# Iron Oxide Mediated Degradation of Mutagen Pyrene and Determination of Degradation Products

Bina Gupta and Himanshu Gupta

Abstract—Photocatalytic degradation of pyrene, a potent mutagen, has been studied on synthesized iron oxide, goethite and on soil surfaces in presence of goethite. The effect of soil pH and UV irradiation wavelength was also investigated. The decay profiles in all the cases follow first order kinetics. The results reveal that photodegradation of pyrene in soil is maximum under UV irradiation of short wavelength. The rate constants for the degradation of pyrene in the presence of optimum photocatalytic dose of goethite were  $1.03 \times 10^{-2}$ ,  $3.52 \times 10^{-3}$  and  $8.99 \times 10^{-3}$  h<sup>-1</sup>, for acidic, neutral and alkaline soils respectively. Samples were analysed using high performance liquid chromatography in an isocratic mode and degradation products were identified using liquid chromatography-mass spectrometry. Present work clearly demonstrates that goethite effectively disintegrates pyrene in soil into smaller non-toxic fragments.

Index Terms—HPLC-MS, iron oxides, metabolites, pyrene.

#### I. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), present ubiquitously in the environment, are pollutants of concern because of their toxic, mutagenic and carcinogenic potential. Natural activities like eruption of volcanoes, forest fires as well as anthropogenic activities like domestic wood burning, cigarette smoke, vehicular traffic exhausts and industrial releases are responsible for the entry of the PAHs in the environment. The USEPA has considered 16 PAHs as harmful pollutants based on their toxicity and exposure to humans. Increased industrialisation, transportation, spillage and improper disposal of crude oils have resulted in PAH contaminated sites around the world [1]-[4] posing ecological and health risks. This necessitates the need to understand the fate of PAHs in the environment. Pyrene, a four ringed PAH is frequently used as a model PAH. Pyrene is a dominant mutagen found in PAH contaminated soils [5], [6]. Despite of not being a carcinogen, it enhances the carcinogenic effects of benzo[a]pyrene [7]. Thus pyrene was selected for the present studies.

Microbial [7]-[10] and photocatalytic [11]-[14] degradation of PAHs has been studied by various researchers. Limitation with microbial degradation is that the rate of degradation is slow for high molecular weight (more than three ring) PAHs [15]. Both TiO<sub>2</sub> and iron oxides have been used as photocatalyst for the degradation of PAHs. Iron oxides in comparison to TiO<sub>2</sub> are cheap and easily synthesized in the laboratory. Moreover, the

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degradation rates achieved with both type of photocatalysts are comparable [16], [17]. Photodegradation of pyrene on soil surfaces in presence of  ${\rm TiO_2}$  [16] and haematite [17] has been studied. Another iron oxide, goethite, has also been used for the photodegradation of pyrene by Wang *et al.* [18]. The authors used solid catalyst surface for the degradation studies and reported pyrene as an intermediate product of photodegradation of pyrene. No other attempts have been made to identify the metabolites of photodegradation of pyrene. In view of this, degradation of mutagenic PAH pyrene was planned in presence of goethite, an iron oxide, in soil under a set of optimised conditions. The effect of various parameters like irradiation wavelength, soil pH and amount of iron oxide on the degradation was studied and degradation products were identified.

## II. MATERIALS AND METHODS

#### A. Chemicals

Pyrene (>99%) was purchased as certified standard from HIMEDIA. HPLC grade Acetonitrile (ACN), ferric nitrate nonahydrate, potassium hydroxide and fluorisil were supplied by RANKEM. Ultrapure water was used throughout the analysis. Three different soil samples collected from different regions of India – Uttarkashi (Uttarakhand), Roorkee (Uttarakhand) and Sriganganagar (Rajasthan), were sieved to less than 75 micrometer and sterilised twice in an autoclave at 121  $^{\circ}$ C for 30 min. A stock solution of pyrene was prepared with a concentration of 1000  $\mu g$  mL $^{-1}$  in ACN and stored in dark at 4  $^{\circ}$ C.

# B. HPLC and LC-MS

Samples of the degradation studies were analysed using Agilent 1100 series HPLC (Agilent Technologies, USA) equipped with UV detector and an Octadecyl end capped RP-C<sub>18</sub> column (250  $\times$  4.6mm, 5  $\mu$ m). A binary solvent system comprising of acetonitrile:water (90:10) in an isocratic mode was used as an eluent at a flow rate of 1 mL min<sup>-1</sup>. Twenty microliter sample was injected for each analysis. Pyrene was eluted at a retention time of 5.8 min under above mentioned conditions. A linear calibration plot was obtained in the concentration range 0.2 to 100 µg mL<sup>-1</sup>. Metabolites were identified using LC-MS (Shimadzu Corporation, Kyoto, Japan) with Labsolutions software version. The instrument is equipped with automatic injection, a binary pump and UV-Vis detector coupled in series with a mass selective detector equipped with an electron spray ionization source. An Octadecyl end capped RP- $C_{18}$  column (100  $\times$  1.6 mm) and a binary solvent system consisting of acetonitrile: water (70:30) with a flow rate of 0.2 mL/min were employed for the analysis. Ionization of analytes was carried out using the following conditions in ESI mode: nebulizer gas flow 10 psi, dry gas 10 Lmin<sup>-1</sup>, dry temperature 250 °C, capillary voltage 4000 V, fragmentor voltage 100 V, vaporiser temperature 250 °C.

# C. Synthesis and Characterisation of Goethite

Goethite was synthesized using precipitation method [19] involving addition of potassium hydroxide to ferric nitrate solution dropwise with constant and rapid stirring to make the solution highly alkaline. A red brown precipitate obtained was diluted with deionised water and kept in an oven for 72 h at 70-75 °C. Goethite was obtained as a light yellow coloured precipitate which was washed with double distilled water and dried in an oven at 50-55 °C. Goethite was characterised by powder X-ray diffraction (XRD) using Bruker AXS D8 powder diffractometer employing Cu  $K_{\alpha}$  radiation (40 kV, 45 mA,  $\lambda$ =1.5418 A°) and goniometer speed of 2 %min.

#### D. Photodegradation Experiments

Pyrene was photodegraded in a UV cabinet and irradiated with a selected wavelength of 254 nm unless mentioned otherwise. All the analysis was carried out in three replicates. Soil samples (1 g) containing pyrene (50  $\mu$ g) were mixed with a definite amount of goethite and irradiated in the UV chamber for 0-120 h. To study the effect of different variables investigations were carried out at varying irradiation wavelength, photocatalytic dose and soil pH. The samples were removed periodically at a regular interval of 24 h. Soil samples removed were extracted through developed method and analysed using HPLC and LC-MS.

## E. Extraction of Pyrene from Soil

Soil samples were extracted with 5 mL ACN in an ultrasonic bath at 20-2 5 °C for 40-45 min. The extract was centrifuged at 3000 rpm for 10 min. A thin layer of anhydrous sodium sulphate followed by 1 g of florisil was introduced in a column in order to perform *in situ* clean up. The final extracts were passed through the column, rinsed with ACN (8 mL) and concentrated to the appropriate volume using rotary evaporator and analysed through HPLC. The percentage recovery of pyrene from soil in all the cases was found to be > 95%.

## III. RESULTS AND DISCUSSION

# A. Characterisation of Goethite

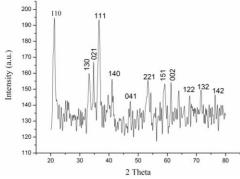


Fig. 1. XRD spectrum of iron oxide goethite.

XRD spectrum of synthesized iron oxide goethite is

shown in Fig. 1. XRD data was concordant to the JCPDS-ICDD (1979). The crystallite size (9.83 nm) was calculated using Scherrer's equation i.e.  $D = (0.9\lambda)/(\beta\cos\theta)$ , where  $\lambda$  is X-ray wavelength,  $\beta$  is line broadening measured at halfheight for the most intense peaks of XRD and  $\theta$  is Bragg angle of the particles.

## B. Effect of Photocatalyst Dose

The effect of photocatalyst (α-FeOOH) dose on the photodegradation of pyrene was investigated at pH 6.8 by mixing varying amounts (1-5 wt%) of goethite to the soil spiked with pyrene. Plots drawn between natural log of pyrene concentration and time gave straight line, suggesting first order rate kinetics (Fig. 2). The first order rate constant values are  $1.24 \times 10^{-3}$ ,  $3.05 \times 10^{-3}$ ,  $3.18 \times 10^{-3}$ ,  $3.52 \times 10^{-3}$ ,  $3.36 \times 10^{-3}$  and  $3.34 \times 10^{-3}$  h<sup>-1</sup> corresponding to catalyst dose of 0, 1, 2, 3, 4 and 5 wt%, respectively. Results further reveal that the photodegradation of pyrene is significantly higher in the presence of photocatalyst goethite and increase in the amount of catalyst further enhances the rate of disintegration of pyrene upto 3 wt% of goethite. The results indicated that the degradation of pyrene in presence of 3 wt% of goethite ( $k = 3.52 \times 10^{-3} \text{ h}^{-1}$ ) is slightly higher than the degradation of pyrene in presence of 3 wt% haematite (k =  $3.4 \times 10^{-3} \text{ h}^{-1}$ ) reported by Zhang *et al.* [17].

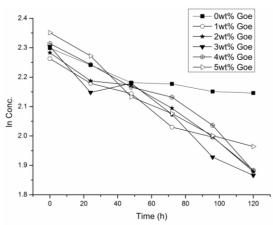


Fig. 2. Effect of dose of  $\alpha\text{-FeOOH}$  on photodegradation of pyrene at pH 6.8.

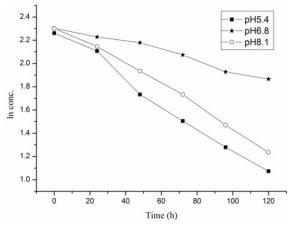


Fig. 3. Effect of soil pH on photocatalytic degradation of pyrene.

## C. Effect of Soil pH

To examine the effect of soil pH, photodegradation of pyrene was carried out in the soils of varying pH (5.4, 6.8 and 8.1). At all the three pH, concentration of pyrene

decreases exponentially with time, suggesting first order rate kinetics (Fig. 3). The values of rate constant corresponding to soil pH 5.4, 6.8 and 8.1 were  $1.03 \times 10^{-2}$ ,  $3.52 \times 10^{-3}$  and  $8.99 \times 10^{-3}$  h<sup>-1</sup>, respectively. It is evident from the results that the rate of degradation of pyrene is faster in acidic and alkaline soils than neutral soil. Zhang and coworkers [16] also reported faster degradation of pyrene in acidic and alkaline soils in presence of photocatalyst  $TiO_2$ .

# D. Effect of UV Wavelength

The disintegration of pyrene was examined under three different wavelengths i.e. 254, 365 and 410 nm. The values of rate constants are  $3.52 \times 10^{-3}$ ,  $3.10 \times 10^{-3}$  and  $2.01 \times 10^{-3}$  h<sup>-1</sup> at wavelengths 254, 365, and 410 nm, respectively. The results suggest that the degradation of pyrene decreases with the increase in irradiation wavelength. Faster degradation at shorter wavelength can be explained on the basis of the fact that the lower is the wavelength, higher is the energy of the incident radiation.

# E. Degradation Products

Fig. 4. Possible pathway for the degradation of pyrene in neutral soil. Structures in brackets are hypothetical intermediates.

Pyrene was degraded photochemically in acidic, neutral and alkaline soils under optimised conditions (3 wt% goethite, 254 nm wavelength) for 120 h. The samples were removed, extracted at regular time intervals (0, 24, 48, 72, 96 and 120 h) and analysed through LC-MS for the identification of different degradation products. The

degradation products and their possible pathways in neutral (pH 6.8), basic (pH 8.1) and acidic (pH 5.4) soils are shown in Fig. 4-Fig. 6, respectively. In all the three cases, unirradiated samples (zero h) gave a prominent peak of pyrene (parent compound) only.

#### F. Neutral Soil

In the beginning (zero h) only the molecular ion peak was observed. Two metabolite peaks at m/z 219 and 100 were observed in addition to the molecular ion peak after 24 h. The metabolite at m/z 219 is due to 1-hydroxypyrene or pyrene-4,5-epoxide and the peak at m/z 100 is due to degradation of pyrene to a simpler compound which could not be identified. After 48 h additional peaks at m/z 224 and 256 were observed which are assigned to 3,4dihydrophenanthrene-4-carboxylic acid and 3,4-dihydroxy-3,4-dihydrophenanthrene-4-carboxylic acid. After 72 h, peaks at m/z 224 and 256 disappeared and new peaks at m/z 105 and 198 appeared. The molecular ion peak at m/z 105 could not be identified and the peak at m/z 198 was assigned to 3-(1-hydroxy-2-naphthalenyl)-2-propenal. After 96 h, the metabolite peaks were observed at m/z 105, 182 and 158. The metabolite peaks at m/z 182 and 158 have been assigned to 3-(2-naphthalenyl)-2-propenal naphthalenedione, respectively. After 120 h, peaks at m/z 146, 129 and 158 persist which are due to 1,2dihydronaphthalene-1-ol, naphthalene and 1,2naphthalenedione, respectively.

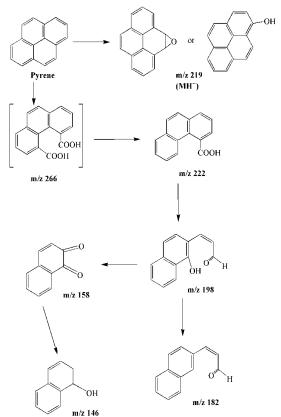


Fig. 5. Possible pathway for the degradation of pyrene in basic soil. Structures in brackets are hypothetical intermediates.

#### G. Basic Soil

A metabolite peak at m/z 100 was observed in addition to the parent ion peak after 24 h. After 48 h, additional peaks at m/z 219 and 198 were observed. After 72 h, the metabolite

peaks observed were at m/z 100, 105, 222, 219 and 158. The metabolite peaks at m/z 222 was assigned to phenenanthrene-4-carboxylic acid. Metabolite peaks at m/z 105, 182 and 158 were observed after 96 h. After 120 h, only the metabolites with m/z 100 and 146 were discernible.

Fig. 6. Possible pathway for the degradation of pyrene in acidic soil. Structures in brackets are hypothetical intermediates.

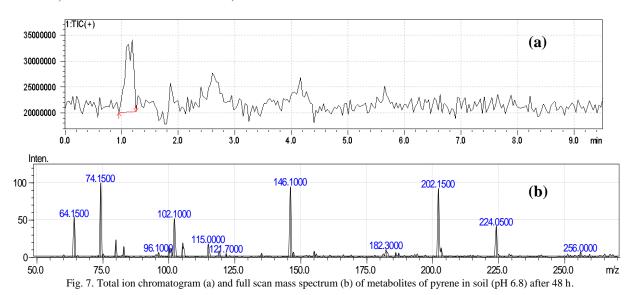
## H. Acidic Soil

After 24 h, two metabolite peaks were observed at m/z 105 and 219, in addition to the parent ion peak. After 48 h, new metabolite peaks at m/z 248 and 220 appeared which were assigned to 2-hydroxypyrene-4,5-dione and 6-hydroxy-4H-cyclopenta[def]phenanthrene-4-one, respectively.

After 72 h, the metabolites with m/z 219, 248 and 220

disappeared and new peaks at m/z 235, 225 and 206 appeared which were assigned to 4,5-dihydroxypyrene, phenanthrene-4-carboxylic acid and 4H-cyclopenta[def]phenanthrene-4-ol, respectively. After 96 h, the detected peaks were at m/z 100, 105, 182, 225 and 122. The metabolite peak at m/z 122 was assigned to benzoic acid. Only the metabolite peaks at m/z 100 and 182 were found persistent after 120 h.

On the basis of the observed metabolites, it can be concluded that the degradation of pyrene occurs through oxidative mechanism. A typical total ion chromatogram and corresponding full scan mass spectrum of pyrene are shown in Fig. 7(a) and 7(b), respectively. Blank experiments for the photodegradation of pyrene in soil in the absence of catalyst were conducted for 0, 24, 48, 72, 96 and 120 h. A prominent peak due to pyrene was observed in all the samples and only a few of the identified metabolite peaks (m/z 224, 182, 105 and 102) were detected till 120 h, which indicates that the degradation of pyrene is slow in the absence of the catalyst and may involve mechanism similar to the degradation occurring in presence of the catalyst. The degradation products may also appear in the form of other possible isomers, in addition to the metabolites shown in the degradation pathways. In different soils, the nature and persistence of the degradation products are different due to dissimilar chemical environment. In neutral and basic soils, the metabolites follow the similar pathway for the photodegradation of pyrene, while in acidic soil, the different metabolic pathway is followed. The metabolites were confirmed by selective ion monitoring (SIM) and product ion scan. The metabolite peaks were detected at [M]<sup>+</sup> or [M + H]<sup>+</sup>. Several identified metabolites have been reported earlier under different experimental conditions [7], [8], [20]-[23]. Metabolites with m/z 158, 146, 129 and m/z 100, 146 (naphthalene and its derivatives) were found to be persistent in basic and neutral soil, respectively after 120 h. In case of acidic soil, metabolites with m/z 100 and 182 (hydrogenated phenanthrene) were found persistent after 120 h. The degradation pathways indicated that the mutagen pyrene is degraded efficiently in presence of goethite into smaller non-carcinogenic/non-mutagenic hydrocarbons (naphthalene, phenanthrene and their derivatives) after 120 h.



#### IV. CONCLUSIONS

Present study reports an inexpensive and efficient approach for the photodegradation of pyrene in the soils of varying pH involving goethite as a photocatalyst. The decay in all the cases follow first order kinetics. The effect of soil pH, wavelength and catalyst dose on the photodegradation suggest fast and efficient degradation of pyrene in presence of 3 wt% goethite. The results further indicate that the photodegradation of pyrene is faster in acidic and alkaline soils than neutral soils. The results reveal that the photodegradation of pyrene occurs through oxidative metabolism. The study provides an efficient method for the remediation of pyrene contaminated soil surfaces where pyrene is degraded to smaller hydrocarbons, naphthalene, phenanthrene and their derivatives. The present work will also provide metabolite data bank for other toxicological studies.

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