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Genus and Species of Bacteria from Domestic Water to Remove Pure Motor Oil

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Abstract

It is reported that pure motor oil (PMO) leaks into domestic water, causing problems for its possible subsequent recycling, especially in the current global water crisis. An alternative solution, to facilitate the reuse of this type of water, is to take advantage of the natural capacity of genera and species of Enterobacteriaceae, as well as other kind of bacteria, that live in this and other environments, different from domestic water, that also oxidize mixtures of hydrocarbons from the PMO. By it is biostimulation, with a detergent that emulsifies hydrocarbons, a mineral solution that induces its oxidation, and H2O2 as a source of oxygen (O2), that accelerates the elimination of PMO. The objectives of this work were: i) analyze the biostimulation of enteric and other bacteria, in the elimination of PMO, in culture medium conditions, ii) identify bacteria capable of oxidizing PMO. In this sense, all these types of bacteria, isolated from domestic water impacted by PMO, were biostimulated by tween 20, a mineral solution and H2O2; Then the concentration of total hydrocarbons, was determined to measure the disappearance of PMO, by mass coupling gas chromatography. All bacteria that showed the ability, to eliminate PMO were identified by biochemical profiling, with automated Vytek equipment. The results showed that Escherichia coli, Acinetobacter iwoffi, Morganella morganii and Staphylococcus aureus, isolated from domestic water, showed the capacity to oxidize PMO, biostimulated by tween 20, mineral solution and H2O2. It was concluded that in domestic water there are bacterial genera, and species, with a natural capacity to eliminate PMO, whose oxidation potential of hydrocarbon mixtures, can be exploited in the treatment of domestic water, allowing it to be reused, for agricultural and industrial purposes, in the face of the current water scarcity.

Keywords: *Environmental, water, attenuation, bacterial diversity, hydrocarbons source of carbon and energy, mineralization.*

1. Introduction

The contamination of domestic water by pure motor oil (PMO), that are mixtures of hydrocarbons: aliphatic, aromatic, etc (1-3), is an environmental problem, for water reuse at this time, facing of the current water scarcity, shortage for various uses (4-6). In domestic water there is a wide diversity of microorganisms, with the potential to oxidize aliphatic, and aromatic hydrocarbons, similar to PMO (2-5), that are common contaminate of domestic water, and prevent it from being recycled, in garden and industrial irrigation (7-9). An alternative solution to eliminate PMO, is biostimulation by tween 20, to emulsify hydrocarbons, followed by mineral solution, to balance the basic nutritional to supply the demand of the microorganisms, that are living usually in domestic water, and H_2O_2, as an oxygen (O_2) source, induces to aerobic, native heterotrophic microbes, to oxidize PMO (10-12). In this sense, it is reported that enteric heterotrophic, facultative anaerobic bacteria, and also some reports about Gram-positive aerobic cocci, inhabitants of air dust, such as *Staphylococcus* spp, can do so (13, 14). Based on these facts, it´s logical to assume that regarding the genetic and natural capacity to mineralize PMO. The objectives of this work were: i) to analyze the biostimulation of bacteria, in the elimination of PMO, and WMO in culture medium conditions ii) to identify the enteric and other bacteria oxidizing PMO.

2. MATERIALS AND METHODS

In this first step 500 ml Bartha flasks, were used with 100 ml of a mineral solution, to biostimulate the oxidation of PMO: 28 mM K_2HPO_4, 29 mM KH_2PO_4, 25 mM MgSO4, 125 mM NH_4NO_3, 10 mM $CaCO_3$, 26 mM KCl, 3 mM $ZnSO_4$, 3 mM $CuSO_4$, 1.3 mM $FeSO_4$, 21 mM EDTA, pH 6, 0.01% tween 20 (w/v). These flasks were inoculated with 5.0 ml containing 1 x 10^6/ml of a pure culture, of a bacteria isolated from domestic water impacted, by PMO on eosin methylene blue agar (EMB). The flasks were incubated for 4 weeks/100 rpm/30º C. The consumption of the total aliphatic hydrocarbons from PMO, were determined by mass-coupled gas chromatography (5,9). Profile of biochemical identification of enteric bacteria and other bacteria, isolated from domestic water contaminated, by PMO was carried out with Vytek automated equipment (10,11).

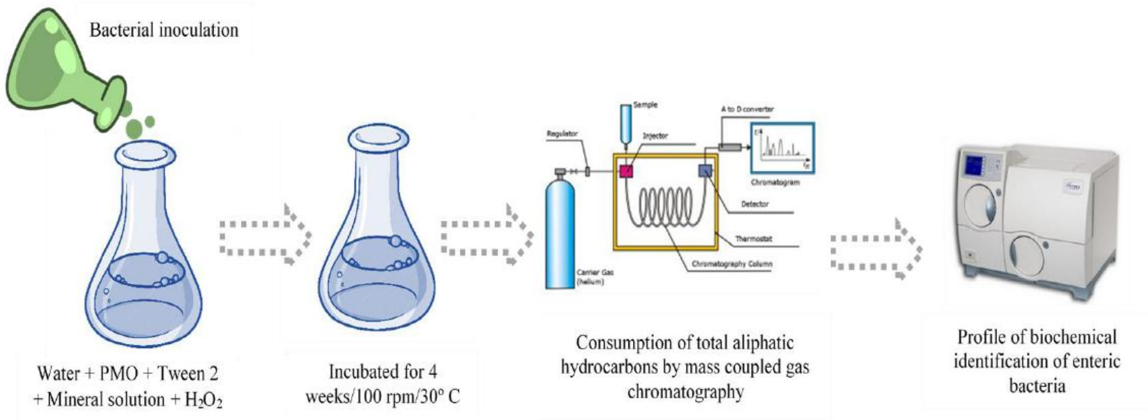

Figure 1. *Diagram to analyze the potential of enterobacteria and a Gram-positive coccus to oxidize pure motor oil by biostimulation.*

3. RESULTS AND DISCUSSION

Figure 2 shows the growth of enteric *E. coli* in pure motor oil (PMO) biostimulated with tween 20 followed by a mineral solution and H_2O_2 at time 0 and after 21 days, it was evident that both actions favored, the use of hydrocarbons as carbon and energy source (3), for effective but slow PMO mineralization (6-8).

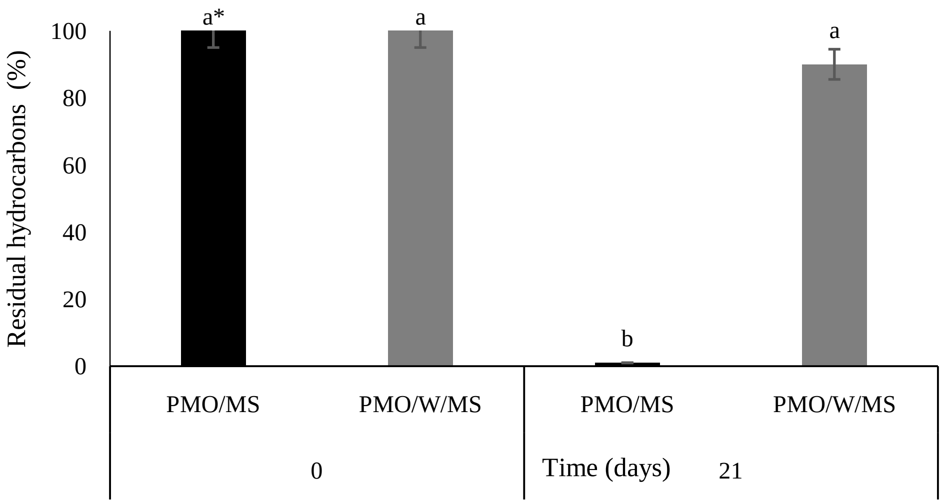

Figure 2. *Response of Escherichia coli biostimulated by tween 20, a mineral solution and H_2O_2 on total hydrocarbon mineralization of pure motor oil.*

Pure motor oil biostimulated by a mineral solution (PMO/MS); Pure motor oil non biostimulated by mineral solution (PMO/W/MS); *Different letters indicate numerical values statistically distinct by ANOVA/Tukey (P<0.05).

Figure 3 shows the growth of another enterica *Morganella morganii* in PMO biostimulated with tween 20 and then with a mineral solution. Here it was evident that this enterica has a capacity limited to the aliphatic but non-aromatic hydrocarbons of the PMO, probably due to the slightest exchange. genetics than *M. morganii* to adapt to environments with hydrocarbons similar to that of the PMO (9,10).

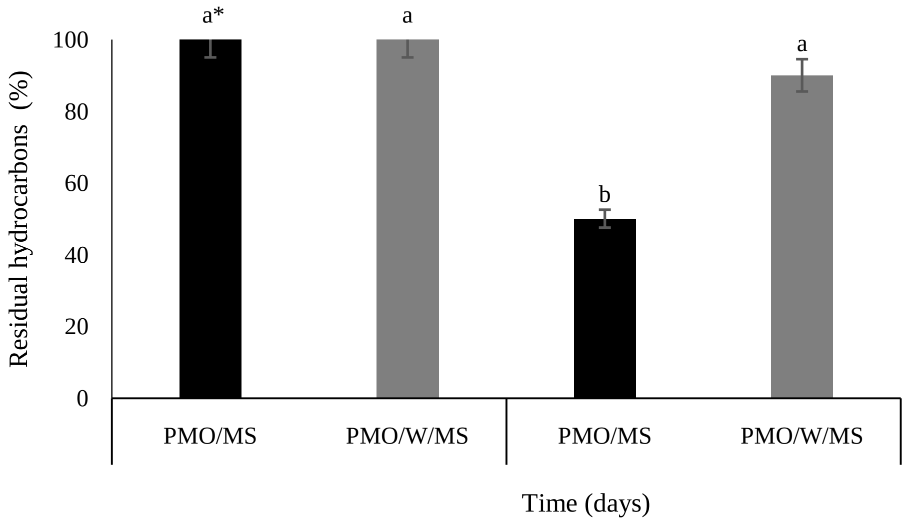

Figure 3. *Response of Morganella morganii to biostimulation by tween 20, mineral solution, H_2O_2 on total hydrocarbon mineralization of pure motor oil.*

(PMO/MS) = Pure motor oil biostimulated by a mineral solution; (PMO/W/MS) =Pure motor oil non biostimulated by mineral solution; *Different letters indicate numerical values statistically distinct by ANOVA/Tukey (P<0.05).

Figure 4 shows the growth of *Acinetobacter uwofii* in PMO biostimulated with tween 20 and a mineral solution. It was evident that *A. uwofii* was able to use most of the hydrocarbons in PMO (11-13), as has been widely reported in both water and in soil impacted by mixtures of crude or refined petroleum hydrocarbons, something interesting is that this type of enteric lacks the virulence genes that are common in the same genus and species isolated from clinical cases in the urinary tract and menginitis (13).

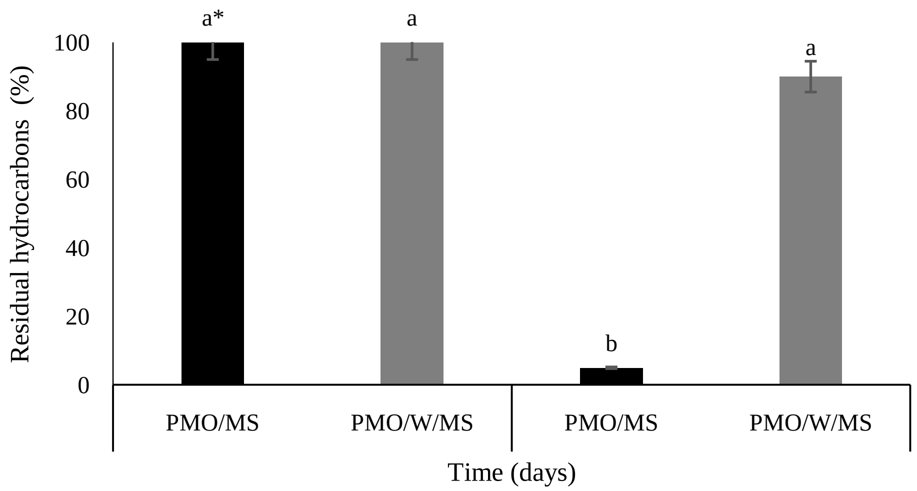

Figure 4. *Response of Acinetobacter iwofii biostimulated by tween 20, a mineral solution and H_2O_2 on total hydrocarbon mineralization of pure motor oil.*

(PMO/MS) = Pure motor oil biostimulated by a mineral solution; (PMO/W/MS) =Pure motor oil non biostimulated by mineral solution; *Different letters indicate numerical values statistically distinct by ANOVA/Tukey (P<0.05).

Figure 5 shows the growth of the only non-enteric bacteria, Gram positive *Staphylococcus aureus*, which biostimulated with tween 20 and a mineral solution, oxidized a part of the PMO hydrocarbons, which indicates that the genetic capacity to use mixtures of hydrocarbons such as PMO (14,15), are widely distributed in the environment, although in the literature this cluster-shaped coccus is associated with infections of the skin and upper respiratory system, it is known that it may be part of the microbiota that exists in nature. as potential to eliminate hydrocarbon mixtures that impact water and soil (6,7).

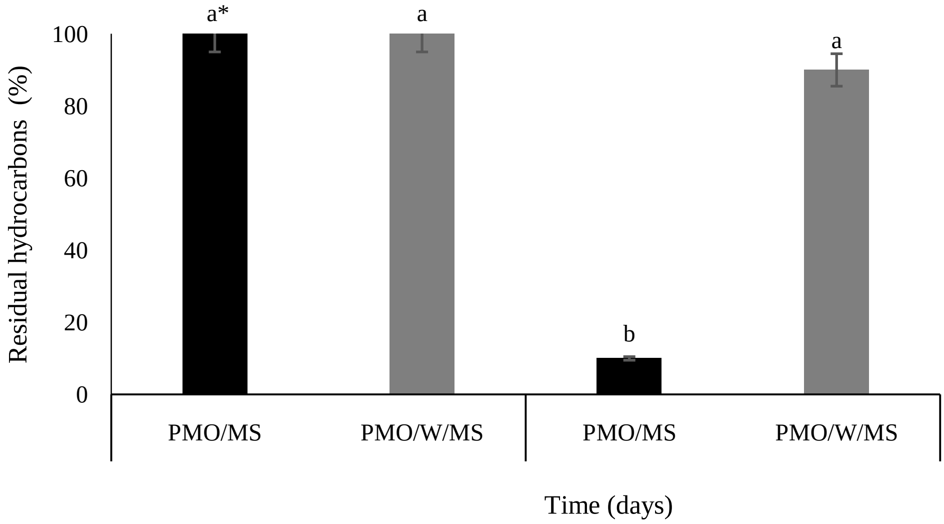

Figure 5. *Response of Staphylococcus aureus to biostimulation by tween 20, a mineral solution and H_2O_2 on total hydrocarbon mineralization of pure motor oil.*

(PMO/MS) Pure motor oil biostimulated by a mineral solution; (PMO/W/MS) = Pure motor oil non biostimulated by a mineral solution. *Different letters indicate numerical values statistically distinct by ANOVA/Tukey ($P<0.05$).

Figure 6 shows the consumption activity of the PMO hydrocarbon mixture, by the consortium of bacterial genera and species biostimulated with tween 20, a mineral solution and H_2O_2, by the consortium isolated from domestic water: *A iwofii, E. coli, M. morganii* and *S aureus*, in which it was evident that there is no type of competition for the consumption of PMO (1-3), while it does demonstrate that despite being diverse genera, also that can act without problem, to use these hydrocarbons as a source of carbon and energy (4,5), useful for the biorecovery of water impacted by hydrocarbon mixtures such as those of PMO (12,15).

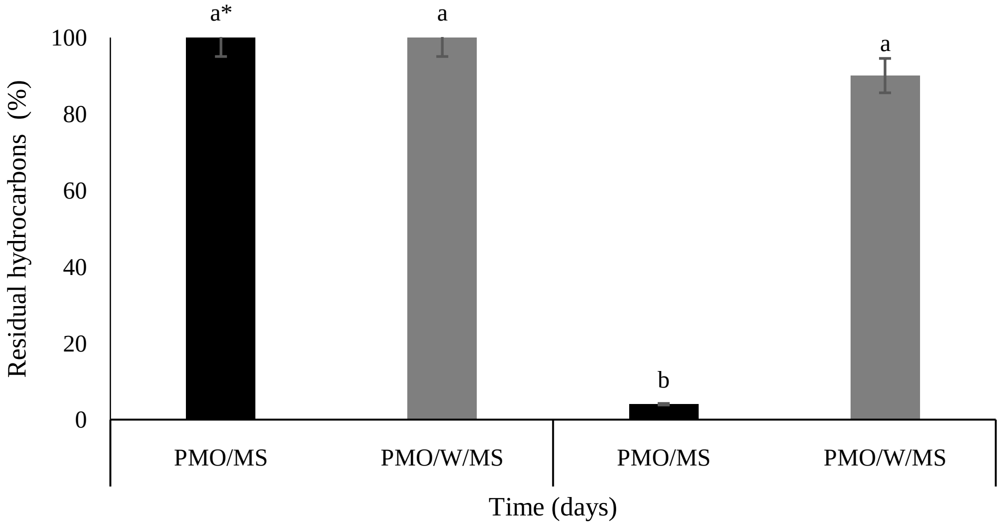

Figure 6. *Response of consortium of: E. coli, M. morganii, A. iwofii and S. aureus to biostimulation by tween 20, a mineral solution and H_2O_2 on total hydrocarbon mineralization of pure motor oil.*

(PMO/MS) =Pure motor oil biostimulated by a mineral solution; (PMO/W/MS) = Pure motor oil non biostimulated by mineral solution; *Different letters indicate numerical values statistically distinct by ANOVA/Tukey ($P<0.05$).

4. CONCLUSION

In domestic water it is possible to induce the elimination of hydrocarbon mixtures such as PMO, based on the fact that in nature there are microorganisms capable of oxidizing hydrocarbon mixtures, which contaminate both water and soil, through ecological bioaugmentation strategies or biostimulation, that allows them to be reutilized for agricultural or industrial purposes.

5. ACKNOWLEDGEMENTS

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6. CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

[1] Brzeszcz J, Kaszycki P .2018. Aerobic bacteria degrading both *n*-alkanes and aromatic hydrocarbons: an undervalued strategy for metabolic diversity and flexibility. Biodegradation 29:359–407. https://doi.org/10.1007/s10532-018-9837-x

[2] Brzeszcz J, Kapusta P, Steliga T, Turkiewicz A. 2020. Hydrocarbon removal by two differently developed microbial inoculants and comparing their actions with biostimulation treatment. Molecules 25:661. https://doi.org/10.3390/molecules25030661

[3] Castro AR, Cavaleiro A., Pereira MA 2019. Oil and hydrocarbon- producing bacteria. In: Stams A, Sousa D (eds). Biogenesis of hydrocarbons. Handbook of hydrocarbon and lipid microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-78108-2_8.

[4] Davoodi SM, Miri S, Taheran M, Brar SK, Galvez-Cloutier R, Martel R 2020. Bioremediation of unconventional oil contaminated ecosystems under natural and assisted conditions: a review. Environ Sci Technol 54:2054–2067. https://doi.org/10.1021/acs.est. 9b00906

[5] Gao H, Wu M, Liu H, Xu Y, Liu Z 2022. Effect of petroleum hydro-carbon pollution levels on the soil microecosystem and ecological function. Environ Pollut 293:118511. https://doi.org/10.1016/j.envpol. 2021.118511

[6] Imam A, Suman SK, Kanaujia PK, Ray A 2022. Biological machinery for polycyclic aromatic hydrocarbons degradation: a review. Bioresource Technol 343:126121. https://doi.org/10.1016/j.biort ech.2021.126121

[7] Jesus F, Pereira JL, Campos I, Santos M, Ré A, Keizer J, Nogueira A, Gonçalves FKM, Abrantes N, Serpa D 2022. A review of polycy- clic aromatic hydrocarbons distribution in freshwater Ecosystems and their toxicity to benthic fauna. Sci Total Environ 820:153282.https://doi.org/10.1016/j.scitotenv.2022.15328

[8] Lima SD, Oliveira AF, Golin R, Lopes VCP, Caixeta DS, Lima ZM, Morais EB. 2020. Isolation and characterization of hydrocarbon- degrading bacteria from gas station leaking-contaminated groundwater in the Southern Amazon, Brazil. Brazilian Journal of Biology. 80(2): 354 — 361.

[9] Medić A, Lješević M, Inui H, Beškoski V, Kojić I, Stojanović K, Karadžić I. 2020. Efficient biodegradation of petroleum *n*-alkanes and polycyclic aromatic hydrocarbons by polyextremophilic *Psedomonas aeruginosa* sunai with multidegradative capacity. RSC Adv 10:14060–14070. https://doi.org/10.1039/C9RA10371F

[10] Moreno R, Rojo F. 2019. Enzymes for aerobic degradation of alkanes in bacteria. In: Rojo F (ed) Aerobic utilization of hydrocarbons, oils, and lipids. Handbook of hydrocarbon and lipid microbiology. Springer, Cham, pp. 117–142. https://doi.org/10.1007/ 978-3-319-50418-6_6

[11] Révész F, Figueroa-Gonzalez PA, Probst AJ, Kriszt B, Banerjee S, Szoboszlay S, Maróti G, Táncsics A . 2020. Microaerobic conditions caused the overwhelming dominance of *Acinetobacter* spp. and the marginalization of *Rhodococcus* spp, in diesel fuel/ crude oil mixture-amended enrichment cultures. Arch Microbiol 202:329–342. https://doi.org/10.1007/s00203-019-01749-2

[12] Semenova EM, Babich TL, Sokolova DS, Ershov AP, Raievska YI, Bidzhieva SK, Stepanov AL, Korneykova MV, Myazin V, Nazina TN. 2022. Microbial communities of seawater and coastal soil of

Russian Arctic Region and their potential for bioremediation from hydrocarbon pollutants. Microorganisms 10:1490. https://doi.org/10.3390/microorganisms10081490

[13] Yang R, Zhang G, Li S, Moazeni F, Li Y, Wu Y, Zhnag Y, Chen T, Liu G, Zhang B, Wu X 2019. Degradation of crude oil by mixed cultures of bacteria isolated from the Qinghai-Tibet plateau and comparative analysis metabolic mechanism. Environ Sci Pollut Res 26:1834–1847. https://doi.org/10.1007/s11356-018-3718-z

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