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Biorestauration of Swage Polluted by Waste Motor Oil with *Pleurotus florida* Crude Extract and Mineral Solution

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GJSFR-H Classification: *DDC Code: 559.429*

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Abstract- In México, swage is likely contaminated by waste motor oil (WMO) that contains aliphatic and aromatic hydrocarbons. An ecological alternative to solve this problem is bio stimulation with indigenous microbiota to eliminate the aliphatic fraction of WMO. However, this aromatic part of WMO contain benzene recalcitrant, an unexplored alternative is bio stimulation with extract of *Pleurotusflorida (ePf)* and mineral solution for its elimination. The objetive of this research were to analyze bio stimulation swage polluted by WMO containing benzene with *ePf* and mineral solution for its elimination. The effect of bio stimulation of *ePf* plus mineral solution on the WMO and benzene was measuring by the amount of CO_2 produced. This result indicated that bio stimulation of swage polluted by WMO with *Pf* and mineral solution eliminated this fraction to allow the reuse of this water.

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I. Introduction

In Mexico and in many other places, environmental contamination related to petrochemicals has been recognized as one of the most serious problems for wastewater, groundwater, surface water and other bodies of water. In Mexico, the annual production of waste motor oil (WMO) is approximately 325 million liters (Soumeya et al., 2022). It is estimated that only 20% of the volume generated receives adequate final treatment.

The composition of WMO includes a wide range of aliphatic and aromatic hydrocarbons with chain lengths ranging from C 15 to C50, (Iqbal et al., 2018), minor amounts of additives, viscosity improvers, oxidation inhibitors, nitrogen, and sulfur compounds, as well as metals such as lead, zinc, barium and magnesium. These contaminants arise from normal wear of engine components and heating and oxidation of lubricating oil during engine operation. WMO may contain higher percentages of polycyclic aromatic hydrocarbons (PAHs) and additives compared to fresh oil, and the concentration of PAHs in WMO may range from 34 to 190 times higher than those in fresh motor oil (American Public Health Association, 2012; Soumeya *et al*., 2022). Therefore, WMO is a mixture of aliphatic and aromatic hydrocarbons that involves a risk to human health and the environment, notably sewage (Chandra *et al*., 2012).

The presence of benzene in WMO-contaminated sewage is particularly problematic, as it has a relatively high-water solubility (1.8 g/l, 15°C), and is easily transferred to groundwater and drinking water supplies(de Oliveira *et al*., 2009; Mitra and Roy, 2011; Iqbal *et al*., 2018). Benzene is challenging to remove because it lacks an activating (O_2) oxygen or N(nitrogen) substituent group, making the oxidation of the ring not energetically feasible. Long-term health effects of benzene exposure include adverse effects on bone marrow and cancer in humans(El-Naas *et al*., 2014).

Various biological remediation schemes have been investigated to treat water, sewage, and industrial effluents containing aliphatic hydrocarbons and benzene (Harms *et al*., 2011; Chandra *et al*., 2018). The most widely applied biostimulation for aliphatic hydrocarbon´s its elimination by the native microbial consortium via enrichment withbasic minerals such as nitrogen, phosphorous, potassium, and others (Demir, 2004). However, biological treatment methods have commonly been limited by the toxicity of these compounds, and the correspondingly low concentrations of the substrates to which the microbes must be exposed (Okolafor and Ekhaise, 2022). Yet, while most of the studies have focused on bacteria, little is known about the contribution of fungi of the bioremediation of the environment polluted by benzene (Gadd, 2001; Dittman *et al*., 2002).

Fungal-mediated mineralization of soil pollutants has mainly been assayed with white-rot fungi (Demir, 2004; Okolafor and Ekhaise, 2022). It has been shown that many species belonging to the white rot fungi group can degrade lignin, which is a natural polymer (Harms *et al*., 2011). Therefore, an environmentally friendly solution to induce full or partial mineralization of WMO in sewage is the biostimulation of native microbiota by enrichment with essential macronutrients (Surajudeen and Benjamin, 2009).

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Removal of the aromatic fraction is possible with an extracellular enzyme extract of *P. florida (ePf)*, a basidiomycete that synthesizes Manganese peroxidase (MnP), Lignin peroxidase (LiP) and a Lactase (LiP)(Gadd, 2001; Dittman *et al*., 2002; Demir, 2004; Harms *et al*., 2011). This is an enzymatic complex with a substrate chemical non-specificity to hydrolyze aromatic rings, similar to those in the composition of WMO (Estebar *et al*., 2012). The objetive of this research were to analyze biostimulation swage polluted by WMO containing benzene with *Pf* and mineral solution for its elimination.

II. Material and Methods

a) *Fungi cultivation and obtaining of enzymatic crude extract*

The fungus *P. florida* was donated by Kamuro Inc. based in Morelia, Michoacán, Mexico. It was grown by preparing malt extract and incubated at 28 °C for seven days. The fungi were inoculated in a flask containing distilled water and 7.5 g of sterile wheat straw as the only source of carbon and energy. The flask was incubated at 28°C for 14 days, according to Demir (2004). At the end of the period, the flask content was centrifuged at 1000 rpm/10 min, and the supernatant was filtered using a Millipore membrane, 0.2 μ. The protein concentration was measured using a curve of bovine albumin as standard. The *ePf* was conserved in glycerol at -20°C until use.

b) *Effect of biostimulation with extract of P. florida sewage polluted by waste motor oil containing benzene*

WMO was diluted (1:100) in distilled water. Immediately, a sample of 10 ml was transferred to a Bartha flask with 500 ml capacity, with H_2O_2: 2 ppm; $MnSO_4$: 2 mM1.0mlande*Pf* 1mg/ml. All Bartha flasks were incubated at 30°C (±2°C), 100 rpm for a three-weeks period. A relative control consisted of 100 ml of sewage with the diluted WMO, with sodium azide, H_2O_2 + MnSO4 and no *ePf* biostimulation. An absolute control composed of 100 ml of sewage, sterilized *ePf*, 10 ml of diluted WMO, Tween 80 and sodium azide was also used to inhibit any biological activity. All assays were carried out using triplicates.

Biostimulation of swage polluted byWMO containing benzene with a mineral solution Six Bartha flasks were used, containing 100 ml of sewage, WMO diluted 1:100, and a mineral solution with the following composition (g/l): K_2HPO_4: 4; $MgSO_4$: 3; NH_4NO_3: 10; $CaCO_3$: 1; KCl: 2; $ZnSO_4$: 0.5; $CuSO_4$: 0.5; $FeSO_4$: 0.2; EDTA 8.0; tween 20 0.01%; H_2O_2: 2 ppm; $MnSO_4$:2 mM;1 and 1 mg/ml of the *ePf* extract. All Bartha flasks were incubated at 30°C (±2°C),100 rpm for another three-weeks period. The experiment was carried out using triplicates (Mathur and Majumder, 2010).

c) *Analysis of aliphatic hydrocarbons*

The analysis of benzene concentration was carried out using a gas chromatograph (Perkin Elmer Autosystem Series) coupled toa FID, using anElite-5 Capillary Column coated with a 5% diphenyl/95% Dimethyl Polysiloxane stationary phase, 30m length, 0.25diameter, 0.25 mm film thickness in a split injection mode. The carrier gas was Helium; the column oven temperature was 40° C for 8 min and was increased from 40-180° C at 6° C min-1. The injector temperature was 250° C (Gosh *et al.,* 2018).

d) *Experimental design*

Five treatments, as shown in Table 1, were used to analyze the effect of ePf on benzene ring breakage in sewage with WMO as well as its mineralization by biostimulation with a mineral solution.

Table 1: Experimental essay on biostimulation of swage polluting by waste motor oil *Pleurotusflorida* and biostimulation with mineral solution

Treatment (T)	Sewage	*P. florida* extract	WMO	Sodium Azide	Tween 80	H_2O_2	$MnSO_4$	Mineral solution
1 (relative control)	+	-	+	+	-	+	+	-
2(absolutecontrol)	+	+*	+	+	+	+	+	-
3	+	+	+	+	+	+	+	-
4	+	+	+*	+	+	+	+	-
5	+	+	+	+	+	+	+	+

**Sterilized, (+) =use; (-) = non use*

III. Results

Figure 1 shows that benzene degradation activity was induced by the biostimulation with ePf in sewage contaminated by WMO. The breakdown of WMO benzene showed a delay, that had been mostly degraded; this suggests the presence of the interaction of this aromatic compound interaction between WMO constituents during its degradation. The analysis of WMO yielded an initial benzene concentration of 34.2 μmol in the Bartha flasks. The biostimulation with *ePf* induced the depletion of benzene concentration in only four days. However, in the same period, there was also an abiotic loss of benzene, probably due to evaporation, since the concentration in the control experiment on the third day was 19.2 μmol. Benzene decreased after four days; in the control treatment without biostimulation with *ePf*, this loss due to evaporation was up to 30% (Figure

1); an abiotic loss of benzene has been reported to increase with incubation time due to its high solubility in water, volatilization and adsorption on the walls of Bartha flasks (Shah, 2017). The aerobic microbial degradation of the WMO had different patterns on CO_2 production(Figure 2).The microbial benzene on WMO breaking increased in 5 days after biostimulation with mineral solution with macronutrients based in mineral salts of N, P, K, and other elements to enriched sewage polluted with WMO; this was followed by a constant decrease over the next 16 days of the issue (Gadd, 2001; Surajudeen and Benjamin, 2009).

While Figure 3 shows the effect of biostimulation of sewage contaminated with WMO by *ePf* and mineral solution on benzene removal that reached up to a concentration of 32.77 μM (according to the data shown in Figure 1) in less than five days compared to the control data, as no further increase in CO_2 production was observed without the biostimulation caused by the mineral solution. The positive effect on CO_2 production rates indicates that the WMO-contaminated sewage contained a sizeable microbial community capable of mineralizing aromatic hydrocarbons.

In figure 4a, shows the chromatogram of benzene before it was broken down by biostimulation with *ePf* in sewage contaminated with WMO diluted 1:100. Figure 4b shows the chromatogram when benzene was eliminated after biostimulation with ePf and mineral solution.

IV. Discussion

This research has been based on biostimulation of the benzene contained in the WMO by *ePf* and its elimination with mineral solution for the indigenous microbial population in sewage polluted WMO (Rajasulochana and Preethy, 2016; Okola for and Ekhaise, 2022). An attempt was made to evaluate the biostimulation by mineralization kinetics of the microbial consortium. The degradation kinetics of WMO benzene, was analyzed and modeled mathematically. This study shows that *ePf* was able to degrade WMO benzene hydrocarbon in a microcosm. These results show that the participation of fungal extract in the biodegradation of aromatic pollutants in sewage is consistent with reports generated by other authors (Demir, 2004; Surajudeen & Benjamin, 2009; Shah, 2017; Chandra et al., 2018). Biostimulation of WMO containing benzene required the *ePf* and mineral solution due to activity of the indigenous microbiota in sewage exhibited the extraordinary capacity of the microbial consortium to mineralize petroleum aromatics hydrocarbons (Dittman et al., 2002; Estebar et al., 2012). These results also confirm that benzene in WMO is more recalcitrant than aliphatic hydrocarbons (Mathur and Majumder, 2010; El-Naas et al., 2014). Currently, bioremediation of WMO-contaminated sewage containing benzene, is carried out through in situ treatments such as bioventing. However, biostimulation of WMO-contaminated sewage with *ePf*and mineral solution are very important to remove aromatic hydrocarbons, including volatilization (Demir, 2004; Surajudeen and Benjamin 2009; Mathur and Majumder, 2010; Mitra and Roy, 2011). Fungi growing on volatile aromatic hydrocarbons have been used successfully for the biofiltration of air containing volatile hydrocarbons (Harms et al., 2011; Iqbal et al., 2018; Okolafor and Ekhaise, 20002).

This preliminary study indicates that biostimulation of benzene-containing WMO-contaminated sewage by *P. florida* extract and mineral solution showed a microbial population capable of mineralizing benzene (Gadd, 2001; Demir, 2004; Chandra et al., 2018). Further studies are still needed to evaluate the biostimulation of benzene-containing WMO-contaminated sewage with *P. florida* extract and mineral solution on a large scale (Rajasulochana and Preethy, 2016).

V. Conclusion

This research concluded that biostimulation of *ePf*¸ and mineral solution in recovering sewage polluted by WMO containing benzene to reuse in irrigation city gardens and industrial issues.

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Conflicts of interest

The authors declared no have conflict interest for the study.

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Figures

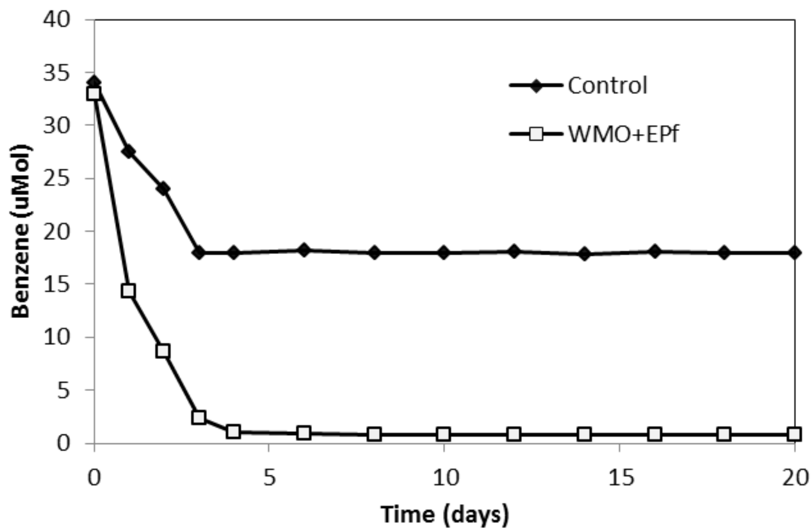

Figure 1: Effect of biostimulation with *Pleurotus florida extract* on benzene hydrolysis in waste motor oil polluting sewage before biostimulation with mineral solution.

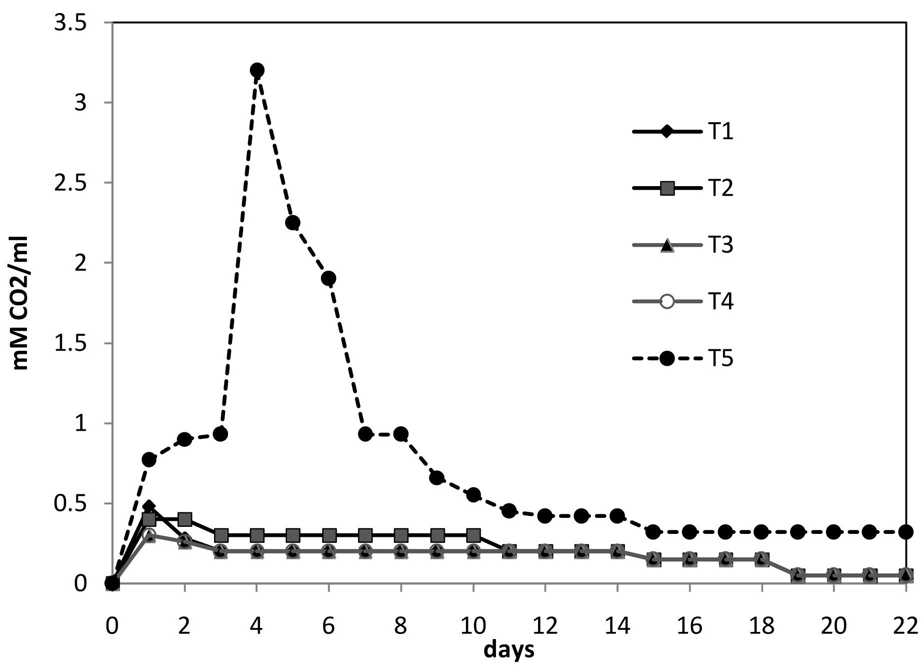

Figure 2: Effect of biostimulation sewage polluted by waste motor oil with *Pleurotus florida* extract and mineral solution

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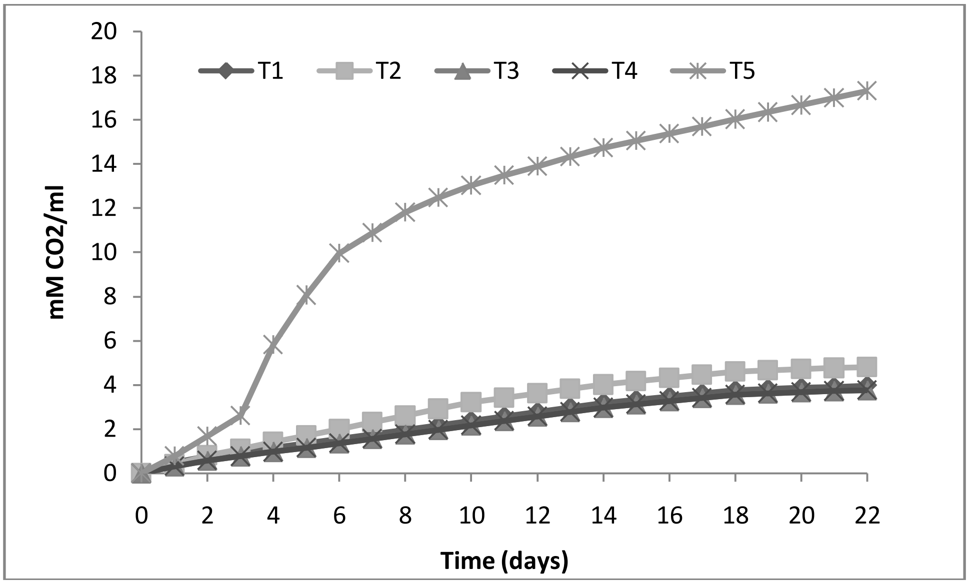

Figure 3: Effect of biostimulation sewage polluted by waste motor oil with *Pleurotus florida* extract and mineral solution on benzene elimination

T1: sewage/WMO (benzene) biostimulated with *P. florida extract* --NaAzide -H_2O_2-$MnSO_4$

T2: sewage/WMO (benzene) biostimulated with Tween 20+ *P. florida extract* +Na Azide+H_2O_2+$MnSO_4$

T3: sewage/WMO (benzene) biostimulated with Tween 20 - *P. florida extract* +Na Azide+H_2O_2+$MnSO_4$

T4: sewage/WMO (benzene) biostimulated with tween 20+ *P. florida extract* sterilized+ Na Azide +H_2O_2+$MnSO_4$

T5: sewage/WMO (benzene) biostimulated with tween 20+ *P.florida extract*+H_2O_2+$MnSO_4$+mineral solution

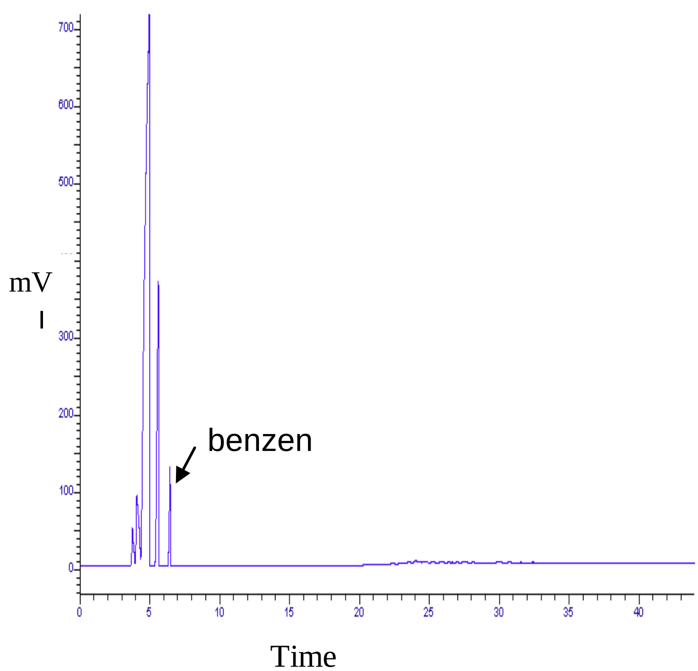

Figure 4a: Chromatogram showing the benzene peak before been breaking by *ePf* insewage sludge polluted with waste motor oil

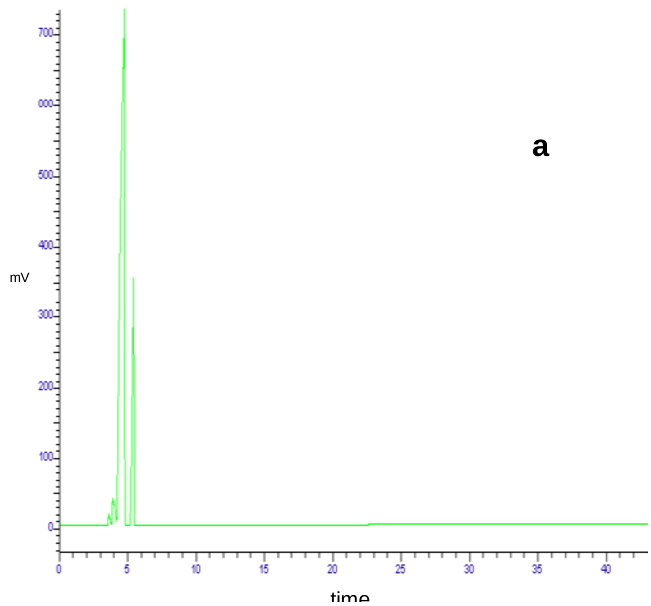

Figure 4b: Chromatogram showed when benzene in waste motor oil was mineralized completely after applying e*Pf* polluted with sewage