

Sustainable and Eco-friendly Method for Oil Recovery from Refinery Sludge Using Froth Flotation and Biosurfactants

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Abstract

Oily sludge resulting from refineries is threatening the environment and human health. Traditional clean-up methods of oily sludge have limitations in efficiency, environmental effects and cost. A combination of froth flotation and biosurfactants method was established for treating oily sludge that generated from the south refineries company, Basrah Governorate, Iraq. This work presents a part of an ongoing project to develop an appropriate low-cost and sustainable method to recover oil from refinery oily sludge. Biosurfactants driven from *Bacillus cereus* were produced, extracted and evaluated for their effectiveness as frothing agent. Flotation parameters including biosurfactants concentrations, air-water ratio, temperature and flotation time were determined for efficient separation of oil from oil-in-water emulsion. The optimum conditions revealed by this study were 0.2 g/L biosurfactant concentration, 15% air-water ratio, 30 °C and 25 minutes flotation time. The obtained results showed that the combined method demonstrated a significant oil removal (90%) in compare with flotation alone (60%). The GC-MS analysis showed near complete removal of hydrocarbons confirming the effectiveness of this method in oil separation and enhancing oil removal. This finding suggests that the combined approach is a promising and sustainable strategy for oily sludge treatment. Further studies are required to validate the efficiency of this method in large-scale applications.

Keywords: Oily sludge; Remediation; Air flotation; Biosurfactant; Oil removal

1. Introduction

According to the ubiquitous use of petroleum products consumed all over the world, large amounts of petroleum hydrocarbons are released into the environment during oil extraction, storage, transportation, refining and waste treatments (Adebiyi, 2022). Petroleum industries bring numerous benefits to society but are globally recognized as an economic activity with substantial environmental impacts, and oily sludge is one of the most significant solid wastes produced in the petroleum and petrochemical industry (Hasan *et al.*, 2023). It has been estimated that 1000 m³ of oily sludge are produced during the petrochemical industry processing for every 500,000 m³ of crude oil (Hu *et al.*, 2013). Oily sludge is a complex emulsion of various petroleum hydrocarbons (PHCs), water, heavy metals, and solid particles. Many components of oily sludge are listed as toxic and priority pollutants by the United States Environmental Protection Agency; EPA (US EPA, 2016) due to their toxicity and their mutagenic and carcinogenic potential towards humans (Singha and Deka, 2024 and Wang *et al.*, 2025). Despite the substantial pollution and toxicity of sludges from the oil sector, the development of an effective and environmentally friendly reduction strategy remains challenging. Increasing pressure by environmental regulatory agencies has provided motivation to develop new clean up technologies

Currently, several techniques have been used for the minimization of the treatment of oily sludge including incineration, microwave irradiation, solvent extraction, Fenton oxidation, photocatalysis, freeze-thaw, dissolved air flotation and bioremediation (Hui *et al.*, 2020). However, in addition to their advantages, these methods have significant limitations. For instance, incineration produce potentially toxic elements and compounds in soils that create risks for the environment. (Liang *et al.*, 2021). The microwave irradiation method is energy intensive, there are few or no industrial-scale magnetrons and limited treatment due to the limited microwave depth penetration (Lee *et al.*, 2020). Solvent extraction is time consuming, labor intensive and requires the use of large volume of organic solvents (Zuloaga *et al.*, 2012),

which are themselves environmentally challenging. Photocatalysis method needs catalyst recovery (Bockenstedt *et al.*, 2021). Flotation is a simple and less expensive method, but it effective when treating sludge is relatively low viscosity (Li *et al.*, 2021a). Bioremediation requires a large area of land, and time-consuming process (Hamidi *et al.*, 2021). Biosurfactants are environmentally friendly and effective in treating oily sludge, however, they are very time consuming. A previous study mentioned that about 30% of total hydrocarbons removed by biosurfactants after 3 months (Rahbari-Sisakht *et al.*, 2017).

The above methods require large quantities of materials, energy, generate secondary pollutants, time consuming and human resources to place devices into contaminated areas, and none are sustainable. The limitations mentioned above may resolve partially by the use of combined methods. The combined methods can use the benefit of two methods and increase the efficiency of the treatment, particularly in relation to energy and cost (Huang *et al.*, 2025). Several studies have been demonstrated that combined treatment methods may recover oil from oily sludge more than single treatment. For example, solvent extraction and freezing/thawing (Hu *et al.*, 2015), Fenton reactions and biomass technology (Guo *et al.*, 2014). Therefore, there is an urgent need to develop new, effective and efficient methods to clean-up oily sludge and its discharge.

Froth flotation is widely used for recovery minerals (Gao *et al.*, 2021) and recently has been used for treating oily sludge (Thong *et al.*, 2021). A previous study has been demonstrated that oil recovery was about 55% when froth flotation applied (Li *et al.*, 2021b). Froth flotation is a complex technique requires group of reagents including frothers, which are crucial in all flotation processes via forming a froth that can increase bubble stability (Pawliszak *et al.*, 2024). Due to synthetic frothers mostly are hazardous to living organisms and entire ecosystem, bio-based frothers, called biosurfactants, have been developed to avoid environmental issues (Asgari *et al.*, 2024). Biosurfactants are biological chemicals produced by bacteria, yeast, plants, or fungi as secondary

metabolites (Bhadra *et al.*, 2023). They have several benefits over synthetic surfactants including high effectiveness, low toxicity, lower surface tension, and enhance interaction with pollutants (Karnwal *et al.*, 2023). In this study, a combined laboratory-scale approach including biosurfactants and froth flotation method has been used to recover oil from oily sludge and reduction of the waste mass.

The aim of this research is to optimize a combined biosurfactant and froth flotation procedure for the treatment of oily sludge under laboratory conditions by i) production of a suitable biosurfactant; ii) assessment of its effectiveness as a frothing agent, and iii) evaluation of its effectiveness alone and in combination with froth flotation

2. Methodology

2.1 Sample collection and screening of biosurfactants producing bacteria

2.1.1 Site description and sample collection

Samples of sludge were collected from an active refinery company (Basrah Oil Refinery Company, Basrah City, Basrah, Iraq). Samples were collected from different places in the refinery such as industrial water treatment tank, skimming division sink and Oil cooling tower (20 g each). Approximately, 20 g, accurately weighed, was collected of each sample. Samples were carefully placed into sterile, dry polyethylene bags using sterile spatulas. All samples were labelled, and stored in a refrigerator for further analyses. Under sterile conditions, 1 g or 1 mL of each sample was added to a test tube containing 9 ml distilled water and then thoroughly suspended using a vortex. Serial dilutions from 10^{-1} to 10^{-4} were conducted DI water. The dilutions were filtered using Millipore filter paper with a nominal pore size of $0.45 \mu\text{m}$ 100 μL of the dilutions was spread on petri dishes containing Lauria-Bertani medium (LB), then incubated at $30 \text{ }^\circ\text{C}$ for 18 hours. Swabs were taken from the developing colonies, which were Gram-stained and examined under the light microscope. Gram positive, spore-forming bacilli were selected because they showed capability for oil degradation.

2.1.2 Identification of bacteria

Bacterial isolates were identified genetically using 16sRNA technique. The bacterial DNA was extracted using NucliSENS EasyMag (bioMérieux) according to the manufacturer's instructions. The strain was identified as the species *Bacillus cereus*.

2.1.3 Isolation and screening of biosurfactants producing bacteria

The collected samples were diluted using serial dilution method and plated on LB media plates. The plates were incubated at $30 \text{ }^\circ\text{C}$ for 24 h. After incubation, some colonies were selected and re-cultured on new LB plates to obtain pure strain. The isolate was inoculated in 50 mL LB broth, and incubated in shaking incubator 140 rpm for 24 h at $30 \text{ }^\circ\text{C}$ for inoculum preparation. Preparation bacterial growth was conducted according to a previous study (Nejad *et al.*, 2020). Briefly, 10 mL of oil was added to 250 mL flask containing 100 mL enrichment culture solution. The enrichment cultures contained (g/L) K_2HPO_4 , 6.3; KH_2PO_4 , 1.8; NH_4Cl , 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1; $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 0.1; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.1 and 1 mL/L of trace elements solution, pH 7.0. The trace elements solution contained (g/L) H_3BO_3 , 0.03; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.02; Na_2MoO_4 , 0.006; $\text{CuSO}_4 \cdot 2\text{H}_2\text{O}$, 0.001 (Ahmadi *et al.*, 2019). The growth media were then incubated and shaken (Excella E24, New Brunswick Scientific, Edison, NJ) at $30 \text{ }^\circ\text{C}$ for 72 h. After this period, the flasks containing the bacterial growth media were removed from the incubator in preparation for the growth and biosurfactant production.

2.1.4 Biosurfactant production assessment

Oil displacement activity method (ODA) considered as the most common, rapid and easy method for preliminary screening of biosurfactant producing bacteria. The presence of biosurfactant was confirmed using this method as described previously (Kumari *et al.*, 2012). In this method, 50 μL of crude oil was added to a 15 cm diameter plate containing 40 ml of distilled water. After that,

15 μL of the cell free culture broth was added to the oil surface. The zone of oil displaced (clear zone) was measured to determine the biosurfactant activity.

2.1.5 Extraction of the biosurfactants

Extraction of the biosurfactants was conducted according to the method described previously (Parthipan *et al.*, 2017). The culture medium was centrifuged at 10,000 rpm for 15 min to remove the bacterial cells and then the pH of clear supernatant was adjusted to approximately 2.0 by adding 2M of HCl. The solution was kept at 4 °C for 24 h to precipitate. The precipitated supernatant was re-centrifuged at 10,000 rpm for 15 min to collect the pelleted precipitates and pH adjusted to 7.0. Crude biosurfactants were then extracted by chloroform: methanol (2:1, v/v) mixture. Evaporation of the organic solvent under vacuum yielded the biosurfactant.

2.1.6 Determination critical micelle concentration

Surface tension changes were measured to determine the critical micelle concentration (CMC) of solution of the isolated biosurfactants. Culture broth were centrifuged at 5000 \times g for 20 min to obtain cell-free broth. A tensiometer (Fisher Scientific Co., Pitts- burgh, PA) was used to measure surface tension at room temperature.

2.2 Optimization of oil recovery using flotation and biosurfactants .

2.2.1 Flotation tank

A (10 L) tank was used for the flotation experiments. The effectiveness of the flotation process depends on differences in densities of the treated materials. The tank is supplied with an air pump at the bottom to generate bubbles, and has a mixer at the bottom (Figure 1). This mixer spins at 1000 rpm which keeps the oil droplets in the suspension and disperses bubbles effectively. After the foam formation, petroleum materials are expected to stick to the bubbles. The foam with oil materials raised to the surface of the tank, while the other materials sedimented, forming a layer at the bottom of the tank. A skimmer, fixed at the surface of the tank was designed to skim the foam into a separate tank.

2.2.2 Flotation Experiment

After determination of the optimal conditions of separation conditions, the experimental was applied to recover oil from sludge design. The experiments were conducted in the flotation tank containing about 1 kg of oily sludge and about 8 L of distilled water was added and the mixture was mixed thoroughly with a mixer at the bottom of the tank in order to grind the semi-solid oil such as stones to achieve an

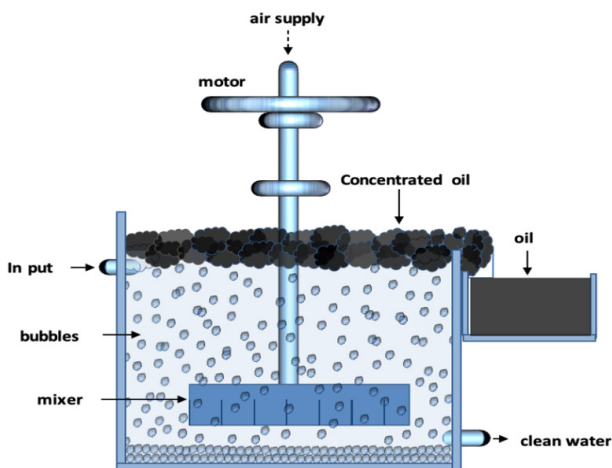


Figure1. A diagram illustrates froth flotation tank

appropriate size. The water level controlled. The water-sludge mixture was maintained by adding water when needed. The tank mixer speed was kept constant at 1000 rpm. Different temperature (20, 25, 30 and 35 °C), biosurfactants concentrations (0.1, 0.2, 0.5, 0.7), flotation time (10, 20, 30, 40 and 50 minutes) and air:water ratio (5, 10, 15, 20%) were examined for maximize separation. The experiments were conducted in duplicate to calculate the experimental error.

2.2.3 Oil Extraction from oily sludge and treated samples

Samples from oily sludge (before and after treatment) weighing one gram were carefully measured and placed into a glass vial with a volume of 45 mL. Subsequently, 30 mL mixture of acetone and hexane in a 1:1 ratio by volume was added to each vial. The vials were sealed using a cap lined with Teflon material and subjected to shaking on a rotary shaker running at a frequency of 120 cycles per minute for 4 hours. Then, the mixture was left in the room temperature to settle forward to the extraction process.

Dissolved oil samples, from treated samples by flotation alone and by flotation and biosurfactant together were extracted using carbon tetrachloride (CCl₄) as described previously (Grimalt, and Olivé, 1993). Briefly, 50 mL of CCl₄ was added to the sample, with vigorous shaking, then separated. After the sample settled, a small amount of Na₂SO₄ was added to remove water. The portion containing CCl₄ was collected then transferred to a separation column containing glass wool and a layer of 20 grams of Florisil to remove impurities. The purified sample was then collected and concentrated to 5 mL using a rotary evaporator. The concentrated sample was transferred to a clean 10 mL flask, and 5 mL n-hexane, was added for further analytical measurements.

The concentrations of oil samples before and after treatments were measured using GC-MS on an Agilent 6890N gas chromatography system or an Agilent 5975 mass spectrometer equipped with an auto sampler (Agilent 7683B) as described in previous study (Alabresm et al., 2018). The analytical GC

column was an Agilent TG-5MS (30 m, 0.25 mm I. D., 0.25 µm film thickness) coated with 5% phenylmethylsiloxane stationary phase. High-purity helium (99.9%) at a constant flowrate of 1.5 mL/min was used as the carrier gas. 1 µL of extracted sample was injected; with the injection port maintained at 250 °C in the splitless mode. Spectra were obtained in the electron impact mode (70 eV) scanning from 40 m/z to 400 m/z. The oven temperature was operated from 60 °C to 300 °C rising at 10 °C/min saturated hydrocarbons were monitored in the oil samples before and after the treatment. The investigation focused on aliphatic molecules within the standard range of carbon chain lengths, specifically C7 to C26. The substance is commonly mL employed as an injectant in gas chromatography for detection. This investigation assessed the number and quality of aliphatic and aromatic pollutants in sediment samples.

2.3 Statistical analysis

A one-way ANOVA was conducted using Prism software (version 7.00 for Windows, GraphPad Software, La Jolla California USA) to analyze for significant differences between treatments. Three replicates were used in each experiment.

3. Results and Discussion

3.1 Biosurfactant production assessment

Biosurfactants are compounds produced by microorganisms to enhance hydrocarbons emulsification by reducing oil-water interfacial tension and thereby increasing their availability for microbial degradation (Sogbanmu et al., 2019). They are considered as a sustainable option for recovering oily sludge compared to physicochemical processes (Silva et al., 2024). The use of these natural surfactants has been extensively explored since they are capable of mobilizing, emulsifying and solubilizing compounds, enhancing biodegradation processes in soil, as well as enabling bioremediation and wastewater treatment.

Several strains were isolated from different sites i.e. sediments, oily sludge

and wastewater. All were screened for biosurfactants production. Among all, *Bacillus* was selected as the best biosurfactants producing strain. Previous studies showed that *Bacillus* sp. is commonly known for producing biosurfactants from various environments and able to survive and adapt to a wide range of conditions (Jiang et al., 2024). The biosurfactant production assessment experiment showed that the purified biosurfactant from the strain was added to the center of a crude oil surface. The results demonstrated a clear zone on the oil surface compare with the control with no biosurfactant. The surface activity was markedly high concentration of biosurfactant (Figure 2). These results confirmed the presence of biosurfactant in the cell free culture supernatant. The high emulsification indicated that the hydrocarbons compounds become more bioavailable to bacteria and support faster degradation of crude oil. Many previous studies have reported that *Bacillus* sp. are efficient in production of biosurfactant and thus they have been used for many applications in oil industry (Parthipan et al., 2017).

3.2 Effect of flotation parameters on separation of oil from oily sludge

3.2.1 Biosurfactant concentration

The critical micelle concentration of biosurfactants test was used to determine the minimum concentration of the biosurfactant required for maximum reduction of water

surface tension of solution. The results showed the surface tension reduced from 70 to 25 mN/m when biosurfactants concentration increased up to 0.2 g/L (Figure 3). However, it remained constant at the same previous measurement. This is indicated that the critical micelle concentration of biosurfactants is 0.2 g/L. It has been reported previously that 0.2 g/L was effective of lower the surface tension between oil and water (Wang et al. 2024). These findings are close to or better than those found in other previous studies. For example, a study indicated 3.0 g/L of a biosurfactant reduced a surface tension to 29.52 mN/m (Chafale, and Kapley, 2022). Another study used 8.5 g/L of biosurfactant to achieve a surface tension of 25 mN/m (Luna et al, 2013). The different in CMC values may be attributed to differences in some factors such as biosurfactants purity and composition, bacterial strain and its cultivation (Silva et al., 2018).

3.2.2 Air-water ratio

Air-water ratio is most common parameter used in the development of flotation air system (Alegria et al., 2021, and Lei et al., 2024). It was indicated in this study that an increase in oil removal with an increased in air-water ratio from 5-20 %. The results showed that 15 % is the optimal ratio for oil removal (Figure 4). It was also noted that a further increase in air-water ratio beyond 15% resulted in a decrease in oil removal. The required air- water ratio is determined by the concentration and size of the sludge particle (Alegria et al., 2021).

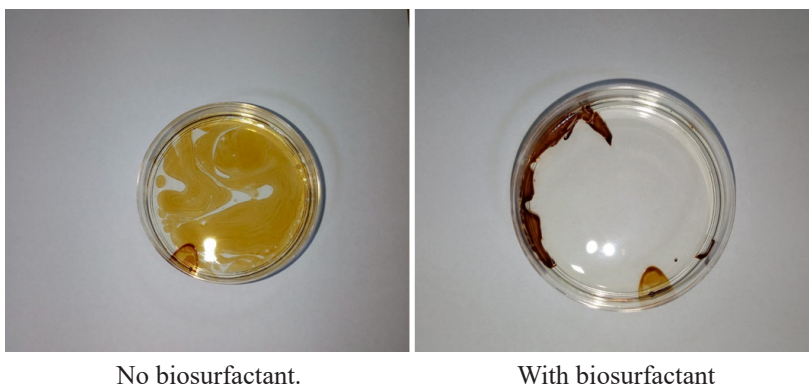


Figure 2. Biosurfactant activity on surface contains oil

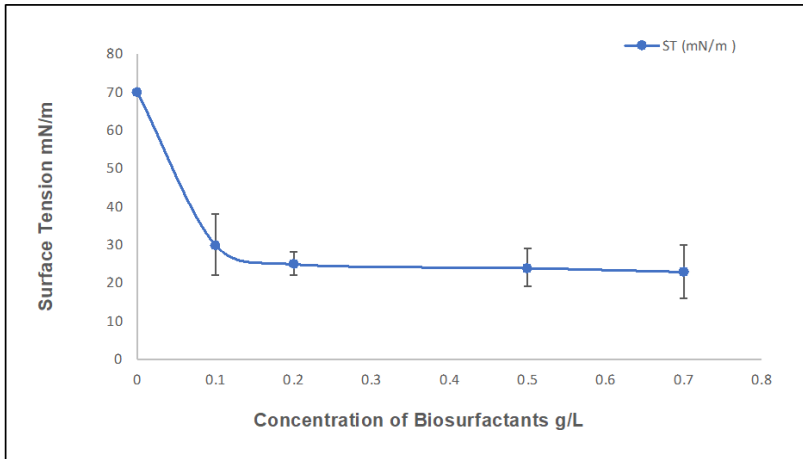


Figure 3. Critical micelle concentration of the biosurfactant

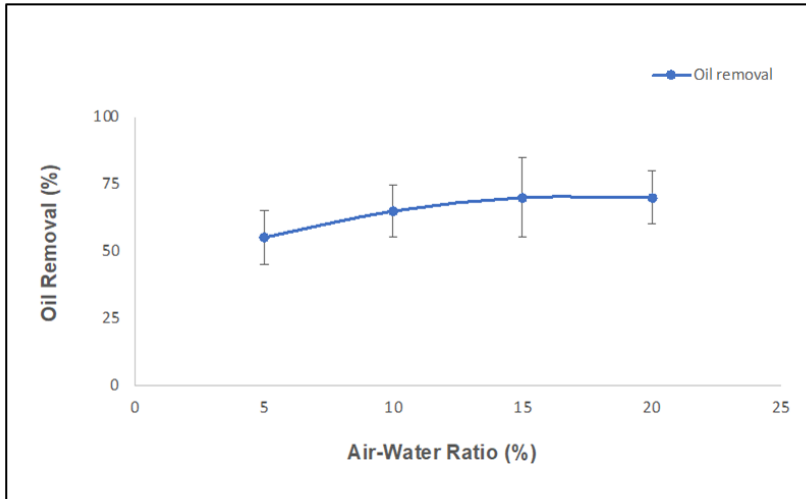


Figure 4. Effects of air-water ratio (%) on oil removal

3.2.3 Flotation Time

The flotation time refers to the contact time between oil droplets and air bubbles. Figure 5 illustrates that a longer flotation time resulted in increasing oil removal dramatically. It was found 25 min to be optimal for oil removal. When flotation time was increased, it enhanced the probability of oil droplets attachment to air bubbles, and increasing movement of the oil to the surface. However, it was noted the longer flotation time beyond 25 min resulted in a decrease in air bubbles and leading to a decrease in oil removal. This decrease could be attributed to the distribution by shear stress in the contact zone between oil droplets and air bubbles

(Yan *et al.*, 2014). Therefore, the flotation time set on 25 min.

3.2.4 Flotation Temperature

The oil removal changed with different flotation temperature. Figure 6 showed that the oil removal increased with the increased temperature of flotation solution. The optimal temperature indicated was 30 °C. However, the removal rate remained constant beyond 30 °C. Increasing the temperature of sludge solution could reduce the of oil viscosity, and it was easier to separate oil from the solid surface (Li *et al.*, 2021). It was also noted high temperature caused evaporation of water and organic substances (Lv X *et al.*, 2020 and Yan *et al.*, 2014).

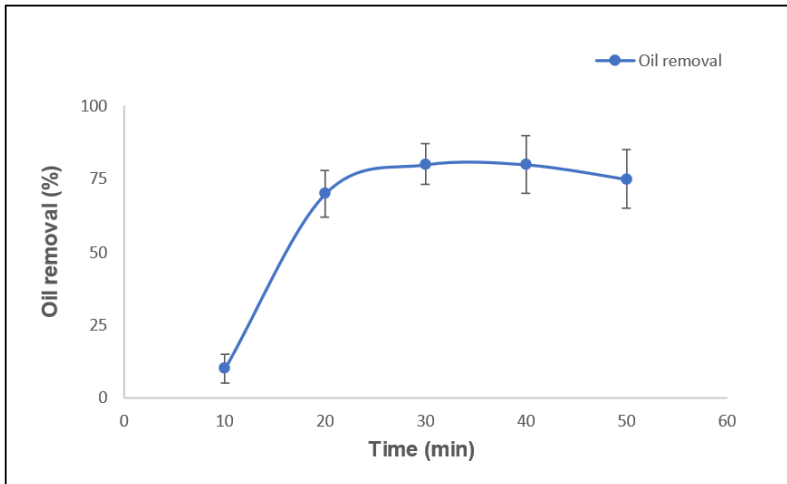


Figure 5. Effects of time on oil removal

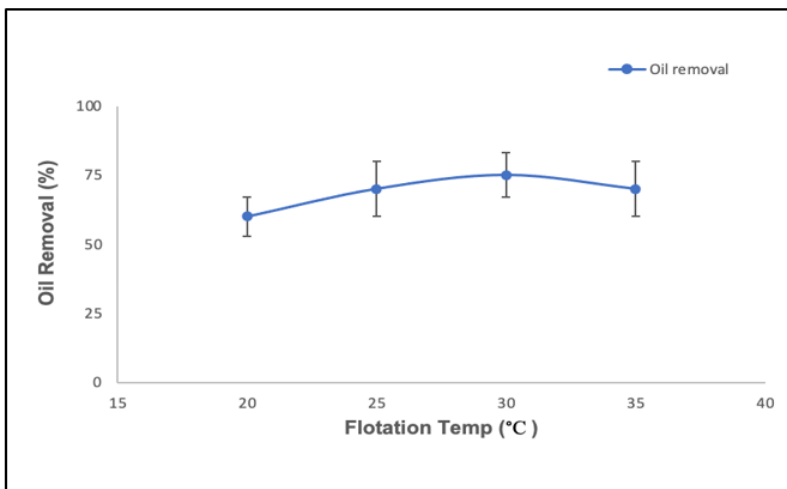


Figure 6. Effects of temperature on oil removal

3.2.5 Optimum Crude Oil Removal using air flotation and biosurfactants

The oil removal from oily sludge was carried out using the air flotation (with and without) biosurfactants after the optimization of the operational conditions. It was noticed that the oil removed by any parameter had an effect on the other parameter. Therefore, the results of this study will be explained based on the parameters have been examined. In this study the optimal parameters were found as 0.2 g/L biosurfactants concentrations, 15% air-water ratio, 25 min flotation time and 30 °C flotation temperature. Considering

these optimum conditions obtained from this study, the experiments were conducting with and without biosurfactant to determine the efficiency of the combination method. The results indicated that when mixing the sludge with water and then treated with biosurfactants led to break oil emulsion, and let the dissolved air flotation removing most of the emulsified oil in addition to the original free oil content. The percent removal of oil compounds was enhanced significantly ($p < 0.05$) when biosurfactants added to the flotation system compared with using flotation alone (Figure 7). The results showed after the treatment that some of the hydrocarbon compounds

(n-alkanes C9 - C22) were completely removed. On the other hand, the other hydrocarbon compounds (n-alkanes C23-C26) the removal rate ranged between 80% and 90%. Whereas, the experiment performed with the optimized conditions without biosurfactant resulted in an oil removal rate of about 60% using the air flotation alone. This finding clearly demonstrated the importance of adding biosurfactant as frothers in the separation process.

Action of the biosurfactant is dissolving crude oil and desorbing it from the sludge surface to the solution. Finally, Figure 8 clearly shows that the oil was removed from oily sludge near completely. Quantitative

measurement of the recovered oil from the sludge indicated more than 90% of the oil can be recovered. This method is a promising technique not only for removing oil but also for oil recovery with little loss. The results also revealed that the combined approach can remove oil compounds at very low concentrations as well as other pollutants such as algae and cyanobacteria under laboratory conditions. This suggests that this technique could be efficient and sustainable alternative to synthetic surfactants. However, further studies need to be performed at field scale in order to provide validation of this method.

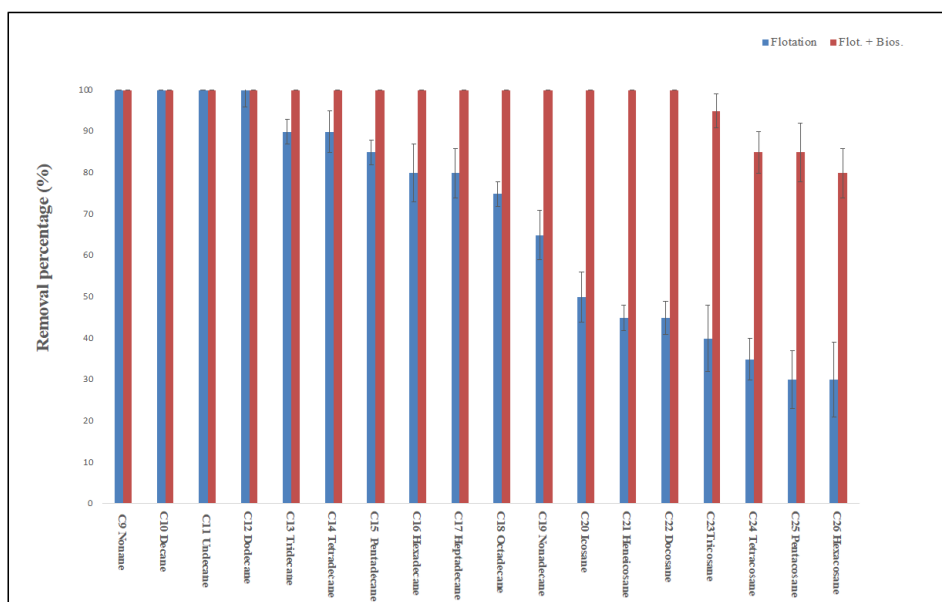
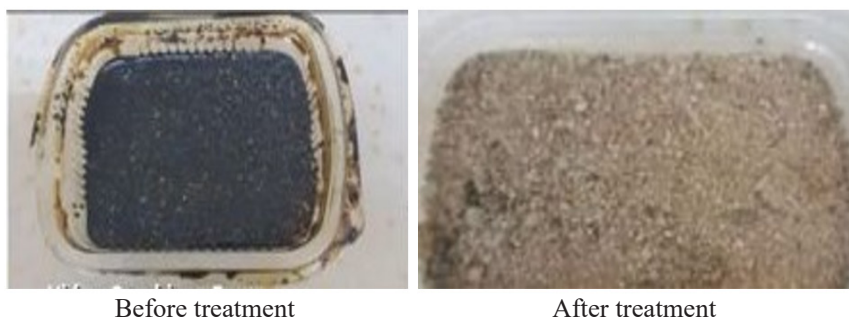


Figure 7. GC–MS results of n-alkane removal by flotation only and flotation + biosurfactants (Error bars represent one standard deviation (n = 3))



Before treatment

After treatment

Figure 8. Oily sludge before and after treatment

4. Conclusion

This study optimized a combined biosurfactant and froth flotation method for oil recovery from oily sludge and compared it with froth floatation alone. The results demonstrated a significant improvement in oil recovery when biosurfactants combined with flotation by reducing surface tension and enhancing the separation of oil. Optimal flotation parameters were determined and led to a significant increase in oil removal efficiency. These findings confirmed the effectiveness of biosurfactants sustainable and environmentally friendly in improvement of flotation method for oily sludge remediation. Further studies are required for large-scale applications and long-term sustainability.

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