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Utilizing Bioflocculants Produced by Bacteria to Remediate Oil Contaminated Water

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ABSTRACT

Bioflocculants are extracellular polymeric substances (EPS) synthesized and released by microorganisms including bacteria, algae and fungi with many applications for wastewater treatment. The current study aimed to produce bioflocculant compounds from bacteria and evaluate their efficiency in the treatment of polluted water with hydrocarbon. The bacteria were isolated from wastewater and oil-contaminated soils in Basrah city, Iraq. The bacteria used in the present study isolated and identified in a previous study, these isolates as *Aeromonas simiae* and *Exiguobacterium profundum*. Both *Aeromonas simiae* and *Exiguobacterium profundum* demonstrated high efficacy in the remediation of wastewater from the Najibiya plant achieving up to 88% turbidity reduction under optimal conditions. *Aeromonas simiae* showed varied performance in removing Total Dissolved Solids (TDS), Total Suspended Solids (TSS), and Total Petroleum Hydrocabons (TPHs), with the best results at 43%, 57%, and 86.04% respectively, under specific pH and dosage conditions. Similarly, *Exiguobacterium profundum* showed excellent results, particularly in removing TDS, TSS and TPHs, reaching up to 52%, 82% and 94.23% respectively. *Exiguobacterium profundum* was highly effective bacterium in removing TPHs from the wastewater of the AL- Najibiya plant reaching 94.23%. The effectiveness of both strains varied with pH levels and dosages, highlighting their potential in targeted wastewater remediation applications.

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INTRODUCTION

Industrialization promotes economic expansion; improper waste management can make it a significant cause of pollution. One major environmental issue that contributes to water contamination is the increasing number of waste-producing companies worldwide (Dlamini *et al*., 2019). Surface water contains a variety of substances that must be removed before it can be used as drinking water. These substances can be divided into three categories: colloidal solids, settleable suspended solids, and dissolved solids (Naidoo & Olaniran, 2014).

Drinking water is made safe to drink by using flocculation, filtration, and disinfection to remove harmful impurities. Adding a coagulant to water destabilizes negatively charged particles, causing them to clump together into larger flocs during the flocculation process, this is known as coagulation (Renault *et al*., 2009). Flocculation is widely used in wastewater treatment technology because it is convenient, inexpensive, energy-efficient, easy to operate, and environmentally friendly (Ma *et al*., 2018). Flocculants can be divided into three types: natural (chitosan, bioflocculant), inorganic (aluminum sulfate and polyaluminum chloride), and organic synthetic (polyacrylic acid and polyacrylamide derivatives). Many organic and

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inorganic flocculants are widely used because they are inexpensive and effective but can cause serious diseases, like neurological disorders, cancer and Alzheimer's disease (Kurniawan *et al*., 2020).

Bioflocculants are a type of flocculant produced by microorganisms that are biodegradable, harmless, and do not cause secondary pollution. These properties have made bioflocculants the subject of much wider attention and research in recent years (Okaiyeto *et al*., 2014). Scientists have recently identified several microorganisms (bacteria, fungi, and yeast) that produce bioflocculants. These bioflocculants have been characterized and found to be mostly composed of polysaccharides, proteins, glycoproteins, nucleic acids, and lipids (Ajao *et al*., 2018; Ang & Mohammad, 2020). The bioflocculant produced by *Rhodococcus erythropolis* (Guo *et al*., 2015) is predominantly protein in nature, while *Bacillus mucilaginosus* (Lian *et al*., 2008), *Proteus mirabilis* (Zhang *et al.,* 2009) and *Bacillus toyonensis* (Okaiyeto *et al*., 2015) have been shown to produce glycoprotein bioflocculants, whereas those of *Paenibacillus elgii* (Li *et al*., 2013), and *Serratia ficaria* (Gong *et al.,* 2008) are mainly polysaccharides. Some of these biomacromolecules, polysaccharide based bioflocculants draw particular attention especially with regard to wastewater treatment. They have a unique structure and some functional properties. They contain ionizable functional groups that enable them to be effective not only in removing suspended solids, heavy metals, dyes, and pathogens but also in reducing the turbidity of different types of industrial wastewater effluents (Patil *et al*., 2011). The aim of the current study is to produce bioflocculant compounds from bacteria and evaluate their efficiency in the treatment of polluted water with hydrocarbons at different concentrations, as well as choosing different speeds and times using the Jar test.

MATERIALS & METHODS

The bacteria utilized in the study

The bacteria utilized in the present study were isolated and identified in a previous study (AL Khafaji *et al*., 2023) from the Hamden wastewater treatment plant and from contaminated soil by oil in the Rumelia area north of Basrah city, Iraq. The molecular techniques using sequence analysis of 16S ribosomal DNA gene were utilized in identification of bacterial isolates.

Extraction of bioflocculant compounds

The crude bioflocculants were produced by inoculating activated bacteria into the production medium adopted from Mathias *et al*. (2017) that was made of g/L Lactose (20), K_2HPO_4 (5), KH_2PO_4 (2), NH_4Cl (2), $MgSO_4 \cdot 7H_2O$ (0.2), $(NH_4)_2SO_4$ (0.2), Urea (0.5) and NaCl (0.1), with conditions pH=7, a 3day incubation period and 5% inoculum size and incubated in a shaker incubator (150 rpm) at a temperature of 30 ºC for 3 days. After 72 h of fermentation, the culture broth was centrifuged for 15 min at 8000 rpm at 4 ◦C. Absolute methanol (2 volumes) was added to the supernatant, shaken vigorously and left at 4 °C overnight for precipitation. The supernatant was discarded and the precipitate was vacuum-dried to obtain a purified bioflocculant (Dlamini *et al*., 2019; Ngema *et al*., 2020).

Characterization of bioflocculants by FTIR analysis

The infrared spectra useful for the identification of chemical bonds and functional groups of the partially purified unknown bioflocculant were performed using a Shimadzu FTIR instrument (Chemistry Department, Science College, University of Basrah). About 1mg of dried bioflocculant was ground with 100 mg of KBr to form a powder and pressed into a thin pellet which could be measured by FTIR in the wave number range of 4000- 400 cm^{-1}**.**

Application of the produced bioflocculants in the remediation of wastewater

The wastewater samples were collected from the Al-Najibiya plant and transferred to the laboratory to perform measurements before and after the wastewater treatment by the bioflocculants from the bacterial species as a biological treatment in the laboratory. The measurements were performed before and after wastewater treatment and included the following:

Turbidity

After calibrating the instrument with standard solutions, turbidity was measured using a turbidity meter (Lovibond) and the reading was recorded in nephelometric turbidity units (NTU) according to APHA, (2005).

Total Suspended Solids

Total suspended solids were measured according to the method described in APHA (2005). A specific volume of the water sample was taken and filtrated through pre-weighted Millipore filter paper (0.45µm), then the paper was dried in an oven at 105°C for 90 minutes to remove all water. The net weight of TSS was calculated by using the following expression:

$TSS\ mg/L = (A - B) * 10000/V$

Where,

A: Weight of filter +dried residue (mg), B: Weight of filter (mg).

Total Dissolved Solid (TDS)

As for the measurement of total dissolved solids, it is done by using TDS meter.

Potential of Hydrogen Ion (pH)

pH was measured by using pH-meter.

The Wastewater remediation by the produced Bioflocculants

After the examination of wastewater, the bacterial bioflocculants were used in the treatment of wastewater. The bacterial bioflocculant containing supernatant was added into wastewater at 8%. A total volume of 800 ml was prepared in a 1 L glass beaker by mixing 712 ml of wastewater, 24 ml of $CaCl_2$ and 64 ml of bioflocculant containing supernatant. The mixing speed of slow time with 40 rpm, pH and time were determined by Experimental Design Matrix (Adnan *et al.,* 2017).

Experimental Design Matrix for Biofocculation Activity

Plackett–Burman (PB) design for screening. The PB design significantly minimizes the trial numbers required to achieve the desired outcome, lowering the resource cost in terms of labor and time. The present study explored 3 independent variables using a PB design to determine which variables significantly affected biofocculation activity. These variables were pH, speed of slow time with 40 rpm and time.

Hydrocarbons Extraction

Hydrocarbons in the water sample were extracted according to Chaillan *et al*. (2004) where 100 ml of the sample was taken and mixed with chloroform (1:1) in a separating funnel and was left to settle where the organic phase was easily separated because it was heavier than water then left to evaporate the solvent. The hydrocarbons were dissolved in 25 ml of normal hexane and passed through a chromatographic separating column containing glass wool at the bottom, silica gel (100-200 mesh), alumina (100-200 mesh), and anhydrous sodium sulfate (Na_2SO_4) respectively. The aliphatic fractions were eluted from the column with n-hexane (25 ml), while the aromatics were eluted with benzene (25 ml). The samples were then air dried and stored until detection with gas-liquid chromatography for aliphatic and aromatic hydrocarbons. The percentage of hydrocarbons removal on each sampling day was determined from the expression

(Al-Baldawi *et al.*, 2013):

Removal % $= \mathrm{TPH_{\circ}} - \mathrm{TPHt} \div \mathrm{TPH_{\circ}} \times 100$

Where,

TPH_0: the initial total petroleum hydrocarbon concentration (0day)

TPHt: total petroleum hydrocarbon concentration in every sampling day during the experiment period.

Statistical analysis

Results were statistically analyzed using SPSS software. Two-way ANOVA was performed to evaluate the differences among the different optimization parameters. $P < 0.05$ was considered statistically significant. The average values presented for emulsification activity and biomass were estimated using 3 replications and expressed as mean ± standard deviation.

RESULTS & DISCUSSION

The bacteria utilized in the study

The bacteria used in the current study and which were isolated and identified in a previous study, these isolates as *Aeromonas simiae* and *Exiguobacterium profundum.*

Characterization of bioflocculants by FTIR analysis

FTIR of purified bioflocculant produced by *Aeromonas simiae*

The infrared spectra of the partially purified bioflocculant produced by *Aeromonas simiae*, as can be seen in Fig. 1 showed structural features of bioflocculant. The band at 3213.51 cm^{-1} is attributed to free -OH stretching groups. The peaks observed at 2341.66 cm^{-1} are characteristic of C=C stretching groups and were identified as carbodiimide. The absorption band around 1662.69 cm^{-1} represent ester and carbonyl groups (C=O) and was identified as amide. The absorption bands between 1500 and 1000 cm^{-1} are due to the presence of unsaturated C–H bonds as well as C–O bonds in the bioflocculant. The peaks around 1000–1100 cm^{-1} were reported as the characteristic peaks of all sugar derivatives. The small band absorptions reported at 756.12, 570.95 and 435.93 cm^{-1} are due to the presence of (C=C) stretching groups which indicated that the bioflocculant of *Aeromonas simiae* was a protein-bound polysaccharide. The FT-IR spectrum confirmed the presence of functional groups, which are groups of atoms within a molecule that determine the chemical properties and characteristics of chemical reactions. We inferred that the bioflocculant produced by *Aeromonas simiae* may be a type of glycoprotein (Xiong *et al.,* 2010; Tremblay *et al.,* 2011; Huang *et al.,* 2019).

FTIR of purified bioflocculant produced by *Exiguobacterium profundum*

The infrared spectra of partially purified bioflocculant produced by *Exiguobacterium profundum,* as can be seen in Fig. 2 showed structural features of bioflocculant. The band at 3132.50 cm^{-1} is attributed to free (-OH) and (–NH) stretching groups. The peaks observed at 2341.66 cm^{-1} characteristic of C=C stretching groups were identified as carbodiimide. The absorption band around 1658.84 cm^{-1} representing ester and carbonyl groups (C=O) was identified as amide. The absorption bands between 1500 and 1000 cm^{-1} are due to the presence of unsaturated C–H bonds as well as C–O bonds in the bioflocculant. The peaks around 1000–1100 cm^{-1} were reported as the characteristic peaks of all sugar derivatives. The absorption bands at 871.85 cm^{-1} can be associated with β-glycosidic linkage between sugar derivatives. The small band absorptions reported at 759.98, 698.25, 570.95 and 435.93 cm^{-1} are due to the presence of (C=C) stretching groups which indicated that the bioflocculant of *Exiguobacterium profundum* bacterium was a protein-bound polysaccharide. We inferred that the bioflocculant produced by *Exiguobacterium profundum* may be a type of glycoprotein (Xiong *et al.,* 2010; Tremblay *et al.,* 2011; Huang *et al.,* 2019).

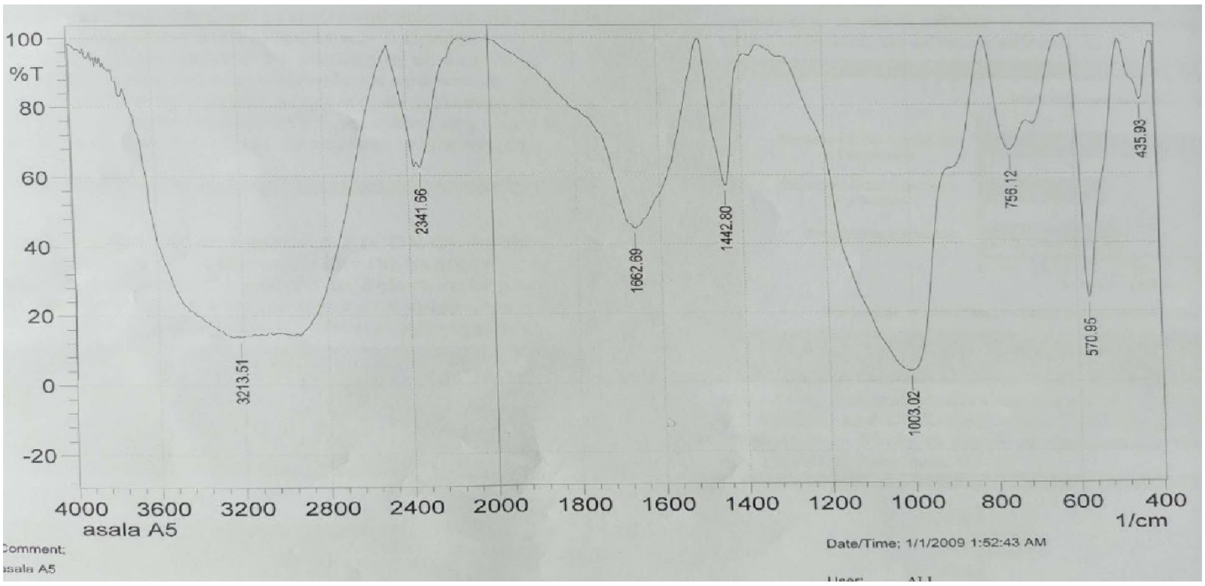

Fig. 1. FTIR spectrum of bioflocculant produced by *Aeromonas simiae*

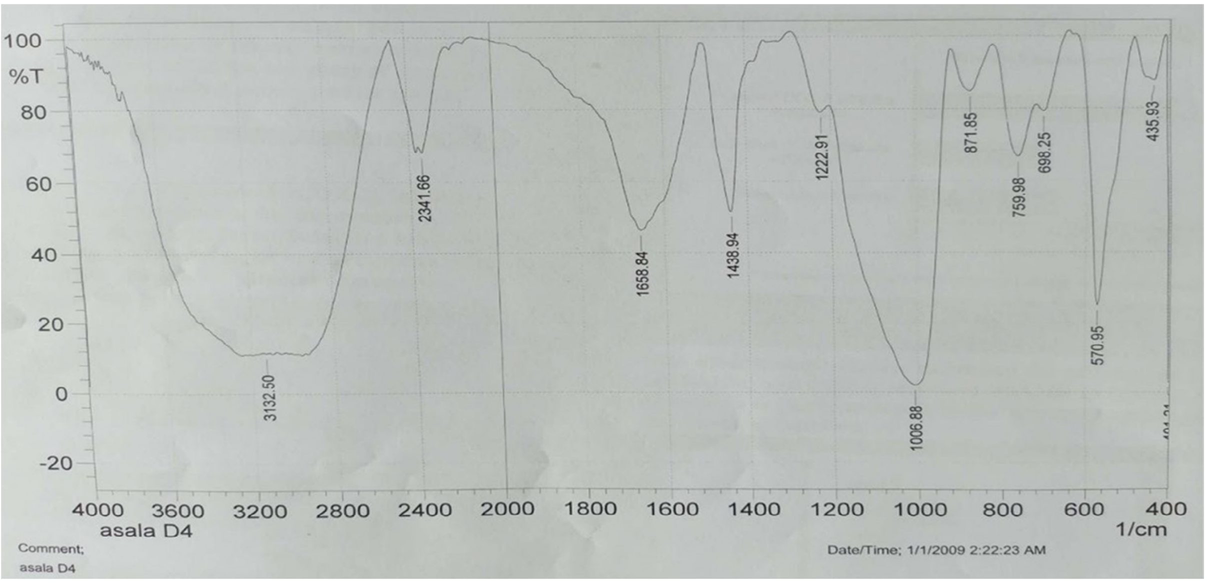

Fig. 2. FTIR spectrum of bioflocculant produced by *Exiguobacterium profundum*

Application of bioflocculants efficiency for remediating industrial water

The physical and chemical characteristics of the polluted water from Al-Najibiya Power Plant were measured to remove pollutants so that it could be released into the environment with the least possible damage, in addition to its importance in various uses. The measurements included turbidity, total suspended solids, total dissolved materials, and total petroleum hydrocarbons. Some characteristics of the polluted water collected from AL-Najibiya Power Plant were measured, such as turbidity, dissolved solids, and suspended solids (Table 1) as well as hydrocarbon compounds before treatment.

1. *Aeromonas simiae*

The bioflocculant compounds extracted from *Aeromonas simiae* isolate were applied to

Table 1. The characteristics of contaminated water from AL-Najibiya Power Plant

Wastewater characteristics	Values
Turbidity	235 (NTU)
TSS	60 (mg/L)
TDS	989 (PPM)
TPH	325.490 (mg/L)

Table 2. The efficiency of bioflocculant produce from *Aeromonas simiae* to remove turbidity, TDS and TSS from wastewater of AL–Najibiya Power Plant

No.	pH	Slow time with 40 rpm (min)	Dose (ml)	Turbidity removal %	TDS removal %	TSS removal %
1	9	4	8	60%	39%	33%
2	5	4	8	51%	26%	46%
3	7	7	8	40%	16%	57%
4	7	3	8	43%	24%	22%
5	7	11	8	35%	40%	37%
6	5	10	8	76%	7.69 %	21%
7	10	7	8	83%	36%	25%
8	4	7	8	55%	14%	28%
9	9	10	8	88%	43%	33%

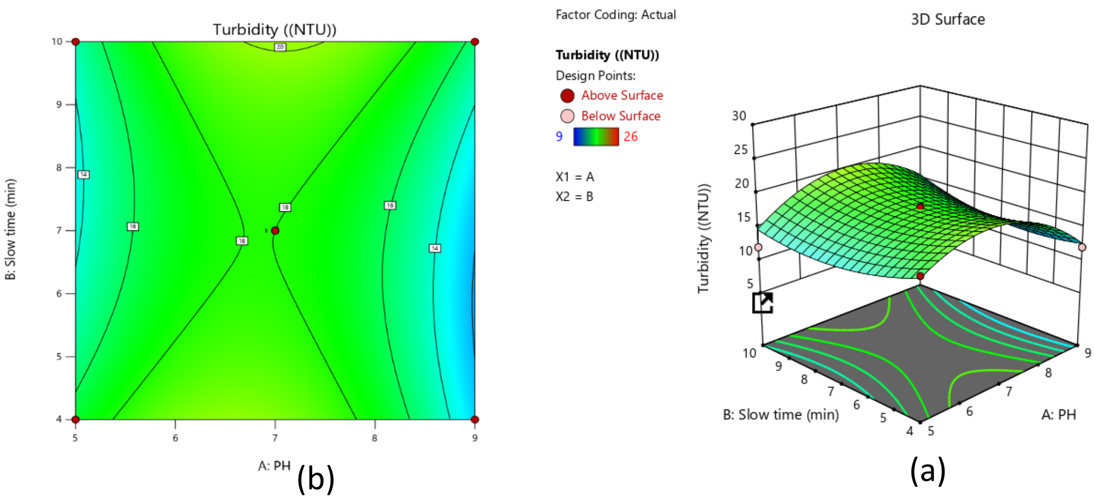

Fig. 3. (a) 3D plot and **(b)** a contour plot diagram showing the relationship of pH and slow time in the removal of turbidity from polluted water using bioflocculant from *Aeromonas simiae*

determine the effectiveness of these compounds in removing the pollutants present in the sample water through test ideal conditions such as the effect of pH as well as slow time with 40 rpm and measurement of these characteristics after treatment by bioflocculants.

The effect of pH and slow time with 40 rpm *on turbidity removal*

The maximum turbidity removal was 88% at a pH of 9 and a dose of 8 ml for 10 min slow time with 40 rpm as shown in Table 2. While the lowest removal rate was 35% at pH 7 and a dose of 8 ml for an 11 min slow time with 40 rpm. The effect of pH on removal efficiency as exhibited in Fig. 3. The result showed the best removal efficiency at pH (9,10), therefore we can conclude that turbidity removal increased with increasing pH because a higher pH tends to promote sedimentation fundamentally (Mohammed, 2015). The pH is an important factor that should be considered when using bioflocculants for wastewater treatment (Pu *et al.,* 2020).

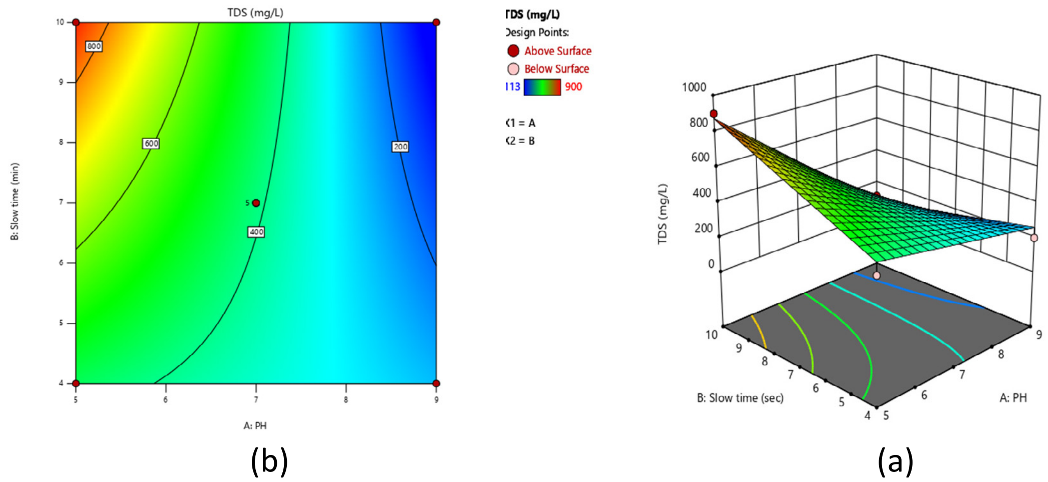

Fig. 4. (a)3D plot and **(b)** a contour plot diagram showing the relationship of pH and slow time in the removal of TDS from polluted water using bioflocculants of *Aeromonas simiae*

The statistical analysis showed that there were statistically significant differences $P \leq 0.05$. The current study concluded, as mentioned by Buthelezi *et al*. (2009) and Siddharth *et al*. (2021) that the bioflocculant activity for drinking water treatment by EPS synthesized from *Bacillus licheniformis* aided with $CaCl_2$ showed good performance on the removal of turbidity and COD, achieving maximum removal efficiencies of 95.6% and 61.2% respectively.

The contour plot and 3D surface plot drawings as shown in Fig. 3 demonstrate the relationship between the pH and slow time with 40 rpm in turbidity removal, as the removal increases within the range of pH (9-10) and slow time with 40 rpm (7–10) min to achieve removal of more than 83 % and the removal percentage decrease to (35%-40%) at pH 7.

The effect of pH and Slow time with 40 rpm on TDS removal

The maximum TDS removal was 43% at pH 9 and a dose of 8 ml for 10 min slow time with 40 rpm, while the lowest removal rate was 7.69 % at pH 7 and a dose of 8 ml for 10 min slow time with 40 rpm (Table 2). The result obtained indicated that the percentage of removal of TDS is low due to the electrical charges carried by the bioflocculants and dissolved particles. The mechanism of bioflocculants for the removal or aggregation of total dissolved solids is attributed to alter or destabilize negatively charged dissolved particles. The positively charged bioflocculants adsorb on the surface of negatively charged particles by neutralizing their charge (Daud *et al.,* 2013). The statistical analysis showed that there were statistically significant differences $P \leq 0.05$.

The contour plot and 3D surface plots drawings (Fig. 4) demonstrated the relationship between the pH and slow time with 40 rpm in TDS removal. The removal of TDS increases within the range of pH (9-7) and slow time with 40 rpm (4–11) min to achieve removal more than 40 %. The removal percentage decreases to (7.67%–16%) at pH 7.

The effect of pH and Slow time with 40 rpm on TSS removal

The maximum TSS removal was 57% at a pH 7 and a dose 8 ml for 7 min slow time with 40 rpm, while the lowest removal rate was 21% at pH 5 and a dose 8 ml for 10 min slow time with 40 rpm (Table 2). The decrease in adsorption capacity at low pH values would be expected because the acidic medium would lead to an increase in hydrogen ion concentration, which would then neutralize the negatively charged bioflocculant surface, reducing the adsorption of positively charged ions due to a reduction in the force of attraction between adsorbent and

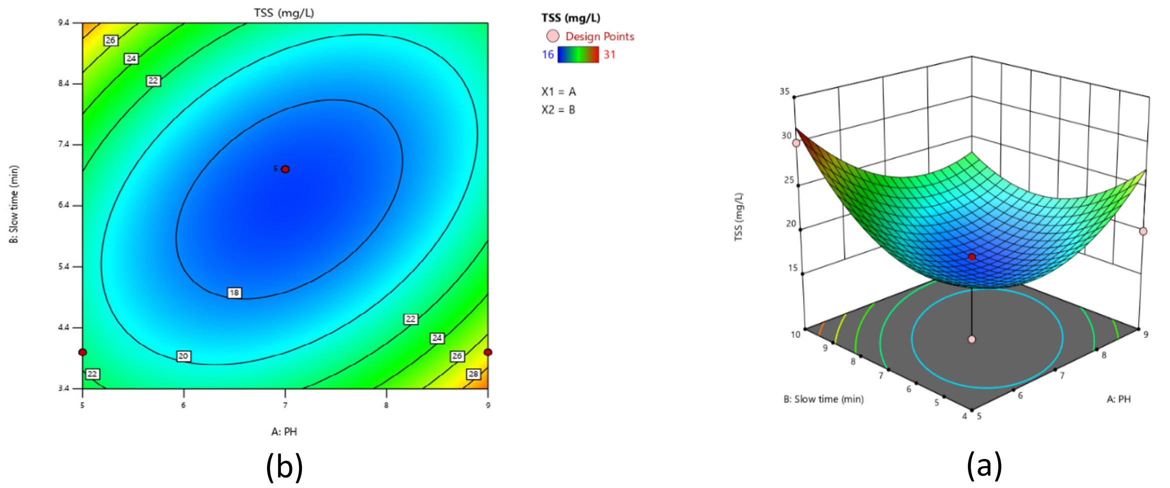

Fig. 5. (a) 3D plot and **(b)** a contour plot diagram showing the relationship of pH and slow time in the removal of TSS from polluted water using bioflocculant from *Aeromonas simiae*

Table 3. The efficiency of biofloculant produce from *Aeromonas simiae* to remove TPHs from wastewater of AL–Najibiya Power Plant

No.	pH	Slow time with 40 rpm (min)	Dose (ml)	TPHs	Removal %
1	9	4	8	260.524	19.95%
2	5	4	8	45.408	86.04%
3	7	7	8	143.859	55.80%
4	7	3	8	179.766	44.70%
5	7	11	8	122.896	62.24%
6	5	10	8	188.678	42.03%
7	10	7	8	200.798	38.30%
8	4	7	8	166.392	48..81 %
9	9	10	8	250.146	23.14%

adsorbate (Desta & Bote, 2021). The statistical analysis showed that there were statistically significant differences $P \leq 0.05$. According to a study by Lian *et al.* (2008) microbial flocculant synthesized from *Bacillus mucilaginosus* was used to treat domestic municipal wastewater, with a removal efficiency of 93.3% for suspended solids.

The contour plot and 3D surface plots drawings (Fig. 5) demonstrate the relationship between the pH and slow time with 40 rpm in TSS removal. The removal of TSS increases within the range of pH (7) and slow time with 40 rpm (4–7) min to achieve removal more than 50 %. The removal percentage decreases to (21%–35%) at pH (5-9).

The effect of pH and slow time with 40 rpm on Total petroleum hydrocarbons removal

The maximum total petroleum hydrocarbons removal using bioflocculants of *Aeromonas simiae* bacteria was 86.04 % at a pH 5 and a dose 8 ml for 4 min slow time with 40 rpm while the lowest removal rate was 19.95% at pH of 9 and a dose of 8 ml for 4 min of slow time with 40 rpm (Table 3). The statistical analysis showed that there were statistically significant differences $P \leq 0.05$.

The contour plot and 3D surface plots drawings (Fig. 6) demonstrate the relationship between the pH and slow time with 40 rpm in TPHs removal, as the removal increases within the range of pH (5-7) and slow time with 40 rpm (4-11) min to achieve removal more than 50 %. The removal percentage decreases to (19.95%-23.14%) at pH 9.

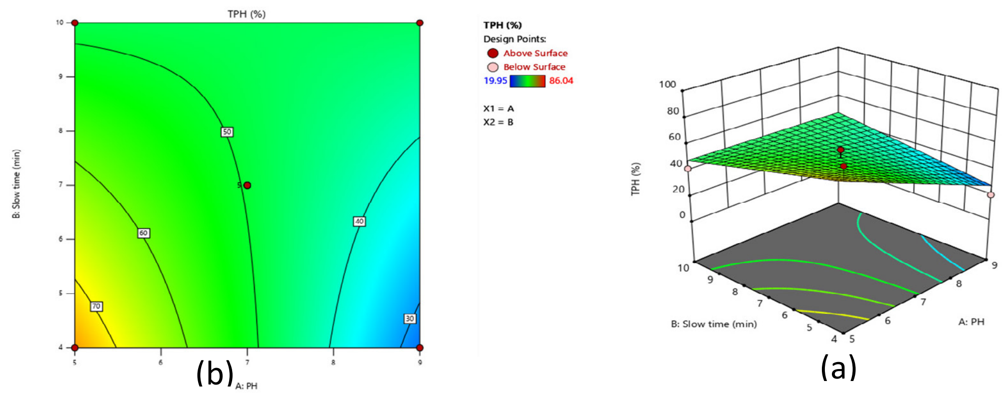

Fig. 6. (a) 3D plot and **(b)** a contour plot diagram showing the relationship of pH and slow time in the removal of TAH from polluted water using bioflocculant from *Aeromonas simiae*

Table 4. The characteristics of contaminated water from AL Najibiya power Plant

Wastewater characteristics	Unit
Turbidity	226 (NTU)
TSS	640 (mg/L)
TDS	5520 (PPM)
TPHs	6867.713 (mg/L)

Table 5. The efficiency of bioflocculant produce by *Exiguobacterium profundum* to remove turbidity, TDS and TSS from water AL–Najibiya Power Plant

No.	pH	Slow time with 40 rpm (min)	Dose (ml)	Turbidity removal %	TDS removal %	TSS removal %
1	9	4	8	88%	16%	82%
2	5	4	8	61%	6.2%	66%
3	7	7	8	81%	52%	63%
4	7	3	8	20%	31%	37%
5	7	11	8	42%	27%	58%
6	5	10	8	40%	18%	56%
7	10	7	8	85%	23%	54%
8	4	7	8	85%	37%	52%
9	9	10	8	81%	28%	80%

2- *Exiguobacterium profundum*

The bioflocculant compounds extracted from *Exiguobacterium profundum* isolate were applied to determine the effectiveness of these compounds in removing the pollutants present in the sample water through testing ideal conditions such as the effect of pH as well as slow time and measurement of these characteristics after treatment by bioflocculants. Some characteristics of the polluted water collected from the AL-Najibiya Power Plant were measured, such as turbidity, dissolved solids, and suspended solids (Table 4) as well as hydrocarbon compounds before treatment.

The effect of pH and Slow time with 40 rpm on Turbidity removal

The maximum turbidity removal was 88% at pH 9 and dose at 8 ml in 4 min slow time with 40 rpm, while the lowest removal rate was 20% at pH 7 and dose at 8 ml for 3 min slow time with 40 rpm. The best removal efficiency, (Table 5) was reported at pH (9,10). The pH change has no influence on the effectiveness of biopolymers, it may have an impact on the characteristics of wastewater, which could indicate a reduction in flocculation efficiency at specific pH levels (Agarwal *et al.,* 2001). The result is consistent with a study showing turbidity removal occurring at a higher pH 6.5 (Kim *et al.,* 2001; Mensah-Akutteh *et al.,* 2022). The statistical analysis showed that there were statistically significant differences $P \leq 0.05$.

The contour plot and 3D surface plots drawings (Fig. 7) demonstrate the relationship between the pH and slow time with 40 rpm in turbidity removal, as the removal increases within the range of pH (9-10) and slow time (7–11) min to achieve removal more than 85 %. The removal percentage decreases to (20%-40%) at pH (5-7).

The effect of pH and slow time with 40 rpm on TDS removal

The maximum TDS removal was 52% at pH 7 and dose of 8 ml for 7 min of slow time with 40 rpm, while the lowest removal rate at 16% at pH 9 and dose at 8 ml fore 4 min slow time with 40 rpm (Table 5). The result obtained, which indicated that the percentage of TDS removal is low, is due to the electrical charges carried by the bioflocculants and dissolved particles. The mechanism of bioflocculants for the removal or aggregation of total dissolved solids is attributed to alter or destabilize negatively charged dissolved particles. The positively charged bioflocculants adsorb on the surface of negatively charged particles by neutralizing their charge (Daud *et al.,* 2013). The statistical analysis showed that were statistically significant differences $P \leq 0.05$.

The contour plot and 3D surface plots drawings (Fig. 8) demonstrate the relationship between the pH and slow time in TDS removal, as the removal increases within the range of pH (7) and slow time with 40 rpm (7) min to achieve removal more than 35 %. The removal percentage decreases to (16%- 18%) at pH (5-9).

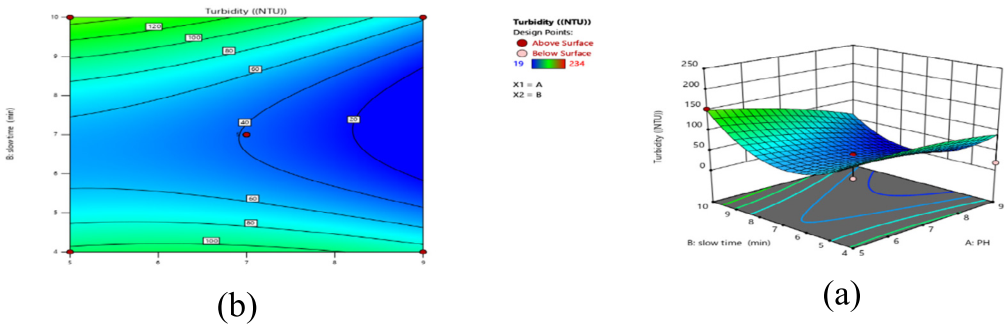

Fig. 7. (a) 3D plot and (b) a contour plot diagram showing the relationship of pH and slow time in the removal of turbidity from polluted water by using bioflocculant from *Exiguobacterium profundum*

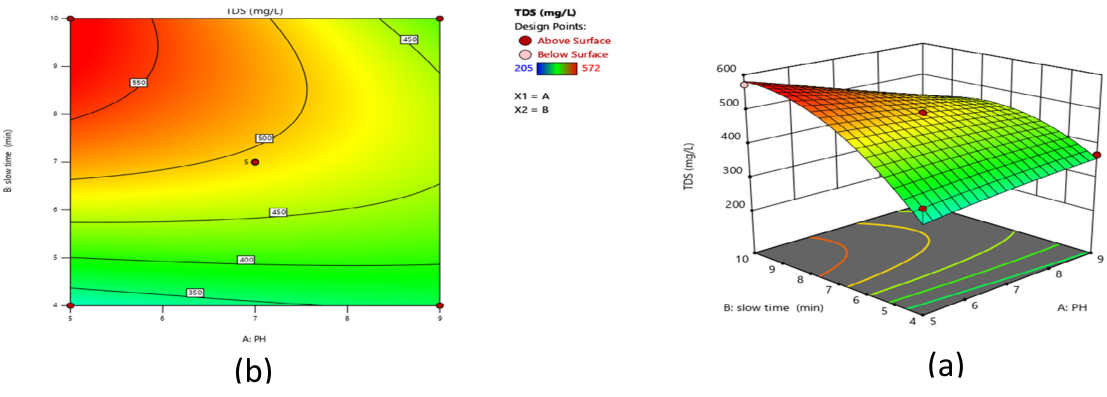

Fig. 8. (a) 3D plot and **(b)** a contour plot diagram showing the relationship of pH and slow time in the removal of TDS from polluted water using bioflocculant from *Exiguobacterium profundum*

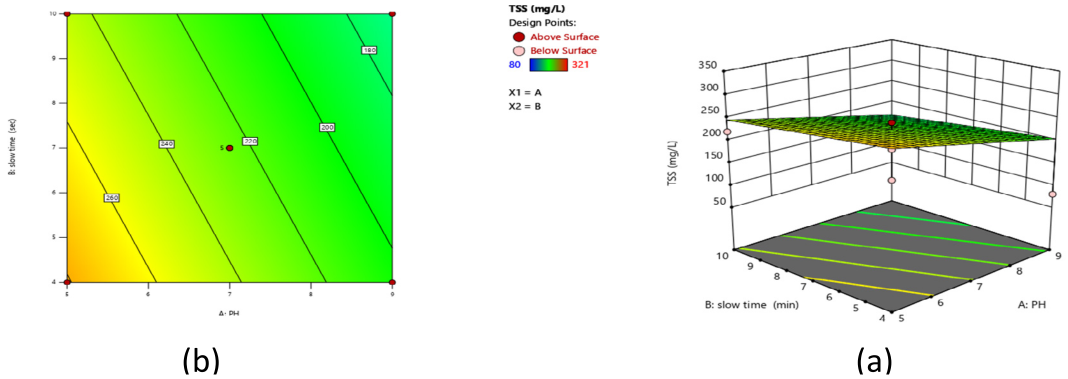

Fig. 9. (a) 3D plot and **(b)** a contour plot diagram showing the relationship of pH and slow time in the removal of TSS from polluted water using bioflocculant from *Exiguobacterium profundum*

Table 6. The efficiency of bioflocculant produce from *Exiguobacterium profundum* to remove TPHs from water AL–Najibiya Power Plant

No.	pH	Slow time with 40 rpm (min)	Dose (ml)	TPHs	Removal %
1	9	4	8	591.21	91.39%
2	5	4	8	395.62	94.23%
3	7	7	8	695.48	89.87%
4	7	3	8	1100.47	83.97%
5	7	11	8	2994.64	56.39%
6	5	10	8	1212.437	82.34%
7	10	7	8	2550.60	62.86%
8	4	7	8	727.505	89.40%
9	9	10	8	977.74	85.76%

The effect of pH and slow time with 40 rpm on TSS removal

The maximum TSS removal was 82% in pH 9 and a dose at 8 ml for 4 min slow time with 40 rpm while the lowest removal rate was 37% in pH 7 and dose at 8 ml in 3 min slow time with 40 rpm (Table 5). The result obtained indicated that the percentage of TSS removal is high due to the use of bioflocculants from *Exiguobacterium profundum.* This is due to a combination of factors, including increased charge density, increased bridging capacity, increased solubility and the specific type of bioflocculant. The composition of the wastewater can also affect the removal of suspended solids (Joshi *et al.*, 2019). The statistical analysis showed that were statistically significant differences at $P \leq 0.05$.

The contour plot and 3D surface plots (Fig. 9) demonstrate the relationship between pH and slow time with 40 rpm in TSS removal, as the removal increases within the range of pH (9) and slow time with 40 rpm (4-10) min to achieve removal of more than 80 %. The removal percentage decreases to (37%-52%) at pH (7-4).

The effect of pH and slow time on Total petroleum hydrocarbons removal

The maximum total petroleum hydrocarbons removal using bioflocculants of *Exiguobacterium profundum* bacteria was 94.23% in pH 5 and dose at 8 ml for 4 min slow time with 40 rpm while the lowest removal rate was 56.39% in pH 7 and dose at 8 ml for 11 min slow time with 40 rpm (Table 6). Total petroleum hydrocarbons before and after processing are compared to determine process ability. The fundamental reason why thrombosis therapy was so successful in lowering the total petroleum hydrocarbons content was that the hydrocarbons that were already present were linked to the particle component that was eliminated throughout the procedure. The statistical analysis showed that there were statistically significant differences $P \leq 0.05$.

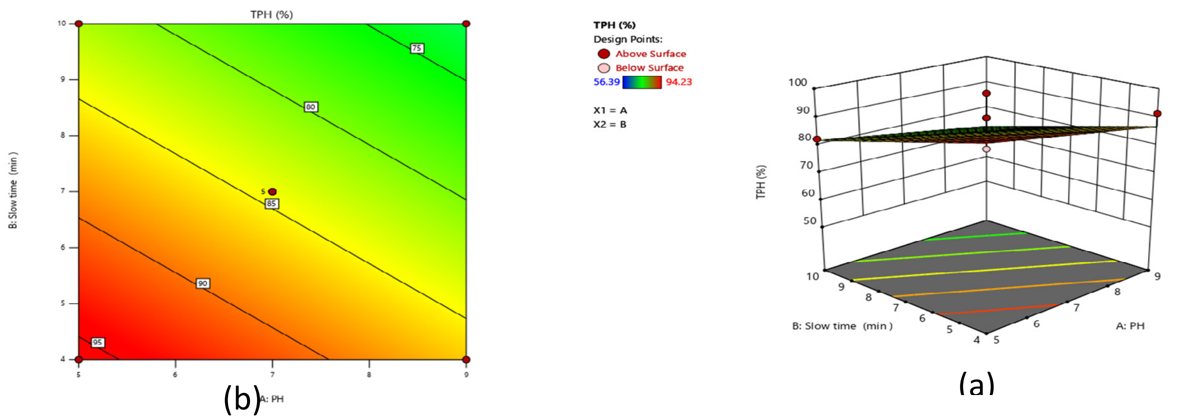

Fig. 10. (a) 3D plot and **(b)** a contour plot diagram showing the relationship of pH and slow time in the removal of TAH from polluted water using bioflocculant of *Exiguobacterium profundum*

The contour plot and 3D surface plots (Fig. 10) demonstrate the relationship between pH and slow time with 40 rpm in TPHs removal, as the removal increases within the range of pH (5-9) and slow time with 40 rpm (4-10) min to achieve removal more than 90 %. The removal percentage decreases to (56.39%-62.86%) at pH (7).

CONCLUSIONS

Aeromonas simiae and *Exiguobacterium profundum* gave the best results when applied with wastewater from the AL- Najibiya plant, especially for Total Petroleum Hydrocarbons (TPHs) with a removal efficiency of about 80% and 90% where the best medium for removal is acidic to neutral pH (5-7) for both bacteria. *Exiguobacterium profundum* was a highly effective bacterium in removing total hydrocarbon compounds from the wastewater of the AL- Najibiya plant reaching 94.23%.

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The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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