POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) AS BIOMARKERS FOR ASSESSING BIODEGRADATION SUSCEPTIBILITIES OF CRUDE OIL SPILLED ON SOILS

(Polisiklik Aromatik Hidrokarbon Sebagai Bioindikator Penentuan Tingkat Kemudahan Biodegradasi Minyak Mentah pada Tanah)

Markus Heryanto Langsa
Jurusan Kimia FMIPA Universitas Negeri Papua
Jl. Gunung Salju Amban Manokwari Papua Barat
Email : herilangsa@yahoo.com

ABSTRAK

Kemudahan mengalami biodegradasi senyawa-senyawa polisiklik aromatik hidrokarbon (PAHs) dalam minyak mentah yang diekstrak dari tanah pada hari ke-0 dan ke-39 telah ditentukan. Sampel tanah yang digunakan dikontaminasikan dengan minyak mentah Barrow sebanyak 5% (w/w) untuk memperoleh sampel tanah yang sama dengan kondisi lingkungannya. Tingkat biodegradasi dari minyak mentah Barrow yang diekstrak dari sampel tanah pada hari ke 0 dan hari ke 39 ditentukan berdasarkan fraksi senyawa PAH yang dianalisis menggunakan GC-MS. Persen perubahan kelimpahan (abundances) terhadap waktu dari beberapa senyawa alkilnaptalena ditentukan untuk mengetahui urutan kemudahan terdegradasinya isomer senyawa-senyawa tersebut. Ditemukan bahwa isomer-isomer 2-metilnaptalena (2-MN) lebih mudah terdegradasi oleh mikrobiologi dibandingkan dengan isomer 1-MN. Analisis GC-MS terhadap sampel minyak mentah Barrow yang asli tidak menunjukkan adanya biodegradasi, namun ketika minyak mentah tersebut ditumpahkan ke tanah dan dibiarkan selama 39 hari pengamatan, minyak mentah tersebut mengalami biodegradasi ke tingkat 2-3.

***Kata kunci**: biodegradasi, Polisiklik Aromatik Hidrokarbon (PAH), tanah terkontaminasi.*

INTRODUCTION

The impact that an oil spill has on the environment depends on many factors, including the chemical and physical nature of the oil or refined product, the local environmental conditions (i.e. wind currents, temperature, salinity and biota). Some oil spills are quickly bioremediaded whereas other spills can have long-term effects spanning several years (Fisher *et.al*., 1998; Peters and Moldowan, 1993).

Bioremediation is a process that encourages microbial populations to degrade hydrocarbon contaminants (Barron, 2004). Microbial degradation is one of the most effective methods to remediate oil from soil or sediment after a spill. The toxicity of the contaminants is reduced because bioremediation transforms xenobiotics introduced into the environment to less toxic or innocuous forms, and/or mineralises them to CO_2, H_2O, O_2 and other inorganic products. Thus migration of contaminants is usually prevented and ecological recovery is usually promoted.

Numerous studies also have been made to measure the effects of biodegradation on aromatic hydrocarbon distributions in soils (e.g. (Bispo *et al.*, 1999; Budzinski *et al.*, 1998; Chang *et al*., 2002; Henner *et al.*, 1999; Loehr, 1991; Pospisil *et al.*, 1996; Sauer *et al.*, 1998; Skiba *et al.*, 1992). Similar trends/ results to those reported above for reservoired oils have been observed for soils. For example, the rate of aromatic family biodegradation decreases with increasing aromatic rings (or molecular weight or boiling point), similar to what would generally be predicted through

evaporation. Naphthalenes degrades faster than the phenanthrenes and dibenzothiophenes and chrysene is one of the most resistant to biodegradation (Sauer *et al.*, 1998). Within individual compound classes, some isomers are more susceptible to biodegradation than others, and this is controlled by the position of the alkyl substituents on the aromatic ring (Huang *et al.*, 2004). In a soil study by Pospisil *et al.*, (1996), it was found that the concentration of the aromatic hydrocarbons with three and four-rings decreased to about 0.5 % of the original oil.

Barrow Island is a region where the climate is arid and the island experiences very high temperatures exceeding 30^{o}C, which means bacterial biodegradation is a slow process and the oil pollutants in that area will take a significantly longer period of time to degrade (Davie, 2004). It has been recognised that because of these climatic conditions, action needs to be taken in order to optimise the bacterial activity. In a study by Watkins (1996b) fertilizers were added to the soil to act as a carbon source for the bacteria and to promote bioremediation in that area.

The main objective of this study is to observe the rate of biodegradation of the Barrow crude oil in soil after production flowline leaks, including polycyclic aromatic hydrocarbons, PAHs and Saturated hydrocarbons (SHC). This will be performed by a mesocosm experiment. GC-MS analyses of the saturates and aromatics fractions from the crude oils before and after contamination will be carried out and changes in abundances of compounds will be rigorously assessed. By testing the effects of crude oil on soil composition, this research will represent how production flowline leaks are affecting the soils on Barrow Island. These results may then be used as a basis for further detailed research and also provide clues on better management of contaminated sites upon the island.

Experimental

Soil Samples

Soil samples provided by the Centre of Land Rehabilitation Laboratory UWA were collected from the top 20 cm of the soil profile on Barrow Island. Several physical properties of the soil were measured by UWA's laboratory. These properties include water holding capacity and soil moisture content. A vessel of contaminated soil was prepared in a 500 mL sealed glass jar containing 100 g of soil. The soil holding water capacity was adjusted to around 50%. The appropriate amount of oil (5% w/w) was applied to obtain an even distribution of oil across the surface of the soil to imitate the similar conditions in the field. Two replicates were used. The vessels were sealed after adding the oil and left to incubate for 39 days at 25^{o}C. The % of extractable hydrocarbons (saturated, aromatic and NSOs) was measured on day 0 and day 39.

Extraction Soil Contaminated Oil

Soil samples were dried at room temperature. The ground soils were extracted by using an accelerated solvent extractor using 9:1 mixture of dichloromethane and methanol. Extracts were dried under a nitrogen purge and weighed

Crude oil

Crude oil was provided by the Centre of Land Rehabilitation Laboratory UWA. The oil was collected from the production flowlines on Barrow Island. The whole crude has a specific gravity of 0.8363, a sulphur content of 500 ppm and a nitrogen content of 320 ppm. The crude oil is composed of 95 % hydrogen and carbon, with smaller quantities of additional elements including sulphur, oxygen, nitrogen and metals.

Separation of Saturated, Aromatic and Polar Hydrocarbons (NSO) by Silica-Gel Chromatography.

In a typical small-scale separation, the crude oil or maltenes fraction (20 mg ~ 2 drops) was applied to the top of a small column (6cm x 0.4cm) of activated (120^{o}C, overnight) silica gel pre-eluted with pentane. The aliphatic hydrocarbon (saturate) fraction was eluted with pentane (2 mL); the aromatic fraction with a solution of dichlromethane (DCM) in pentane (2 mL, 30%); and the polar fraction with a solution of equal parts of DCM and methanol (2mL). The resulting saturate (10 drops) and aromatic fractions (20 drops) were diluted with hexane for analysis by GC-MS.

Results and Discussion

1. Identification of Naphthalene and Alkylnaphthalenes

Naphthalene and alkylnaphthalenes are some of the major components of the aromatic fraction. These compounds were identified using their molecular ion mass chromatograms as shown in Figure 1. Naphthalene (*m/z* 128) was assigned based on its mass spectrum and by comparison of retention time with that of a reference sample The alkyl isomers, i.e. methyl-(*m/z* 142), dimethyl-(*m/z* 156), trimethyl-(*m/z* 170) and tetramethylnaphthalenes (*m/z* 184) were identified by comparing retention times and mass spectra with those reported in the chemical literature (Brown & Maher, 1992; Jiang, 1998; Volkman *et al.*, 1984).

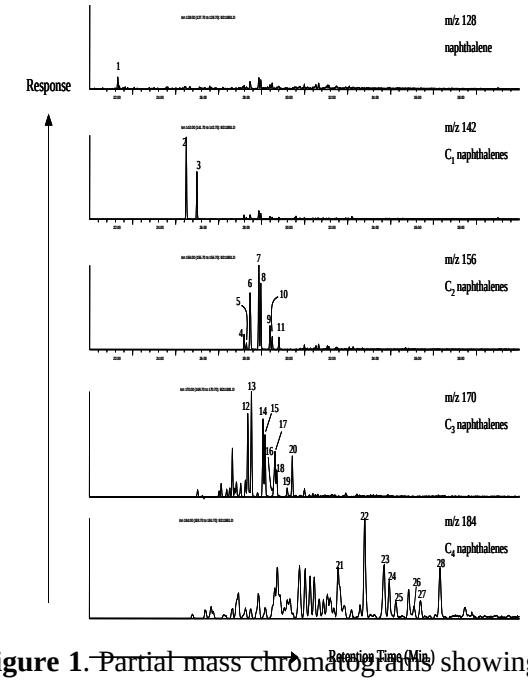


Figure 1. Partial mass chromatograms showing the distribution of naphthalene and its alkylated derivatives in the original Barrow crude oil

2. The Effect of Biodegradation on Individual Alkylnaphthalene Isomers of Extracted Oil from Soil

Selected mass chromatograms reveal the presence of naphthalene, the MNs, the DMNs, TMNs and TeMNs in the aromatic hydrocarbon fraction from the original oil, extracted oil at day 0 and 39 shown in Figure 2. Inspection of these chromatograms depicts that only naphthalene is almost completely removed from the oil extracted from biodegraded oil contaminated for 39 days whereas the MNs, the DMNs, the TMNs and TeMNs isomers are slightly altered.

Table 1. Aromatic compounds identified in samples.

Peak No.	Compound	Molecular ion
1	Naphthalene	128
2	2-Methylnaphthalene	142
3	1-Methylnaphthalene	142
4	2-Ethylnaphthalene	156
5	1-Ethylnaphthalene	156
6	2,6-Dimethylnaphthalene	156
7	1,3-Dimethylnaphthalene	156
8	1,6-Dimethylnaphthalene	156
9	1,4-Dimethylnaphthalene	156
10	1,5-Dimethylnaphthalene	156
11	1,2-Dimethylnaphthalene	156
12	1,3,7-Trimethylnaphthalene	170
13	1,3,6-Trimethylnaphthalene	170
14	1,4,6-Trimethylnaphthalene	170
15	2,3,6-Trimethylnaphthalene	170
16	1,2,7-Trimethylnaphthalene	170
17	1,6,7-Trimethylnaphthalene	170
18	1,2,6-Trimethylnaphthalene	170
19	1,2,4-Trimethylnaphthalene	170
20	1,2,5-Trimethylnaphthalene	170
21	1,3,5,7-Tetramethylnaphthalene	184
22	1,3,6,7-Tetramethylnaphthalene	184
23	1,2,4,7-Tetramethylnaphthalene	184
24	1,2,5,7-Tetramethylnaphthalene	184
25	2,3,6,7-Tetramethylnaphthalene	184
26	1,2,3,7-Tetramethylnaphthalene	184
27	1,2,3,6-Tetramethylnaphthalene	184
28	1,2,5,6-Tetramethylnaphthalene	184

The peak areas for the alkylnaphthalene isomers were determined and plotted as % change in relative abundance calculated from the contribution of each alkylnaphthalene isomer to the total sum of the isomers. The results for these treatments are presented graphically in Figure 3. It is evident that 2-MN is decreasing in % abundance from day 0 to 39 whereas 1-MN is increasing in % abundance. Thus the 2-MN is easily biodegraded and therefore most susceptible to biodegradation.

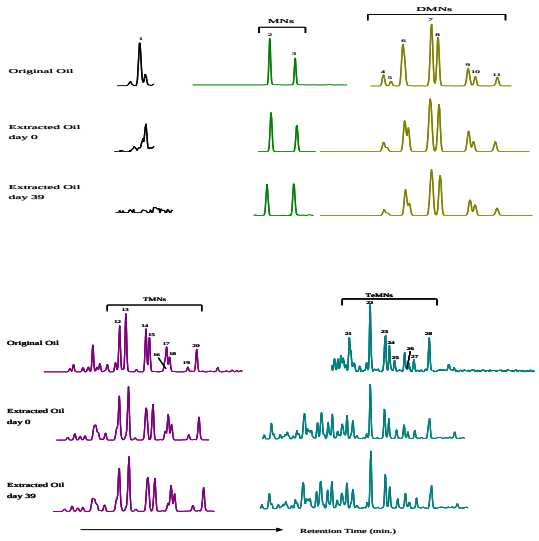
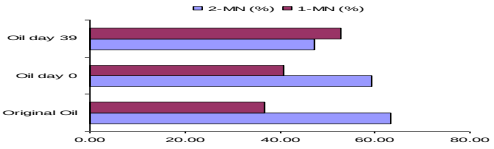


Figure 2. Mass chromatograms showing naphthalene (*m/z* 128), methylnaphthalenes (MNs, *m/z* 142), dimethylnaphthalenes (DMNs, *m/z* 156), trimethylnaphthalenes (TMNs, *m/z* 170), and tetramethylnaphthalenes (TeMNs, *m/z* 184) present in oil samples (refer to Table 2.2 for peak identification).

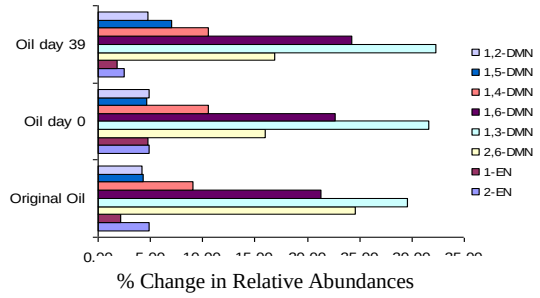
As can be seen from Figure 3(b) that 2,6-DMN is also decreasing in % abundance to a greater extent than the other DMNs suggested that this isomer is far more susceptible to biodegradation than the other DMNs. 1,3-DMN, 1,5-DMN and 1,6-DMN appear to be fairly resistant to biodegradation indicated by increasing in % abundances, while 1,2-DMN and 1,4-DMN are intermediate. Susceptibility in ENs is also evident with 2-EN appears to have been easily removed relative to 1-EN shown by increasing in % abundances of this isomer after being contaminated with the soil for 39 days.

The TMNs were examined and results of % abundances are presented in Figure 3(d). It is immediately apparent that 2,3,6-TMN shows a decrease in % abundance where as 1,4,6-TMN displays anincrease in % abundance. This indicates that 2,3,4-TMN is the most susceptible isomer to biodegradation in the TMN isomers whereas 1,4,6-TMN is the least resistant to biodegradation. The % for 1,2,7-TMN, 1,2,5-TMN, 1,2,6-TMN and 1,2,4-TMN are intermediate.

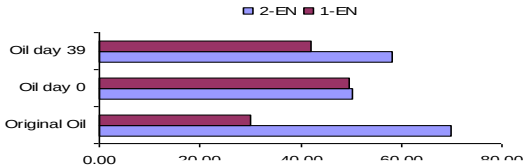
(a) Methylnaphthalenes



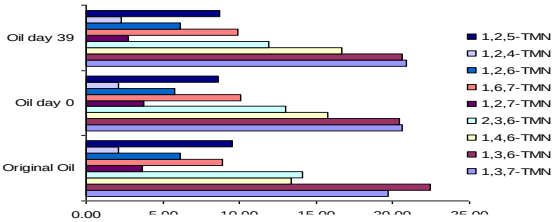
(b) Dimethylnaphthalenes



(c) Ethylnaphthalenes



(d) Trimethylnaphthalenes



(e) Tetramethylnaphthalenes

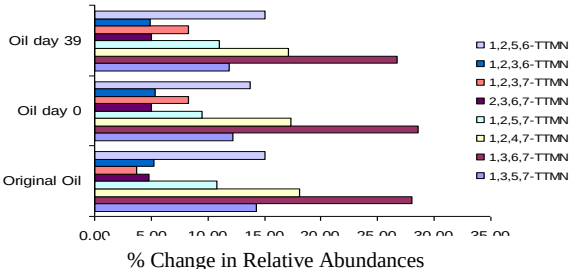


Figure 3. Bar graph showing % changes in abundance of MNs (a), DMNs (b), ENs (c), TMNs (d), and TeMNs (e) from original oil and oil samples taken from soil in mesocosm at day 0 and day 39.

The results from the analysis of the TeMN are presented in Figure 3(e). It is evident that most of the isomers remain relatively unaltered indicated by almost parallel the bar graph of the isomers from the oil samples. The only slight decrease in % abundance is for 1,3,5,7-TeMN perhpas indicating this isomer is easily removed by biodegradation. 1,2,3,6-TeMN, 1,2,5,6-TeMN, 1,2,5,7-TeMN, and 2,3,6,7-TeMN are relatively constant while 1,2,3,7-TeMN is slightly increasing in % abundance showing that this compound is most resistant to biodegradation.

From the findings presented by % change in abundances of each alkylnaphthalene relative to the sum of the isomers shows the Barrow oil has been biodegraded over time. The depletion of naphthalene in samples at day 0 and 39 is probably due to evaporation of this compound. The decrease in % abundance of 2-MN relative to the 1-MN, the changes in the distributions of DMNs and TMNs with time indicate the oil has been biodegraded. The% abundances of TeMNs remain relatively unaltered.

Based on these results and previously reported data the oil at day 0 and 39 are equivalent to a biodegradation level 2 to 3. The following is a summary of susceptibility to biodegradation of the various alkylnaphthalene isomers.

Most susceptible Intermediate Most resistant

Naphthalene and MNs

N > 2-MN >> 1-MN

DMNs

2,6 > 1,2 ~ 1,4 > 1,3 ~ 1,5 ~ 1,6

ENs

2 >> 1

TMNs

2,3,6~1,3,6>>1,2,7~1,2,5~1,2,6~1,2,4 >1,6,7~1,3,7>1,4,6

TeMNs

1,3,5,7~1,3,6,7~1,2,4,7>1,2,3,6~1,2,5,6~1,2,5,7~2,3,6,7>1,2,3,7

Conclusions

The results from this study show that the extent of biodegradation of Barrow crude oil contaminated soil could be determined on the basis of changes in the composition of the saturated hydrocarbons and aromatic hydrocarbon distributions over 39 days by GC-MS analyses. These changes were accompanied by the alteration of the polycyclic aromatic hydrocarbons, in particular the alkylnapthalenes. It was evident that the 2-MN was more susceptible to biodegradation than the 1-MN.

As a result of the above observations, the biodegradation level of oil from the contaminated soils at day 0 and day 39 was assigned at 2-3.

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