.

#### **Consent for publication**

Not applicable.

#### **Competing interests**

The authors declare that they have no competing interests that affect this study.

Received: 4 April 2024 Accepted: 12 March 2025 Published online: 02 April 2025

#### References

- Abbas SZ, Yong Y, Khan MA, Siddiqui MR (2020) Bioflocculants produced by bacterial strains isolated from palm oil mill effluent are used to remove eriochrome black T dye from water. Polymers 12(7):1545. https://doi.org/10.3390/polym12071545
- Abu Bakar SNH, Hasan HA, Abdullah SRS, Kasan NA, Muhamad MH, Kurniawan SB (2021) A review of the production process of bacteria-based polymeric flocculants. J Water Process Eng 40:11915. https://doi.org/10. 1016/j.jwpe.2021.101915
- Abu Tawila ZM, Ismail S, Dadrasnia A, Usman MM (2018) Production and characterization of a bioflocculant produced by Bacillus salmalaya 139SI-7 and its applications in wastewater treatment. Molecules 23(10):2689. https://doi.org/10.3390/molecules23102689
- Akhter F, Soomro SA, Siddique M, Ahmed M (2021) Plant and nonplant based polymeric coagulants for wastewater treatment: a review. Jurnal Kejuruteraan 33(2):175–181. https://doi.org/10.17576/ jkukm-2021-33(2)-02
- Alias J, Hasan HA, Abdullah SRS, Othman AR (2022) Properties of bio flocculant-producing bacteria for high flocculating activity efficiency. Environ Technol Innov 27:102529. https://doi.org/10.1016/j.eti.2022. 102529
- Al khafaji AM, Almansoory AF, Alyousif NA (2023) Isolation, screening, and molecular identification of bioflocculants–producing bacteria. Biodiversitas 24(8):4410–4417. https://doi.org/10.13057/biodiv/d240822
- Alyousif NA (2022) Distribution, occurrence, and molecular characterization of *Bacillus*-related species isolated from different soil in Basrah Province, Iraq. Biodiversitas 23(2):679–686. https://doi.org/10.13057/biodiv/ d230209
- Andrew M, Jayaraman G (2020) Structural features of microbial exopolysaccharides about their antioxidant activity. Carbohydr Res 487:107881. https://doi.org/10.1016/j.carres.2019.107881
- Bakar SNHA, Hasan HA, Abdullah SRS, Kasan NA, Muhamad MH, Kurniawan SB (2021) A review of the production process of bacteria-based polymeric flocculants. J Water Process Eng 40:101915. https://doi.org/10. 1016/j.jwpe.2021.101915
- Berekaa MM, Ezzeldin MF (2018) Exopolysaccharide from Bacillus mojavensis DAS10–1; production and characterization. J Pure Appl Microbiol 12:633–640. https://doi.org/10.22207/JPaM.12.2.21
- Bhasheer SK, Umavathi S, Banupriya D, Thangavel M, Thangam Y (2014) Diversity of diesel degrading bacteria from a hydrocarbon contaminated soil. Int J Curr Microbiol Appl Sci 3(11):363–369. https://doi.org/10.1038/ onc.2008.69
- Biswas T, Banerjee S, Saha A, Bhattacharya A, Chanda C, Gantayet LM, Bhadury P, Chaudhuri SR (2022) Bacterial consortium based petrochemical wastewater treatment: from strain isolation to industrial effluent treatment. Environ Adv 7:100132. https://doi.org/10.1016/j.envadv.2021. 100132
- Chen S, Cheng R, Xu X, Kong C, Wang L, Fu R, Li J, Wang S, Zhang J (2022) The structure and flocculation characteristics of a novel exopolysaccharide from a Paenibacillus isolate. Carbohydr Polym 291:119561. https:// doi.org/10.1016/j.carbpol.2022.119561
- Cheng R, Wang X, Zhu H, Yan B, Shutes B, Xu Y, Fu B, Wen H (2020) Isolation and characterization of a salt-tolerant denitrifying bacterium *Alishewanella* sp. F2 from seawall muddy water. Sci Rep 10(1):10002. https:// doi.org/10.1038/s41598-020-66989-5
- Divakaran R, Pillai VS (2001) Flocculation of kaolinite suspensions in water by chitosan. Water Res 35(16):3904–3908. https://doi.org/10.1016/S0043-1354(01)00131-2
- Daud NM, Rozaimah S, Abdullah S, Hasan HA (2017) Response surface methodological analysis for optimizing acid-catalyzed transesterification biodiesel wastewater pre-treatment using coagulation-flocculation process. Process Saf Environ Prot 113:184–192. https://doi.org/10.1016/j. psep.2017.10.006
- Edokpayi JN, Odiyo JO, Durowoju OS (2017) Impact of wastewater on surface water quality in developing countries: a case study of South Africa. Water Qual 10(66561):10–5772. https://doi.org/10.5772/66561
- Feng X, Gong Y, Ye MQ, Du ZJ (2021) Antibiotic modulation of capsular exopolysaccharide in *Pelagicoccus enzymogenes* sp. nov. Isolated from marine sediment. Front Mar Sci 8:655735. https://doi.org/10.3389/fmars. 2021.655735

- Gouveia JG, dos Silva ALS, dos Santos ECL, Martins ES, López AMQ (2019) Optimization of bioflocculant production by *Bacillus* spp. from sugarcane crop soil or sludge of the agroindustrial effluent. Braz J Chem Eng 36:627–637. https://doi.org/10.1590/0104-6632.20190362s20180360
- Han Z, Dong J, Shen Z, Mou R, Zhou Y, Chen X, Fu Xi, Yang C (2019) Nitrogen removal of anaerobically digested swine wastewater by pilot-scale tidal flow constructed wetland based on in-situ biological regeneration of zeolite. Chemosphere 217:364–373. https://doi.org/10.1016/j.chemo sphere.2018.11.036
- He H, Zhang X, Yang C, Zeng G, Li H, Chen Y (2018) Treatment of organic wastewater containing a high concentration of sulfate by crystallization-Fenton-SBR. J Environ Eng 144(6):04018041. https://doi.org/10.1061/ (ASCE)EE.1943-7870.0001387
- 22. He J, Zou J, Shao Z, Zhang J, Liu Z, Yu Z (2010) Characteristics and flocculating mechanism of a novel bioflocculant HBF-3 produced by deep-sea bacterium mutant *Halomonas* sp. V3a'. World J Microbiol Biotechnol 26:1135–1141. https://doi.org/10.1007/s11274-009-0281-2
- Jie G, Hua-ying B, Ming-xiu X, Yuan-xia L, Qian L, Yan-fen Z (2006) Characterization of a bioflocculant from a newly isolated *Vagococcus* sp. W31. J Zhejiang Univ Sci B 7:186–192. https://doi.org/10.1631/jzus.2006.B0186
- Jing L, Zong S, Li J, Ye M, Surhio MM, Yang L (2017) Potential mechanism of protection effect of exopolysaccharide from *Lachnum* YM406 and its derivatives on carbon tetrachloride-induced acute liver injury in mice. J Funct Foods 36:203–214. https://doi.org/10.1016/j.jff.2017.06.057
- Joshi N, Rathod M, Vyas D, Kumar R, Mody K (2019) Multiple pollutants are removed from industrial wastewaters using a novel bioflocculant produced by *Bacillus licheniformis* NJ3. Environ Prog Sustain Energy 38(s1):S306–S314. https://doi.org/10.1002/ep.13027
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096
- Kerbauy G, Perugini MRE, Yamauchi LM, Yamada-Ogatta SF (2011) Vancomycin-dependent *Enterococcus faecium* van A: characterization of the first case isolated in a university hospital in Brazil. Braz J Med Biol Res 44:253–257. https://doi.org/10.1590/S0100-879X2011007500006
- Liu C, Wang K, Jiang JH, Liu WJ, Wang JY (2015) A novel bioflocculant produced by a salt-tolerant, alkaliphilic, and biofilm-forming strain *Bacillus agaradhaerens* C9 and its application in harvesting *Chlorella minutissima* UTEX2341. Biochem Eng J 93:166–172. https://doi.org/10.1016/j.bej.2014. 10.006
- McFarlane AJ, Bremmell KE, Addai-Mensah J (2005) Optimising the dewatering behaviour of clay tailings through interfacial chemistry, orthokinetic flocculation and controlled shear. Powder Technol 160(1):27–34. https://doi.org/10.1016/j.powtec.2005.04.046
- Miyoshi T, Iwatsuki T, Naganuma T (2005) Phylogenetic characterization of 16S rRNA gene clones from deep-groundwater microorganisms that pass through 02-micrometer-pore-size filters. Appl Environ Microbiol 71(2):1084–1088. https://doi.org/10.1128/AEM.71.2.1084-1088.2005
- Obi LU, Atagana HI, Adeleke RA (2016) Isolation and characterisation of crude oil sludge degrading bacteria. Springerplus 5:1–13. https://doi.org/ 10.1186/s40064-016-3617-z
- Ogunlaja A, Ibidunni BJ, Oyende K, Ogunlaja OO (2020) Optimization of bioflocculant production by bacteria isolated from oil-polluted soil and fermented maize effluent. IFE J Sci 22(2):201–210. https://doi.org/10. 4314/ijs.v22i2.18
- Okaiyeto K, Nwodo UU, Mabinya LV, Okoh AI (2014) Evaluation of the flocculation potential and characterization of bioflocculant produced by *Micrococcus* sp. Leo. Appl Biochem Microbiol 50:601–608. https://doi.org/ 10.1134/S000368381406012X
- Okaiyeto K, Nwodo UU, Okoli SA, Mabinya LV, Okoh AI (2016) Implications for public health demands alternatives to inorganic and synthetic flocculants: bioflocculants as important candidates. MicrobiologyOpen 5(2):177–211. https://doi.org/10.1002/mbo3.334
- Poli A, Finore I, Romano I, Gioiello A, Lama L, Nicolaus B (2017) Microbial diversity in extreme marine habitats and their biomolecules. Microorganisms 5(2):25. https://doi.org/10.3390/microorganisms5020025
- Ram G, Melvin Joe M, Devraj S, Benson A (2019) Rhamnolipid production using *Shewanella seohaensis* BS18 and evaluation of its efficiency along with phytoremediation and bioaugmentation for bioremediation of hydrocarbon contaminated soils. Int J Phytorem 21(13):1375–1383. https://doi.org/10.1080/15226514.2019.1633254

- Rizvi FZ, Kanwal W, Faisal M (2016) Chromate-reducing profile of bacterial strains isolated from industrial effluents. Polish J Environ Stud. https://doi. org/10.15244/pjoes/61881
- Salehizadeh H, Vossoughi M, Alemzadeh I (2000) Some investigations on bioflocculant producing bacteria. Biochem Eng J 5(1):39–44. https://doi. org/10.1016/S1369-703X(99)00066-2
- Shahadat M, Teng TT, Rafatullah M, Shaikh ZA, Sreekrishnan TR, Ali SW (2017) Bacterial bioflocculants: a review of recent advances and perspectives. Chem Eng J 328:1139–1152. https://doi.org/10.1016/j.cej.2017.07. 105
- Sharma BR, Dhuldhoya NC, Merchant UC (2006) Flocculants—an ecofriendly approach. J Polym Environ 14(2):195–202. https://doi.org/10. 1007/s10924-006-0011-x
- Shinde G, Jadhava A (2023) Exploring extracellular hydrolytic enzyme and bio flocculant-producing bacterial isolates for bioremediation of vegetable oil refinery effluent. J Chem Health Risks 13(4):503–510. https://doi. org/10.52783/jchr.v13.i4.850
- Tang J, Qi S, Li Z, An Q, Xie M, Yang B, Wang Y (2014) Production, purification and application of polysaccharide-based bioflocculant by *Paenibacillus mucilaginosus*. Carbohydr Polym 113:463–470. https://doi.org/10. 1016/j.carbpol.2014.07.045
- Tekin N, Demirbaş Ö, Alkan M (2005) Adsorption of cationic polyacrylamide onto kaolinite. Microporous Mesoporous Mater 85(3):340–350. https://doi.org/10.1016/j.micromeso.2005.07.004
- Wan C, Zhao XQ, Guo SL, Alam MA, Bai FW (2013) Bioflocculant production from *Solibacillus silvestris* W01 and its application in a cost-effective harvest of marine microalga *Nannochloropsis oceanica* by flocculation. Biores Technol 135:207–212. https://doi.org/10.1016/j.biortech.2012.10. 004
- Wang Z, Shen L, Zhuang X, Shi J, Wang Y, He N, Chang YI (2015) Flocculation characterization of a bioflocculant from *Bacillus licheniformis*. Ind Eng Chem Res 54(11):2894–2901. https://doi.org/10.1021/ie5050204
- Warwick C, Guerreiro A, Soares A (2013) Sensing and analysis of soluble phosphates in environmental samples: A review. Biosens Bioelectron 41:1–11. https://doi.org/10.1016/j.bios.2012.07.012
- Yaeger RG (2011) Protozoa: structure, classification, growth, and development. Review from In: Medical microbiology, 4th ed. University of Texas Medical Branch at Galveston, Galveston (TX), 1996
- Yang H, Xiao J, Wang F, Zhang L (2013) Adsorption–flocculation of *Rhodo-coccus erythropolis* on micro-fine hemalitic. J Cent South Univ 44:874–879
- Zaki S, Farag S, Elreesh GA, Elkady M, Nosier M, El Abd D (2011) Characterization of bioflocculants produced by bacteria isolated from crude petroleum oil. Int J Environ Sci Technol 8:831–840. https://doi.org/10. 1007/BF03326266
- Zhang ZQ, Bo L, Xia SQ, Wang XJ, Yang AM (2007) Production and application of a novel bioflocculant by multiple-microorganism consortia using brewery wastewater as carbon source. J Environ Sci 19(6):667–673. https://doi.org/10.1016/S1001-0742(07)60112-0

## **Publisher's Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.