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Potential resistance of asphalt, tar and polyethylene coatings in deep pipelines to native soil microorganisms

Abstract

The protection of the alloys of deep pipelines in the oil industry and similar coated with derivatives of aliphatic and aromatic hydrocarbons such as asphalt, tar and polyethylene are necessary to prevent damage to the different types of alloy in the pipes that are established in liquid environments such as water, or solid environments such as soil under abiotic and biotic conditions that cause corrosion and considerable economic losses in the restoration of fuel and water conduction systems: such as temperature, pH, concentrations of mineral salts, oxygenation level, etc. in which aerobic heterotrophic microbial activity. Therefore, the objectives of this work were i) to analyze the resistance to biodegradation of asphalt, tar and polyethylene tapes in the soil ii) to analyze the capacity of soil microorganisms to use these tapes as a source of carbon and energy iii) to identify the biochemistry of the microorganisms that attack these coatings. To this end, asphalt, tar, and polyethylene tapes were introduced into non-sterile soil to analyze what types of microorganisms use as a source of carbon and energy to biodegrade its destruction leading to corrosion. The microorganisms responsible for this biodegradation were then identified. The results show that a wide variety of microorganisms exist in the soil that biodegrade these tapes, utilizing them as a source of carbon and energy. These results support the need for chemical modifications to increase resistance to biodegradation and prevent damage to thousands of kilometers of deep-water pipelines used for various important human-industrial activities.

Keywords: hydrocarbons, soil, protection, corrosion, microbial potential, underground pipe

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Introduction

The petrochemical industry, such as gas pipelines, uses deep-seated pipes coated with asphalt, tar, or polyethylene in the ground. These pipes, depending on their chemical composition, are resistant to biodegradation, with the resulting corrosion of the alloys and economic losses.[1,2] This is a consequence of the main physical, chemical, and biological effects that are constant in the diverse environments where deep-seated pipes are installed.[3–5] In the soil where these pipelines are installed, there is a wide variety of aerobic heterotrophic microorganisms capable of utilizing asphalt, tar, or polyethylene as a source of carbon and energy.[6–8] The susceptibility of pipeline coatings makes it necessary to increase the resistance of the various types of coatings to deterioration to reduce the high cost of replacing pipelines that cover thousands of kilometers worldwide.[9–12] Therefore, the objectives of this work were i) to analyze the resistance to biodegradation of asphalt, tar and polyethylene tapes in the soil ii) to analyze the capacity of soil microorganisms to use these tapes as a source of carbon and energy iii) to identify the biochemistry of the microorganisms that attack these coatings.

Material and methods

To analyze the resistance of asphalt, tar and polyethylene coats. These tapes of each 10 x 5 cm were buried in each soil at a depth of 20 cm and of different types. For 4 months, the humidity was maintained in each case, at the capacity of field of each soil at ambient temperature trying to simulate the common conditions in tropical environments minimum temperature 15 ºC during the night maximum of 40 ºC during the day. Each month the coats were weighed based on the initial value of the same coat left in the laboratory according to standard recommendations indicated in the literature on the matter. From the adhered soil, the following were isolated on agar and enriched tape broth (g/L): asphalt, tar and polyethylene pulverized cut dissolved in a solvent when was possible 10.0, peptone 5, yeast extract 1.5, NaCl 1.4, K_2HPO_4 3.0, $MgSO_4$ 2.5, with 20 ppm/L of yeast extract as growth factor, pH 6.5 with and without agar 18.0.[9–12] When the experiment was carried out in 250 ml Erlenmeyer flasks with 150 ml of enriched coats broth, were incubated at 30ºC with shaking at 200 rpm for four months using dry weight of the final cells and protein concentration as a variable response to measure microbial capacity to growth under control conditions in asphalt, tar and polyethylene coats dissolved as was explain before to add as a carbon and energy source.[13,14] The petri dishes with the enriched coats agar were incubated for 15 days up to a month, the colonies of the microorganisms were reseeded in selective agar for *Pseudomonas* or Sabouraud for fungi. Axenic cultures were identified by morphology and type of sporulation in the case of fungi and biochemistry for bacteria.[15–18] All experimental data were analyzed by ANOVA and Tukey ($P \leq 0.05$), to establish the minimum significant difference.

Results and discussion

Table 1 shows the physicochemical analysis of the soils used for the isolation of aerobic heterotrophic microorganisms: genera of bacteria and microscopic fungi native to those sites, from the south of the Mexican Republic: 4 soils from Tabasco and 12 soils from Veracruz, México with 4 from each site: Coatzacoalcos, Minatitlan and Pajaritos, w which showed a humidity that varied from 8.8% to 9.65%, values of soils from semi-desert areas by the type of sandy loam texture, with an organic matter content that was poor to medium 3 to 4.2% which reduces the diversity and activity of microorganisms that can have a degrading effect on the coatings of the deep pipe.[2–5] While the pH fluctuation was recorded from 4.8 to 7.3, the acid value to slightly alkaline, products of the type of organic matter in these soils, where the deep pipe is installed, coated with asphalt, tar and/or polyethylene of the abiotic and biotic factors with covers derived from petroleum asphalt, tar, or polyethylene.[6,8,9]

Table 1 Physico-chemical analysis of soils in contact with asphalt, tar and polyethylene coats of the buried pipe from some specific states of Mexican Republic

Origen of soil sample	Number of soil simples	Texture	pH	Humidity (%)	Organic matter concentration Per cent (%)
Villahermosa, Tabasco, México	4	sandy	4.8	8.8	3 (poor)
Minatitlán, Veracruz (Ver) México	4	sandy	7	9.5	4 a 3.3 (medium to poor)
Pajaritos, Ver, México	4	sandy	6.7	9.	4. 2 (medium)
Coatzacoalcos, Ver, México	4	sandy	7.3	9.65	4. (medium)

Table 2 shows the solubility of different types of asphalt coating whose chemical composition is divided into asphaltenes and maltenes; asphaltenes are high molecular weight, where asphaltenes are mixtures of aromatic hydrocarbons, in addition to carbon and hydrogen, they contain nitrogen, oxygen, sulfur, traces of aluminum, iron, nickel and vanadium, while maltenes contain naphthenic aromatics, with some paraffin chains, compounds insoluble in organic solvents such as ethanol, xylene and ethyl ether but soluble in benzene, toluene and chloroform, while vegetable pitch or tar, a mixture of aromatic hydrocarbons, was partially soluble in toluene, with no solubility problems in xylene, chloroform and benzene. Meanwhile, high-density polyethylene is a thermoplastic polymer composed mainly of repeating units of ethylene (C_2H_4); for deep pipelines was insoluble in all solvents used: benzene, chloroform, ethyl ether, ethanol, toluene and xylene. However, given the capacity of soil microorganisms that can synthesize biodetergents and lipases to use them as a source of carbon and energy, it was demonstrated that they have the capacity to destroy it.[1,3,4–6]

Table 2 Solubility of coatings in organic solvents of asphalt, tar and polyethylene for deep pipe

Type of coating used in deep pipe	Toluene	Xilene	Choloform	Ethyl ether	Ethanol	Benzene
Asphalt	+	-	+	-	-	+
tar	+/-	+	+	-	-	+
Polyethylene	-	-	-	-	-	-

All of them were tested in a ratio of 1:10 + = Dissolves well ± = Slightly soluble - = insoluble

Table 3 shows the potential capacity of *Pseudomonas putida, Fusarium oxysporum, Trichoderma harzianum* and *Penicillium chrysogenum*. It shows that *P. putida, F. oxyporum,* and *T. harzianum,* due to their genome, synthesize biodetergents, lipases, and enzymes that oxidize aliphatic and aromatic hydrocarbons to use asphalt and polyethylene as a source of carbon and energy.[13,15–19] While it was evident the difficulty that these native microorganisms from soils where deep pipelines exist had in utilizing pitch as a source of carbon and energy.[20–23] However, in all the soils analyzed it is possible to find microorganisms that, under certain external environmental conditions can oxidize these coatings,[24–28] and then expose the metallic alloys of this pipeline to the environment of each soil, causing corrosion and the consequent damage that is expensive to repair, in that sense better chemicals improvement should be made to protect deep pipelines inside the different types of soil.[27–31]

Table 3 Use of asphalt, tar and polyethylene coats as sources of carbon and energy in culture medium, by microorganisms' soil natives

		A		
Type of coating used in deep pipe	***Pseudomonas putida***	***Fusarium oxyporum***	***Trichoderma harzianum***	***Penicillum chrysogenum***
Asphalt	+++	+++	++	+
Tar	++	+	+	-
Polyethylene	+++	+++	+++	+++

+++ abundant growth ++ medium growth + poor growth

Table 4 shows that *P. putida* and *F. oxysporum* grew without problems in the asphalt and tar covers, observing that they solubilized both hydrocarbon compounds, which implies the synthesis of biodetergents and lipases and then, through a depolymerization of this mixture, oxidize them by using them as a source of carbon and energy,[21–23] a situation that in the soil, according to the specific properties of each type, is possible to occur, which leads to the degradation of these covers with the risk of subsequent corrosion of the metallic alloys of the deep pipe.[24–26] While *T. harzianum* was registered to grow on asphalt hydrocarbons but with difficulty on pitch hydrocarbons indicating that they have genetic limitations to synthesize biodetergents and lipases as well as other enzymes laccase types to use them as a source of carbon and energy, in contrast to *P. chrysogenum* which was unable to oxidize pitch hydrocarbons[27–30] due to do not have complex vitamins B as growth factor. However, it should be noted that in soils the combination of metabolisms of

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microorganisms that attack hydrocarbons is by a consortium action so this inability of *P. chrysogenum* does not limit the deterioration of pitch covers[31–33] hence the need to improve the resistance capacity of these hydrocarbon covers to the natural biological action of the native microbiota of the environment.[34]

Table 4 Use of asphalt and tar as the only source of energy and carbon by soil microorganisms

Soil microorganisms	Culture medium using asphalt and tar as only energy and carbon source	
	Asphalt	Tar
Pseudomonas putida	+++	+++
Fusarium oxyporum	+++	++
Trichoderma harzianum	++	+
Penicillum chrysogenum	++	-

Dissolved with sodium bentonite at a surface area of 3.1 wt/wt.

(+++) = abundant growth, (++) = regular growth, (+) = poor growth, (-) = negative growth

Table 5 shows the maximum degradation capacity of the pitch with 50.62% by *F. oxysporum* through the synthesis of biodetergents, lipases, moonoxygenases: lignin-peroxidase, laccase and manganese-peroxidase that allowed it to use this mixture of aromatic hydrocarbons as a source of carbon and energy,[26–34] in clear contrast to the limited degradation capacity of *P. putida* which reached 10.7%, as well as *P. chrysogenum* with 6.56% and *T. harzianum* with 5.36% as soon as they grew on the pitch.[35–37] In relation to the resistance of asphalt another mixture of aliphatic and mainly aromatic hydrocarbons the percentage of degradation of *P. putida* was 11.3%, followed by *F. oxysporum* with 9.64%, *P. chrysogenum* 5.0% and the lowest of *T. harzianum* with 4.8% here it was evident that individually the destruction of the asphalt-based coating is not possible but in the soil in an open environment the existence of microbial consortia shows that under a multiple action the asphalt can be degraded.[38–40] Finally, when *P. putida* grew on the polyethylene-based coating it reached 21.5% degradation, then *P. chrysogenum* with 5.0%, while *F. oxysporum* and *P. chrysogenum* barely reached 3.5 and 3.4 respectively.[22–26]

Table 5 Potential percentage of biological degradation by soil microorganisms of the types of coatings for deep pipes: asphalt, tar and polyethylene used under laboratory conditions

Microorganisms Islotes from soil	Coating								
	Tar			Asphalt			Polyethylene		
	weight 1	weight 2	%	weight 1	weight2	%	weight 1	Weight 2	%
Pseudomonas putida	0.5[c**]	0.44[c]	10.7	0.5[c]	0.44[c]	11.3	0.5[c]	0.39[a]	21.5
Fusarium oxyporum	0.5[c]	0.47[a]	50.62	0.5[c]	0.45[b]	9.64	0.5[c]	0.47[b]	3.5
Trichoderma harzianum	0.5[c]	0.47[a]	5.36	0.5[c]	0.47[a]	4.8	0.5[c]	0.48[b]	3.4
Penicillium chrysogenum	0.5[c]	0.46[b]	6.56	0.5[c]	0.47[a]	5.0	0.5[c]	0.47[b]	5.0

*All data were average of four repetitions * *ANOVA and Tukey (P ≤ 0.05).

Table 6 shows the growth of *P. putida,* which was selected based on its genome that has been shown to synthesize a wide variety of biodetergents to solubilize hydrocarbon fractions, lipases, monooxygenases, lignin peroxidases in culture medium,[24–28] in which protein concentration was used as an indirect measure of the genetic capacity of *P. putida* to degrade asphalt coatings with an increase in protein from the initial value to the final of 13.2%, with 22.0% in pitch and 19.2% with polyethylene when using different hydrocarbons first the linear short chain aliphatic type and then continue with the simple aromatics and subsequently the polycyclic as a source of carbon and energy.[29–33] Which shows that in the soils analyzed as reported in the literature there is a wide diversity of microorganisms that potentially have the capacity to degrade deep pipe coatings,[35–40] especially because in open environments the physical and chemical environmental conditions can facilitate or partially or totally stop the destruction of the coatings that protect the deep pipe,[35–39] which is why it is important to analyze the biological activity of the microorganisms that exist naturally as well as the physicochemical properties of the environments for the selection of coatings with greater resistance to deterioration that prevent the possible corrosion of deep pipes of high economic and industrial value.[41–44]

Table 6 Growth of *Pseudomonas putida* as a function of the increase in protein* by using coating used in deep pipe asphalt, tar and polyethylene, the only source of carbon and energy

Dynamic of growth *P. putida* in different coats used in deep pipe measured by protein increase per cent	asphalt	tar	polyethylene
		+*Protein (µg/ml)	
Initial	160.5[b**]	65.5[b]	119.0[b]
final	173.7[a]	87.5[a]	128.2[a]
Increase per cent	13.2	22.0	19.2

+All data were average of four repetitions * *ANOVA and Tukey (P ≤ 0.05)

Table 7 shows the morphological and biochemical profile of *P. putida*, a genus and species with a notable cosmopolitan character, with the potential capacity to oxidize all types of complex and simple organic compounds, such as sugars and hydrocarbons found in asphalt, tar and polyethylene under aerobic conditions.[45–50] This is why it synthesizes the enzymes catalase and oxidase and can also use inorganic compounds with NO_3 as the final electron acceptor and reduce it in anaerobiosis.[2,15,22] The genera and species of microscopic soil fungi are identified based on the reproductive structures they generated in selective media for each one; in some cases, fungicide sensitivity tests were performed to demonstrate the species (data not shown).

Table 7 Morphology and biochemical behavior of the *Pseudomonas putida* with potential ability to use asphalt, tar and polyethylene as sources of energy and carbon

Biochemical test	*Pseudomonas putida**
Shape	Rods
Motiitiy by flagella	+
Gram	Negative -
Oxidase	+
Catalase	+
Use as energy and carbon source	
Sucrose	+
Lactose	-
Glucose	+
Maltose	+
Citratates	+
Succinates	+
Acetates	+
L-arginine	+
Reduction of $NO^{-3} \rightarrow NO^{-2}$	+

(+) Positive response, Negative response (-).[44–50]

Conclusion

It was clear that the resistance of asphalt, tar, and polyethylene coatings depended on their chemical composition, as well as on the combinations of aliphatic, aromatic, and polycyclic hydrocarbons found in asphalt and tar. In contrast to the large, long-chain hydrocarbon polymers in polyethylene, these types of coatings are resistant to a wide range of abiotic and biotic environmental conditions. It was evident that all soils analyzed contained an aerobic heterotrophic microbial population capable of degrading asphalt, tar, and polyethylene coatings. This is common, independent of the physicochemical properties of the soils of Tabasco and Veracruz, southwest Mexico, sites where large extensions of deep pipelines are located due to oil industry activities. Laboratory results indicate that individual soil microorganisms can utilize asphalt, tar, and polyethylene coatings as carbon and energy sources. This demonstrates that, due to the nature of the coatings, the degradation of each coating requires intrinsic chemical modifications so that the deep pipeline in the various environments where they are installed has sufficient resistance to protect it from both physical and chemical actions, given the high value that water and fuel represent for the economy of transport.

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Conflicts of interest

The authors declare that there is no type of conflict of interest in its planning, execution and writing with the institutions involved, as well as those that financially supported this research.

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