

## BIO RECOVERING OF WASTE WATER AND AGRICULTURAL SOIL POLLUTED BY WASTE INDUSTRIAL OIL OR WASTE MOTOR OIL

---

*Maribel Leal-Castillo*

Unidad Académica, Multidisciplinaria,  
Reynosa- Rodhe, Universidad Autónoma  
de Tamaulipas, Carretera Reynosa San  
Fernando, Reynosa, Tamaulipas, México

*José Luis Hernández-Mendoza*

Centro de Biotecnología Genómica  
Experimental

*Guider Martos-Dominguez*

Laboratorios Clínicos y Bacteriológicos  
Juárez, Jalisco, México

*Juan Manuel Sánchez-Yañez*

Environmental Laboratory Microbiology,  
Research Institute in Chemistry and  
Biology, Morelia, Michoacán, Universidad  
Michoacana de San Nicolas de Hidalgo  
Morelia, Michoacán, México

\*corresponding author: [syanez@umich.mx](mailto:syanez@umich.mx)

All content in this magazine is  
licensed under a Creative Com-  
mons Attribution License. Attri-  
bution-Non-Commercial-Non-  
Derivatives 4.0 International (CC  
BY-NC-ND 4.0).



**Abstract:** Water and soil are natural resources that can be contaminated by waste industrial oil (WIO) or waste motor oil (WMO). This causes loss of soil fertility and makes it difficult to reuse water for various purposes. The aim of this work was to analyze the bio-remediation of water and soil impacted by 20,000 ppm of WIO or WMO. For this purpose, wastewater and agricultural soil contaminated by WIO or WMO were biostimulated by Tween 20, followed by biostimulation with 2 mineral solutions with different concentrations of monobasic and dibasic phosphates,  $H_2O_2$  with and without agitation. An experimental design with two controls and four treatments was used. The response variables used to measure the removal of WIO and WMO were the production of  $CO_2$  and the disappearance of aliphatic hydrocarbons by Soxhlet. The experimental results were analyzed by ANOVA-Tukey. The results showed that in both agricultural soil and waste water, the first biostimulation by Tween 20 was necessary to emulsify the aliphatic hydrocarbons of WIO and WMO, followed by biostimulation with mineral solution 1 with the highest amount of phosphate salts, which induced a rapid mineralization of WIO and WMO, both in waste water and soil, which was followed by biostimulation with  $H_2O_2$  with and without agitation to increase the removal amount of hydrocarbons from WIO and WMO, due to the high hydrocarbon oxidation activity by native aerobic heterotrophic microorganisms in soil and waste water. The results of biostimulated soil and waste water were statistically different compared to non-biostimulated waste water and agricultural soil where WIO or WMO did not disappear. It was indicated that in both waste water and agricultural soil, the aliphatic hydrocarbons of WIO and WMO were the ones that were removed faster with mechanical agitation and  $H_2O_2$ . It was detected that in both soil and

water, the concentration of WIO and WMO was reduced from 20,000 ppm to 1000 ppm. It is concluded that biostimulation is a useful strategy for the recovery of water waste and agricultural soil impacted by WIO and WMO to allowed to reuse with not risk for humans or animals.

**Keywords:** water, soil, aerobic heterotrophic microorganisms, mineralization, environmental, health.

## INTRODUCTION

Environmental pollution related to petrochemical products is recognized as one of the most serious problems in soil, waste water, groundwater and superficial and other water bodies. In México as is in many developing countries, the annual production of waste industrial oil and waste motor oil (WMO) is approximately 325 million liters (1-3). It is estimated that only 20% of the generated volume under goes proper final treatment (4-6). The composition of WIO and WMO is aliphatic with chain lengths ranging from C15 to C50. WOI or WMO may also (4); Nitrogen (N) and sulfur (S) compounds, and metals as lead (Pb), zinc (Zn), barium (Ba) and magnesium (Mg) as well as other inorganic and organic compounds (7-9). All these contaminants arise from normal wear of engine components and from heating and oxidation of lubricating oil during engine operation (10,11). Therefore, WIO and WMO as a mixture of aliphatic hydrocarbons involves a risk to the human health and the environment, particularly waste water or WIO and WMO groundwater and drinking water supplies (7,12) including agricultural soil (8-10). An ecological alternative of solution es bioremediation schemes is investigated for the treatment of waste water and agricultural soil containing WMO and WMO with aliphatic hydrocarbons (11-13). Bioremediation of soil and waste water polluted by WIO or WMO

could be used for aliphatic hydrocarbons mineralization by biostimulation of the aerobic native heterotrophic microbial consortium with a mineral solution having in balance basic inorganic compounds such as N, phosphorous (P), potassium (K) and others important minerals for microbial metabolism (10,14,16). The aim of this work was to analysis the bio-recovery of waste water and agricultural soil impacted by WIO or WMO.

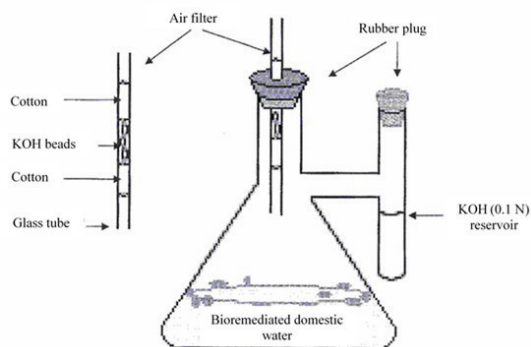
## MATERIAL AND METHODS

Biostimulation of waste water polluted by waste industrial oil (WIO) or waste motor oil (WMO).

This experiment were used Bartha flask 500 mL Bartha flasks were used (Figure 1), with 100 mL of waste water polluted by WIO or WMO automotive mechanic workshop of Monterrey, N.L. México diluted 1:100, equivalent to 12,000 ppm of WIO or WMO biostimulated with 0.01% of Tween 20, following by mineral solution 1 (MS1) with this chemical composition (g/L):  $K_2HPO_4$  10.0;  $KH_2PO_4$  8.0;  $MgSO_4$  3.0;  $NH_4NO_3$  10.0;  $CaCO_3$  1.0;  $KCl$  2.0;  $ZnSO_4$  0.5.0;  $CuSO_4$  0.5;  $FeSO_4$  0.2, and EDTA 8. and mineral solution (MS2) with this chemical composition (g/L):  $K_2HPO_4$  5.0  $KH_2PO_4$  4.0;  $MgSO_4$  3.0;  $NH_4NO_3$  10.0;  $CaCO_3$  1.0;  $KCl$  2.0;  $ZnSO_4$  0.5;  $CuSO_4$  0.5;  $FeSO_4$  0.2, and EDTA 8. The flasks show in figure 1 (, were shaken at 100 rpm and incubated at  $30 \pm 2^\circ C$ /3 weeks, the experiment was carried out in 5 repetitions. As an absolute control was used: a flask only with mineral solution 1 (MS1), other flask only with mineral solution 2 (MS2): treatment 1, a flask with waste water polluted by WIO, biostimulated by Tween 20 and MS1; as treatment 2 a flask with waste water polluted by WMO, biostimulated by Tween 20, and MS1, treatment 3 a flask with waste water polluted by WIO biostimulated by Tween 20 and MS1, treatment 4 a flask with waste water polluted

by Tween 20 and MS2. All experimental data of this trial were analyzed by ANOVA-Tukey:  $P < 0.01\%$  (3,8,13,22).

While agricultural soil was classified as sandy loam, medium cation exchange capacity, rich in organic matter 4.0, slightly alkaline pH 7.2, then passed through a 2 mm light sieve to be artificially contaminated by 20,000 ppm WMO dissolved in Tween 20 detergent, 0.5 mL for 99.5 mL of WMO for a concentration equivalent to 20,000 ppm/ 100 g of soil. Then the following biostimulation was by  $H_2O_2$  at 1,0% 10 ml/100g of waste water or agricultural soil/per week during 5 weeks. To demonstrate in waste water polluted by WIO or WMO by biostimulation in each of the arms 10 mL of 0.1 N KOH was added to the flask to capture the  $CO_2$ , every 24 h the 0.1 N KOH was taken from each flask, the  $CO_2$  production was quantified by titration using 0.1 N HCl (16-18). At the beginning and end of the experiment, the concentration of WIO and WMO was quantified by the Soxhlet method (15).



**Figure 1.** Bartha flask (respirometer) to measure biostimulation of waste water (domestic water) polluted by waste motor oil or waste motor oil

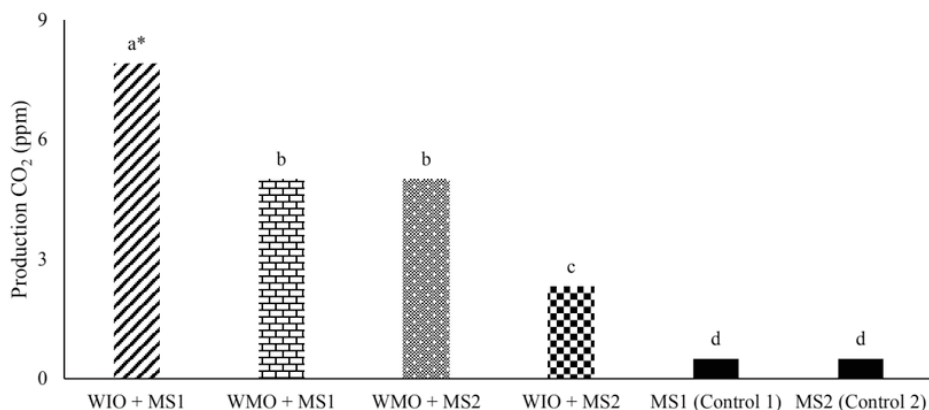


Figure 1. CO<sub>2</sub> production in waste water and agricultural soil polluted by WIO and WMO, biorecovery by mineral solution with two concentrations of: K<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub> \*n=5 WIO = Waste industrial oil. WMO = waste motor oil. Tween 20 was the first biostimulated and the next mineral solution 1 s (MS1) =g/L: NH<sub>4</sub>NO<sub>3</sub>, 10.0; K<sub>2</sub>HPO 10.0; KH<sub>2</sub>PO<sub>4</sub> 8.0; MgSO<sub>4</sub>, 3.0; CaCO<sub>3</sub>, 1.0; KCl, 2.0; ZnSO<sub>4</sub>, 0.5.0; CuSO<sub>4</sub>; 0.5; FeSO<sub>4</sub>, 0.2, and EDTA 8. Mineral solution 2 (MS2) =Mineral solution (g/L) NH<sub>4</sub>NO<sub>3</sub>, 10.0; K<sub>2</sub>HPO 5.0 KH<sub>2</sub>PO<sub>4</sub> 4.0; MgSO<sub>4</sub>, 3.0; CaCO<sub>3</sub>, 1.0; KCl, 2.0; ZnSO<sub>4</sub>, 0.5.0; CuSO<sub>4</sub>; 0.5; FeSO<sub>4</sub>, 0.2, and EDTA 8, \* Values with different letters are statistically different (ANOVA-Tukey: P<0.01%).

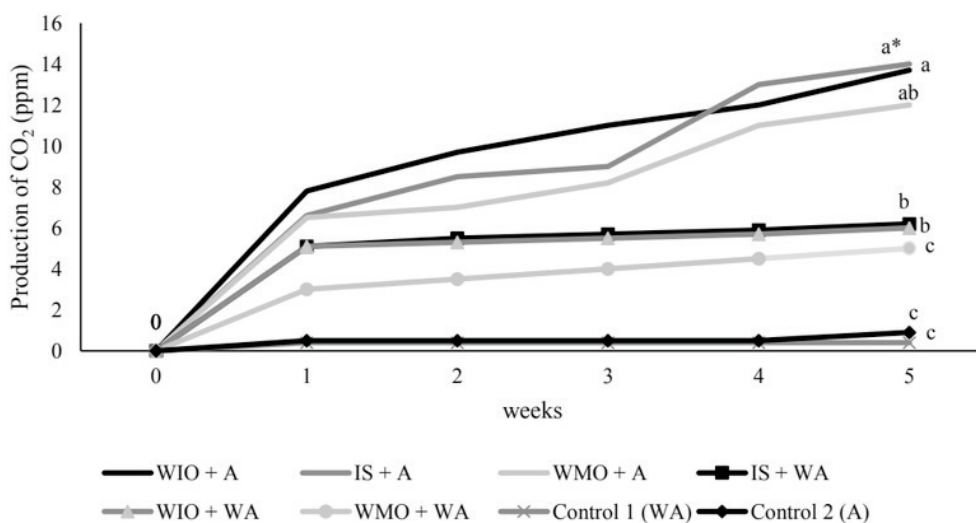


Figure 2. CO<sub>2</sub> production by the aerobic heterotrophic microbiota of waste water polluted by WIO, WMO, and agricultural soil biostimulated by mineral solution 1 with and without agitation. WIO = Waste industrial oil. WMO = waste motor oil. IS= impacted agricultural soil by WMO. Tween 20 was the first biostimulated and the next mineral solution 1 (MS1) =g/L: NH<sub>4</sub>NO<sub>3</sub>, 10.0; K<sub>2</sub>HPO 10.0; KH<sub>2</sub>PO<sub>4</sub> 8.0; MgSO<sub>4</sub>, 3.0; CaCO<sub>3</sub>, 1.0; KCl, 2.0; ZnSO<sub>4</sub>, 0.5.0; CuSO<sub>4</sub>; 0.5; FeSO<sub>4</sub>, 0.2, and EDTA 8 WA =without agitation A=Agitation \* Values with different letters are statistically different (ANOVA-Tukey: P<0.01%). A= agitation

## RESULTS AND DISCUSSION

Figure 1 shows that the removal of WMO and WIO was dependent on the first biostimulation by Tween 20 that emulsified them and made them available, so that the following biostimulation by mineral solution 1 (MS1), with a higher concentration of mono- and dibasic phosphates than mineral solution 2 (MS2), supports that due to the narrow K<sub>ps</sub> range of the phosphates, a higher amount of both phosphates was necessary (17-19). It was evident that CO<sub>2</sub> generation resulted from the oxidation of both WIO and WMO by the activity of native aerobic heterotrophic microorganisms naturally occurring in wastewater, which proves that toxic hydrocarbons from WIO or WMO can be mineralized by the native microbiota of the wastewater (20,21). According to the literature, there is a wide diversity of aerobic heterotrophic microorganisms capable of oxidizing various types of hydrocarbons (22), which are common in wastewater, that have the potential to remove WIO or WMO induced by a comprehensive bioremediation strategy, based on a detergent such as Tween 20, a mineral equilibrium solution, as a source of O<sub>2</sub> as applied in this research work (16).

Figure 2. Shows that waste water with WIO or WMO as well as agricultural soil impacted by WMO was possible to bioremediation by biostimulation first with Tween 20, followed by biostimulation with mineral solution 1 containing the highest amount of mono- and dibasic potassium phosphates (14,20). Especially when the waste water as well as the soil was biostimulated with the agitated that supports that the maximum mineralization by the native heterotrophic aerobic microorganisms of the waste water and soil (12,21,22) in addition to the biostimulation with the mineral solution and/or air input to remove the WMO, WIO or soil (16-18). In both environments the positive impact of

agitation, in stark contrast to the lack of where the maximum oxidation of WMO, WIO in waste water or soil was lower and slower when was not agitated. This is supported by the numerical values given the statistical difference in each case compared to when only the mineral solution without WMO or WIO was present (13,20).

Figure 3 shows that both the CO<sub>2</sub> production derived from the bio-recovery of agricultural soil and waste water contaminated by WMO and WIO (12,14), through the biostimulation by Tween 20 that by emulsifying the hydrocarbons of WMO and WIO made the aliphatics available to the aerobic heterotrophic microorganisms native to the waste water and soil, that with biostimulation with mineral solution 1 induced the efficient mineralization of WMO and WIO especially by biostimulation of both environments with H<sub>2</sub>O<sub>2</sub> as a source of oxygen (O<sub>2</sub>). That accelerated and increased the amount of WMO and WIO that was oxidized (19-21): the numerical values in this case were statistically different compared to the numerical values of CO<sub>2</sub> released when instead of H<sub>2</sub>O<sub>2</sub> soil and waste water were subjected to mechanical agitation (10,13). The minimum values of CO<sub>2</sub> production were detected when water or soil without biostimulation were subjected only to biostimulation with H<sub>2</sub>O<sub>2</sub> or without biostimulation were subjected only to mechanical agitation (1,5,7). The above made it evident that CO<sub>2</sub> production in waste water or soil was dependent on the hydrocarbon mixture of WMO and WIO biostimulated integrally, by Tween 20, mineral solution 1, and/or mechanical agitation, indicated that in both agricultural soil and waste water the concentration of both cases ranged from 20,000 to 1000 as measured by Soxhlet (4,8,15) Ongoing research supports the existence in agricultural soil and waste water of native aerobic heterotrophic

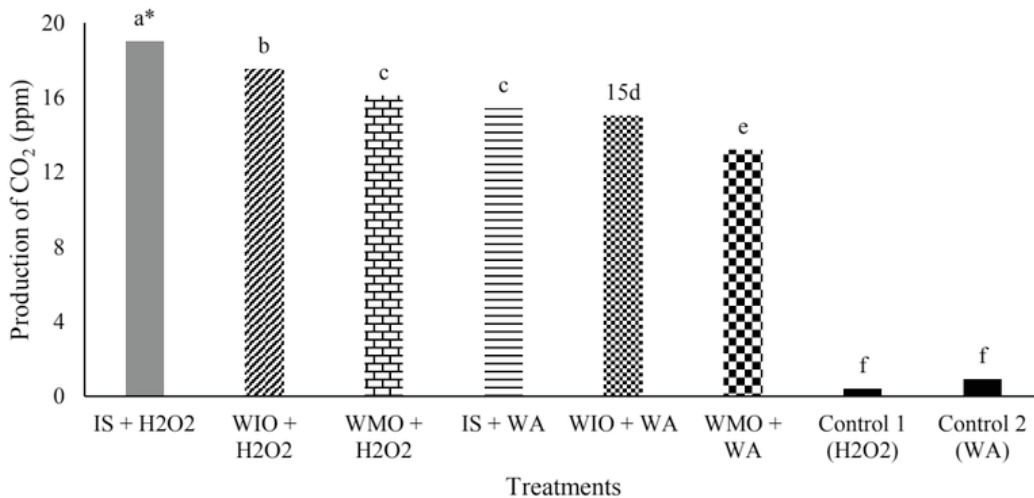


Figure 3 shows the production of CO<sub>2</sub> derived from the bio-recovery of waste water and agricultural soil polluted by WIO or WMO biostimulated by Tween 20, mineral solution 1, H<sub>2</sub>O<sub>2</sub> and/or mechanical agitation. WIO = Waste industrial oil. WMO = waste motor oil. IS= impacted agricultural soil by WMO. Tween 20 was the first biostimulated and the next mineral solution 1(MS1) =g/L: NH<sub>4</sub>NO<sub>3</sub>, 10.0; K<sub>2</sub>HPO 10.0; KH<sub>2</sub>PO<sub>4</sub> 8.0; MgSO<sub>4</sub>, 3.0; CaCO<sub>3</sub>, 1.0; KCl, 2.0; ZnSO<sub>4</sub>, 0.5.0; CuSO<sub>4</sub>; 0.5; FeSO<sub>4</sub>, 0.2, and EDTA 8. WA =without agitation A=Agitation H<sub>2</sub>O<sub>2</sub> Values with different letters are statistically different (ANOVA-Tukey: P<0.01%).

microorganisms capable of mineralizing up to 90% of WIO and WMO aliphatic short chain hydrocarbons main hydrocarbons responsible for WIO or WMO toxicity for any life, in that sense waste water and agricultural soil are free for these pollutants, to reuse them. According national and international regulations this concentration allowed to reuse waste water or agricultural soil with no risk for humans or animals (9,17,22).

## CONCLUSION

This confirms that the possible recovery of agricultural soil and waste water impacted by WIO or WMO requires a biostimulation that integrates a detergent, a mineral solution with sufficient amount of monobasic and dibasic phosphates, an O<sub>2</sub> source of H<sub>2</sub>O<sub>2</sub>, better than mechanical agitation, constitutes an ecological strategy for the bio-recovery of waste water and agricultural soil for reuse without risk to the health of humans and animal.

## ACKNOWLEDGEMENTS

To Unidad Académica, Multidisciplinaria, Reynosa- Rodhe, Universidad Autónoma de Tamaulipas, Carretera Reynosa San Fernando 88779, Reynosa, Tamaulipas, México, Project 2.7 (2024) supported by the Scientific Research Coordination-UMSNH: “Aislamiento y selección de microorganismos endófitos promotores de vegetal para la agricultura y biorecuperacion de suelos, To Phytónutrientes de México and BIONUTRA S.A de CV, Maravatio, Mich, México. To MC Juan Luis Ignacio de la Cruz for his technical support.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## FUNDING

This manuscript received no external funding.

## REFERENCES

1. Varjani, S., Pandey, A., Taherzadeh, M., Ngo, H.H., Tyagi, R.D. (Eds.) 2022. Biomass, Biofuels, Biochemicals: Circular Bioeconomy: Technologies for Waste Remediation. Elsevier. London UK.
2. Roy A, Dutta A, Pal S et al., 2018. Biostimulation and bioaugmentation of native microbial community accelerated bioremediation of oil refinery sludge. *Bioresource Technology* 253: 22–32.
3. Imam, A, Suman SK, Ghosh D, and Kanaujia, K J. 2019. Analytical approaches used in monitoring the bioremediation of hydrocarbons in petroleum-contaminated soil and sludge. *TRAC Trends in Analytical Chemistry* 118:50–64.
4. Ossai LC, Ahmed A, Hassan A, and Hamid FS, 2020. Remediation of soil and water contaminated with petroleum hydrocarbons: a review. *Environmental Technology and Innovation* 17: 1–42
5. Ramadass K, Megharaj M, Venkateswarlu K, et al. 2018. Bioavailability of weathered hydrocarbons in engine oil-contaminated soil: Impact of bioaugmentation mediated by *Pseudomonas* spp. on bioremediation. *Science of The Total Environment*. 636:968–974.
6. Roy A, Dutta A, Pal Set al. 2018. Biostimulation and bioaugmentation of native microbial community accelerated bioremediation of oil refinery sludge. *Bioresource Technology*, 253:22–32
7. Nasr M. 2019. Environmental perspectives of plant-microbe nexus for soil and water remediation. *Microbiome in Plant Health and Disease: Challenges and Opportunities*, 403-419.
8. Maulin P Shah. 2017. Bioremediation Waste Water Treatment. *Journal Bioremediation & Biodegradation*. 9 (1):3-10
9. Birch H, Hammershøj R, Comber M, et al. 2017. Biodegradation of hydrocarbon- mixtures in surface waters at environmentally relevant levels—effect of inoculum origin on kinetics and sequence of degradation. *Chemosphere* 184:400–407.
10. Ruikar, A., & Pawar, H. S. 2022. Diversity and Interaction of Microbes in Biodegradation. *Microbial Community Studies in Industrial Wastewater Treatment*, 185-213.
11. Basuki W. 2017. Biodegradation of used synthetic lubricating oil by *Brevundimonas diminuta* AKL 1.6. *Makara Journal of Science*. 136–142.
12. Rizzardini, C.B., Goi, D., 2014. Sustainability of domestic sewage sludge disposal. *Sustainability*. 6,2424–243.
13. Sambandum A, Ponnusamy V K and Ashokkumar M. 2020. Review on hybrid techniques for the degradation of organic pollutants in aqueous environment. *Ultrasonic Sonochemistry* 67: 105130, <https://doi.org/10.1016/j.ultsonch.2020.105130>.
14. Fijalkowski, K., Rorat, A., Grobelak, A., Kacprzak, M.J., 2017. The presence of contaminations in sewage sludge—The current situation. *J. Environ Manag.* 203,1126-1136
15. Norma Oficial Mexicana NMX-F.089-S-1978. Determinación de extracto etéreo (método Soxhlet) en alimentos. foodstuff-determination of ether extract (Soxhlet). DOF Secretaria de Gobernación [en línea]. 2013 [Acceso 20 de May 2018]. Disponible en: <http://www.colpos.mx/bancodennormas/nmexicanas/NMX-F-089-S-1978.PDF>
16. Surajudeen, A and Benjamin OA. 2009. Comparison of biostimulation and bioaugmentation techniques for the remediation of used motor oil. *Brazilian Archives of Biology and Technology* 52: 747-754
17. Usman, K., Khan, S., Ghulam, S., Khan, M.U., Khan, N., Khan, M.A., Khalil, S.K. 2012. Sewage sludge: an important biological resource for sustainable agriculture and its Journal Pre-proof environmental implications. *American Journal of Plant Sciences* 3 (12), 1708-1721, doi: 10.4236/ajps.2012.3122
18. Kochhar, N., Shrivastava, S., Ghosh, A., Rawat, V. S., Sodhi, K. K., & Kumar, M. 2022. Perspectives on the microorganism of extreme environments and their applications. *Current research in microbial sciences*, 3, 100134.
19. Enerijiofi K.E. 2020. Bioremediation of environmental contaminants a sustainable alternative to environmental management in: Bioremediation for environmental Sustainability: Toxicity mechanisms of contaminants, degradation, detoxification challenges, Sexena G, Kumar V, and Shah MP (eds). Elsevier, USA 2: 461-480
20. Mora-Ravelo, SA, Alarcón A, Rocandio-Rodriguez M and. Vanoye-Eligio V. 2017. Bioremediation of wastewater for reutilization in agricultural systems: a review. *Applied Ecology and Environmental Research* 15 (1): 33
21. Sajad R, Gupta M, and Richa MKS. 2018. Bioremediation of Naphthalene and Other PAH Contaminants: An Approach for Cleaning of Environment,” *Journal of Environmental Research and Development* 12: (3): 292–297.
22. Huang X, Zhou H, Liang Y, et al.2020. Enhanced Bioremediation of Hydraulic Fracturing Flowback and Produced Water Using an Indigenous Biosurfactant-Producing Bacteria *Acinetobacter* sp. Y2. *Chemical Engineering Journal*. 397:125348.