International Journal of GEOMATE, Aug, 2017, Vol.13, Issue 36, pp.75-79
Geotec., Const. Mat. & Env., ISSN:2186-2990, Japan, DOI: http://dx.doi.org/10.21660/2017.36.2842

ENHANCED OIL RECOVERY USING BIOTRANSFORMATION TECHNIQUE ON HEAVY CRUDE OIL

Abdullah Al-Sayegh[1], *Yahya Al-Wahaibi[1], Saif Al-Bahry[2], Abdulkadir Elshafie[2], Ali Al-Bemani[1] and Sanket Joshi[3]

[1]Department of Petroleum and Chemical Engineering, Sultan Qaboos University, Oman
[2]Department of Biology, Sultan Qaboos University, Oman
[3]Central Analytical and Applied Research Unit, Sultan Qaboos University, Oman

*Corresponding Author, Received: 08 June 2016, Revised: 14 August 2016, Accepted: 12 Oct 2016

ABSTRACT: Abundance of heavy crude oil resources and costly enhanced oil recovery (EOR) techniques necessitate development of inexpensive heavy oil recovery methods. Microbial EOR (MEOR) techniques are environmentally friendly and need little input of energy to produce MEOR agents. One potential application of MEOR is in the biotransformation of heavy oil where bacteria break heavier fractions of heavy crude oil to lighter compounds; thus, improving oil recovery. In this study, two spore forming bacteria: Bacillus subtilis AS2 and Bacillus licheniformis AS5, which were isolated from heavy oil (13.3 °API) contaminated soil samples from a heavy oil field, Oman, were tested for their biotransformation abilities. Bacterial growth was analyzed by optical density measurements and heavy crude oil recovery was determined by core flooding experiments. At aerobic biodegradation flask experiments, M2 medium spiked with glucose had the highest bacteria growth and crude oil biodegradation in comparison with: (1) M2 medium with no added chemicals and (2) M2 medium spiked with sodium thiosulfate. At anaerobic in situ conditions Berea sandstone core flooding experiments, additional 2.9% and 3.1% of residual oil saturation was recovered by B. subtilis AS2 and B. licheniformis AS5 respectively after one week of incubation. By increasing the incubation time and inoculation percentage for isolate AS5, the oil recovery increased to 5.0%. When glucose was added to M2 medium, AS5 oil recovery increased to 16.4%.The results showed that locally isolated bacterial strains have the potential for biotransformation of heavy oil and enhanced oil recovery.

Keywords: Enhanced Oil Recovery, Heavy Oil, Biotransformation, B. subtilis, B. licheniformis

1. INTRODUCTION

Low quality heavy crude oil resources are estimated at seven times that of conventional crude oils [1] and are hard and costly to produce since expensive enhanced oil recovery (EOR) techniques are needed to extract the viscous crude oil to surface. In comparison to other EOR techniques, microbial EOR (MEOR) needs little input of energy to produce oil recovering agents, i.e. the microorganisms and their metabolic products at such technique. Also, they do not directly depend on the global crude oil price [2]. MEOR is environmentally friendly compared to conventional EOR [3]-[4]. Biotechnological applications at the petroleum industry include a very wide range of applications such as biotransformation, biosulfurization, biodenitrogenation and biocatalysis [5].

Microbial biotransformation of crude oil include all activities that make it easier to produce and transport, as well as the chemical changes that increase the value of the oil [6]. Crude oil biotransformation involves utilization of crude oil as a substrate for introduced microbiological population [7] and alteration of physical properties through bioproducts [4]. Molds, yeasts, algae and protozoa are not suitable for *in situ* reservoir conditions because of their size or inability; only bacteria are applicable at such conditions [5]. There are more than 175 genera of bacteria that are able to grow using hydrocarbons as sole or major carbon source [8]. Crude oils are essentially complex mixtures of hydrocarbons and other organic and inorganic compounds. Carbon is the main constituent of crude oils representing 85% to 90% of the crude, followed by hydrogen (10% to 14%) and non-hydrocarbon elements such as nitrogen, sulfur and oxygen and organo-metallic compounds [9-12]. Heavy crude oil contains substantial quantities of complex hydrocarbons, heteroatoms and metal contents, which are costly to process. [13, 12].

Soil contamination with crude oil drastically changes the soil's physical and chemical properties to the extent that it jeopardizes the safety of constructions on contaminated soil [14]. It also causes microbial (archaea, bacteria and fungi) community shifts [15] in favor of microbes which could feed on or at least adapt to the type of crude

oil contaminant. Thus, oil contaminated soils are rich sources for biotransformation bacteria.

At an earlier study, 15 spore forming Bacillus species bacteria were isolated from contaminated soil samples at an Omani heavy oil field and identified [16]. Soil samples were heated at 80°C at 100 ml of distilled water at 250 ml flasks in order to eliminate all bacteria that cannot withstand the heat and retain only the spore forming bacteria since these could survive the harsh *in situ* reservoir conditions. All bacteria growth media did not contain carbon, which is an essential element for the growth of living organisms. The only carbon source was is the heavy crude oil; thus, obliging bacteria to utilize the heavy crude oil and biodegrade it. M2 medium was proven the most suitable medium and biotransformation results were encouraging.

Many strains of *Bacillus subtilis* and *Bacillus licheniformis* species were reported as effective crude oil biodegraders. *Bacillus subtilis* DM-04 strain that was isolated from North-East India was used effectively for in situ bioremediation [17]. Also, a bacterial consortium of five strains including a *Bacillus licheniformis* strain achieved 48% biodegradation of asphaltene [18]. Asphaltene is the most difficult crude oil compound to biodegrade. Generally, biodegradability of crude oil components decreases in the following order: n-alkanes, branched-chain alkanes, branched alkenes, low molecular weight n-alkyl aromatics, monoaromatics, cyclic alkanes, polycyclic aromatic hydrocarbons (PAHs) and asphaltenes [19].

For biodegradation to occur, bacteria should gain direct cell contact with the substrate which could be facilitated by producing biosurfactant in order to increase substrate bio-availability [20]. Bacillus species could produce biosurfactants that aid degradation process by increasing oil emulsification and its accessibility [21-23] if suitable nutrients and growth conditions are present.

In this study, *Bacillus subtilis* AS2 and *Bacillus licheniformis* AS5 bacteria were tested for their biotransformation abilities at aerobic flask conditions (which could be used for bioremediation purposes if properly optimized at later stages) considering the M2 medium spiked with different chemicals. The bacteria were also tested at anaerobic conditions using Berea sandstone core flooding experiments to test the pursued *in situ* MEOR biotransformation objective.

2. MATERIALS AND METHODS

2.1. Minimum Salt Medium and Crude Oil

The composition of the used minimum salt medium (MSM) M2 was (g/l): KH_2PO_4, 1.0; K_2HPO_4, 1.0; KNO_3, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; yeast extract, 0.5. The concentrations of trace elements of M2 were (g/l): $ZnSO_4 \cdot 7H_2O$, 2.32; $MnSO_4 \cdot 4H_2O$, 1.78; H_3BO_3, 0.56; $CuSO_4 \cdot 5H_2O$, 1.00; $Na_2MoO_4 \cdot 2H_2O$, 0.39; $CoCl_2 \cdot 6H_2O$, 0.42; EDTA, 1.00; $NiCl_2 \cdot 6H_2O$, 0.004 and KI, 0.66. The viscosity and density of the sampled heavy crude oil were 968 cP and 0.961 g/cc, at 40 °C respectively. The crude oil had 13.3 °API and 5.5% asphaltene content. Oil contaminated soil samples were collected from heavy crude oil field oil-sludge pits (Fig. 1).

2.2. Aerobic Flask Experiments

The experiments were conducted in order to evaluate bacterial growth of each bacteria at M2 medium when other compounds are added. 3% glucose was added to M2 medium as a supplementary source of carbon since M2 does not contain a carbon source to kick start bacterial growth and biotransformation. At another set, 3% of sodium thiosulfate was added to M2 medium to oxidize the PAH present at the crude oil into a more bioavailable form [24]. 2 ml of bacteria in Luria Bertani (LB) seed medium were inoculated in 250 ml flasks containing 100 ml of M2 medium and 1 g of the heavy crude oil. M2 flasks were incubated at 40°C in shaker set at 160 rpm. 1 ml samples were taken daily from each flask to measure optical density at 660 nm (OD_{660}).

2.3. Anaerobic Core Flooding Experiments

Core flooding experiments were run to evaluate the ability of the isolated bacteria to biotransform heavy crude oil at *in situ* anaerobic conditions using Berea sandstone cores. Enhancement in oil recovery was quantified from the residual oil production.

The cores were cleaned by using the soxhlet extraction method with methanol and dried at 65 °C for 24 hours before usage. The cores were saturated with filtered reservoir water (i.e. formation brine) using vacuum desiccators and pore volume (PV) was determined using the dry and wet weights of the cores. The cores were flooded with oil at 0.4 cm^3/min until no more water was produced. The oil initially in place (OIIP) which was determined by measuring the displaced volume of water. The core was subjected to water flood (WF) at 0.4 cm^3/min. The residual oil was calculated by measuring the amount of oil produced from the water flood. Bacteria were inoculated from LB seed medium to M2 production medium and then the mixture of bacteria and nutrients were injected into the cores (i.e. bacteria and nutrients flooding; BNF). The tertiary recovery of extra oil was calculated by measuring oil that was produced by water flooding the core after incubation of bacteria (i.e. WF after BNF). Core floods were conducted at 40°C.

3. RESULTS

3.1. Minimum Salt Medium and Crude Oil

The MSM M2 performed as expected showing rapid bacterial growth of small circular colonies in less than 3 days. Other media were tested however resulted in slow growing rhizoidal colonies that needed at least a week to grow and had low biodegradation level. Figure 2 shows samples of observed bacterial cultures at M2 medium (circular colonies) and M4 medium petri dishes (rhizoidal colonies).

Fig. 1: The oil-sludge pit where the soil samples were collected.

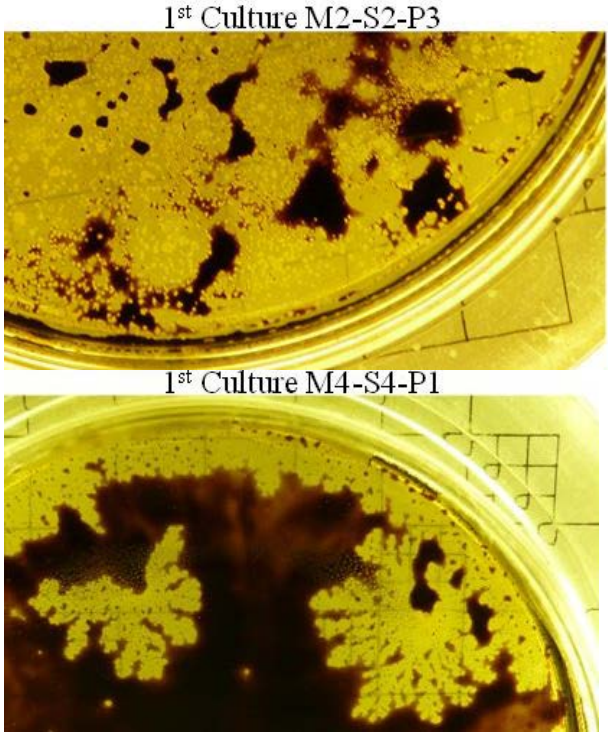

Fig. 2: Samples of observed bacterial cultures of M2 medium (top) and M4 medium (bottom) at petri dishes.

3.2. Aerobic Flask Experiments

AS2 and AS5 growth curves are shown at Fig. 3. M2 medium spiked with 3% glucose had the highest bacteria growth and crude oil biodegradation in comparison with M2 medium with no added chemicals and M2 medium spiked with 3% sodium thiosulfate.

The sodium thiosulfate that was supposed to enhance biodegradation, inhibited bacterial growth in comparison to the M2 medium bacterial growth where no chemicals were added.

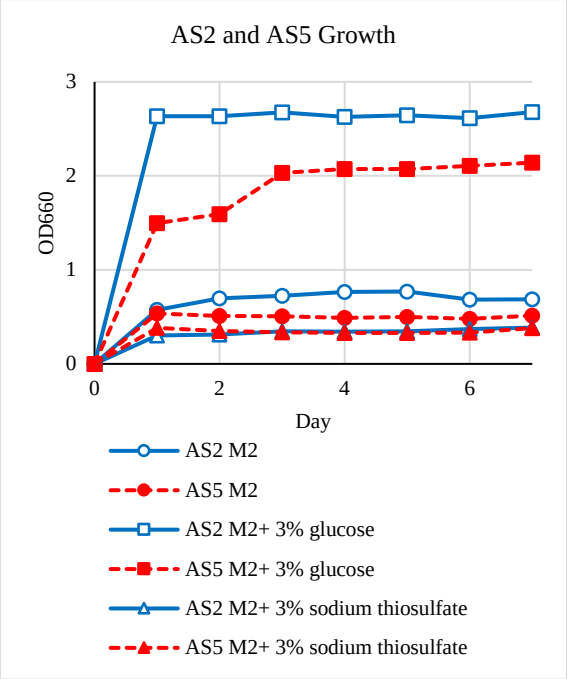

Fig. 3: AS2 and AS5 growth curves.

3.3. Anaerobic Core Flooding Experiments

At anaerobic *in situ* conditions Berea sandstone core flooding experiments, additional 2.9% and 3.1% of residual oil saturation was recovered by *B. subtilis* AS2 and *B. licheniformis* AS5 respectively after one week of incubation at the core and 5% inoculation from LB seed medium as shown at Fig. 4 and Fig. 5 respectively. Since it had a higher percentage of oil recovery, further experiments focused on the AS5 bacteria.

By increasing the incubation time of AS5 bacteria from 1 week to 2 weeks and increasing inoculation percentage from 5% to 20%, oil recovery increased to 5.0% as shown at Fig. 6. When glucose was added to M2 medium, AS5 oil recovery increased to 16.4% after 2 weeks of incubation and to 18.3% after 6 weeks of incubation (Fig. 7).

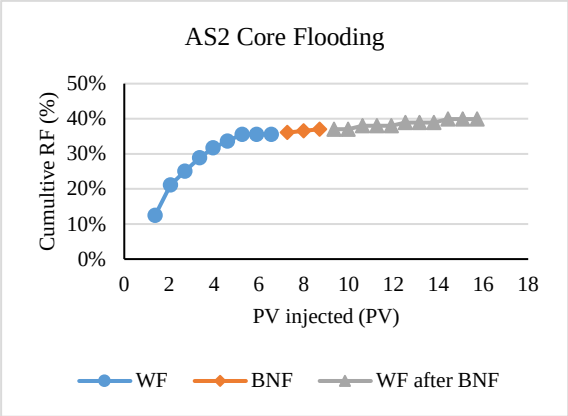

Fig. 4: AS2 core flooding (1-week incubation; 5% inoculation).

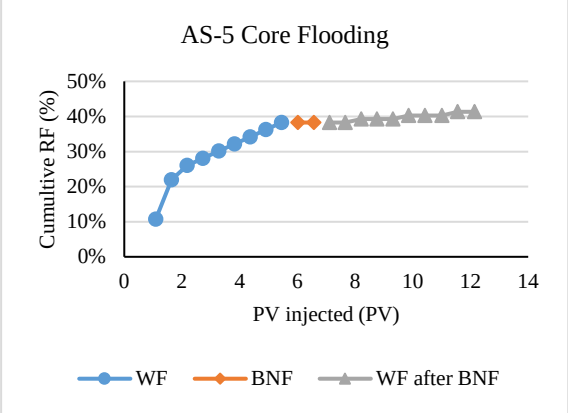

Fig. 5: AS5 core flooding (1-week incubation; 5% inoculation).

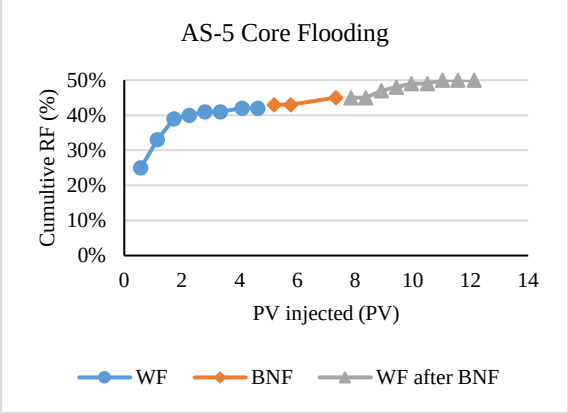

Fig. 6: AS5 core flooding (2-weeks incubation; 20% inoculation).

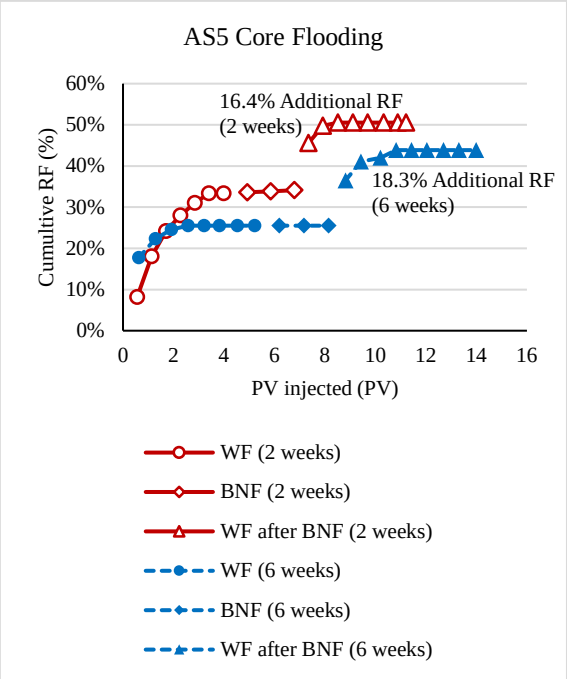

Fig. 7: AS5 core flooding (20% inoculation and glucose was added).

4. CONCLUSION

B. subtilis AS2 and *B. licheniformis* AS5 bacteria isolated from soils contaminated with heavy crude oil were successfully able to biotransform heavy crude oil at both aerobic and anaerobic conditions.

Addition of glucose at aerobic conditions was found beneficial to the bacterial growth as AS2 and AS5 bacterial growth increased by 3.9 and 4.1 folds respectively. However, addition of sodium thiosulfate was detrimental as AS2 and AS5 bacterial growth decreased by 40% and 37% respectively in comparison to the base M2 growth.

The anaerobic *in situ* core flooding results confirmed the effectiveness of the glucose addition as oil recovery by the AS5 bacteria increased more than 3 folds in comparison to the base M2 recovery. Increase of incubation time increased recovery; however, the increase was limited to below 3% recovery at all cases.

The results confirm that environmentally friendly and reasonably priced biotransformation process is an effective method for utilizing heavy crude oil resources if suitable medium and optimum growth conditions are taken into account.

5. REFERENCES

[1] Ramérez-Corredores MM, Abhijeet PB. Chapter 3 Emerging biocatalytic processes. Studies in Surface Science and Catalysis. Elsevier; 2007. p. 65-226.

[2] Sen R. Biotechnology in petroleum recovery: The microbial EOR. Progress in Energy and Combustion Science. 2008;34(6):714-24. doi:http://dx.doi.org/10.1016/j.pecs.2008.05.001.

[3] Lazar I, Petrisor IG, Yen TF. Microbial Enhanced Oil Recovery (MEOR). Petroleum Science and Technology. 2007;25(11):1353-66. doi:http://dx.doi.org/10.1080/10916460701287714.

[4] Bachmann RT, Johnson AC, Edyvean RGJ. Biotechnology in the petroleum industry: An overview. International Biodeterioration & Biodegradation. 2014;86, Part C(0):225-37. doi:http://dx.doi.org/10.1016/j.ibiod.2013.09.011.

[5] Singh A, Singh B, Ward O. Potential applications of bioprocess technology in petroleum industry. Biodegradation. 2012;23(6):865-80. doi:10.1007/s10532-012-9577-2.

[6] Leon V, Kumar M. Biological upgrading of heavy crude oil. Biotechnology and Bioprocess Engineering. 2005;10(6):471-81. doi:10.1007/bf02932281.

[7] Bubela B. Geobiology and Microbiologically Enhanced Oil Recovery. In: Donaldson EC, Chilingarian GV, Yen TF, editors. Microbial Enhanced Oil Recovery. vol Developments in Petroleum Science. April 1989: Elsevier Science Ltd 1989. p. 75-97.

[8] Prince RC, Gramain A, McGenity TJ. Prokaryotic Hydrocarbon Degraders. In: Timmis K, editor. Handbook of Hydrocarbon and Lipid Microbiology. Springer Berlin Heidelberg; 2010. p. 1669-92.

[9] Donaldson EC, Knapp RM, Yen TF, Chilingarjan GV. The Subsurface Environment. In: Donaldson EC, Chilingarian GV, Yen TF, editors. Microbial Enhanced Oil Recovery. vol Developments in Petroleum Science. April 1989: Elsevier Science Ltd 1989. p. 15-36.

[10] Mansoori GA. A unified perspective on the phase behaviour of petroleum fluids. International Journal of Oil, Gas and Coal Technology. 2009;2(2):141. doi:10.1504/ijogct.2009.024884.

[11] Moustafa YM, Morsi RE. Biomarkers. In: Dhanarasu DS, editor. Chromatography and Its Applications: InTech; 2012.

[12] Chaudhuri UR. Crude Petroleum Oil (Chapter 1). Fundamentals of Petroleum and Petrochemical Engineering. Chemical Industries: CRC Press; 2010. p. 1-23.

[13] Akmaz S, Iscan O, Gurkaynak MA, Yasar M. The Structural Characterization of Saturate, Aromatic, Resin, and Asphaltene Fractions of Batiraman Crude Oil. Petroleum Science and Technology. 2011;29(2):160-71. doi:10.1080/10916460903330361.

[14] Abousnina RM, Manalo A, Shiau J, Lokuge W. An Overview on Oil Contaminated Sand and Its Engineering Applications. International Journal of GEOMATE. 2016;10(19):1615-22.

[15] Morais D, Pylro V, Clark IM, Hirsch PR, Totola MR. Responses of microbial community from tropical pristine coastal soil to crude oil contamination. PeerJ. 2016;4:e1733. doi:10.7717/peerj.1733.

[16] Al-Sayegh A, Al-Wahaibi Y, Al-Bahry S, Elshafie A, Al-Bemani A, Joshi S. Microbial enhanced heavy crude oil recovery through biodegradation using bacterial isolates from an Omani oil field. Microbial Cell Factories. 2015;14(1):1-11. doi:10.1186/s12934-015-0330-5.

[17] Das K, Mukherjee AK. Crude petroleum-oil biodegradation efficiency of Bacillus subtilis and Pseudomonas aeruginosa strains isolated from a petroleum-oil contaminated soil from North-East India. Bioresource Technology. 2007;98(7):1339-45. doi:http://dx.doi.org/10.1016/j.biortech.2006.05.032.

[18] Tavassoli T, Mousavi SM, Shojaosadati SA, Salehizadeh H. Asphaltene biodegradation using microorganisms isolated from oil samples. Fuel. 2012;93(0):142-8. doi:http://dx.doi.org/10.1016/j.fuel.2011.10.021.

[19] Tyagi M, da Fonseca MMR, Carvalho CCCR. Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. Biodegradation. 2010;22(2):231-41. doi:10.1007/s10532-010-9394-4.

[20] Wentzel A, Ellingsen T, Kotlar H-K, Zotchev S, Throne-Holst M. Bacterial metabolism of long-chain n-alkanes. Applied Microbiology and Biotechnology. 2007;76(6):1209-21. doi:10.1007/s00253-007-1119-1.

[21] Joshi S, Bharucha C, Jha S, Yadav S, Nerurkar A, Desai AJ. Biosurfactant production using molasses and whey under thermophilic conditions. Bioresource Technology. 2008;99(1):195-9.

[22] Al-Sulaimani H, Al-Wahaibi Y, Al-Bahry S, Elshafie A, Al-Bemani A, Joshi S et al. Optimization and Partial Characterization of Biosurfactants Produced by Bacillus Species and Their Potential for Ex-Situ Enhanced Oil Recovery. SPE Journal. 2011;16(3):672-82.

[23] Chandankere R, Yao J, Choi MMF, Masakorala K, Chan Y. An efficient biosurfactant-producing and crude-oil emulsifying bacterium Bacillus methylotrophicus USTBa isolated from petroleum reservoir. Biochemical Engineering Journal. 2013;74:46-53. doi:http://dx.doi.org/10.1016/j.bej.2013.02.018.

[24] Dhamodharan D, Jayapriya J. Integrated Approach for Polycyclic Aromatic Hydrocarbon Solubilization from the Soil Matrix to Enhance Bioremediation. Bioremediation Journal. 2015;19(4):287-95. doi:10.1080/10889868.2015.1066303.