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Evaluation of biosurfactant potential from *Brevibacillus* sp AVN13 for remediation of hydrocarbons and heavy metals

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Abstract: Bioremediation with the help of biosurfactant is one of the promising technologies in treating the hydrocarbon and heavy metal contaminated sites. Biosurfactant was produced naturally by microorganism and hence biodegradable, which is alternative to chemical surfactant for various applications. In this study the biosurfactant producing strain Brevibacillus sp. AVN 13 was used for hydrocarbon and heavy metal remediation. The biosurfactant enhanced biodegradation assay of used engine oil by Brevibacillus sp. AVN 13 was carried out using Erlenmeyer flask experiment and the degradation of hydrocarbon removal rate was analyzed by gas chromatography. The Brevibacillus sp. AVN 13 is having ability to degrade 72 % of hydrocarbon. Then biosurfactant assisted chromium removal process was carried out. The effect of initial chromium concentration, effect of pH, incubation time and concentration of biosurfactant were studied. The removal rate of chromium using biosurfactant was found to be 72.54 % under optimized conditions. Overall this study proved that, biosurfactant from Brevibacillus sp. AVN13 can be effectively used for degradation of oil spilled and heavy metals contaminated sites.

Keywords: Biosurfactant, hydrocarbon, heavy metals, gas chromatography, *Brevibacillus* sp.

1. Introduction: The heavy metals and petroleum hydrocarbons adversely affect the quality and fertility of soil and vegetation thriving over there. The polycyclic, n-alkane and aromatic petroleum hydrocarbons are omnipresent in the environment today and are considered as persistent organic pollutants that have even been reported as potential carcinogens involving considerable public health hazards **[1, 2]**. In general, engine oil is used for lubricating various types of automotive engines. During engine wear, engine oil prefers a number of additional components such as chlorinated hydrocarbons, naphthalene, sulfur and other materials such as heavy metals (chromium, lead and cadmium). After a period of time, engine oil should be changed for automotive engine due to change in viscosity of engine oil. Any such oil rich in contamination and unsuitable for original use is known as used engine oil **[3]**.

In the present study, used engine oil is the subject of interest. A large amount of used engine oil generating from workshops and automobiles are not properly discharged into the environment. Improper disposal of oil percolates into soil and cause contamination of soil and ground water. This contamination in turn affects the biological cycle and inhibits the plant development. Further, when the contaminated ground water mixes with water resources, it causes a severe damage to the marine environment. According to US EPA, one gallon of used engine oil contaminates one million gallons of fresh water (US.EPA, 1994) **[3]**. These pollutants present in the aquatic and terrestrial environment can cause hazards to socio-economic and public health.

Currently, various technologies like physical, chemical and biological methods are available to treat oil spilled sites. Chemical and physical methods were not efficient and having many drawbacks **[4 - 6]**. In biological method, during biodegradation of hydrocarbon, the microbe produces surface active molecules which are known as Biosurfactant. A surface active molecule accelerates the degradation of hydrocarbon by emulsifying the hydrocarbon present in the solution and also increases the surface area for microbial attack. Biosurfactant is an emerging tool for remediation of hydrocarbon and oil contaminated soil **[7, 8]**. Moreover biosurfactants are used for remediating the heavy metals (lead, cadmium, uranium) contaminated sites. At present, all surfactants are chemically derived from petroleum and used for different purpose. Due to increasing environmental concerns, low toxicity, biodegradability and low production cost, microbial surfactants have gained interest in recent decades. So biosurfactants are considered as alternative source to chemically synthesized surfactants **[9]**.

2. Experimental:

2.1 Microbial source

In the previous study, a biosurfactant producing organism was isolated from crude oil contaminated soil and identified as *Brevibacillus sp*. AVN 13. The same bacterial culture was used in this study also **[10]**.

2.1 Biosurfactant enhanced biodegradation of used engine oil

The degradation efficiency of hydrocarbons presented in used engine oil using biosurfactant producing bacteria and purified biosurfactant was conducted. In order to evaluate the same, the degradation study was carried out using MS media adapting optimized parameters and conditions. Four sets of Erlenmeyer flasks were filled equally with 500 ml of optimized MSM containing 5 % (v/v) of used engine oil. After sterilization at 120 °C for 20 minutes and cooling the broth, microbial culture solution (2 %, v/v at OD600 of 1.0, precultured in MSM supplemented with 1 % v/v of used engine oil) and purified biosurfactant (40 mg) produced by biosurfactant producing bacteria were added into the corresponding shake flasks. The mineral salt medium (MSM) containing following components (g/l): KH_2PO_4, 0.7; Na_2HPO_4, 0.9; $NaNO_3$, 2.0; NaCl, 4; $MgSO_4.7H_2O$, 0.4; $CaCl_2.2H_2O$, 0.1; $FeSO_4.7H_2O$, 2.0; $MnSO_4.H_2O$, 1.5; $(NH_4)_6MO_7O_{24}.4H_2O$, 0.6; engine oil 1% (v/v) was used as sole carbon source. The medium was sterilized by autoclaving at 121 ºC for 15 min **[11]**.

The experimental design is as follows.

Experiment 1 Mineral salt medium + Used Engine Oil (5 %, v/v)

Experiment 2 Mineral salt medium + Heat Killed Bacterial cells (2 %, v/v) + Used Engine Oil (5 %, v/v)

Experiment 3 Mineral salt medium + Bacterial cells (2 %, v/v) + Used Engine Oil (5 %, v/v)

Experiment 4 Mineral salt medium + Bacterial cells (2 %, v/v) + Used Engine Oil (5 %, v/v) + Biosurfactant (80 mg/L)

The flasks were kept incubated at optimum temperature with shaking at 45 rpm for 20 days. The residual used engine oil that remained in the culture medium was extracted using hexane by liquid – liquid extraction process. Further the concentration of residual oil was estimated by gas chromatography analysis **[12]**. The n-alkane distributions presented in the residual fractions was analyzed by Agilent technologies 7890, A Gas Chromatograph, coupled Mass Spectrometer model No -5975 C with triple-axis high energy diode (XL) electron ionization detector and polar capillary column RTX-5 (30 mm length, 0.32 mm internal diameter and 0.25 µm film thickness). The hexane extracts of residual used engine oil (1 µL) was injected into the sample port using a Hamilton syringe. Nitrogen was used as a

carrier gas. The initial column temperature was programmed at 70 ºC; the injector temperature was maintained at 290 ºC and was held for 2 min, then the temperature was ramped at a rate of 10 ºC per minute upto 320 ºC. Further the detector temperature was maintained at 300 ºC. The relative degradation of used engine oil was calculated by the differences in the total peak area obtained for each petroleum hydrocarbon.

2.2 Hexavalent chromium removal from synthetic wastewater using biosurfactant

2.2.1 Preparation of chromium wastewater and determination of Cr (VI) concentration

The hexavalent chromium stock solution is prepared by dissolving potassium dichromate (K2Cr2O7) crystals in distilled water. A stock solution of 1000 mg/L is prepared by dissolving 2.835 g of potassium dichromate in one liter of distilled water. Further, the working solution of various concentrations was prepared by diluting the stock solution with required amount of distilled water. The hexavalent chromium concentration was analyzed by spectrophotometric analysis using DPC (1,5-Diphenylcarbazide) at wavelength of 540 nm. A standard calibration curve for hexavalent chromium (Cr VI) was constructed by using the standard hexavalent chromium (Cr VI) at various concentrations.

2.2.2 Batch experiments for hexavalent chromium Cr (VI) removal

The biosurfactant assisted hexavalent chromium (Cr VI) removal process is carried out in an Erlenmeyer flask of 250 mL capacity. A volume of 100 mL of hexavalent chromium (Cr VI) was taken in flask and agitated in incubatory orbital shaker at 37 °C by varying the different experimental parameters like initial (Cr VI) concentration (20 to 100 mg/L), pH (2 to 10), biosurfactant concentration (20 to 140 mg/L), time (15 to 105 min). The pH of the solution is adjusted using 0.1 M HCl and 0.1 M NaOH in pH meter. The residual concentration is determined at regular interval of time. The residual sample is collected and subjected to the U-V analysis by 1,5 diphenyl carbazide method at 540 nm. The percentage removal of hexavalent chromium (Cr VI) is calculated using the following equation:

$$\%\ removal = \frac{C_i - C_F}{C_i} \times 100$$

C_i is the initial Cr VI concentration and C_f is the final Cr VI concentration.

3. Results and discussion:

The applications of biosurfactant produced by *Brevibacillus* sp. AVN13 in hydrocarbon degradation and bioremediation of heavy metals were studied and their results are discussed in this section.

3.1 Biosurfactant enhanced biodegradation of used engine oil

The GC fingerprints of biodegradation studies clearly display the significant changes that have happened within the hydrocarbon components during the period of this study. The period of study lasted for 20 days, comprising of 4 sets of experiments as mentioned before in section 2.1. From the GC results, it has been observed that the used engine oil contained abundant alkane compounds with high molecular weight of carbon numbers from C24-C29 and while those in the low molecular weight range with carbon number of C18–C25. The GC fingerprints, Figure (1) of the untreated used engine oil showed range of hydrocarbon fractions from C19–C40, with intense peaks in range of C23-C27.

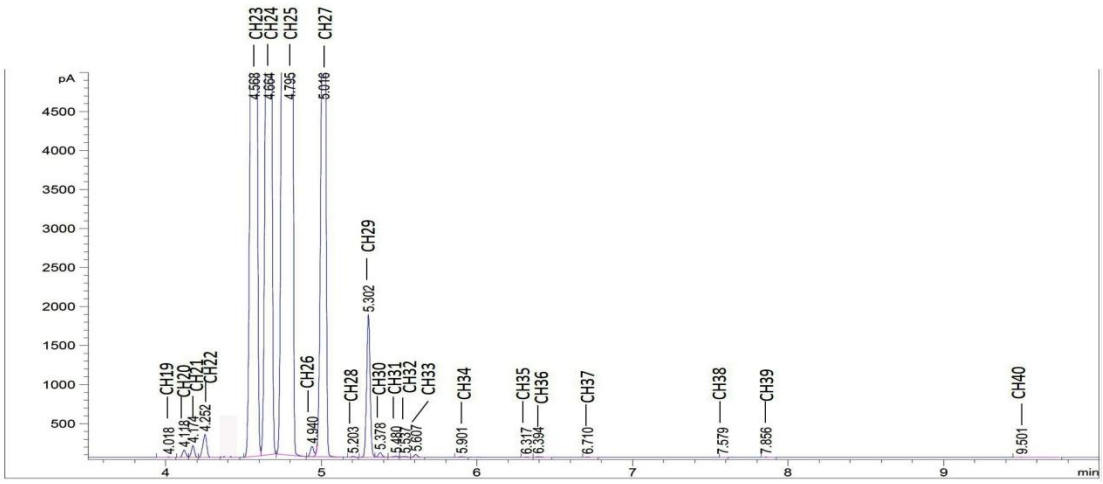

Figure (1): Gas chromatogram of untreated used engine oil (Sterile Control).

In the experiment-2, the used engine oil was treated with heat killed bacterial cells in order to know whether the bacterial cell surface substances have any influence on biodegradation process. The results displayed in Figure (2) indicates that no significant difference between the untreated and heat killed *Brevibacillus* sp. AVN13 cells treated used engine oil, whereas a minor elevation in C_{21} peak might be due to the presence of n-alkanoic fatty acids present in the cell wall of most methanogic and halophilic bacteria in order to maintain cell-wall fluidity .

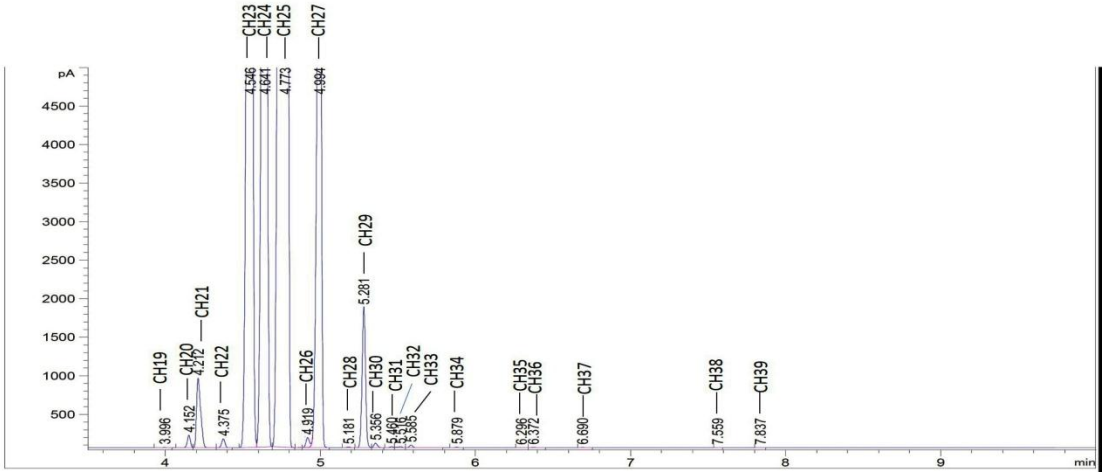

Figure (2): Gas chromatogram of used engine oil treated with heat killed *Brevibacillus* sp. AVN13 cells.

In the chromatogram of experiment-3, a visible reduction in C_{10}-C_{36} alkanes was observed in Figure (3). The highest peaks ranged from C_{25} – C_{27} have reduced to 28 % than compared to the untreated batch. The considerable reduction in C_{19} - Nonadecane, C_{20} - Icosane, C_{21} – Heinecosane was seen. The prominent hydrocarbons C_{28}, C_{38} and C_{39} peaks had completely disappeared with the treatment of *Brevibacillus* sp. AVN13.

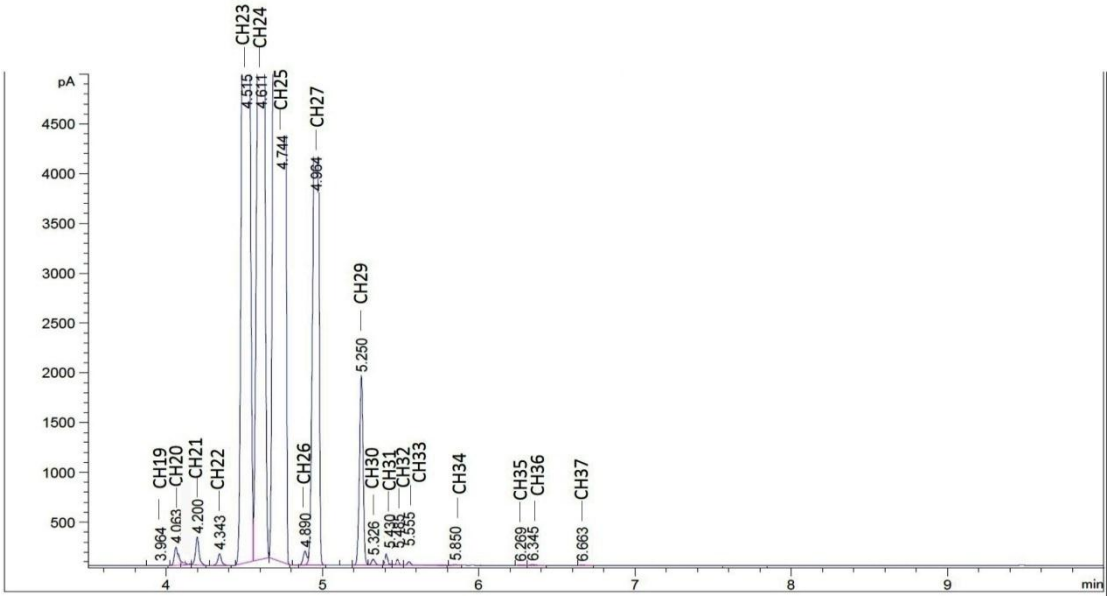

Figure (3): Gas chromatogram of used engine oil treated with *Brevibacillus* sp. AVN13 for 20 days.

The GC fingerprints of experiment-4 showed drastic reduction in most of the peaks and are shown in Figure (4). The prominent C_{23}, C_{24}, C_{25} peaks had reduced to less than 65 % of their original height. The mono- and dimethyl-alkanes C_{30}, C_{31}, C_{32} and C_{33} remained in traces. The Heinecosane, Docosane and hopane series (C_{35} – C_{37}) have completely disappeared. Except the tetracyclic terpene hydrocarbons (C_{24} – C_{27}) remained prominent in almost all chromatograms and remained least in the degradation trend. In general the overall degradation level is greater than 70 percent.

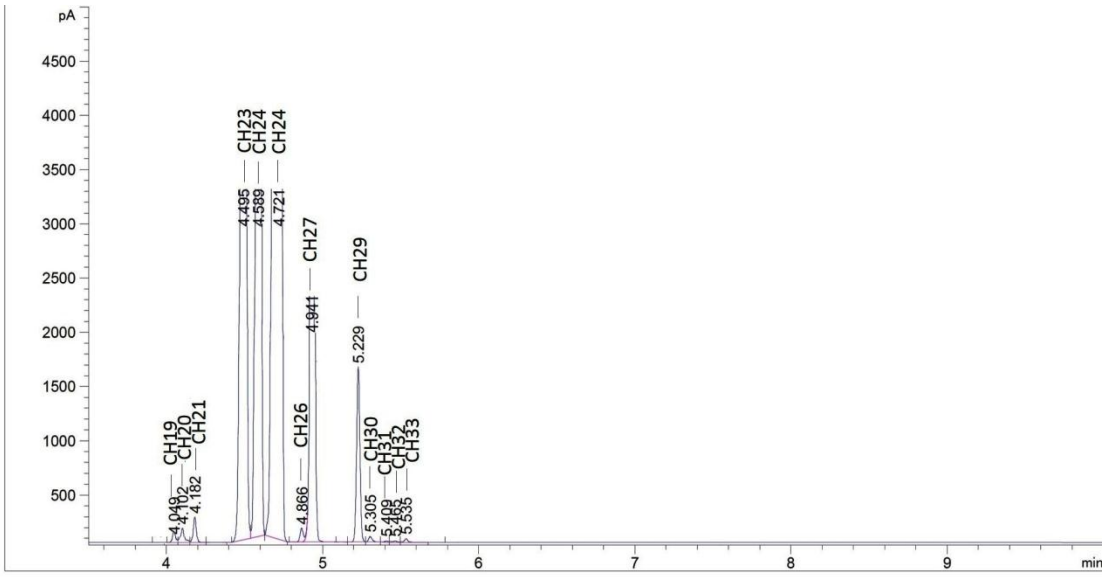

Figure (4): Gas chromatogram of used engine oil treated with *Brevibacillus* sp. AVN13 and supplemented with purified biosurfactant (80 mg/L).

Obayori **[13]** observed biodegradation of hydrocarbons in fresh and used engine oils by *Pseudomonas aeruginosa* LP5 for 21 days. The hydrocarbon peaks greater than C20 were found to be completely disappeared on Day 21, whereas the medium fraction ranges of C14, C15, and C17 remained discernible. The degradation rates were also found to differ on oil types. M. Bhattacharya **[14]** performed degradation studies using waste engine oil. The waste engine oil degradation was observed in Bushnell-

Haas medium. 71.52 % degradation was observed in 7 days. H. Mehdi **[15]** has reported the relationship between alkane degradation and cell surface hydrophobicity in crude oil-degrading bacteria isolated from Persian Gulf.

3.3 Hexavalent chromium removal from synthetic wastewater using biosurfactant

3.3.1 Effect of initial Cr (VI) concentration:

The effect of initial Cr (VI) concentrations on removal of metal ions from aqueous solution by the novel lipoprotein biosurfactant produced by *Brevibacillus* sp AVN13 was experimentally determined by varying the Cr (VI) concentrations from 20 to 100 mg/L. Figure (1) shows the percentage removal of chromium ions using lipoprotein biosurfactant. The percentage removal of chromium ions was found to be decreased from 72.9 % to 65.36 % as the concentration of Cr (VI) increased from 20 to 100 mg/L Figure (5). The results obtained suggested that at higher concentration, the metal ions were larger which are competing for the functional available in the biosurfactant **[16, 17]**. Also, there is a repulsion which retards the binding of metals ions **[18, 19]**. In addition, molar ratio of lipoprotein biosurfactant to metal ions is low at an elevated initial metal ions concentration. This condition reveals that biosurfactant is strong enough to bind with metals with threshold capacity. However, it loses its capacity on elevated level of critical concentration **[18]**. Thus, removal was found to be lower at higher Cr (VI) concentration; however, at lower Cr (VI) concentration the number binding groups are proportion, hence the removal was maximum **[19, 20]**.

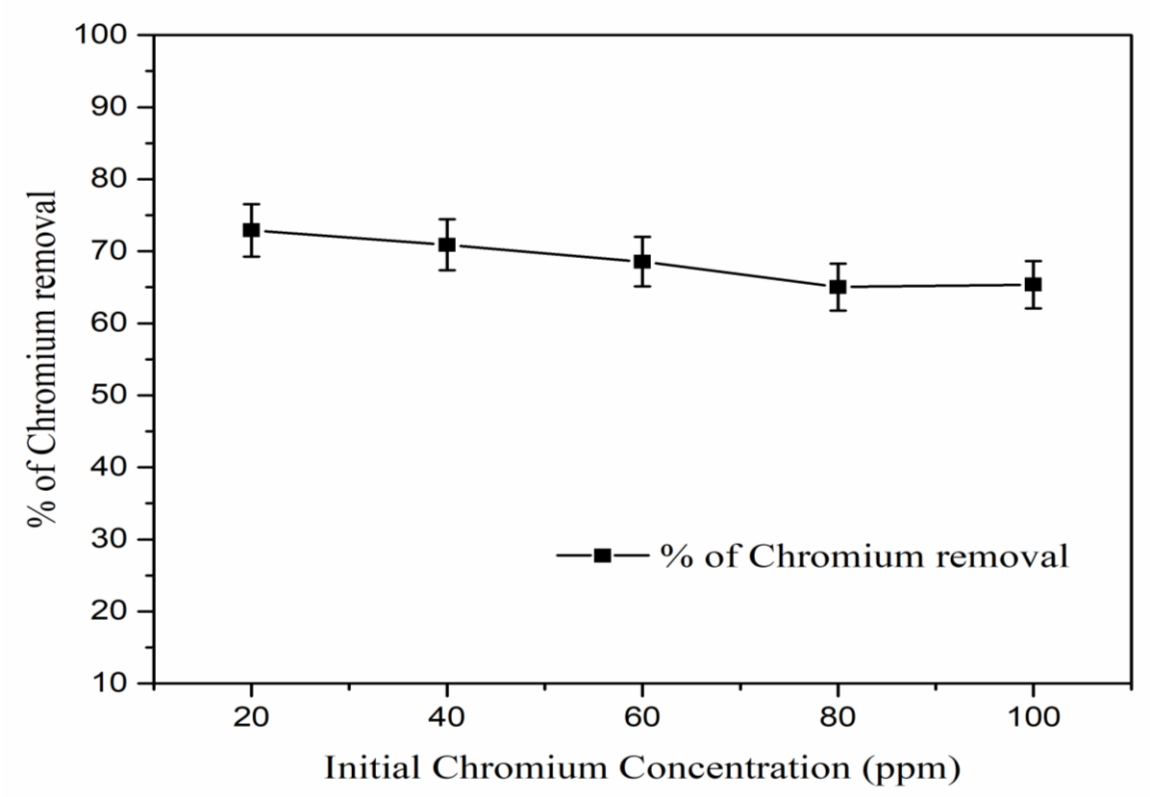

Figure (5): Effect of Initial Cr (VI) Concentration (V: 100 mL;
Biosurfactant Concentration: 50 mg/L; Agitation: 250 rpm; Temp: 37˚C; pH: 6.0).

3.3.2 Effect of time on Cr (VI) removal:

The effect of time on removal of Cr (VI) metal ions from aqueous solution is shown in Figure (2). The results showed that there is a gradual increase in the percentage removal of Cr (VI) from 27 % to 73.72 % between the time intervals of 15 min to 60 min respectively as in Figure (6). Further, increase in time (105 min) has led to decrease in the percentage removal (68.11 %). Thus, the optimum for the removal of metal ions was found to be 60 min. The previous study report showed that chromium has high affinity with lipopepetide biosurfactant when compared with other heavy metals **[18]**. A similar kind of trend is followed in our present study which indicates high binding capacity of our novel biosurfactant produced from *Brevibacillus* sp AVN13 **[10]**.

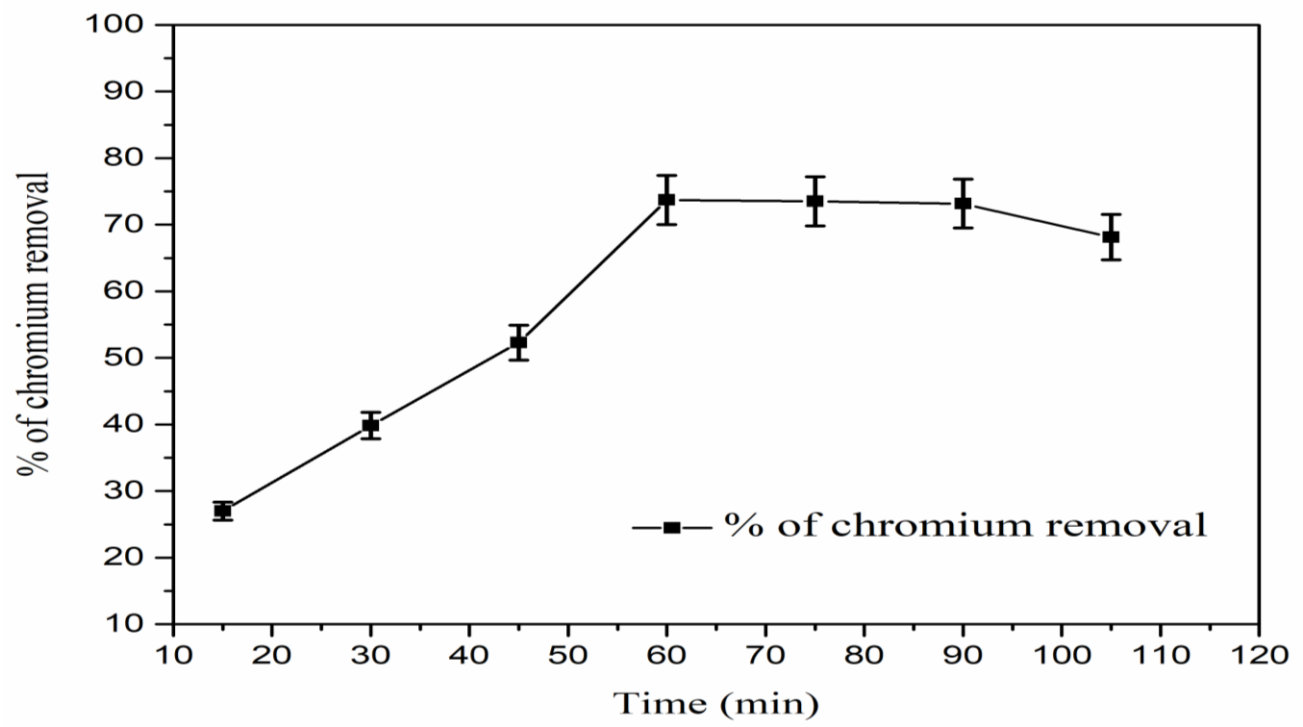

Figure (6): Effect of Time on Cr (VI) removal (C_O = 100 mg/L V: 100 mL; Biosurfactant Concentration: 50 mg/L; Agitation: 250 rpm; Temp: 37°C; pH:6.0)

3.3.3. Effect of pH on Cr (VI) removal

A vital role is played by pH in the solution chemistry of Cr (VI) during the sequestration using novel lipopeptide biosurfactant produced from *Brevibacillus* sp AVN13 **[10]**. In the present study, the pH experiment was performed to check the optimum pH by varying the pH between 2 to 10. Figure (7) shows the effect of pH on removal of Cr (VI) using lipopeptide biosurfactant produced from *Brevibacillus* sp AVN13. From the Figure (3), it is observed that as the pH was increased from 2 to 6 there was an increase of Cr (VI) removal percentage from 40.28 % to 74.11 % respectively. However, at pH 10 the Cr (VI) removal percentage was recorded as 67.33 %. This result indicates that removal was more or less maximum at neutral condition **[18]**. The previous FTIR characterization study report the lipopeptide biosurfactant produced from *Brevibacillus* sp AVN13 showed that the active site contains amino groups **[10]**. So, at low pH, the functional groups get protonated and therefore there is a repulsion of metal ions **[18]**. Therefore, metal ion removal was poor at highly acidic condition. Perhaps, at alkaline condition, there is a completion between the metal ions and chelating ion (OH^-) to bind with the active surface groups of lipopeptide biosurfactant molecules, eventually, the hydroxyl ions occupy

the active sites and leave behind the Cr (VI) ions back into solution **[21, 22]**. Hence, the Cr (VI) removal percentage was higher at pH 6.0 close to neutral pH level **[18]**.

3.3.4. Effect of biosurfactant concentration on Cr (VI) removal:

The effect of biosurfactant concentration on the removal of Cr (VI) ions was studied at different concentrations of lipopeptide biosurfactant produced from *Brevibacillus* sp AVN13 ranging from 20 to 140 mg/L. The removal efficiency of Cr (VI) ions was increased (31.56 % to 73.78 %) with increasing

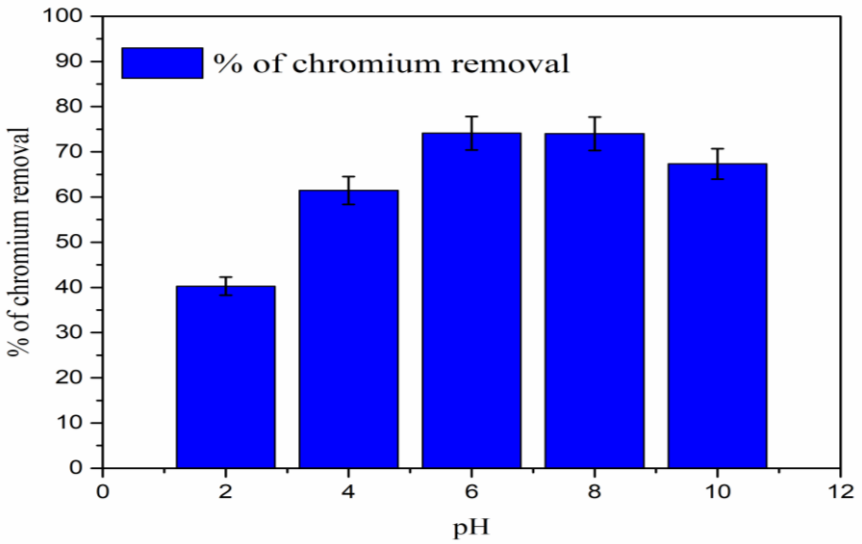

Figure (7): Effect of pH on Cr (VI) removal (C_O = 100 mg/L V: 100 mL; Agitation: 250 rpm; Biosurfactant Concentration: 50 mg/L; Temp: 37 ºC).

biosurfactant concentration (20 to 60 mg/L) as shown in Figure (8). But, a further increase in biosurfactant concentration from 80 to 150 mg/L has a negative impact on removal percentage of 73.56 % to 60.83 %. This is attributed by splitting the effect of flux between the lipopeptide biosurfactant and Cr (VI) ions **[23, 24].** The low level dosage of lipopeptide biosurfactant is favorable for the heavy metal sequestration which might be due to the critical micelle concentration **[25]**.

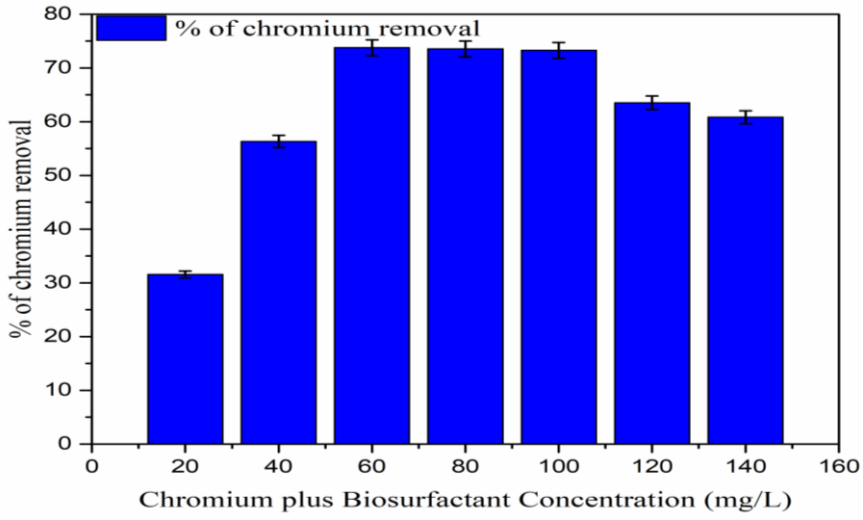

Figure (8): Effect of biosurfactant concentration on Cr (VI) removal (C_O = 100 mg/L V: 100 mL; Biosurfactant Concentration: 20 to 140 mg/L Agitation: 250 rpm; Temp: 37˚C).

4. Conclusions: In this study, the biosurfactant enhanced biodegradation assay of used engine oil by *Brevibacillus* sp. AVN 13 was carried out using Erlenmeyer flask experiment and the degradation of hydrocarbon removal rate was analysed by gas chromatography. The *Brevibacillus* sp. AVN 13 is having ability to degrade 72 % of hydrocarbon. The biosurfactant assisted chromium removal process was investigated. The experimental studies showed that effect of initial chromium concentration, effect of pH, incubation time and concentration of biosurfactant had a strong effect on chromium removal process. The removal rate of chromium using biosurfactant was found to be 72.54 % under optimized conditions. These results suggested that *Brevibacillus* sp. AVN 13 could be effectively employed for biosurfactant production. From the biodegradation assay of hydrocarbon and heavy metal removal process, it could be concluded that *Brevibacillus* sp. AVN 13 can be used for the degradation of oil spill effluents and also to remediate heavy metal contaminated sites.

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