

PREDICTION OF THE BIODEGRADATION OF PETROLEUM THIOPHENE IN THE ENVIRONMENTAL

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Abstract

Thiophene is a tar component and is often present at sites contaminated by benzene that is one of the most widespread contaminants in soil and groundwater. Earlier research shows that thiophene is resistant to biological degradation, but it can be degraded cometabolically with benzene or other aromatic hydrocarbons as a primary substrate. This study summarises the available knowledge about the thiophene degradation (observed and predicted). This work considered the probable biodegradation of thiophene from petroleum spills under environment. The obtained results from the prediction of the biodegradation for the petroleum thiophene by the OECD (Q)SAR Application Toolbox system are restricted. Metabolites of the heterocyclic compound as thiophene based on observed microbial metabolism were not demonstrated, but six metabolites in the microbial metabolism simulator were predicted.

Keywords: thiophene; petroleum; biodegradation.

1. Introduction

The microorganisms possess the greatest enzymatic diversity found on earth and metabolize millions of organic compounds to capture chemical energy for growth. This metabolism, called catabolism or biodegradation, is the principal driving force in the degradative half of the earth's carbon cycle [1]. Microorganisms are increasingly used in engineered systems to biodegrade hazardous, xenobiotic compounds, an application commonly known as bioremediation [2].

The ability to predict biodegradation pathways accurately has practical implications that go beyond functional genomics. Through such efforts, companies save the capital expended in developing an unusable compound and mitigate against the negative publicity engendered for marketing an environmentally unsound product. Thus, the improved accuracy of industrial biodegradation predictions can benefit both individual companies and society as a whole. Regulatory agencies likewise could do a better job of regulating industry and protecting the public if they possessed more accurate models for predicting the environmental fate of organic chemicals [3].

There have been numerous efforts to predict both the biodegradability and the pathway(s) of biodegradation for a given compound under a given set of conditions, typically either aerobic or anaerobic [4-9]. Most of the efforts have been rule based, drawing general conclusions about what structures would or would not be readily biodegraded. Some only address whether a compound will be biodegraded and, if so, will biodegradation proceed slowly or quickly. The expert system projects, META [7] and BESS [9], also seek to determine at least one plausible biodegradation pathway.

Biodegradation pathway prediction requires the use of biochemical knowledge sometimes called metabolic logic. This requires knowledge of:

- organic functional groups to match a new chemical structure to one whose metabolism is already known;
- intermediary metabolism pathways to deduce how a new biodegradation can funnel a metabolite into a common pathway most efficiently;
- microbial enzymatic reactions to match a given reaction with a known enzyme;

- organic chemistry reactions to deduce what new reactions are chemically plausible to decompose a compound when precedents are not available.

Scientists studying biodegradation acquire this knowledge and these skills through many years of study and experimentation. This requires a means of organizing biodegradation reactions in some systematic fashion [3].

The University of Minnesota Biocatalysis/ Biodegradation Database (UM-BBD) began in 1995 and now contains information on almost 1200 compounds, over 800 enzymes, almost 1300 reactions and almost 500 microorganism entries. Besides these data, it includes a Biochemical Periodic Table (UM-BPT) and a rule-based Pathway Prediction System (UM-PPS) that predicts plausible pathways for microbial degradation of organic compounds [10].

Petroleum oil biodegradation by bacteria can occur under both oxic and anoxic conditions [11], albeit by the action of different consortia of organisms. In the subsurface, oil biodegradation occurs primarily under anoxic conditions, mediated by sulphate reducing bacteria [12] or other anaerobes using a variety of other electron acceptors as the oxidant. On a structural basis, the hydrocarbons in crude oil are classified as alkanes (*normal* or *iso*), cycloalkanes, and aromatics. Alkenes, which are the unsaturated analogs of alkanes, are rare in crude oil but occur in many refined petroleum products as a consequence of the cracking process. Increasing carbon numbers of alkanes (homology), variations in carbonchain branching (*iso*-alkanes), ring condensations, and interclass combinations e.g., phenylalkanes, account for the high numbers of hydrocarbons that occur in crude oil. In addition, smaller amounts of oxygen – (phenols, naphthenic acids), nitrogen- (pyridine, pyrrole, indole), and sulfur- (alkylthiol, thiophene) containing compounds, collectively designated as “resins” and partially oxygenated, highly condensed asphaltic fraction occur also in crude but not in refined petroleum [13].

The inherent biodegradability of these individual components is a reflection of their chemical structure, but is also strongly influenced by the physical state and toxicity of the compounds. Therefore, the physical state is that strongly influences their biodegradation [14].

Depending on its origin, crude oils may contain high quantities of organic sulphur compounds. When the organically bound sulphur is not sufficiently removed during the refining process, SO₂ will be formed during combustion. To minimize this environmental concern, stringent regulations on the sulphur content of fuels will come in place worldwide [15]. Heterocyclic aromatic hydrocarbons such as thiophenes and thiophene derivatives are typically found at high concentrations in crude oils. These compounds are often detectable in products like diesel fuels, but at much lower levels [16].

Aerobic microbiological conversion of thiophenes has been studied extensively [17-20]. However, only limited data are available in the literature concerning the sulphur specific anaerobic conversion of thiophenes. Furthermore, clear evidence for significant anaerobic desulphurisation is scarce. It is proposed that thiophene molecules can be used as alternative electron acceptor leading to the formation of the remaining hydrocarbon molecule and H₂S [21]. The main advantage of this reaction is the selective removal of the sulphur atom, thus retaining the caloric value of the hydrocarbon. The sulphate reducing bacterium *Desulfovibrio desulfuricans* M6 was reported to desulphurise various sulphur-containing organic compounds present in crude oils and distillates [22]. Armstrong et al. (1997) [23] tested several pure cultures of SRB and a sulphate reducing community on their ability to convert thiophenes using the method of Kim et al. (1995) [22] and by using growing conditions in the absence of methyl viologen. None of these methods led to significant reductions in the sulphur content of dibenzothiophene or in total sulphur content of vacuum gas oil, deasphalted oil or bitumen.

Until now, no pure cultures were isolated, which can use S-heterocycles like thiophene and benzothiophene as sole source of energy and carbon. However, the formation of H₂S has been detected during the anaerobic degradation of thiophene [24, 25]. Furthermore desulfurization was also found by Kim et al. (1990) [21] and Marcelis et al. (2003) [15]. Studying a naphthalene degrading enrichment culture, the cometabolic transformation of benzothiophene leading to carboxybenzothiophene derivatives (2-carboxy-, 5-carboxy- and dihydrocarboxy-benzothiophene) was found [26]. The carboxylation is a non-specific reaction, including the attack of the benzene- as well as the thiophene-ring. Although the transformation in the laboratory studies was incomplete, the mentioned metabolites were found in groundwater showing the relevance of this transformation in the environment [26-28]. The cometabolic transformation of benzothiophene in a naphthalene and 2-methylnaphthalene degrading

pure culture resulted in the formation of benzothiophene methylsuccinic acid and benzothiophene methylenesuccinic acid [28], which indicated the analogy to anaerobic degradation of a huge number of compounds.

Thiophene was transformed into metabolites that were not degraded further. Mass spectrometry and nuclear magnetic resonance analysis allowed the identification of the two main thiophene conversion products called C₄ (78% of the converted thiophene) and C₅ (20% of the converted thiophene). C₄ and C₅ are two diastereoisomers of thiophene sulphoxide dimer, which is the result of a Diels-Alder condensation between two thiophene S-oxides. Two minor metabolites (C₂ and C₃) were partially identified. C₂ and C₃ were shown to be the adducts of a Diels-Alder condensation of thiophene or thiophene sulphoxide derivatives. A fifth metabolite (C₁) could not be identified because it was lost during the identification procedures. About 0.05% of the converted thiophene was transformed into benzothiophene. The presence of, or the concentration of benzene did not have any influence on the distribution of the metabolites. Dose-response experiments showed that the compounds C₁-C₅ had no toxic effect on the benzene-degrading ability of the microorganisms. Consequently, microbial death resulting from thiophene degradation could not be due to the toxicity of these compounds. Thiophene itself or other more toxic metabolic intermediates might be responsible for the microbial death [29].

Biodegradation of crude oil in subsurface petroleum reservoirs has adversely affected the majority of the world's oil, making recovery and refining of that oil more costly. The prevalent occurrence of biodegradation in shallow subsurface petroleum reservoirs has been attributed to aerobic bacterial hydrocarbon degradation stimulated by surface recharge of oxygen-bearing meteoric waters. This hypothesis is empirically supported by the likelihood of encountering biodegraded oils at higher levels of degradation in reservoirs near the surface. More recent findings, however, suggest that anaerobic degradation processes dominate subsurface sedimentary environments, despite slow reaction kinetics and uncertainty as to the actual degradation pathways occurring in oil reservoirs [30].

This work considered prediction of the microbial metabolism (biodegradation) of petroleum thiophene in the environmental through the CATABOL simulator which is implemented in the OECD (Q)SAR Application Toolbox system.

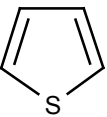
2. Material and methods

Compound data. Thiophene is a S-containing heterocyclic compound with CAS number 110-02-1. A model system was composed as follow: A – thiophene – n-hexadecane-naphthalene. The content of thiophene, was selected by way that the content of sulphur in the model system to be 1400 ppm and the content of naphthalene – 34 % (m/m).

EcoSAR classification. EcoSAR is a user-friendly computer programme developed and routinely applied by the US EPA for predicting aquatic toxicity to fish, Daphnis and algae [31]. This software was used for grouping of the chemicals (Table 1).

Log P. Data for the logarithm of the 1-octanol-water partition coefficient (log P) were obtained from the KOWWIN software [32] (Table 1).

Table 1. Data of thiophene

CAS number	Name	Chemical Structure	Mode of action	EcoSAR classification	log P
110-02-1	Thiophene		Non-polar narcotics	Thiophenes	1.81

Mode of action. For environmental toxicants four broad classes of mode of action have been identified – from class I to class IV [33-36]. The result is summarized in Table 1.

OECD (Q)SAR Application Toolbox. The software used in this study for prediction of the microbial metabolism (biodegradation) of petroleum thiophene is the OECD (Q)SAR Application Toolbox. The Toolbox is a software application intended to be used by governments, chemical industry and other stakeholders in filling gaps in (eco) toxicity data needed for assessing the

hazards of chemicals. The Toolbox incorporates information and tools from various sources into a logical workflow [37].

Observed microbial catabolism. Degradation pathways used by microorganism to obtain carbon and energy from 200 chemicals are stored in a special file format that allows easy computer access to catabolic information. The collection includes the catabolism of C₁-compounds, aliphatic hydrocarbons, alicyclic rings, furans, halogenated hydrocarbons, aromatic hydrocarbons and haloaromatics, amines, sulfonates, nitrates, nitro-derivatives, nitriles, and compounds containing more than one functional group. Most of pathways are related to aerobic conditions. Different sources including monographs, scientific articles and public web sites such as the UM-BBD [38] were used to compile the database.

Simulated microbial simulator. The original CATABOL simulator of microbial metabolism is implemented in the OECD (Q)SAR Application Toolbox [39-41]. Single pathway catabolism is simulated using the abiotic and enzyme-mediated reactions via the hierarchically ordered principal molecular transformations extracted from documented metabolic pathway database. The hierarchy of the transformations is used to control the propagation of the catabolic maps of the chemicals. The simulation starts with the search for match between the parent molecule and the source fragment associated with the transformation having the highest hierarchy. If the match is not found search is performed with the next transformation, etc. When the match is identified, the transformation products are generated. The procedure is repeated for the newly formed products. Predictability (probability that the metabolite is observed, given that the metabolite is predicted) evaluated on the bases of documented catabolism for 200 chemicals stored in the database of "Observed microbial catabolism" is 83%.

3. Results and discussion

Condensed thiophenes comprise a significant portion of the organosulfur compounds in petroleum and in other products from fossil fuels. Thiophene is a five atom heterocyclic aromatic compound consisting of four carbon atoms and a sulphur atom. This compound is part of the NSO-fraction of coal tar. Although NSO-compounds frequently have a strong smell and a high solubility, they are seldom reported. When SO compounds are measured, thiophene is most often not included in the analysis of the environmental samples. Therefore, only a few reports on thiophene contamination are available [42].

The lack of documentation does not mean that thiophene is not present as an environmental contaminant. Thiophene is expected to be present as a minor contaminant on sites contaminated by tar. The industrial production of thiophene and its use in organic synthesis or as a solvent might lead to its release in waste streams. Thiophene might also be present as a major contaminant at industrial sites [43].

Toxicity refers to the potential of a substance to produce an adverse or harmful effect on a living organism. A toxicant is an agent that can produce an adverse effect in a biological system, by either damaging its structure or function or by causing death. The adverse response may be expressed as abnormal mortality, reproduction or growth [44]. Thiophene was shown to be moderately toxic [42].

Many potentially toxic chemicals are present in the environment, and each acts on ecologic targets via their own specific mode of action. Because of time and costs, ecologic risk assessment is mostly based on toxicity data obtained from standardized laboratory tests. Usually, an extrapolation factor is used to derive environmental quality criteria to assure the safety of populations in the field and, consequently, of the ecosystem itself. It has been long known that chemicals affecting an organism in the same way have the same "mode of action". However, it was not known which chemicals have the same mode of action, or even how many modes of action exist. Hermens [33] have used quantitative structure-activity relationship studies to estimate the acute aquatic toxicity of chemicals by classifying them into four classes. These classes are: inert chemicals (baseline toxicity), less inert chemicals, reactive chemicals and specifically acting chemicals. In addition, recently, Verhaar et al. [34] classified the chemicals into one of the previous classes according to their structural characteristics.

Non-polar narcosis is a general, non-specific toxic mechanism of action, which can cause stupor and anesthesia. It is the minimum (acute) toxicity exhibited by organic compounds, as it does not require any specific interactions. Previous studies have shown that this toxic mechanism of action can be accurately modeled by log P alone for classic narcotics such as

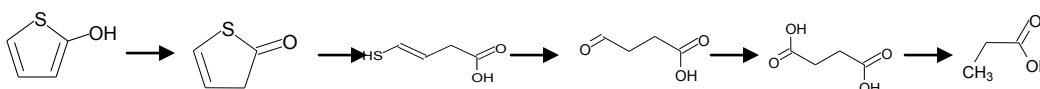
saturated alcohols and ketones [45-47]. This is due to the fact that narcosis is a non-specific reversible physiological effect independent of structure and relies on accumulation in cellular membranes [37].

All organic chemicals have the potential to cause narcosis. Their ability to do so is mainly governed by their concentration and their ability to cause more serious toxic effects, which would mask any narcotic effect the chemical, might cause [48]. In generally, chemicals, which have a more specific mode of action, especially hydrophilic ones, produce greater toxicity than that expected from baseline non-polar narcosis. These chemicals often contain specific structural fragments responsible for their mechanism of action [49]. In this study thiophene was classified by the EcoSAR software (thiophenes) and defined its mode of action (non-polar (neutral) organics) (Table 1) by the OECD (Q)SAR Application Toolbox system.

The study of the biodegradation or biotransformation of organosulphur compounds has two main focuses, each with a different goal. One is the development of a microbial process, known as biodesulfurization, for the specific removal of sulphur from petroleum, leading to a product that yields less sulphur dioxide when it is burned. Ideally, only C-S bonds are broken during biodesulphurization. The other focus is the understanding of how organosulphur compounds are biodegraded so that their fates in contaminated environments can be predicted and assessed. In this case, the pathways of interest involve extensive oxidation with breakage of C-C and C-S bonds, ideally leading to mineralization of the sulphur heterocycles [50].

The possible causes for the harmful effects of thiophene in the environmental can be different. For example, it's hydrophobic, the toxicity of the parent structure or its possible metabolites. In this work will be researched the possible metabolites (observed and predicted). For this aim we will use the OECD (Q)SAR Application Toolbox system. Based on the information found in the UM-BBD, the UM-PPS predicts microbial catabolism of organic compounds [10]. The UM-PPS [51] is becoming a widely recognized pathway prediction system used to improve bioremediation [52]. Predictions are based on biotransformation rules that, in turn, are derived from reactions found in the UM-BBD and the scientific literature. The UM-PPS most accurately predicts compounds that are similar to compounds with known biodegradation mechanisms, for microbes under aerobic conditions and when the compounds are the sole source of energy, carbon, nitrogen or other essential elements for these microbes.

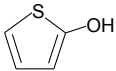
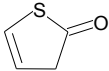
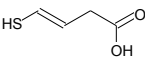
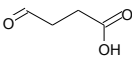
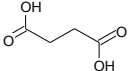

Results in the OECD (Q)SAR Application Toolbox system are shown that observed microbial metabolism for thiophene was not demonstrated, but six metabolites (2-hydroxy thiophene, 2(3H)-thiophenone, 4-Mercaptobut-3-en-1-oic acid, 4-Oxobutanoic acid, Monosodium succinate, Propionic acid) were found in Microbial metabolism simulator



Scheme 1. Predicted metabolites of thiophene

The reactions should be considered as an approximation of the real catabolism by mixed cultures of bacteria. Each chemical transformation includes source fragment (transformation target) and its biodegradation products. The catabolic reactions of thiophene are aromatic ring hydroxylation, isomerism, aromatic ring cleavage, desulfurization, hydrolysis, decarboxylation. The EcoSAR classification of all metabolites of thiophene are: 1) Phenols, Thiophenes, 2) Neutral organics, 3) Thiols (mercaptans)-acid, 4) Aldehydes-acid, 5) Neutral organics-acid and 6) Neutral organics-acid. Some of these metabolites are demonstrated protein binding as Disulfide formation, Schiff base formation. Therefore, part of the metabolites is able to have toxic action on the microorganisms (Table 2).

Table 2. Predicted metabolites, catabolic reactions, EcoSAR classification and toxicity

Name of metabolite	Structure	Catabolic reaction	EcoSAR classification	Protein binding (toxicity)
2-Hydroxy thiophene		Aromatic ring hydroxylation	Phenols, Thiophenes	
2(3H)-Thiophenone		Isomerism	Neutral organics	
4-Mercaptobut-3-en-1-oic acid		Aromatic ring cleavage	Thiols (mercaptans)-acid	Disulfide formation
4-Oxobutanoic acid		Desulfurization	Aldehydes-acid	Schiff base formation
Monosodium succinate		Hydrolysis	Neutral organics-acid	
Propionic acid		Decarboxylation	Neutral organics-acid	

4. Conclusions

Microbial diversity, the richness of species in environmental sites, provides a huge reservoir of resources, which we can utilize for our benefit. However, little is known about the true diversity of bacterial life. Despite the acknowledged value of microorganisms, our understanding of their diversity and many of their key roles in sustaining global life support systems is still very scarce. The results of the biodegradation of S-containing compounds as thiophene are shown that the knowledge about petroleum thiophene are restricted, which can be explained by the structure of this compound and its physical, chemical and toxic properties. Exploring the range of microbial biodiversity is the key to developing effective and environment friendly 'green' technologies. Bioremediation is one such process that exploits the catabolic abilities of microorganisms to degrade harmful and toxic xenobiotics.

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