Marina Yusoff · Nor Hayati Abdul Hamid
Mohd Fadzil Arshad · Ahmad Kamil Arshad
Ahmad Ruslan Mohd Ridzuan · Haryati Awang
Editors

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Part I
Bioremediation and Engineering

Removal of Hexavalent Chromium by Magnetite in Groundwater

Nurul Aqilah Abdul, Jalina Kassim and Amnorzahira Amir

Abstract Hexavalent chromium (Cr^{6+}) is a toxic contaminant that contaminates soil and groundwater and is listed as a priority contaminant. This study investigates removal of Cr^{6+} by magnetite (Fe_3O_4) in groundwater. The removal of Cr^{+6} is significantly dependent on the amount of reactive surface area on the surface of Fe_3O_4. Approximately 20 % of Cr^{+6} was removed by Fe_3O_4 in 20 min of reaction time. The removal of Cr^{+6} increases by 2 times as the concentration of Fe_3O_4 increases from 0.1 to 0.5 g. Results from this study provide a basic understanding of Cr^{6+} by Fe_3O_4 and can be suggested to be implemented at the real site contaminated with Cr^{+6}.

Keywords Cr^{6+} · Magnetite · Iron bearing soil mineral · Removal efficiency

1 Introduction

Chromium is listed by the USEPA as the top priority contaminant [1] which usually originates from anthropogenic sources. The wide spread of chromium is due to its intensive use in industries such as leather tanning, wood preservative, electroplating, and metal finishing [1–4]. Chromium is released from these industries through effluents [5] directly to the environment and thus contaminates the water sources, especially the groundwater environment.

In the environment, chromium exists in several oxidation states, e.g., Cr^{+0}, Cr^{+2}, Cr^{+3}, Cr^{+6} [6].

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However, it predominantly exists as trivalent chromium Cr^{+3} and hexavalent chromium Cr^{+6} [2]. Each of this speciation metal has its own characteristics such as bioavailability, toxicity, transport characteristic, and solubility in the natural environment [7]. Hexavalent chromium, (Cr^{+6}), is highly toxic and mobile in the environment [3, 5, 6, 8] and can cause skin irritation, liver damage, edema, and others [1]. Due to this characteristic, even a small amount of Cr^{+6} released into the environment can accumulate and become harmful for life.

Trivalent chromium (Cr^{+3}) is a trace element which is important in living organisms. Lack of chromium in the human body may have several impacts such as impaired glucose tolerance and glycosuria, while in animals it causes impaired growth and decrease in longevity.

Moreover, Cr^{+3} is less toxic and immobilized in the environment and can become a nutrient to the environment at a certain concentration [5, 6]. Both cannot be degraded once they are released to the environment. Hence, it is important to reduce Cr^{+6} to Cr^{+3} in the soil and the groundwater system [1].

In the natural environment, the toxic metal can be reduced to nontoxic encouraged by microorganisms [9] and also by abiotic natural reductants. The reductant serves as an electron donor which reduces Cr^{+6}. In the anoxic condition, Cr^{+6} attenuation can be obtained by dissolved Fe (II), FeS_2, FeS, and other reduction species [10]. From the previous research, it has been proved that Cr^{+6} in the groundwater system can be reduced to Cr^{+3} by natural organic matter such as fulvic acid and humic acid [8]. Besides, it has also been shown that toxic Cr^{+6} can be reduced by iron bearing soil mineral, magnetite, pyrite, mackinawite, and green rust [2, 7, 8]. Hence, in order to develop remedial technologies, the redox chemistry to reduce Cr^{+6} to Cr^{3+} needs to be applied to treat the contamination of soil and groundwater.

Previous research has shown that ferrous iron is important to reduce Cr^{+6} in the environment as the amount of ferrous iron is abundant in suboxic and anoxic. One of the sources of ferrous iron is magnetite, Fe_3O_4 [7], which has the ability to reduce Cr^{+6} to Cr^{+3}. Fe_3O_4 is one of the iron bearing minerals in the geosphere which has received much attention as a natural reductant for Cr^{+6} [7]. In soil, ferrous iron Fe (II) accumulates as iron oxide mineral, Fe_3O_4.

The reduction of the contaminant in the environment usually occurs through surface reaction [6, 11]. In soil and groundwater, the fate of Cr^{+6} is influenced by complexation and redox reaction. Reductive degradation occurs on the surface of Fe_3O_4 which is influenced by the density of reactive site. Cr^{+6} absorbed to the Fe_3O_4 surface will interact with each other by accepting the electron from ferrous iron which is available at the Fe_3O_4 surface.

Thus the redox reaction between ferrous iron and the Cr^{+6} was important to remediate the soil and groundwater system [6].

The aim of this study is to provide the fundamental knowledge to understand the reduction of Cr^{+6} by Fe_3O_4. We investigate the removal of Cr^{+6} by Fe_3O_4 and the effect of concentration of Fe_3O_4 on the removal of Cr^{+6} by Fe_3O_4.

2 Materials and Methods

2.1 Chemicals and Reagents

The chemicals utilized in this research were potassium dichromate ($K_2Cr_2O_7$) (Merck), Magnetite (Fe_3O_4) (99 %, Sigma Aldrich), Biological buffer Trizma, 2-Amino-2-(hydroxymethyl)-1, 3-propanediol (99 %, Sigma Aldrich) in order to maintain the pH solution and hydrochloric acid, HCl (98 %, ChemAR) to adjust the buffer to the desired pH. The following chemicals were used for colorimetric method: 1,5 diphenylcarbazide, H_2SO_4, acetone (99 %, Merck), anhydrous Na_2CO_3, and NaOH pallet (99 % Merck). Deaerated deionized water (DDW) was prepared using ultrapure water (18 Ω cm) purged by N_2. Anaerobic chamber was maintained purged with 95 % N_2 and 5 % H_2. All reagents and solutions used in the experiments were prepared using DDW.

2.2 Batch Experiment

Batch experiments were conducted to determine removal of Cr^{6+} by Fe_3O_4. To investigate the effectiveness of Cr^{6+} removal by Fe_3O_4, experiments were conducted in 40 mL amber glass vials. TRIS buffer solution (50 mM) was prepared using TRIS sodium salt and DDW. pH of buffer solution was adjusted to the desired pH using 0.1 M HCl acid. The exact amount of Fe_3O_4 0.10 g was weighted and transferred to each vial and TRIS buffer solution (50 mM) was poured into each vial without headspace to keep the pH suspension constant at pH 7.2. Cr^{6+} with a concentration of 1.0 mg/L was then introduced into each vial to initiate removal reaction between Fe_3O_4 and Cr^{6+}. Vials were then rapidly capped, mounted on a tumble mixer, and rotated at 7 rpm at room temperature (25 ± 0.5 °C) for 30 min.

The removal of Cr^{6+} was determined by measuring aqueous concentration of Cr^{6+} in the Fe_3O_4 suspension at each sampling time. At sampling time, samples were centrifuged for 5 min at 5000 rpm, and then aliquots of aqueous solution were collected to measure the Cr^{6+} concentration. Concentration of Cr^{6+} was determined by HACH Spectrophotometer DR5000, Method 8023. Samples and controls were prepared in duplicate. Samples were prepared by following the same procedures as the batch test described above.

To investigate the effect of the contact time by Fe_3O_4, various times (2, 5, 10, 15, 20, 30 and 45 min) were set up. 2.5 g/l of Fe_3O_4 concentration was used and the initial concentration of the Cr^{+6} was constant at 1 mg/l at pH 7.

To study the effects of the Fe_3O_4 concentrations on the removal of Cr^{+6} different concentrations of Fe_3O_4 (0.10, 0.20, 0.30 and 0.50 g) were weighted and transferred into vials. Initial concentration of Cr^{6+} was set at 1.0 mg/L at pH 7.2.

2.3 Analytical Procedure

Chromium, Cr^{6+} Concentration

Concentration of Cr^{6+} was determined using HACH Spectrometer DR5000, following Method 8023. Measurement wavelength of Cr^{6+} is 540 nm. The optimum detection range for this method was 0.010–0.7 mg/L. Aliquots of aqueous solution collected from samples and controls were transferred to 10 ml sample cell and ChromaVer® 3 Reagent Powder Pillow was added for Cr^{6+} determination.

3 Results and Discussion

3.1 Effect of Contact Time on Removal of Cr^{+6} by Fe_3O_4

Figure 1 shows the effect of contact time on the removal of Cr^{+6} by Fe_3O_4 at pH 7 by varying the contact times (2, 5, 10, 15, 20, 30 and 45 min) while the other parameters were constant. The result shows the removal of Cr^{+6} increase as the contact time was increased before reaching the equilibrium state and after reaching the equilibrium state the removal was constant [12]. Approximately 20 % of Cr^{+6} was removed by Fe_3O_4 in 20 min. This indicates that at the initial time, high density of reactive site was available on the surface of Fe_3O_4 for removal of Cr^{+6}.

However, no significant removal of Cr^{+6} by Fe_3O_4 was observed at the end of the contact time. These results show that the reactive site of Fe_3O_4 becomes saturated and is not able to remove Cr^{+6}. The reactive site was exhausted caused by the repulsive force among bulk phase and solute molecules of solid [12].

This finding suggests that reactive chemical species (e.g., Fe^{2+}) on the surface of Fe_3O_4 strongly controls the kinetic removal of Cr^{+6} in this system.

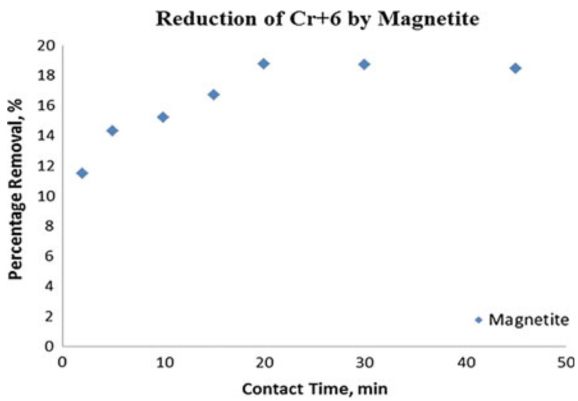

Fig. 1 The effect of contact time on the removal of Cr^{+6}. Magnetite concentration = 0.1 g, initial concentration of Cr^{+6} = 1 mg/L, pH = 7, agitation speed: 7 rpm

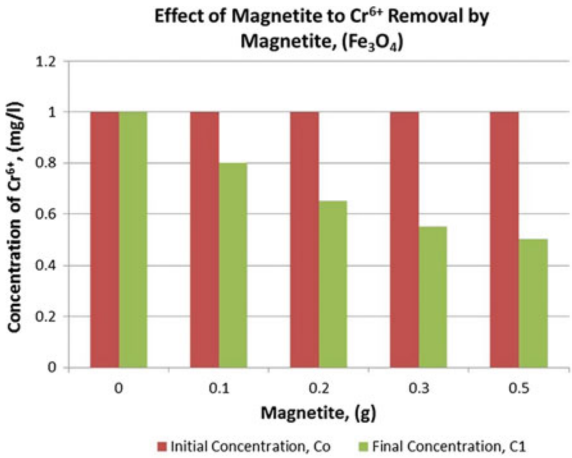

Fig. 2 The effect of magnetite dosages on the removal of Cr^{+6}. Initial concentration of Cr^{+6} = 1 mg/L, pH = 7, agitation speed = 7 rpm

3.2 Effect of Magnetite Concentration on Removal of Cr^{+6} by Fe_3O_4

Figure 2 shows the effect of magnetite removal of concentration on Cr^{+6} by Fe_3O_4.

Experimental results show that the removal percentage of Cr^{+6} increases with increasing Fe_3O_4 concentration. High kinetic removal of Cr^{+6} by Fe_3O_4 was observed with the increasing concentration of Fe_3O_4 (0.1, 0.2, 0.3 and 0.5 g) at pH 7.

The removal of Cr^{+6} increases by 2 times as the concentration of Fe_3O_4 increases. This suggests that the active site available increases as the concentration of Fe_3O_4 increases from 0.1 to 0.5 g.

This result is consistent with previous studies of Cr^{6+} removal using nano zerovalent iron (nZVI). Yu et al., Liu et al. and Fu et al. report that efficiency of Cr^{6+} removal significantly increased with the increased in nZVI loading [13–15].

4 Conclusion

From this study the use of Fe_3O_4 as the absorbent for Cr^{+6} removal from soil and groundwater system was investigated. With respect to the biogeochemical condition, the removal of Cr^{+6} increased with the increase of contact time and magnetite concentration. Increasing the amount of magnetite concentration provides more reactive surface area and thus the removal of Cr^{+6} will continuously increase until it becomes saturated. It is proven that the reactive surface area on the surface of Fe_3O_4 plays a significant role to control kinetic removal of Cr^{+6}. Experimental results from this study can be used as a reference to identify potential reaction mechanisms that may involve during removal of Cr^{+6} by Fe_3O_4 in groundwater. This method can be suggested to be implemented at the real site contaminated with Cr^{+6}.

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Screening of Medium with Different Range of Waste Frying Oil (WFO), Sodium Nitrate ($NaNO_3$) and Sodium Chloride (NaCl) for Biosurfactant Production by Thermophilic *Anoxybacillus* sp. Using Fractional Factorial Design (FFD)

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Abstract In this study, culture medium was optimized for economic production of biosurfactant by *Anoxybacillus* sp. using different waste frying oil, sodium nitrate, and sodium chloride concentrations. Screening step was performed using the Design-Expert software (2 level full factorial design). The response variables are of value for surface tension reduction in the cell-free-culture medium as it indicates the biosurfactant production. The yield of biosurfactant was found to be the highest when surface tension was at the lowest value (42.30 mN/m) at a temperature of 55 °C, agitation 130 rpm, 9 % (v/v) waste frying oil (WFO), 0.5 % (w/v) sodium nitrate ($NaNO_3$), and 0.02 % (w/v) of sodium chloride (NaCl). The biosurfactant was observed to stable in the face of exposure to extreme temperature changes, pH conditions, and salinity. These physiochemical properties demonstrate the potential for using waste frying oil as an inexpensive material for biosurfactant production.

Keywords Biosurfactant · Surface tension · Waste frying oil · *Anoxybacillus* sp. · Full factorial design · MEOR

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1 Introduction

Due to economic concerns, environmental issues, and restrictive law, the demand for biologically produced chemicals is steadily increasing. Microbial surfactant or commercially known as biosurfactant are biomolecules that are synthesized by a variety of microorganisms. Biosurfactants are amphiphilic molecules that have two domains, hydrophobic and hydrophilic [1]. The accumulations of these molecules at the interface induce the formation of micelles which can lead to the reduction of surface and interfacial tensions. This enhances the solubility and mobility of the insoluble or hydrophobic compounds [3, 22]. Biosurfactants are usually synthesized under specific growth conditions either on water miscible or oily substrate [5, 22]. Biosurfactants have huge potential to replace synthetic (chemically-produced) surfactants that are currently used, which will cause bad side effects with long-term use. Unlike most synthetic surfactants, many biosurfactants function effectively at extremes of temperature, salinity, wide range of pH, low toxicity, better foaming (useful in mineral processing), and environmental friendly nature [20]. For these reasons, biosurfactants have gained importance in various commercial applications in biological industries, food processing, pharmaceuticals, biomedical, cosmetics, and agricultural industries. Moreover, they are also suited for petrochemical and environmental application such as bioremediation of polluted sites, oil spill management, and enhanced oil recovery [2].

Pakpitcharoena et al. [14] claim that thermophilic *Anoxybacillus* sp. is a biosurfactant producer, but studies of biosurfactant production using this genus are scarce. In addition, there are no reports on the production of biosurfactant by *Anoxybacillus* sp. using waste frying oil. The uses of thermophilic organisms for biotechnological processes are of great importance as their biochemical pathway can adapt easily to industrial conditions, especially at high temperatures. Most of them are nonpathogenic with high secretion capacity. The genus *Anoxybacillus* belongs to the order *Bacillales* under the *Firmicutes* phylum in the domain bacteria. The first strict anaerobic *Anoxybacillus* sp., *Anoxybacillus pushchinensis*, was isolated from manure [16]. In addition to *A. contaminans* [9], which was isolated from contaminated gelatine from a manufacturing plant, other newly described species originated from various geothermal sites around the globe. Examples of these species include *A. flavithermus*, *A. gonensis*, *A. ayderensis*, *A. kestanbolensis* and *A. amylolyticus* [18]. Recently, *Anoxybacillus salavatliensis* was isolated from a well pipeline [7].

Although the advantages of biosurfactant are well known, only a few biosurfactants are produced on a large scale for commercial application, mainly due to their considerable production and recovery costs. Therefore, aiming at the use of these *Anoxybacillus* sp. in producing large-scale biosurfactants, the yields must be improved which can be achieved through optimization of the culture media. In this work, biosurfactants produced by previously isolated *Anoxybacillus* sp. were optimized through proper manipulation of various ranges of carbon (waste frying oil, WFO), nitrogen (sodium nitrate, $NaNO_3$), and salinity (sodium chloride, NaCl) using fractional factorial design (FFD) for screening more than 2 factors which varied over 2 levels and identified interaction among the factors toward the response.

2 Materials and Methods

2.1 Microorganisms

The biosurfactant-producing bacteria (*Anoxybacillus* sp.) previously isolated from a natural hot spring located in Sungai Klah, Tanjung Malim, Perak, Malaysia was used. The isolate was preserved at −80 °C in an NB medium supplemented with 20 % (v/v) glycerol solution. The composition of NB medium was (g/l): D(+) glucose, 1: Peptone, 15; NaCl, 6; yeast extract, 3. The pH was adjusted to 7.0.

2.2 Media Preparation and Culture Conditions

The cultivation was performed with a 250 mL Erlenmeyer flask containing 100 mL of minimal salt medium (MSM) supplemented with 1 % (v/v) trace element and 10 % (v/v) of inoculum (10^{-7} of cell density). The WFO, $NaNO_3$, and NaCl were added separately. The composition of the MSM (g/L): KH_2PO_4-0.2; K_2HPO_4-0.3; $MgSO_4.7H_2O$-0.5; $CaCl_2$-0.15; $NaCl_2$-0.5; $NaNO_3$-1. The composition of trace element was (mg/L): $ZnSO_4.7H_2O$-50; $MnCl_2.4H_2O$-400; $CoCl.6H_2O$-1; $CuSO_4.5H_2O$-0.4; H_3BO_2-2; $NaMoO_4.2H_2O$-500 [4]. The medium was cultured at temperature 55 °C and shaken at 130 rpm. Sampling was done after 4 days of cultivation period for analysis.

2.3 Determination of Surface Tension Activity

All the measurements were made on culture supernatant after cell removal by centrifugation at 7500 rpm for 15 min in a centrifuge (Heraeus Biofuge) at 4 °C. The surface tension was then analyzed at room temperature using Drop shape Analyzer, DSA 100 (KRUSS, Germany). The experiments were performed in duplicate.

2.4 Experimental Design: Fractional Factorial Design (FFD) and Data Analysis

A preliminary screening was carried out based on FFD with 3 factors which included waste frying oil (WFO), sodium nitrate ($NaNO_3$), and sodium chloride

Table 1 Experimental range levels of the independent variable using 2^3 fractional factorial design

Independent variables	Code levels		
	−1	0	+1
A: WFO % (v/v)	1	5	9
B: $NaNO_3$ % (w/v)	0.1	0.3	0.5
C: Salinity % (w/v)	0.02	0.07	0.13

(NaCl); the design matrix for the experiments are shown in Table 1. A 2^3 full FFD was conducted to determine the factors and their range of composition in the media that most influenced the response, which was surface tension. The experimental setting with 16 duplicated runs varied over 2 concentration levels (−1, +1) with 5 replicated runs at center points in order to estimate the pure error and thus give the prediction of the model [12]. The statistical experimental design and regression analysis were carried out using the Design-Expert software (Stat-Ease Inc., MN, USA, version 6.0.6).

An analysis of variance (ANOVA) was performed to further evaluate the model in order to determine the significant factors on surface tension.

2.5 Determination of Biosurfactant Stability

Cell-free broth obtained after harvesting the culture supernatant at 7500 rpm for 15 min was used for stability studies of the surface tension (mN/m) reduction. Five milliliters of cell-free culture supernatant at 4 days of incubation were exposed to various temperatures (55–25 °C, 25–4 °C, 25–70 °C, 25–100 °C, 25–121–25 °C, 25–121–4 °C) and at different ranges of pHs (2–12). The electrolyte effect was also tested at different range of salinity ((w/v): 2–10 %). The stability of the biosurfactant was measured based on the value of surface tension reduction (mN/m).

3 Results and Discussion

3.1 Fractional Factorial Design (FFD) and Data Analysis

The factorial design enables the identification of the medium components that play a significant role on cell growth as well as the ranges within the medium components vary. A 2^3 FFD was employed and for each of these factors, a wide range of concentrations was selected as shown in Table 1, whereas factor *A* (WFO) ranging from 1 to 9 % (v/v), *B* ($NaNO_3$) ranging from 0.1 to 0.5 % (w/v), and *C* (NaCl) ranging from 0.02 to 0.13 % (w/v).

Results of the experimental design performed to achieve the optimum medium condition response for surface tension reduction are shown in Table 2. For each run,

Table 2 Screening of variables using factorial design with surface tension reduction as the response

Run	WFO % (V/V) *A*	$NaNO_3$ % (w/v) *B*	NaCl % (w/v) *C*	Response surface tension (mN/m)
1	+1	+1	+1	47.47
2	+1	+1	+1	47.77
3	+1	+1	−1	43.09
4	+1	+1	−1	44.02
5	+1	−1	+1	54.48
6	+1	−1	+1	54.50
7	+1	−1	−1	49.15
8	+1	−1	−1	49.36
9	−1	−1	−1	58.35
10	−1	−1	−1	57.45
11	−1	−1	+1	58.32
12	−1	−1	+1	56.13
13	−1	+1	−1	53.12
14	−1	+1	−1	53.33
15	−1	+1	+1	55.20
16	−1	+1	+1	55.12
17	0	0	0	42.30
18	0	0	0	45.62
19	0	0	0	45.68
20	0	0	0	46.07
21	0	0	0	47.09

the surface tension reduction was measured as a response that is proportional to the production of biosurfactant [17, 21]. The experimental setting with 16 duplicated runs varied over 2 concentration levels (−1, +1) with 5 replicated runs at center points (0). Based on the results obtained, the value of surface tension reduction varied from 58.35 to 42.30 mN/m after 4 days of cultivation.

The effects of the medium composition on surface tension were examined in Table 2. Based on the result obtained, the lowest value of surface tension was achieved when *A, B,* and *C* were at the middle level (0). WFO and $NaNO_3$ were used by *Pseudomonas aeruginosa* zju.um1as raw materials for fermentation of rhamnolipids [23], whereas Liu et al. [13] reported that *Alcaligenes* sp. S-XJ-1 produced the highest yield of biodemulsifier achieved with increases of WFO. According to Bergey's manual, a common characteristic of all *Anoxybacillus* sp. is independence from NaCl and a comparatively low resistance to salt (5–6 % NaCl inhibit growth). The results prove that the growth of isolated *Anoxybacillus* sp. is influenced by the increased and decreased concentrations of WFO, $NaNO_3$, and salinity. The value of surface tension was varied from 42.30 to 58.35 mN/m after cultivation for 4 days.

Table 3 Anova results of the first-order model for 2^3 full factorial design

Source	DF	Sum of square	Mean of square	*F* or *t* value	Significant (prob > *F*)
Model	6	353.67	58.95	45.41	<0.0001
Curvature	1	184.10	184.10	141.81	<0.0001
Residual	13	16.88	1.30	–	–
Lack-of-fit	1	0.52	0.52	0.38	0.5490
Pure error	12	16.36	1.36	–	–
Correlation error	20	554.65	–	–	–
$R^2 = 0.9545$	Adjusted $R^2 = 0.9334$		–	–	–

Table 4 Regression analysis of the 2^3 full factorial design

Variable	DF	F value	*p*-value
A	1	157.41	<0.0001
B	1	71.81	<0.0001
C	1	21.47	0.0005
AB	1	6.55	0.0238
AC	1	12.45	0.0037
ABC	1	2.75	0.1211

The analysis of variance (ANOVA) of the first-order model is shown in Table 3, while regression analysis is shown in Table 4. The *p*-value was used to determine the significance of each coefficient and the degree of interaction between each independent variable [6]. The independent variables are more significant with greater *F*-value and smaller *p*-value (less than 0.005) [6, 12]. If *p*-value is greater than 0.1000, it indicates that they are insignificant [6, 12]. From the result, the model and several factors interaction (*BC* (data not shown) and *ABC*) were not significantly different ($p > 0.005$) and the R^2 value obtained was more than 90 % (data not shown). The quality of fit of the equation is expressed by the determination coefficient R^2. The coefficient of determination, R^2, is an indicator of fitting the model to the experimental data [10].

The insignificant factors were removed from the experimental design in order to improve the result. In this study, only factor *CB* was removed because of its influence on the response (surface tension) since the bacterial was unable to produce biosurfactant in the absence of carbon source in the medium to support the bacterial growth [3]. Although factor *ABC* is insignificant, it must be considered in the medium optimization since the value of the regression coefficient was attained with a very high coefficient of determination, $R^2 = 0.9545$ and adjusted $R^2 = 0.9334$. The value of 0.9545 obtained indicated that the model could be explained with ~95 % of the variability in response by the first-order model. The adjusted model showed no significant lack-of-fit, meanwhile the *p*-value of the model was <0.0001, thus indicating that the model is highly significant and the relationship between the surface tension and the factors is adequately represented [12].

As a result, final-order Eq. (1) was generated based on the first-order model to determine the surface tension response (y_1) to the medium composition consisting of WFO (*A*), $NaNO_3$ (*B*), and NaCl (*C*) factors which gave:

$$y = 42.30 - 3.57A - 2.41B + 1.32C - 0.73AB + 1.00AC - 0.47ABC \quad (1)$$

For every unit increased in *C* and *AC*, an increase of 1.32 and 1.00 units was observed, respectively, in *y*. In contrast, for every unit increase in *A*, *B*, *AB,* and *ABC*, *y* will decrease by 3.57, 2.41, 0.73, and 0.47 units respectively.

The response surface plot of interaction between *A* and *B* on surface tension is shown in Fig. 1a. The lowest value of surface tension was achieved when *A* and *B* were at the maximum level. The use of high concentrations of *A* and *B* were carbon and nitrogen source function as a growth supporter to the bacteria and later contribute to the synthesis of biosurfactant and thus reduce the surface tension [19]. The response surface plot of interaction between *A* and *C* shown in Fig. 1b indicates that the value of surface tension is reduced at the lowest concentration of *C* and at the highest concentration of waste frying oil (A). From this result, it is proved that the higher and the lower value of each variable affects the growth of *Anoxybacillus* sp.

3.2 *Study of Biosurfactant Stability*

The stability of the biosurfactant was checked by subjecting the fermentation broth at 4 days of incubation to conditions of high stress, which includes temperature, pH, and salinity. The surface tension showed little variation and remained nearly constant at around 42–43 mN/m when the temperature was varied from 4 to 121 °C. From the results obtained in Table 5, it is shown that the biosurfactant is stable when it is introduced to extreme temperature changes.

With respect to pH variation from 2 to 12 as shown in Table 6, the values of surface tension centered around 42 mN/m without large deviations. The lowest surface tension was recorded when the sample was at pH 7 and the highest surface tension value was recorded at acidic condition which was at pH 2, 42.97 mN/m respectively. The surface activity of the sample relatively remained stable between pH 10 and 12 indicating preference for alkaline conditions.

The salinity was varied over the range of 0–10 % (w/v). As shown in Table 7 the effect on surface tension was around 42 mN/m; the result was observed to be similar to the effect of pH with largely no changes but the lowest value of surface tension reduction was recorded when introducing the biosurfactant at concentration of salinity at 6 % (w/v) which was 42.09 mN/m. According to Bergey's manual, at 5–6 % (w/v) NaCl the growth of *Anoxybacillus* sp. is inhibited, but from the result, the surface tension activity was stable within that range of NaCl [15].

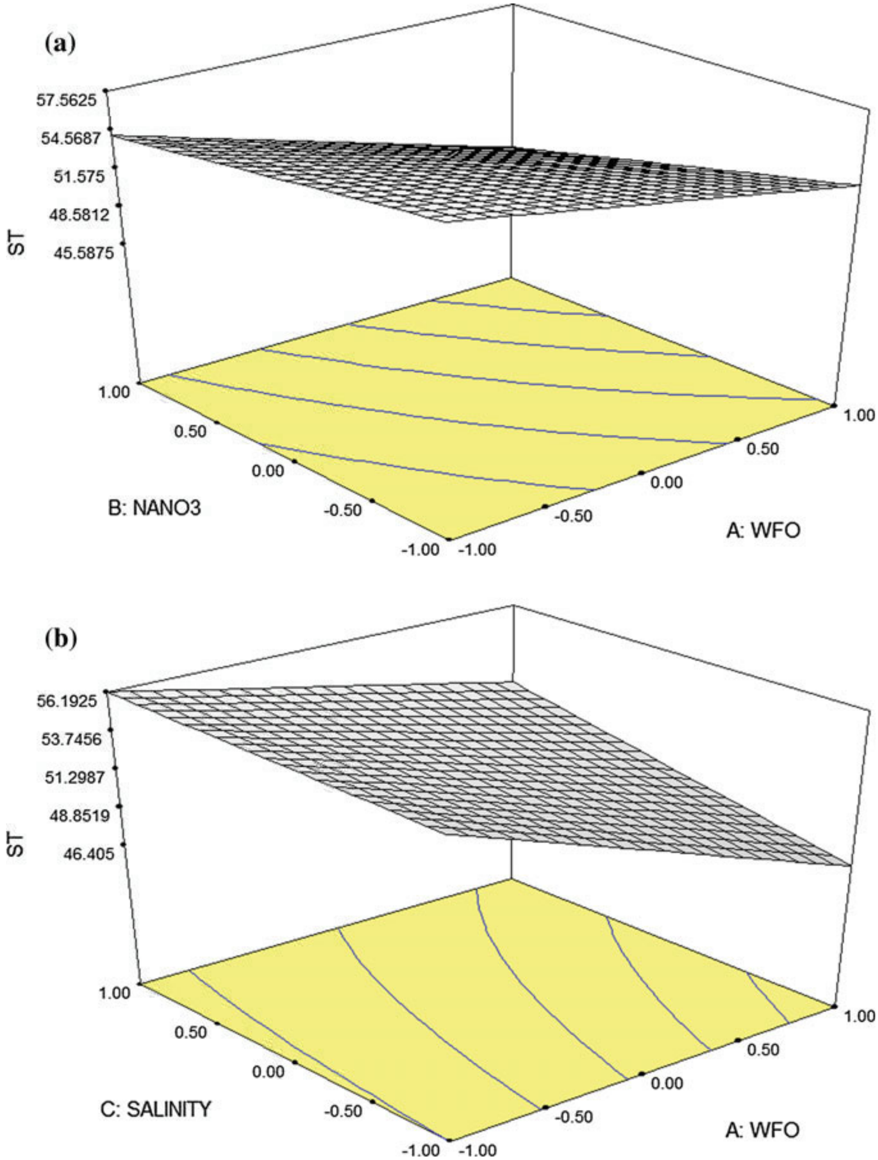

Fig. 1 Response surface plot of the interaction between **a** carbon source (WFO) and nitrogen source ($NaNO_3$) **b** carbon source (WFO) and salinity (NaCl) at 4 days cultivation period

The production of biosurfactant by microorganisms has been a subject of increasing interest in recent years, especially due to their increasing potential application. In the present study, results showed that *Anoxybacillus* sp. producing biosurfactant was stable at different temperature, pH, and salinity. It agrees with the

Table 5 Effect of surface tension on temperature changes

Temperature changes (°C)	Surface tension (mN/m)
From 55 to 25 °C	42.47
From 55 to 25 °C then to 4 °C	42.69
From 55 to 25 °C then to 70 °C	42.81
From 55 to 25 °C then to 100 °C	43.03
From 55 to 25 °C then to 121 to 25 °C	42.52
From 55 to 25 °C then to 121 to 4 °C	42.82

Table 6 Effect of surface tension on pH changes

pH	Surface tension (mN/m)
2	42.97
4	42.76
6	42.81
7	42.37
8	42.47
10	42.50
12	42.76

Table 7 Effect of salinity on surface tension

Salinity % (w/v)	Surface tension (mN/m)
0	42.20
2	42.35
4	42.47
6	42.09
8	42.45
10	42.46

stability results showed by *Bacillus sphaericus* EN3 and *Bacillus azotoformans* EN16 [11]. There are several reports on the stability of biosurfactants at extreme conditions [8, 9]. Taking into cognizance the optimum conditions for the biosurfactants' activity, one can suggest the potential applicability of these surfactants in microbial enhanced oil recovery (MEOR) since these conditions (high temperature, pH, and salinity) prevail in oil reservoirs.

4 Conclusion

In conclusion, through the 2^3 full factorial design, it was observed that the range of waste frying oil, sodium nitrate, and sodium chloride at concentrations of 1–9 % (v/v), 0.1–0.5 % (w/v), and 0.02–0.13 % (w/v), respectively, were the most significant range for biosurfactant production by *Anoxybacillus* sp. In addition, the produced biosurfactant with high stability at different temperature changes, pH, and salinity makes these biosurfactants potential candidates to be used in bioremediation of contaminated sites and in the petroleum industry (MEOR) where drastic conditions are very common.

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