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Petroleum asphaltenes: generated problematic and possible biodegradation mechanisms

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ABSTRACT. Petroleum asphaltenes are hydrocarbons that present an extremely complex molecular structure. They are conformed by different proportions of nitrogen, sulfur and oxygen. These compounds cause diverse problems like the blockage of crude oil extraction and transport pipes, the reduction of their economic use and the pollution of ecosystems. Biodegradation of asphaltenes is an important process that can eliminate these compounds and reduce the problems they cause. However, it is a process that occurs naturally in very reduced proportions. The purpose of this revision is to show the chemical structure of these compounds, the problems they cause and to represent their possible biodegradation mechanisms, based on the processes known for other hydrocarbons of complex structure.

Elimination of the micellar structure, through the application of non-polar solvents, and fragmentation of the asphaltenes through photo-oxidation are the initial processes necessary to be able to degrade these compounds. The produced structures, such as the heteropolycyclic and aromatic, lineal and ramified hydrocarbons, could be degraded in this order through biochemical reactions, such as omega oxidations, beta oxidations and aromatic oxidations respectively. These processes are distributed in an important variety of microorganisms. The elimination period's length can vary from one week, for the simplest structures, to 990 days for those with several condensed aromatic rings.

Key words. Asphaltenes, biodegradation, petroleum.

INTRODUCTION

Petroleum is a non-renewable resource, which is mostly constituted of hydrocarbons. Among these, a conglomerate of very high molecular weight and complex structure compounds exists, with different proportions of nitrogen, sulfur and oxygen, known in conjunction by the generic name of Asphaltenes.

These compounds have a great influence upon the quality of crude oil, in such a way that they can lower its price and the price of its products if their concentration is too elevated. On the other hand, their complex molecular structure does not allow an easy degradation by the microorganism populations of the ecosystems, which produces an accumulation of asphaltenes in places where they get in touch with both petroleum and the environment.

At present, the function that microorganisms have in the degradation of petroleum asphaltenes is unknown. This

RESUMEN. Los asfaltenos del petróleo son hidrocarburos que presentan una estructura molecular extremadamente compleja, los cuales están conformados por diferentes proporciones de nitrógeno, azufre y oxígeno. Estos compuestos ocasionan diversos problemas como el bloqueo de tuberías de extracción y transporte de crudo, reducción de su aprovechamiento económico y contaminación de los ecosistemas. La biodegradación de los asfaltenos es un proceso que constituye un importante método para eliminar a estos compuestos y tratar de reducir los problemas que ocasionan, sin embargo es un proceso que ocurre en proporciones muy reducidas. El propósito principal de esta revisión es mostrar la estructura química de estos compuestos, los problemas que ocasionan y proponer algunos mecanismos de biodegradación posibles, basados en los procesos conocidos para otros hidrocarburos de estructura compleja. La eliminación de la estructura micelar por la aplicación de solventes no polares y la fragmentación de los asfaltenos por fotooxidación son los procesos iniciales necesarios para poder degradar a estos compuestos. Las estructuras producidas como los hidrocarburos lineales y ramificados, heteropolíaromáticos y aromáticos podrían degradarse en este orden a través de reacciones bioquímicas como oxidaciones omega, beta y aromáticas respectivamente, que son procesos distribuidos en una variedad importante de microorganismos. El tiempo de eliminación puede variar de una semana para las estructuras más simples, hasta 990 días para aquéllas con varios anillos aromáticos condensados.

Palabras clave. Asfaltenos, biodegradación, petróleo.

constitutes a biotechnological process that could be an alternative method, helpful in diminishing problems caused by these compounds, in terms of economical profit as well as crude leaking. Therefore, the aim of this revision is to deliver a general view of the asphaltenes' chemical structure and the problems they cause. Another aim is to provide some possible theoretic mechanisms of microbial elimination of such resistant compounds, for they apparently represent the limit of microorganisms' biodegradation capability.

PETROLEUM ASPHALTENES, CHEMICAL STRUCTURE AND PROPERTIES

In general terms, asphaltenes are considered organic compounds that may or may not be associated with petroleum. Organic sources of these compounds, not related to petroleum, such as charcoal, schist, and those contained in sedimentary rocks, probably constitute the most abundant form of carbon on Earth.⁴⁶

J. Boussingault originally defined the term "asphalte" in 1837, as the residue of crude distillation. In present days, petroleum asphaltenes are considered to be the high-

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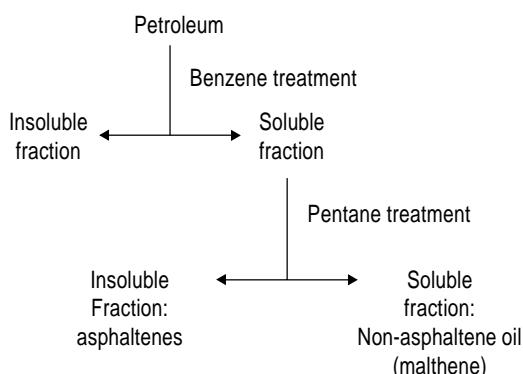


Figure 1. Petroleum asphaltenes solubility (Modified from Long, 1981²⁵).

hest in molecular weight compounds that belong to the group of heterocyclic compounds, which are composed of carbon, hydrogen, nitrogen, sulfur and oxygen.²⁹ These compounds can be described according to two viewpoints:^{4,31} operational (based upon solubility) and molecular (according to its chemical structure).

With respect to solubility, in Fig. 1 a diagram is shown, in which asphaltenes are considered as the most polar fraction of petroleum, soluble in benzene and toluene, but not in pentane and heptane.^{9,25,27,39} The appearance of asphaltenes after being submitted to the effect of pentane or heptane is that of solid, amorphous, friable particles of brown or black colour.^{29,46}

Previously, it was possible to make a simple classification of asphaltenes, according to the solvent applied to precipitate them, in such a way that pentane-, hexane-, and heptane-asphaltenes could be obtained. Nowadays, the tendency is simply to consider as asphaltenes the fraction obtained when using heptane, due to minimal composition differences existent when comparing heptane and hexane usage.^{8,43}

In relation to chemical structure, some authors comment that there is not an accurate asphaltene conformation yet,¹ nevertheless, most authors coincide in representing them as the highest molecular weight hydrocarbons in petroleum, with reported values^{22,45} ranging from 600 to 3×10^5 , and from 1000 to 2×10^6 .

The chemical structure of these compounds is based mainly on carbon and hydrogen, followed by sulfur and nitrogen. Besides, they contain minor proportions of nickel and vanadium in porphyrines included in the general structure.³¹

There are different models of asphaltene structure, that coincide in showing them as a system of aromatic and aliphatic structures of 100 to 300 carbon atoms, with alkyl substitutes and sulfur, nitrogen and oxygen covalently linked, conforming heterocyclic structures.⁴⁶ Nevertheless, the number and disposition of the latter differ in a significant way, as in the model suggested by Speight and Moschopedis⁴⁵ (Fig. 2) where asphaltenes are represented as systems of 6 to 20 or more condensed aromatic structures, linked via alkyl chains. Compared to the model of Strausz⁴⁶ and Murginch³¹ it differs in a very important factor: instead of

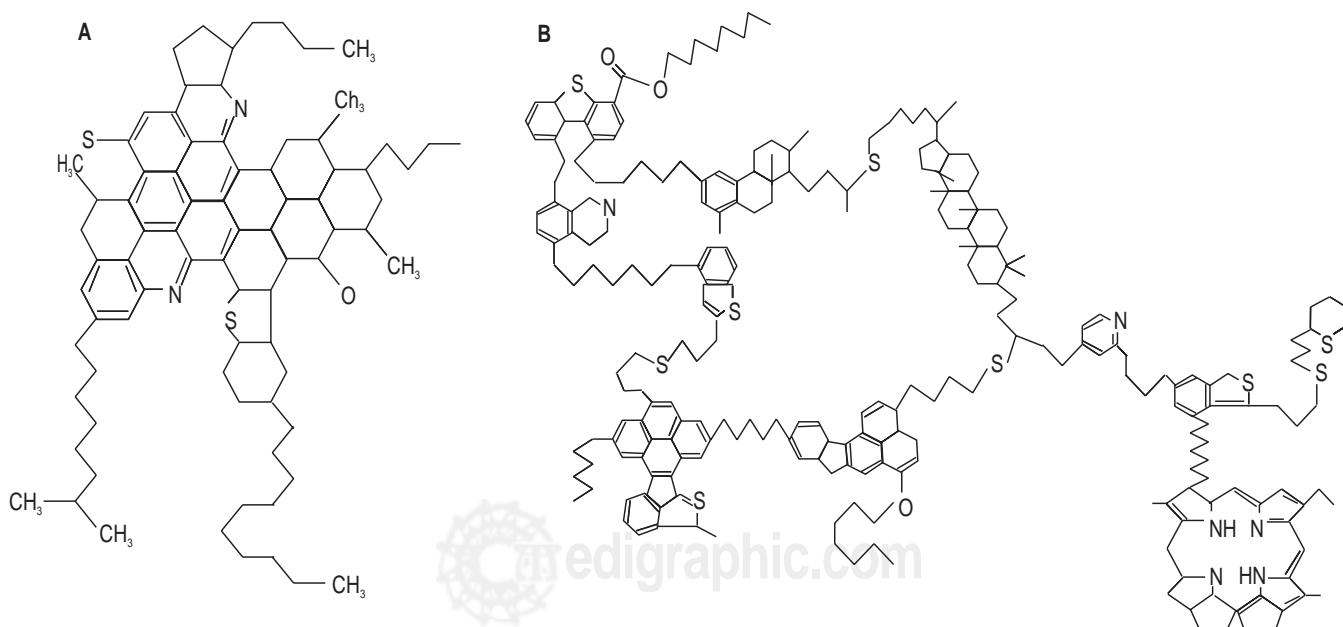


Figure 2. Molecular structures of asphaltenes, proposed by Speight and Moschopedis (A) and Strausz y Murginch (B). (From (A) Speight and Moschopedis, 1981⁴⁵ and (B) Murginch et al., 1999³¹).

showing asphaltenes as a system of numerous aromatic and heterocyclic structures, they propose them as small units of this kind, with a low condensation development and linked by aliphatic chains, with a varying longitude of 4 to 6 or more carbon atoms, and these are linked to aromatic carbons, meaning those included in an aromatic ring.^{1,5} Nitrogen, sulfur and oxygen atoms are incorporated in the structure as internal constituents and functional groups.³⁵

The structure and properties of asphaltenes are characteristics of great importance, determining, in a large proportion the problems they cause in both wells and leakings,⁹ as much as their susceptibility to microbial degradation.

PROBLEMS GENERATED BY ASPHALTENES

It is possible to classify the problems associated to asphaltenes in five general groups:^{24,32} extraction, transport, processing, crude economical profit and leakings.

With respect to extraction, asphaltenes have a large capability of blocking the porous spaces of the deposit, provoking a reduction of the permeability and a remarkable diminishment of the crude's exit flux.^{5,32,55}

When transporting petroleum through pipelines and metallic equipment in general, these compounds might be precipitated by the presence of ferric ions combined with acidic conditions, thus provoking the formation of a solid known as "asphaltenic mud" which deposits in conducts, blocking them and obstructing the free flow of crude.^{1,19} When this kind of mud develops, solvents, such as toluene and xylene are applied in order to dissolve them. This process increases production costs and generates residues of a high toxicity degree.¹⁹

As far as processing implies, asphaltenes affect petroleum refining, causing sulfur elimination to be decreased by developing a catalytic deactivation of the process through the formation of asphaltenic mud,^{5,55} which causes a general limitation in the maximal conversion of less-sulfured petroleum.^{29,39,43}

Regarding crude exploitation, economical profits related to it depend on its chemical composition, in such a way that crude with a high content of asphaltenes (18-22%) is considered as "heavy" and a low quality product. Since this represents major difficulties in its extraction and refining, economical profit notably diminishes.³⁴

Environmental petroleum leakings are the most evident way by which asphaltenes and microorganisms get in touch. If we refer specifically to microorganisms, these compounds present an influence upon their distribution and activity, as they might either have a toxic effect upon the microorganisms or serve them as a source of carbon and energy.^{5,6}

One of the gravest problems related to these compounds in the environment, resides in their resistance to biodegradation

by microbial metabolic activity.^{2,16} Due to this fact, metabolic routes involved in this process are the less known ones in these days, although, there is some evidence suggesting that some microorganisms have the potential capability of transforming asphaltenes, and in the best case, eliminating them.

POSSIBLE MECHANISMS OF BIODEGRADATION

An alternative way to decrease the effects caused by these compounds lies on the metabolic activity of microorganisms that either reside in crude or inhabit different natural ecosystems.³⁸ When these microorganisms develop their full metabolic potential, they might have the capability of using asphaltenes as a source of carbon and energy, or of degrading them by co-metabolism mechanisms. This being possible once these compounds contain carbon, hydrogen, sulfur, nitrogen and oxygen, which are necessary elements for the development of any organism.³³

According to references related to asphaltene degradation, it would appear that these compounds are not susceptible to being degraded by microbial activity,^{10,18,36,37} although it is possible to propose some biodegradation mechanisms, based upon the chemical structure they present and the processes of biological disposal known for other complex structure hydrocarbons.

Biological degradation of asphaltenes is a process that indeed occurs in nature, but in very low percentages, from 5 to 35%. It is mediated by microbial consortia, isolated from sediments and other sources polluted with hydrocarbons,^{48,52} because they present a complex molecular structure and conform mycelia as they are surrounded by paraffins, resins and other hydrocarbons. These factors provoke a low availability for microorganisms from natural ecosystems and petroleum wells.²⁶ Therefore, in order to increase their degradation percentage it is necessary to apply procedures other than microbial ones: first, existent mycelia must be destroyed applying polar solvents (like heptane), so later they can be broken into smaller fragments.

As alkane chains of different longitude interconnect asphaltenes, photo-oxidation could become a phenomenon that would help achieving a break down of these molecules. It has been demonstrated that this process can oxidize alkanes of 15 to 35 carbon atoms, as much as poliaromatic and polyheteroaromatic structures such as phenanthrene, fluorene and dibenzothiophene respectively.¹¹

Although the addition of non-ionic tensoactives might stimulate the degradation of other poliaromatic hydrocarbons, in the case of these compounds such application does not have a significance, it is better to employ abundant microbial consortia to favour degradation, than to employ tensoactives.^{48,51}

Continuing with alternatives related to biological mechanisms, addition of co-metabolic substrates could favo-

ur asphaltene degradation, especially if adding related compounds, such as alkanes, alkylbiphenyles, propane and methane, and those not related like succinate and salicilate, since they have been shown to favour biodegradation of complex molecular structure hydrocarbons like fluorantene and benzopyrene.^{21,47,49}

Combining events like fragmentation, addition of co-metabolic substrates and abundant microbial consortia acclimated with petroleum as only source of carbon and energy, it is quite possible that the obtained structures (now smaller), would be more susceptible to being degraded in the following way.

Due to the relatively simple structure of lineal alkanes, these would be the first biodegraded compounds through ω -oxidation and β -oxidation processes, which are biochemical processes widely distributed in microorganisms, both belonging or not to environments with the presence of petroleum.³⁰

In general terms, degradation of ramified alkanes is a slower process than that of lineal ones, nevertheless, it has been demonstrated that they are also susceptible to biodegradation in relatively short times, especially those compounds that lack quaternary substituted carbons.⁴⁰ Pristane was considered as a general reference of a ramified hydrocarbon, for which the biochemical processes involved in degrading it are ω y β -oxidation, according to Fig. 3. But for this kind of compounds, elimination goes from two to three carbons at a time, three when dealing with a ramification of the molecule.

Microorganisms like *Brevibacterium* sp., *Corynebacterium* sp. and *Rhodococcus* sp.³⁰ realize these processes.

On the other hand, it has been demonstrated that Hetero-PolyAromatic Hydrocarbons (HPAH) might inhibit biodegradation of some PolyAromatic Hydrocarbons (PAH), espe-

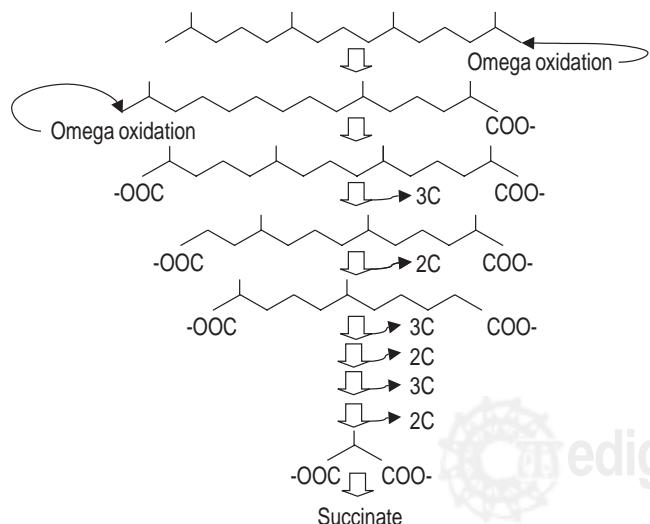


Figure 3. Metabolic route involved in pristane biodegradation (Adapted from Morgan and Watkinson, 1994⁴⁰).

lly of benzopyrene up to 37%, in a way that it is possible that HPAH could be the first to be degraded. Initially those conformed by nitrogen would be degraded, then those conformed by oxygen, and finally those conformed by sulfur, since 100% biodegradation percentages of these compounds within 50, 100 and 200 days respectively, has been reported.²⁸

Oxygenated HPAH are the least studied petroleum hydrocarbons so far. The degrading route best known for these compounds corresponds to dibenzofuran, in which an initial oxidation of the compound occurs which transforms it into trihydroxibiphenyl, which is afterwards oxidized into cathecol or gentisic acid by way of salicilic acid. Both compounds accumulate in the culture medium until the microbial growth has diminished notably, but later they are used as carbon and energy sources as an alternative substrate.¹³ *Pseudomonas* sp. is a microbial genus that may use dibenzofuran as only source of carbon and energy.

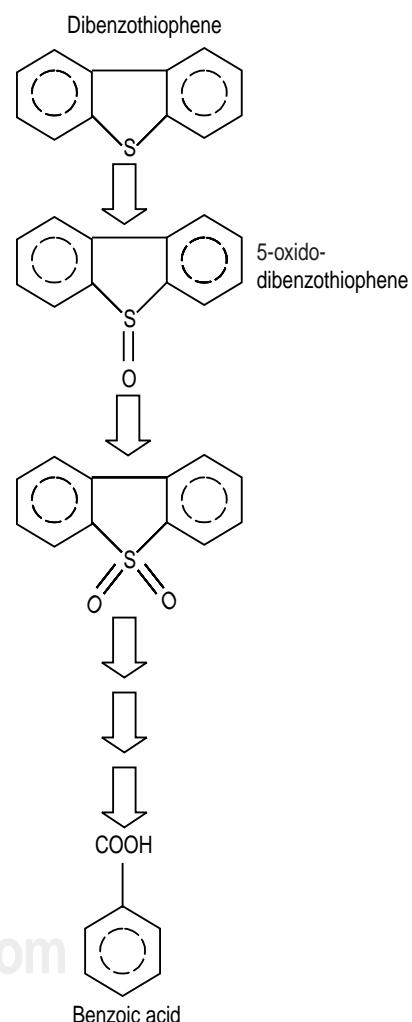


Figure 4. Metabolic route involved in dibenzothiophene biodegradation (Adapted from van Afferden et al., 1990⁵⁰).

The dibenzothiophene degradation route is one of the best characterized for a HPAH (Fig. 4).

There are two routes known for such compound: the di-ol route, and the 5-oxide-dibenzothiophene⁵⁰ route. In the first one, the metabolite 3-hydroxy-2-formylbenzothiophene is produced, which, once it has accumulated is quite toxic for microorganisms. In the case of the second route, when the molecule's sulfur oxidation is produced, formation of toxic intermediaries is avoided, thus allowing a complete mineralization of the compound via benzoic acid.^{30,50}

It has been demonstrated that when a sulfured HPAH presents alkyl substitutes, it may be degraded, if these substitutes possess a length of at least 30 carbon atoms.¹²

In the case of nitrogenated and sulfured HPAH, it has been shown that *Ralstonia* sp. may use carbazol (N-HPAH) as only source of carbon and energy,⁴² as much as *Brevibacterium* sp., *Pseudomonas* sp. and *Rhodococcus erythropolis* may use dibenzothiophene (S-HPAH) and benzothiophene (N-S-HPAH), also as only sources of carbon and sulfur, and of carbon, sulfur, nitrogen and energy respectively.^{14,53}

Once the concentration of HPAH is decreased, PAH biodegradation would begin to increase in an important

proportion, first for those possessing three aromatic rings, then those with four and so on.^{11,21}

Anthracene and phenanthrene are the most studied three-ringed PAH regarding their biodegradation, which might be larger than 70% within 11 days for both compounds, although, several steps throughout the degradation route are still rather proposed than demonstrated.⁴⁴

In the case of phenanthrene, the initial attack to the molecule is carried out by dioxygenases, in order to produce a dihydroxyphenanthrene,⁷ which later suffers a breakage at meta position. Resultant molecules then are opened in whichever rings are left, thus producing compounds that will be transformed along the tricarboxylic acid path.¹⁵

With respect to compounds with more than three rings, elimination time increases. In the cases of benzoanthracene and crisene (both with four rings) biodegradation scores up to 89% and 91% are reached respectively within 105 days, this being carried out by microorganisms resident in composta.⁵⁴ Pyrene is a four-ringed PAH, that is degraded up to 60% by microorganisms belonging to hydrocarbon-polluted soils. In this process (Fig. 5), the opening of the first ring is carried out by the activity of dioxygenases and mo-

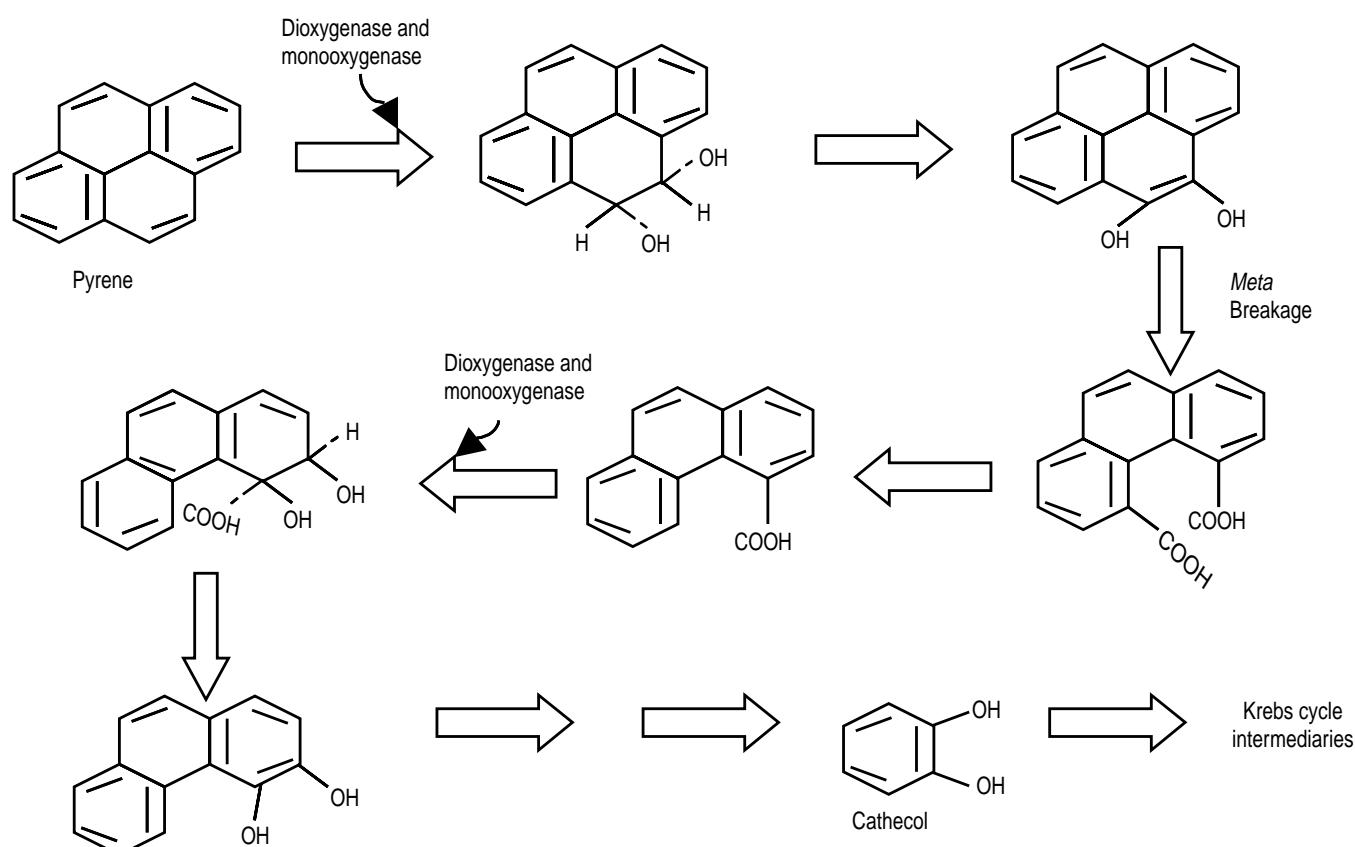


Figure 5. Metabolic route involved in pyrene biodegradation (Adapted from Heitkamp et al., 1988¹⁷).

nooxygenases at ortho position, to later continue with the rupture of the three rings left by a mechanism similar to that of phenanthrene.¹⁷

The compound benzo(a)pyrene is the most complex PAH studied this far. It is conformed by five rings. It has been demonstrated that it can be eliminated up to an 83% within enormous lapses of time of approximately 990 days by soil microorganisms.^{20,23} Nevertheless, benzo(a)pyrene has not been proven to be used as only source of carbon and energy. This compound is also oxidized in an initial step by dioxygenases (Fig. 6), where the breakage is at meta position in the first ring, to later continue through steps similar to those in the pyrene degradation route.⁴¹

Microbial genera responsible for PAH degradation are identified as *Alcaligenes denitrificans* and *Sphingomonas paucimobilis* in the case of fluorantene, and *Mycobacterium* sp. for pyrene and benzo(a)pyrene.²¹

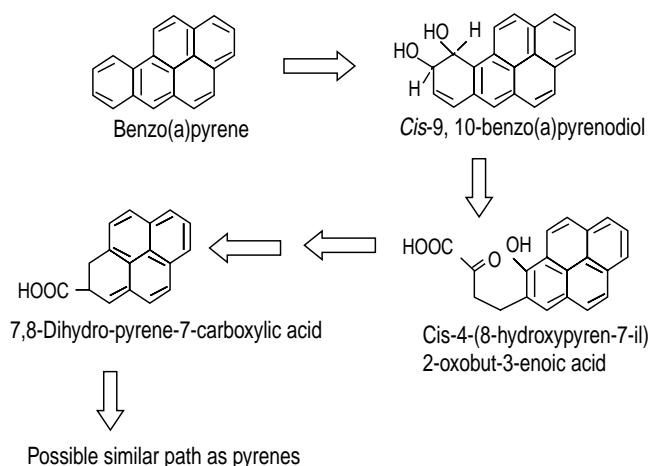


Figure 6. Metabolic route involved in benzo(a)pyrene biodegradation (Adapted from Schneider et al., 1996⁴¹).

- Co-metabolic substrates addition
- Inoculation with microbial consortia acclimated with petroleum as only source of carbon and energy

Time and biodegradation percentage obtained:

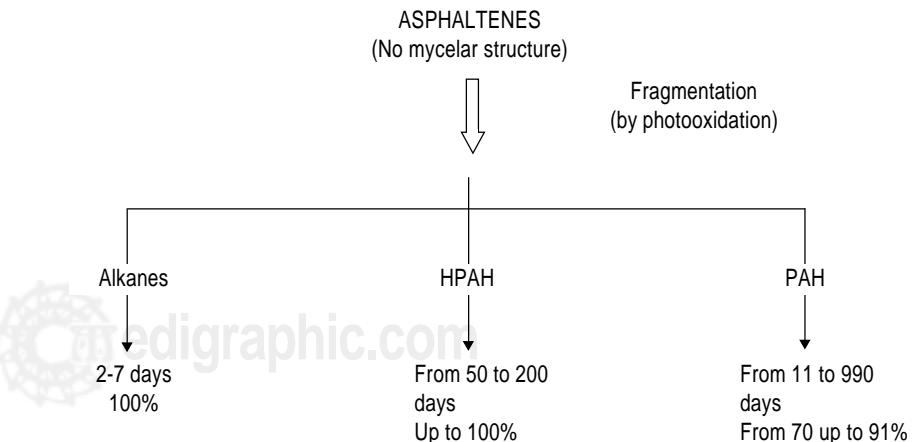


Figure 7. Proposed process for favoring biodegradation of petroleum asphaltenes.

In a general way, degradation of PAH of four or more rings does not yield a remarkable increase to the biomass of the employed consortia,²¹ which means that these compounds are not preferentially used as a carbon source, but as an energy source.

In Fig. 7 the whole process proposed to favor asphaltene degradation is shown, and in Fig. 8 asphaltenes' structure and regions susceptible of being degraded by the processes mentioned before are shown. Here it is noticeable that approximately 50% of the molecule could be eliminated by the activity of microorganisms, this being whenever the structure lacks the rest of the petroleum hydrocarbons.

CONCLUSIONS

Microbial consortia, acclimated to petroleum as their only source of carbon and energy, could be an applicable alternative for degrading petroleum asphaltenes. Nevertheless, it is necessary to implement other procedures such as photo-oxidation and addition of co-metabolic substrates, in order to accomplish such goal.

According to related references, after the first fragmentation, the lineal fraction would be the first to be degraded, followed by ramified alkanes, HPAH and finally PAH, throughout times ranging from several days to approximately 33 months.

Biodegradation of these compounds is a process that might be developed from a theoretical point of view, but it is necessary to present experimental evidences, which may serve as solid fundaments for the proposed process.

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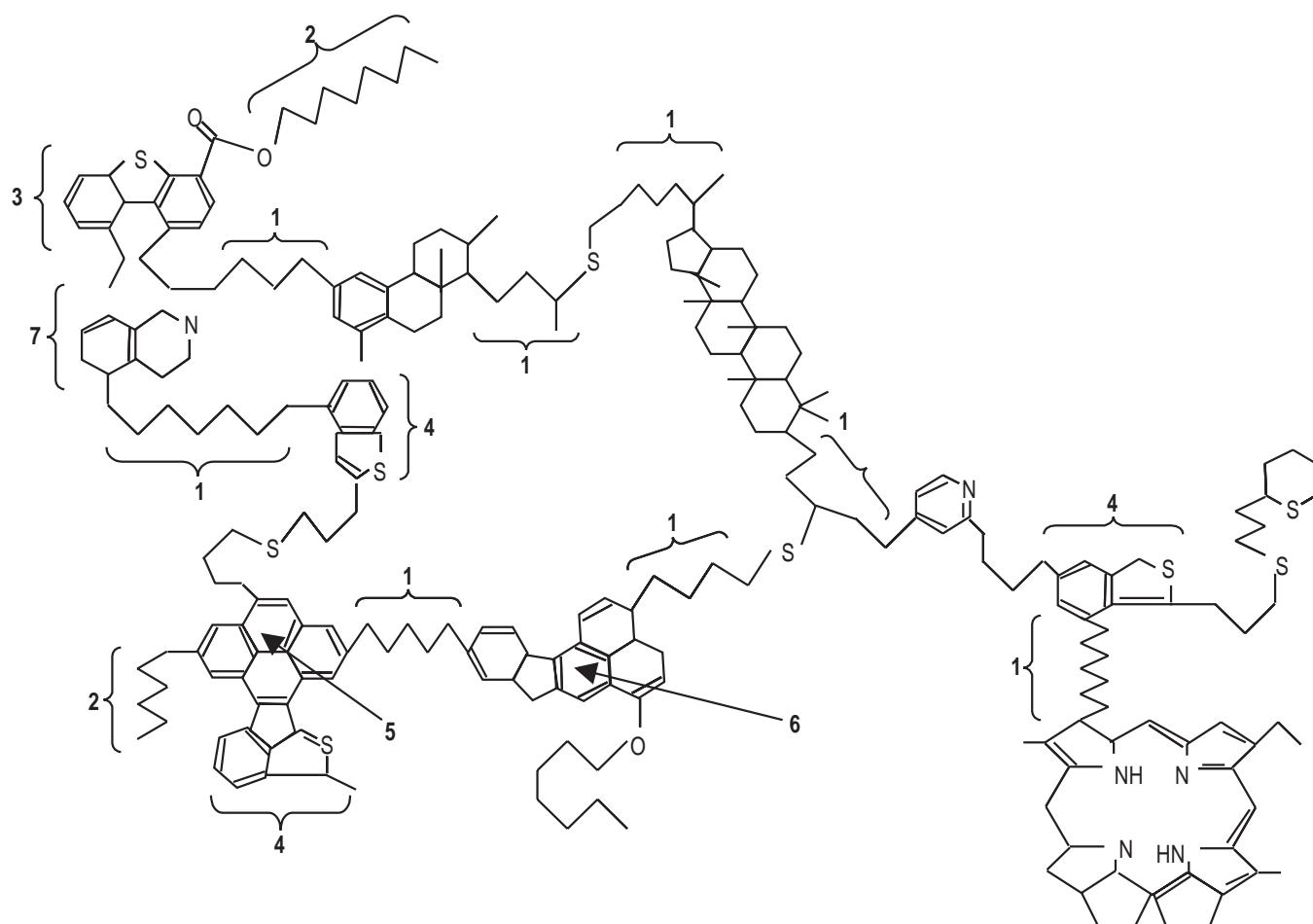


Figure 8. Regions susceptible of fragmentation and biodegradation in an asphaltene molecule. 1: Photooxidation, 2: beta-oxidation, 3: Dibenzothiophene metabolic path, 4: Path similar to dibenzothiophene, 5: Pyrene path, 6: Path similar to benzo(a)pyrenes, 7: similar to carbazols.

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