A novel biosurfactant producing Kocuria rosea ABR6 as potential strain in oil sludge recovery and lubrication

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Abstract

At various stages of crude oil refining, solid and semi-solid wastes, known as petroleum sludge, are produced. Accumulation of oil waste in the refinery leads to reduced efficiency of oil refining and its release causes environmental pollution Biosurfactant-producing isolates were isolated from the oil reservoirs of the Isfahan refinery, Iran, and screened by oil expansion test, droplet collapse, and surface tension reduction measurement. Oil recovery from oil sludge was measured under constant conditions. The effect of factoring biosource lubrication on crude oil in pipelines was investigated in vitro. Also, the optimization of biosurfactant production in different conditions was measured as a single factor and Response surface Methodology. The best biosurfactant-producing bacterium was identified as *Kocuria rosea* ABR6, and its sequence was registered in the gene bank with access number of MK100469 registered. Chemical analysis proved that the biosurfactant produced was a lipopeptide. 7% of crude oil was recovered from petroleum sludge by biosurfactant obtained from *Kocuria rosea* ABR6.Also, the speed of crude oil transfer in pipelines was reduced from 64 seconds to 35 seconds. The highest biosurfactant production was measured at pH 9, aeration rate of 120 rpm and 96 hours after incubation. The use of biosurfactants produced by *Kocuria rosea* ABR6 is recommended to remove oil sludge and lubricate oil in pipelines recommended in the oil industry

Introduction

In these recent years, the control and prevention of chemical pollutants in the petroleum industry always have been a worldwide issue. (Varjani 2017) There are three processes physical, chemical, and use of Biosurfactants for the treatment of these pollutants. However, these mechanisms have numerous problems and the final product is usually toxic. in addition, there is an urgent need for cognition sustainable and eco-friendly methods which require a lesser amount of chemicals, are economically viable, and produce No toxic nature final products. One of the chemical pollutants in the petroleum industry is a compound emulsion of different petroleum hydrocarbons, solid particles, heavy metals, and water with highly toxic to the environment, named oil sludge, removal of these components is a costly and time-consuming process.. Due to its hazardous nature and increased generation quantities around the world, it's necessary to clarify this problem. (Lima 2011) Expulsion of oily sludge from storage tanks can be performed by using biosurfactants to decline viscosity and recovery and refinery of oil. (Banat 1991) Other treatments of oily sludge such as composting and land farming may have considerable applicability and a low operating budget for large-scale treatment, but their microbial degradation process is time-consuming. The detection of oily sludge cure technique depends on sludge features, disposal regulatory requirements, costs, time constraints, and cure capacity(Guangji Hu 2013) The use of Biosurfactants in the industry is the most important Achievement of the 21st century.(Singh 2018) They are amphiphilic surface-active agents Secreted from microorganisms. Bioactive molecules are able to decrease the surface tension between an aqueous mixture and hydrocarbon. (Shekhar et al 2015) In recent years, the application of biosurfactants has increased significantly, due to the non-toxic, Inexpensive economic value, biodegradable nature Bioremediation process is based on an integrated

approach employing microbial communities such as fungi, actinomycetes, bacteria, and earthworms. (Suganthi 2018) On the other hand, the global market for surfactant is amazing and, the market is also, expected to grow to USD 39·86 Billion by 2021 (Markets and Markets 2017). It is considered as a viable process for administration of organic pollutants-rich solid wastes and wastewater. Many studies have suggested using bacteria as a biosurfactant to recovery and removing oily sludge. Many studies have used bacteria as biosurfactant to recovery oily sludge, *Pseudomonas aeruginosa F-2* (Ping Yan 2012) from refinery oily sludge, *Bacillus subtilis 3KP* (Ni'matuzahroh 2016) from the petroleum sludge, *Pseudomonas balearica strain Z8* (Soltani Nejad, 2020) from oily sludge wastes. According to many studies, Oily sludge is one of the most important solid wastes generated in the petroleum industry, and understanding the mechanism of degradation of it would be significant, so we decided to investigate biosurfactants as the best choice. (Hu 2013) The utilize of environmental nanotechnologies (E_nano) is a complex process to resolve the problems as best as associated with the petroleum industry, (Younis 2020) we carried out ZnO as E-Nano in our project.There aren't many documents to investigate about biosurfactant production by *Kocuria rosea*. The main aim of this study is introduce Bacteria as a safe and cost-effective option among various technologies. In the current study; *Kocuria rosea strain ABR6* was isolated from Refinery oil tanks, located in Esfahan, Iran.

Material And Method

Sample collection:

The sample was crude oil, collected from storage tanks located in Isfahan Petroleum Refinery, Isfahan, Iran. Afterwards, transferred to the laboratory under controlled conditions.

Culturing selected Bacteria:

In order to, isolation of the selected bacteria, Bushnell Hass culture was used, cultures contained (g L−1): KH2PO4, 1; 0.2; K2HPO1; NH4NO3, MgSO4. 7H2O 1; FeCl3, 0.002; CaCl2, 0.02; and crude oil, 1% (v/v), and the pH was adjusted to 7. The culture medium was sterilized by autoclave; 10 mL of each sample were inoculated in a 500 mL medium and incubated for 10 days at 30 C and 100 rpm. Then 1 ml of inoculum was added to the culture medium (1.8 g L−1 agar). In the next step, bacteria grown on the specific medium were transferred to an olive broth with the following compounds (g L−1): MgSO4. 7H2O, 0.2; K2HPO4, 1; NH4NO3, 1; FeCl3, 0.002; CaCl2, 0.02; yeast extract and olive oil, 1% (v/v). Olive oil was added, and the pH was adjusted to 7. Afterwards autoclaving, 1% of pre culture was shifted to the olive broth and incubated for 4 days at 30 ° C and 100 rpm (Cameotra and Singh, 2008; El-Sheshtawy and Doheim, 2014).

Identification of bacterial isolate:

To better and certain identification base on the other studies, carried out positive or negative to gram stain test and some routine biochemistry tests (Jessim 2020), to identify isolated bacteria, like the

urease, oxidase, utilization of inulin, citrate utilization test , phosphatase tests, gelatinase, nitrate reduction tests arabinose, and N-acetyl-L-glutamic acid amylase.(Kandi 2016)

And to molecular test, Bacterial genomic DNA was extracted from the bacterial isolate grown on nutrient broth using standard protocol. the PCR was carried out as formerly described (Akbari et al. 2016). 16S rDNA amplicon from isolated strain was sequenced, and the information was searched using NCBI-BLAST search tool for identification of the strain type. The sequence was submitted to the NCBI Gene Bank. (Akbari et al 2018)

Evaluation of biosurfactant production by isolated bacteria:

Extraction of biosurfactant from isolated bacteria was performed according to the technique mentioned by Jorfi et al. (2013) .after centrifugation at 10,000 g for 15 min in order to eliminate the bacterial cells, and then the pH was adjusted by adding 2.0 ml of HCL to the biosurfactant. The precipitate was separated by centrifugation (10,000 g for 20 min) and then extracted with chloroform: methanol (2:1, v/v) mixture. In order to have crude biosurfactant, solvent evaporation under vacuum was used. By the displacement method the possibility of biosurfactant presence was investigated. In short, 50 μL of crude oil was mixed to a 15 cm diameter plate containing 40 ml of distilled water. The cell free culture broth (15 μL) was then added to the oil surface. The cultures with no clear zone were scored as negative indicative of the lack of biosurfactant production (Mousavi et al. 2015 ;Soltani Nejad 2020).

Treatment of oily sludge with biosurfactant:

To preparation the crude oil from oil sludge in laboratory conditions, the present process was performed according to the method performed by Lima et al. 40 g of petroleum sludge obtained from the bottom of crude oil storage tanks autoclaved from Khark refinery Then 50 ml of sterilized distilled water was added to it. Then 10 ml of the medium culture of biosurfactant were inoculated after 96 hours of incubation. Positive control was prepared by adding 10 ml of Twin 80 and negative control was prepared without adding chemical surfactant and biosurfactant. Then 5 days Incubation at ambient temperature and aeration 100 rpm, oil in emulsified petroleum sludge then emulsion by adding One ml of magnesium nitrate solution was broken, the aqueous phase was separated and the amount of recycled oil was measured.(Lima et al. 2011; Cameotra, S.S et al. 2008)

Crude oil lubrication by Biosurfactant:

In the present study, a strain isolated was cultured in Bushnell Hass medium at 30 ° C and 100 rpm. After 96 hours incubation, in order to sure to produce biosurfactant, emulsification index, and oil displacement method done. The pipeline was designed under laboratory conditions, some crude oil in the above tank along with gross biosurfactant in the culture medium it was poured in a ratio of one-fifth as the optimal ratio and it was performed under mixing with a specified speed, and the system output was then passed through a 40 cm duct at a 65 degree angle. Duct passage time was recorded before and after mixing (Amani et al. 2016).

Chemical analysis TLC and FTIR:

Product analysis by thin-layer chromatography (TLC):

In order to chromatograph, a thin layer of silica 60 paper with dimensions of 15.5 cm was arranged. 0.1 Mg of dried biosurfactant dissolved in 10 µl of 90% ethanol and 5 microliters of sample was dotted at a distance of 1 cm from the edge of the paper. The solvent system that was used as the mobile phase including chloroform, methanol, acetic acid with a ratio of 65/15/2 v / v / v was selected. For staining solution containing 15 /. Ursinol and 8.2 ml of 60% sulfuric acid in 42 ml of distilled water sprayed on paper and dried at 100 ° C for 10 minutes and the spots were examined. (Camacho-Chab JC 2013)

Product analysis by Fourier-transform infrared spectroscopy (FTIR):

FTIR (BRUKER,) was performed to show the presence of chemical functional groups in bio surfactant produced by isolated bacteria. Lyophilize was performed to extracted biosurfactant and biosorfactant analysis was performed in assay range of $400-4000$ cm^{-1}.(Yaraguppi 2020)

Biodegradation analysis of crude oil:

BH media selected to evaluate the ability of bacteria isolated from crude oil (100 mL) supplemented with 1% crude oil. Upon incubation at pH 7 and 30 ° C, 160 rpm for 72 h, the grown cultures were centrifuged at..rpm, 4 ° C for 10 min to pellet down the cells. Isolated bacteria use crude oil as a source of carbon this was investigated by the method weighing the residual crude oil based on a gravimetric method wherein the residual crude oil in the cell-free supernatant was extracted in an equal volume of n-hexane and separated using a rotary vacuum evaporator. (Sharma 2019)

Optimization of culture conditions for biosurfactant production:

In order to determine biosurfactant production ability of the isolated bacteria, we decided to carry out single factor optimization and multi-factor optimization by Response surfaces methodology (RSM). In the first section, we overviewed 40 experiments, and according to the results, we detected 15 experiments, which showed in Table 1. In this study, to single factor optimization biosurfactant production, the five impacts of factors which included pH, temperature, carbon and nitrogen sources, agitation and time condition were considered. The culture conditions were based on pH (7, 8, 9, and 10), temperature and carbon source (oil olive, glucose, tribotirin, oil crude) and nitrogen source (peptone, NH4NO3, NaNO3, triptone, yeast extract) , the biosurfactant production was also evaluated under agitation (80, 100, 120, 140 rpm) and time conditions. During the experiments, when each factor was examined, the other four were maintained as constant. To determine the importance of the factors and the better understand interactions between them were analyzed using analysis of variance ANOVA. (chong 2020)

Result

In the present study, several bacteria strains were isolated and because most growth in enrichment culture Bushnell Hass medium was related to W11 isolate, bacterial production was examined.The screening revealed W11 produced a considerable amount of biosurfactant, and reduced the surface tension from 72 mN/m to 31.6 mN/m. also; the diameter of the oil expansion halo was measured at 6 cm. Drop collapse in less than a minute pointed up the presence of biosurfactant in the Bushnell Hass medium .the drop collapse test to survey biosurfactant producing W11 was positive. Isolated bacteria were measured by standard screening technique like, oil displacement method, oil drop collapse method (DCM), surface tension (SFT) measurement and emulsification index. (Soltanighias 2019). This proved its ability to produce biosurfactants and selected for further oil recovery analysis, optimization, and Biodegradation analysis.

Isolation of Biosurfactant-Producing Bacteria:

The isolated bacteria showed a positive growth in the selective cultures medium (olive broth and Buschnel Hass) and we named this strain, W11.To certain identification, biochemistry tests were helpful, the PCR of purified bacterial DNA with universal primers presented the 1500 bp band in gel electrophoresis. .NCBI under the accession number of MK100469 the analysis of the genomic sequence of 16srDNA using Finch TV and thence BLASTN approved that W11 from oil sludge sample has been associated with *Kocuria rosea* and was named *Kocuria rosea* ABR6.also this strain deposited in Petroleum Biotechnology Culture Collection as *kocuria rosea* PBCC1167.

Treatment of oily sludge with biosurfactant:

The medium culture of *Kocuria rosea ABR6* after 72h, 100 rpm and incubation at 30 °C was performed to decrease the oily sludge with sharp viscosity in crude oil storage tank. As a result of investigating the treatment of petroleum sludge with biosurfactant from the isolate *Kocuria rosea ABR6*, 50% of crude oil was recycled in laboratory conditions. In the positive control sample, 75% of crude oil was recovered from petroleum sludge, provided, in the negative control sample, only 3% of the crude oil is recycled.

Crude oil lubrication by Biosurfactant:

For a better description the effect of Crude oil lubrication by Biosurfactant, we designed an experiment on crude oil in pipelines in vitro. The Biosurfactant produced by isolated bacteria accelerated the movement of crude oil, as the crude oil movement time decreased from 66 seconds to 39 seconds.

Chemical analysis TLC and FTIR:

Thin-layer chromatography (TLC) was used to separate non-volatile mixtures. After accomplishment of 72 h, the rest of oil and biosurfactant in the supernatant was controlled with chromatographic technique. This study was performed with abiotic sample introduced as a control. As observed in figure1 Rf 0.81 was observed.

FTIR analysis was performed to characterize the biosurfactant type from *Kocuria rosea ABR6*.According to spectrum FTIR, A stretch around 1380 cm^{-1} corresponds to the presence of $-CH_3$ and $-CH_2$ groups in aliphatic chains of lipids. A broad band at 2926 cm^{-1} represents the $O-H$ stretching vibrations from free hydroxyl groups. Regions around 2926 cm^{-1} signify alcohols and phenols. Peak in the region of 518 cm^{-1} may be likely due to the presence of disulfides in the molecule. Peak around 2402 cm^{-1} may be representing the P-H in the phosphine. Peak around 948 cm^{-1} may be due to occurrence of P-O-R stretch of ester group. Peak around 3398 cm^{-1} reveals the presence of RCONH2 related to amino acids. Peak near 1659 cm^{-1} indicate to the C=C from alkene of bacteria protein, also, peak near 832 cm^{-1}, 616 cm^{-1} and 716 cm^{-1} due to the presence of alkene. Peak around 936 cm^{-1} may be attributed to OH, Carboxylic group. The FTIR analysis demonstrated the biosurfactant produced by the *Kocuria rosea ABR6* was the lipopeptide.

Biodegradation analysis of crude oil:

Following the research, we found out the biosurfactant production using various carbons as energy source. In this study, the ability of *Kocuria rosea ABR6* in utilizing crude oil as a carbon source and produce a biosurfactant was explored. The percentage of crude oil biodegradation by biosurfactant sounds fast. This may be because that the microorganisms in the oil sludge have efficiency ability to use the remaining crude oil as a source of carbon and energy. In sum, degradation of crude oil was reached as 22%.

Optimization of bacterial growth in order to maximize biosurfactant production:

Single factor test:

Impact of pH: To increase the amount of biosurfactant from the *Kocuria rosea ABR6* during the selective condition, five parameters need to be optimized. According to the results, the highest emulsification was related to pH 9 and among 80% (Figure 2 A) .respectively, emulsification of the isolated bacteria in pH 7, 8, 9, and 10 were 70%, 75%, 80%, and 65%. Impact of carbon sources: The most product of biosurfactant, of carbon sources, was related oil olive about 80%, also fermentation of glucose, tributyrin, oil crude by isolated sample were 40%, 53%, 75% (Figure 2 B). Impact of nitrogen sources: In order to find the best nitrogen sources, numbers indicated yeast extract with 75% was at the top. Other sources include, peptone, NH4NO3, NaNO3, and tryptone were 70%, 73%, 52%, and 55%. (Figure 2 C).) Impact of agitation: results show the best agitation was 120rpm, the rest of the results of agitation under 80 rpm, 100rpm, 120rpm, and 140 rpm were, 75%, 78%,83%, and 80% (Figure 2 D).

Response surface analysis:

The response surface analysis results are showed in figure3.The highest growth was related to 72 hours after incubation; hence, the highest emulsification was around 96 percentages. According to graph after 96 hours, isolated strain showed the most biosurfactant production. It shows, *Kocuria rosea* had

maximum biosurfactant production in stationary phase. According to the follow graph pH 9 provided the best condition to biosurfactant production in this case study. Based on the emulsification activity around 97.38, aeration speed 120rpm optimally reported. Looking to increase at incubation time conditions elevated. In order to find the highest level of biosurfactant production by *Kocuria rosea ABR6*, a two-stage experiment was designed. In the first stage, 40 tests were performed and after that 15 tests. Variable factors in these tests were pH, aeration speed and incubation. Based on these results, if the conditions are pH 9, aeration speed 120rpm and incubation72 hours, the production of biosurfactant will be 100%.

The effect of different factors in three dimensions:

Statistical analysis and analysis of variance ANOVA in the second stage of optimizing biosurfactant production by *Kocuria rosea* ABR6. The Figure 4 show, the statistical analysis and analysis of variance ANOVA that for each variable, the corresponding correlation coefficient is P-value and F-value. Based on regression analysis, based on the evidence, it was found that pH and incubation time were positive factors and the P-value was less than 0.05. In general, the model was significant and significantly affected the production of biosurfactants. Actual and predicted amounts of biosurfactant production are given the linear form is predicted. The Figure 6 shows, the effect of pH, agitation rate and incubation time factors on producing biosurfactant and emulsification index. As the slope of the invoice line increases, the effect of that factor on the response will be a steeper, as well as, the Smoother line slope shows, the effect of that factor on response was less. In this experiment, A factor Representative pH had a steep line slope and other factors like incubation time and agitation rate had slower line slope, therefore, they have less effect on the production of biosurfactants. Also, the production equation of biosurfactant based on the tested factors is as follows. According to this equation and the selection of other values, the amount of production can be predicted.

$Y = 93/71-71/0A+26/3 B -77/8C-29/4AB+48/7 A^2$

Y=   Biosurfactant production   A= pH   B= Incubation time   C= Agitation rate

Discuses

Nowadays, the importance of oil pollution is obvious. Oil, and especially oil sludge, has polluted many water and soil areas. For this purpose, a biological solution to remove oil sludge seems necessary and logical way. The diversity of bacteria is able to degradation oil and oil sludge, but obviously the bacteria that are native to the refinery they carry out this process better. Therefore, in this study, we isolated the native bacteria of the reservoir and checked out the ability to produce biosurfactants, biodegradation and lubrication. In the present study, a pipeline was designed in laboratory condition, biosurfactant that produced by *Kocuria rosea* ABR6 led to a doubling of the crude oil movement in the pipelines. There are not many articles about effects of biosurfactant on crude oil lubrication by bacteria. In present study, we succeed to isolated 30 strains from tanks located in Isfahan Petroleum Refinery, which 11 strains had the ability to produce biosurfactant. The Isolate named W11 was known as the best producer biosurfactant.

The result of 16s rDNA shows, isolated W11 had been *Kocuria rosea*. The accession number of MK100469 confirmed by gene bank global and named *Kocuria rosea* ABR6. In order to identify species of bacteria, some routine biochemistry test was done, as well as, Jessim, (2020). the biochemistry test were helpful but they weren't determiner, and because has also been observed that various species of *Kocuria* react differently to routine biochemical tests, so we had needed an advance molecular method and selected 16s rDNA .(Kandi, (2016) Advance molecular method amplicon 16s rDNA from isolated strain was sequenced, and the information was searched using NCBI-BLAST search tool to identify of the strain type. Based on results TLC and FTIR and the Variety of materials present in the biosurfactant, its chemical nature was investigated and these results pointed to the lipopeptide nature. Dhasayan et al (2015) demonstrated that in analysis FTIR, the biosurfactant obtained from B. amyloliquefaciens MB-101 was similar to the lipopeptide structures. Nature of biosurfactant demonstrate characteristics molecular and different applicant, In this study, we introduce *Kocuria rosea* ABR6, as lubrication for Crude oil, which reduced movement of Crude oil and Ren et al (2019) biosurfactant SWPUEN- 1introduced as a cleaning agent to treat oily sludge. During different experiments, we found there are the correlations among variable factors and decided to perform a statistical technique, named RSM to analyze them. Analysis RSM demonstrated, efficiency of biodegradaton depends on the pH, agitation, aeration, carbon and nitrogen sources. The present of heavy mental in oil sludge can be a hazardous and negative effect on the environment and human health, in addition, some sludge treatments such as biosurfactant and incineration can increase these risks, so, we suggest applying other methods like the Electrokinetic process to remove contaminants. (Hu 2013)The potential of bacterial isolate in Crude oil lubrication indicates the importance of biological usage *Kocuria rosea* ABR6 in petroleum industry. In this research, the designed pipeline was to enhance the lubrication of crude oil through pipelines was explored this method employed to reduce viscosity and pressure drop to aid pipeline lubrication of crude oil , and it was successful. However, design a pipeline to crude oil transport is depend on some factors, the properties of the crude oil, distance dimension, cost, environmental problems and the regulation in countries.(Abarasi 2014).Many studies was isolated the biosurfactant-producing bacteria from sea sample (*Bacillus amyloliquefaciens SH20* and *Bacillus thuringiensis SH24*) by Barakat et al(2017), soil sample (*Acinetobacter junii B6*) by Ohadi et al (2017) and solar salt works (*Kocuria marina BS-15*) by Sarafin et al (2014).Due to the fact that the oil industry has a lot of bio-pollution, so it is necessary to use biosurfactants,The Biosurfactant production ability of this bacterial isolate has an appropriate aspect in the bioremediation of contaminated sites. The results of this study demonstrated, *Kocuria rosea ABR6* as a native bacterium isolated from oil refinery of Esfahan by produce biosurfactant that is able to recover crude oil to 35 percentages and reduce the incidence of environmental pollution by crude oil. Producing biosurfactant in the mix with crude oil caused to accelerate movement in the pipeline from 64 seconds to 39 seconds in the laboratory condition. Optimize conditions will leading to significant increase biosurfactant. Therefore the isolated bacterium in the recent study is able to be a reasonable candidate to produce lipopeptide biosurfactant in industrial consumptions.

Declarations

Conflict of Interest

There is no any conflict in this paper between authors.

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Table

Table 1.Experiments of second step designed by Response Surface Analysis

Experiment	Factor number1 Agitation rate	Factor number2 Incubation	Factor number3 pH
1	140	120	9
2	140	72	8
3	140	96	10
4	100	120	9
5	100	96	10
6	100	72	8
7	120	96	9
8	120	96	8
9	120	120	10
10	120	72	10
11	120	96	9
12	120	96	8
13	120	72	9
14	120	96	9
15	120	120	10

Figures
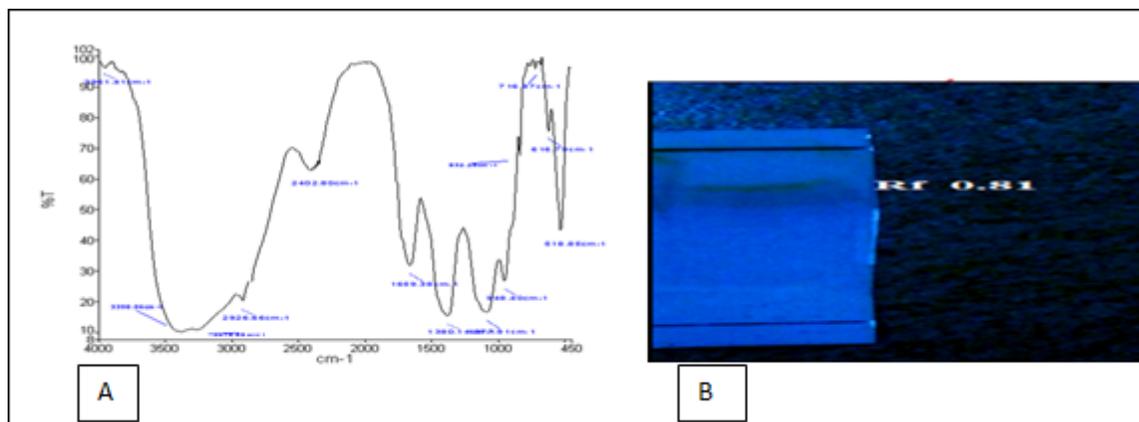
Figure 1

1A)Characterization of biosurfactan using FTIR analysis from Kocuria rosea ABR6 1B) chromatogram biosurfactant produced by Kocuria rosea ABR6
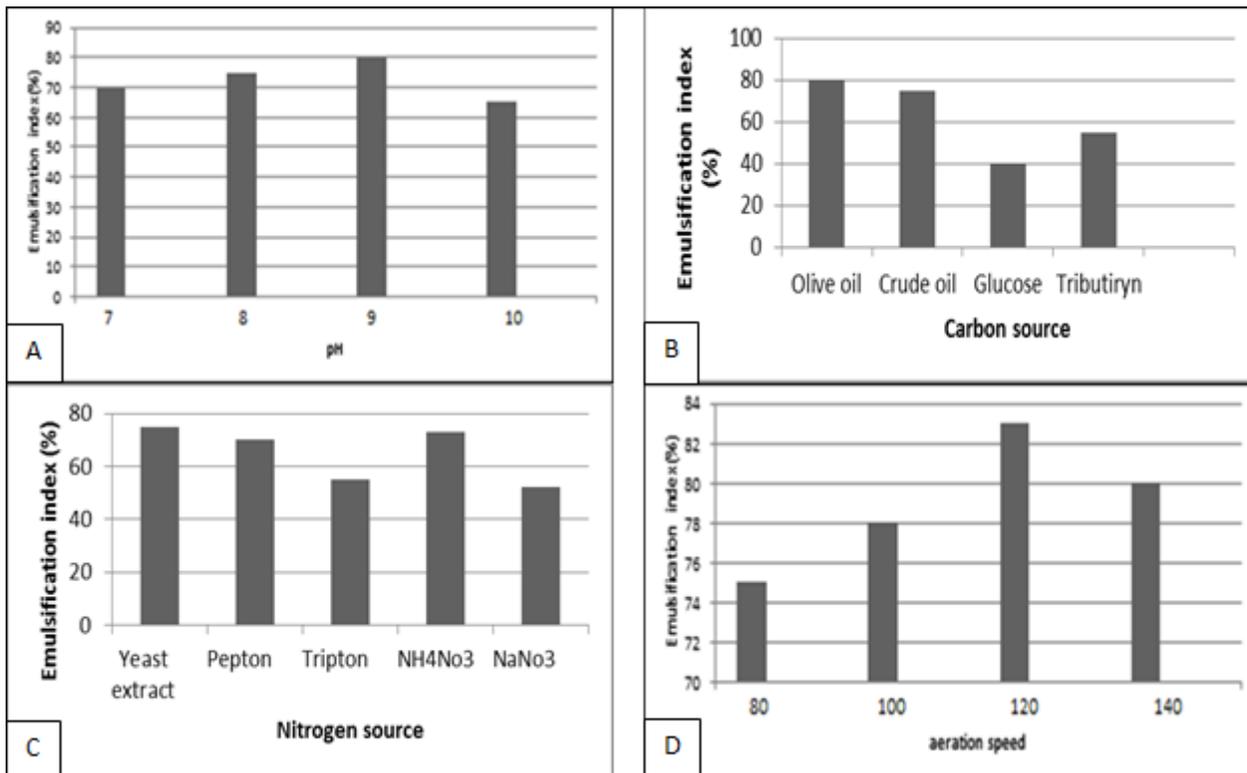
Figure 2

A) Impact of pH in biosurfactant produced by Kocuria rosea ABR6, B) Impact of carbon sources biosurfactant produced by Kocuria rosea ABR6 C) Impact of nitrogen sources biosurfactant produced by Kocuria rosea ABR6 D) Impact of agitation biosurfactant produced by Kocuria rosea ABR6
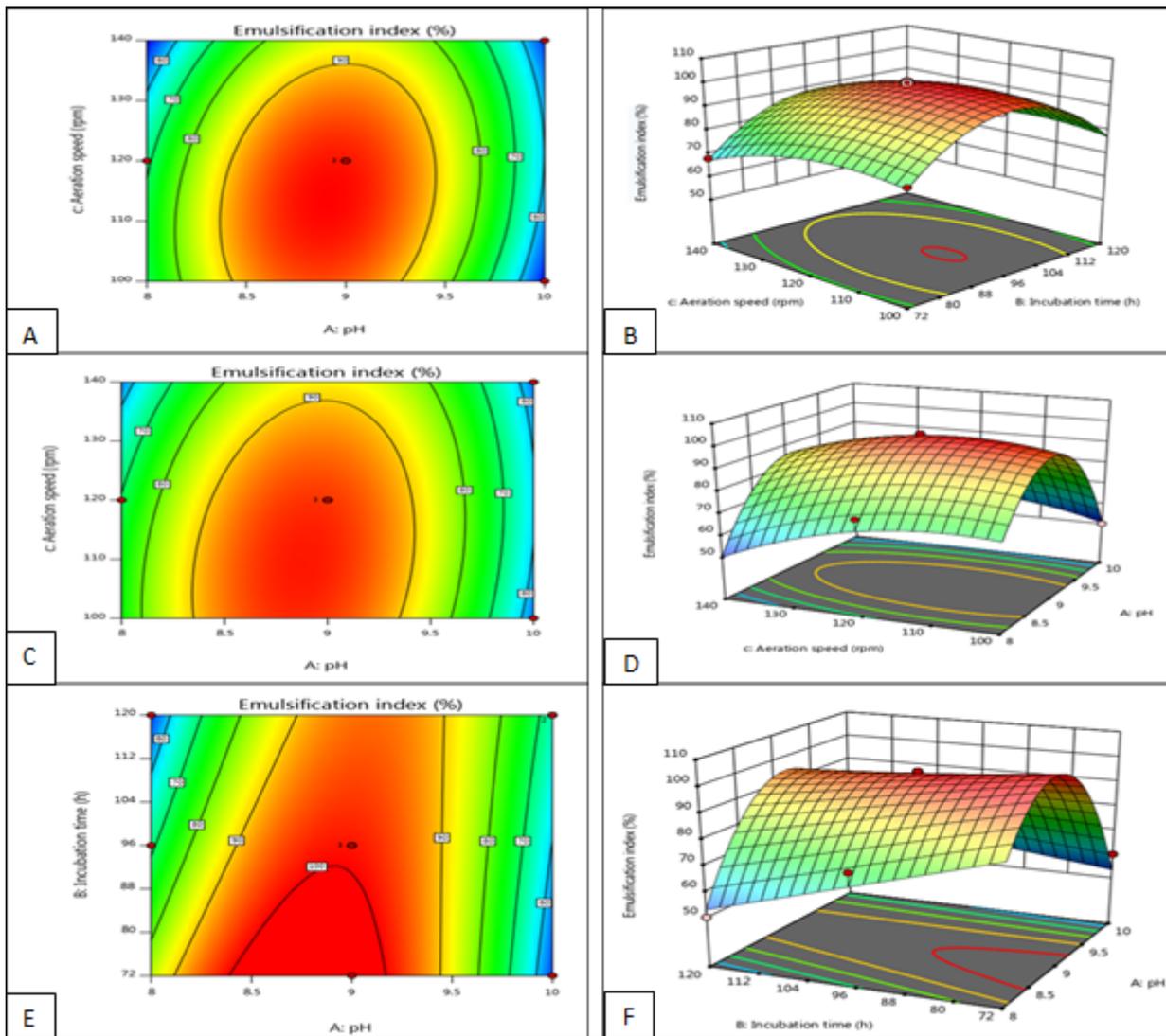
Figure 3

3A) Contour curve the effect of aeration speed factors and incubation time kocuria rosea ABR6 , B) Three-dimensional (3D) curve of the effect of aeration velocity factors and incubation time, C) Contour curve the effect of pH and aeration rate factors kocuria rosea ABR6, D) Three-dimensional (3D) curve of the effect of pH and aeration factors kocuria rosea ABR6 , E) Contour curve the effect of pH factors and incubation time kocuria rosea ABR6, F) Three-dimensional (3D) curve of the influence of pH factors and incubation time.

Source	Term df	Error df	F-value	p-value	
Subplot	4	6.25	42.41	0.0001	significant
A-pH	1	6.11	47.86	0.0004	
B-Incubation time	1	6.41	6.68	0.0391	
AB	1	6.37	15.12	0.0072	
B^2	1	6.12	103.81	< 0.0001	

Figure 4

the analysis of variance ANOVA in biosurfactant production by kocuria rosea ABR6

Figure 5

Actual and predicted values of biosurfactant production in RSM software by Kocuria rosea ABR6
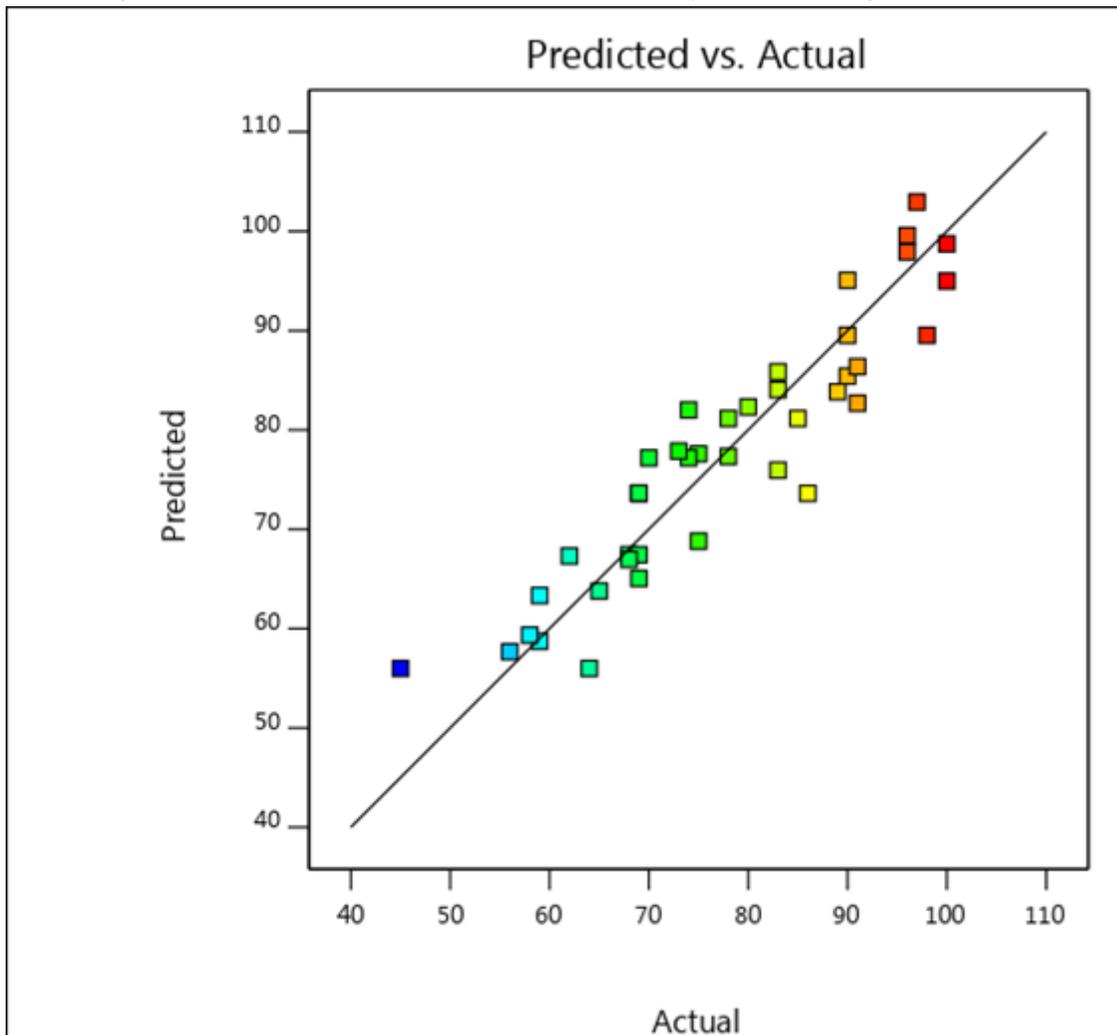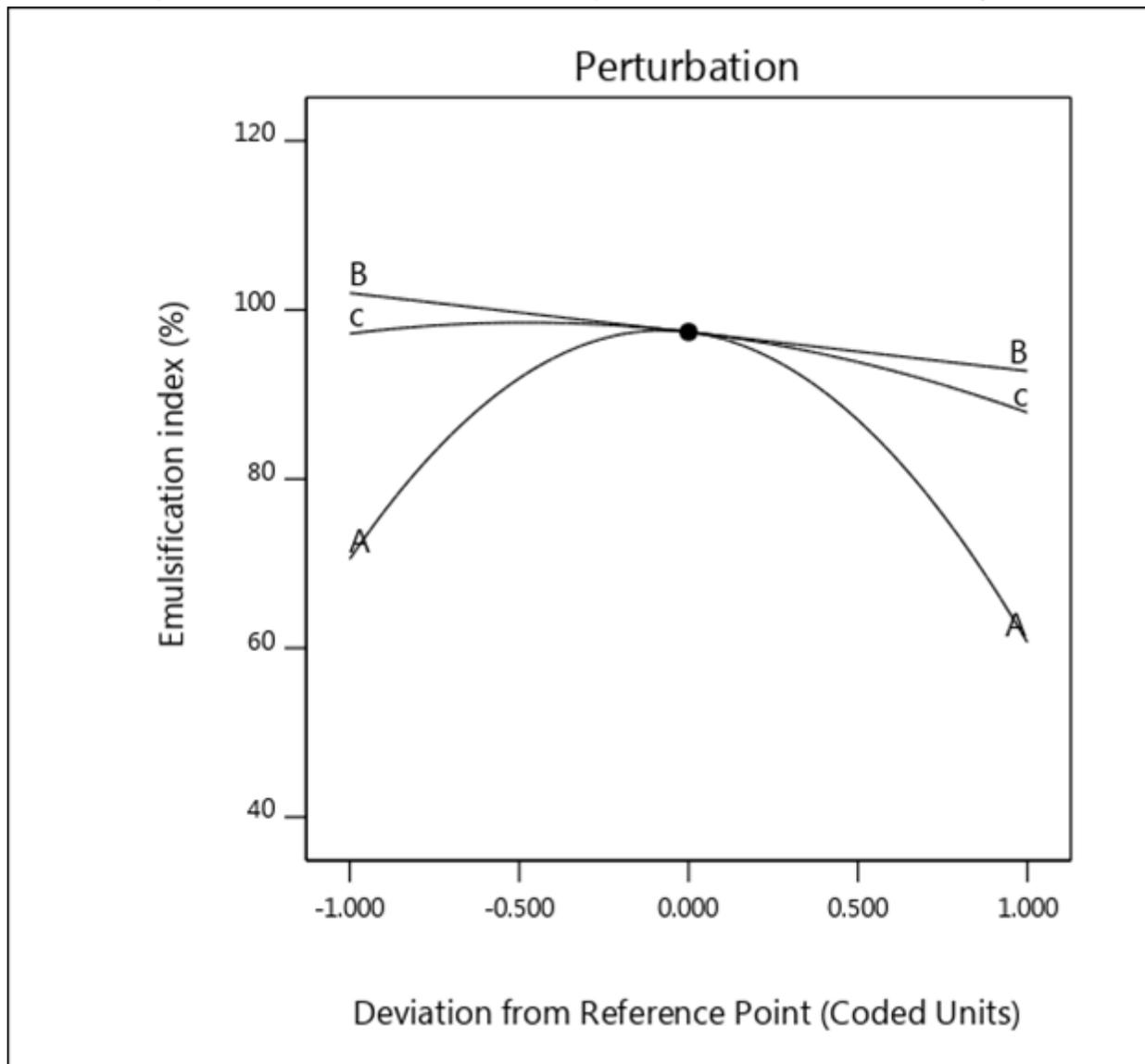

Figure 6

Impact of factors, A: pH, B: Incubation time, C: Agitation rate