

Comparative study of PAH removal efficiency under absence of molecular oxygen: effect of electron acceptor and hydrodynamic conditions

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Abstract: A series of experiments were made in order to compare the removal efficiency of a mixture of four PAHs (naphthalene, phenanthrene, pyrene, and benzo[a]pyrene), under different electron acceptor (NO_3^- , SO_4^{2-}) and hydrodynamic conditions (stagnation and high shear). In all cases naphthalene showed the highest removal efficiency (from 69% up to 100%) as compared with the other PAHs. The fastest rate was obtained for the denitrifying-high shear condition followed by denitrification-no shear, sulfate-reduction-high shear and the lowest for sulfate reduction-no shear. However, most of the perceived removal of the heavier PAHs could be due to aging. No lag time was observed for the denitrifying experiments, and the denitrification rate was the same regardless of the hydrodynamic condition. A lag time of 64 d was observed under conditions of sulfate reduction and high shear. Sulfate reduction did not commence under no shear conditions. No toxic effect was observed for the four PAH mixture under all the conditions tested.

Key words: anaerobic systems, biodegradation, denitrification, hydrodynamic conditions, PAH, sulfate reduction.

Résumé : Des essais ont été effectués pour comparer l'efficacité d'élimination d'un mélange de quatre HAP (naphtalène, phénanthrène, pyrène et benzo[a]pyrène) sous différentes conditions d'accepteur d'électrons (NO_3^- , SO_4^{2-}) et hydrodynamiques (stagnation et fort cisaillement). Dans tous les cas, le naphtalène montrait la meilleure efficacité d'élimination (de 69 % à 100 %) par rapport aux autres HAP. Le taux le plus rapide a été obtenu pour la condition de dénitrification à fort cisaillement, suivie par la dénitrification sans cisaillement, la sulfatoréduction à fort cisaillement et, le plus lent, pour la sulfatoréduction sans cisaillement. Cependant, la plus grande part de l'élimination détectée des HAP les plus lourds pourrait être causée par le vieillissement. Aucun temps mort n'a été observé lors des expériences de dénitrification et le taux de dénitrification était le même, peu importe la condition hydrodynamique. Un temps mort de 64 jours a été remarqué en conditions de sulfatoréduction avec fort cisaillement. La sulfatoréduction doit présenter des conditions de cisaillement pour être effective. Aucun effet toxique n'a été observé pour le mélange des quatre HAP sous toutes les conditions d'essais.

Mots-clés : systèmes anaérobies, biodégradation, dénitrification, conditions hydrodynamiques, HAP, sulfatoréduction.

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Introduction

Polycyclic aromatic hydrocarbons (PAH) are byproducts of incomplete combustion, petroleum distillation, and coal gasification (Bishop 2000; LaGrega 2001; Watts 1998). They have been found underground (Hatzinger and Alexander 1995; Kilbane-II 1998), in river sediments (Chang et al. 2001; Johnson and Ghosh 1998; Quantin et al. 2005), and in marine sediments (Coates et al. 1997; Galushko et al. 1999; Hayes et al. 1999; MacRae and Hall 1998; Rockne et al. 1998), and their importance as hazardous materials has been made evident by their toxicity (Bispo et al. 1999; Randerath et al. 1999; White 2002).

Anaerobic or anoxic biodegradation has been shown to be a viable, inexpensive alternative remediation procedure. Several studies made under anoxic conditions have shown significant PAH removals, mostly for naphthalene and sometimes for phenanthrene (Chang et al. 2001; Eriksson et al. 2003; McNally et al. 1999; Mihelcic and Luthy 1988; Quantin et al. 2005; Rockne et al. 2000; Schmitt et al. 1996). Others have tried sulfate reducing conditions and found them to be capable of removing even heavier PAHs (Bedessem et al. 1997; Coates et al. 1996; Meckenstock et al. 2000; Rothermich et al. 2002; Zhang et al. 2000). However, there are also studies that have not reported such positive results, most of them arguing that lack of bioavailability is the main problem for anaerobic or anoxic biodegradation (Grosser et al. 2000; Lei et al. 2005; Tabak et al. 2003). It has also been shown that PAHs can be strongly adsorbed onto solid organic matter, and, as time goes by, their extraction becomes harder. This phenomenon is known as aging (Hatzinger and Alexander 1995). The aim of this study was to compare the removal efficiency of a mixture of four different PAHs under two different electron acceptor conditions, subjected to two different hydrodynamic conditions. The PAHs were the only source of organic carbon present, other than the

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Table 1. Initial amounts of PAH coated on the crushed pumice.

| Component | Initial amount Denitrifying series (mg/kg dry pumice) | Initial amount Sulfate-Red. series (mg/kg dry pumice) | Initial amount CBRs (NO ₃ ⁻ & SO ₄ ⁻²) (mg/kg dry pumice) |
|----------------|---|---|--|
| Naphthalene | 484 | 490 | 417 |
| Phenanthrene | 450 | 450 | 405 |
| Pyrene | 480 | 500 | 350 |
| Benzo[a]pyrene | 350 | 350 | 327 |

Table 2. Composition of anaerobic medium.

| Component | Initial concentration mg/L | Component | Concentration mg/L |
|--------------------------------------|----------------------------|---|--------------------|
| MgCl ^a ·6H ₂ O | 1000 | NaHCO ₃ | 4200 |
| CaCl ^a ·2H ₂ O | 143 | NaCl | 1000 |
| KCl | 1200 | NaNO ₃ | 2740 ^a |
| KH ₂ PO ₄ | 205 | Na ₂ S ^a ·9H ₂ O | 340 ^b |
| Na ₂ SO ₄ | 2840 | Rezasurin | ~1 |
| NH ₄ Cl | 270 | Trace nutrients (See Table 3) | 1 mL/L |

^aSulfate reducing medium did not contain nitrates

^bReducing agent

Table 3. Composition of trace nutrients in the stock solution.

| Component | Initial conc. mg/L | Component | Initial conc. mg/L |
|---|--------------------|---|--------------------|
| C ₆ H ₉ NO ₆ | 3500 | CuCl ₂ ·6H ₂ O | 130 |
| H ₃ BO ₃ | 62 | ZnCl ₂ | 68 |
| MnCl ₂ ·4H ₂ O | 98 | Na ₂ MoO ₄ ·2H ₂ O | 24 |
| FeCl ₂ ·4H ₂ O | 1500 | Beef Extract | 1000 |
| CoCl ₂ ·6H ₂ O | 120 | NiCl ₂ ·6H ₂ O | 240 |

biomass itself, and there was no previous exposure to the PAHs of either the biomass or the solid phase in order to evaluate the aging process.

Materials and methods

Zero shear experiments (serum bottles)

For the stagnant hydrodynamic condition, two series of 32 serum bottles were prepared (120 mL, amber borosilicate glass, Supelco Co.). Each series consisted of: sample (8 bottles), replicate (8 bottles), dead control (8 bottles), and non-seeded control (8 bottles). The four PAHs (naphthalene, phenanthrene, pyrene, and benzo[a]pyrene) were all reagent grade and supplied by Supelco (a division of Sigma-Aldrich Co, St Louis, MO, USA). Crushed pumice stone with a diameter of 0.14 mm (Hess Pumice Inc., Malad City, ID, USA) was used as the solid phase. The seed came from an anaerobic digester with an average sludge age of 20 d, TVSS = 15 g/L, TKN = 1 g/L, and dissolved COD (CODd) = 2 g/L. The PAHs were coated onto the surface of the pumice stone by pouring a dichloromethane (DCM) solution of the PAHs over it and letting the solvent evaporate. Table 1 shows the proportions of each PAH for all the experiments. The anaerobic medium used in the experiments has been described previously (Zhang and Young 1997) and its composition is shown in Table 2. For all bottles, approximately

20 g of coated pumice and 60 mL of the anaerobic medium were added. For the sample and replicate group, 10 mL of the seed sludge was added. For the dead control, 10 mL of auto-claved sludge was added. No sludge addition was made for the no-seed control. All bottles were capped and maintained inside an anaerobic chamber under N₂ atmosphere at 36 °C.

In every sampling event, four bottles were taken from the chamber (1 sample, 1 replicate, 1 dead control, and 1 no-seed control). Aliquots of the liquid phase were taken from each bottle, filtered through a 0.45 µm filter and used for the determination of dissolved COD, electron acceptor, and PAH concentrations. After the removal of the excess liquid, aliquots of the solid phase were taken from each bottle and used to determine water content (of the wet solid) and COD. The remaining solid was left inside the bottle and was extracted for PAH analysis.

High shear experiments (closed batch reactors, CBR)

For the experiments using continuous mixing conditions, three 8 L reactor vessels were established; one to work under denitrifying conditions, one under sulfidogenesis, and one as a control (no seed). The reactors were designed to work in parallel as shown in Fig. 1. Each reactor was comprised of 1 kg of coated pumice (see Table 1), 7 L of anaerobic media (see Table 2) and, for the case of CBR NO₃⁻ and CBR SO₄⁻², 1 L of seed sludge with the same characteristics as that used for the

Fig. 1. Schematic of the CBR set up.

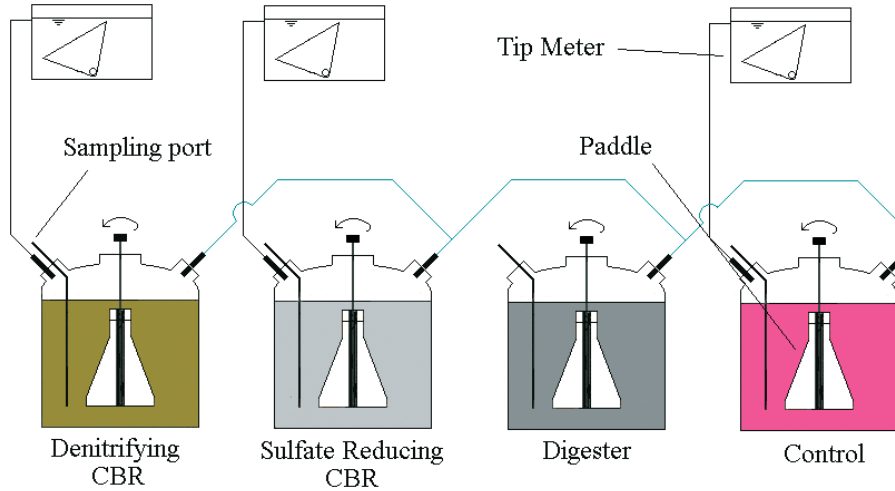
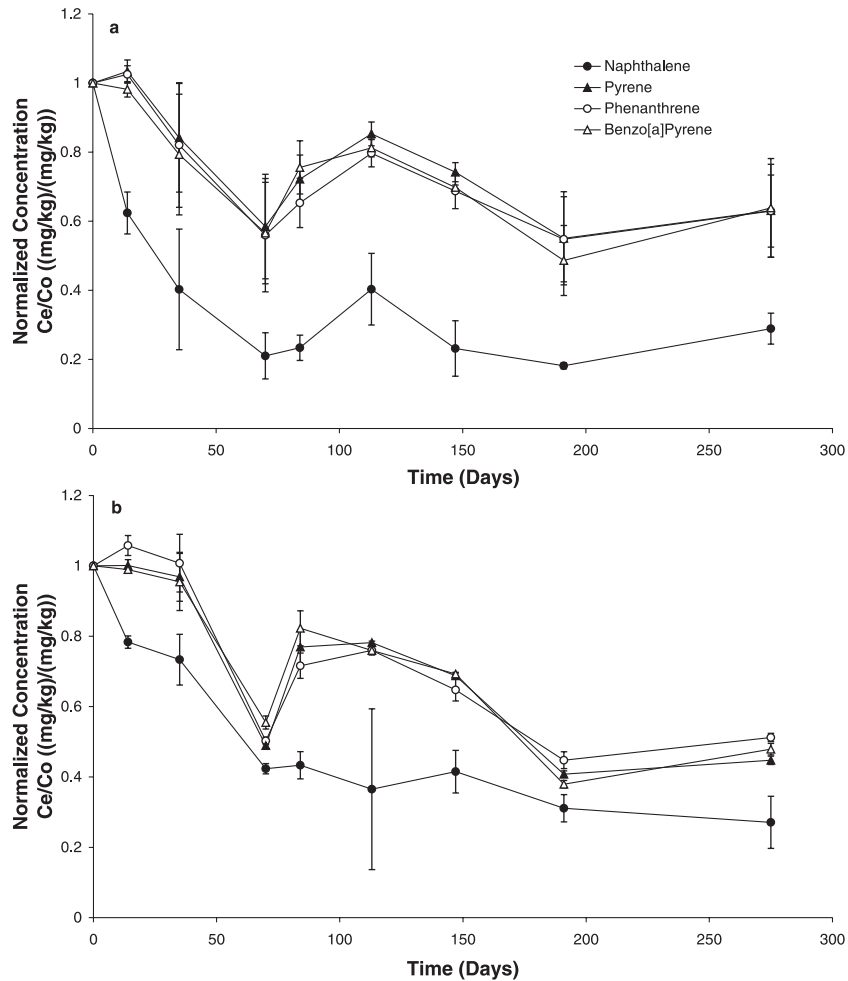


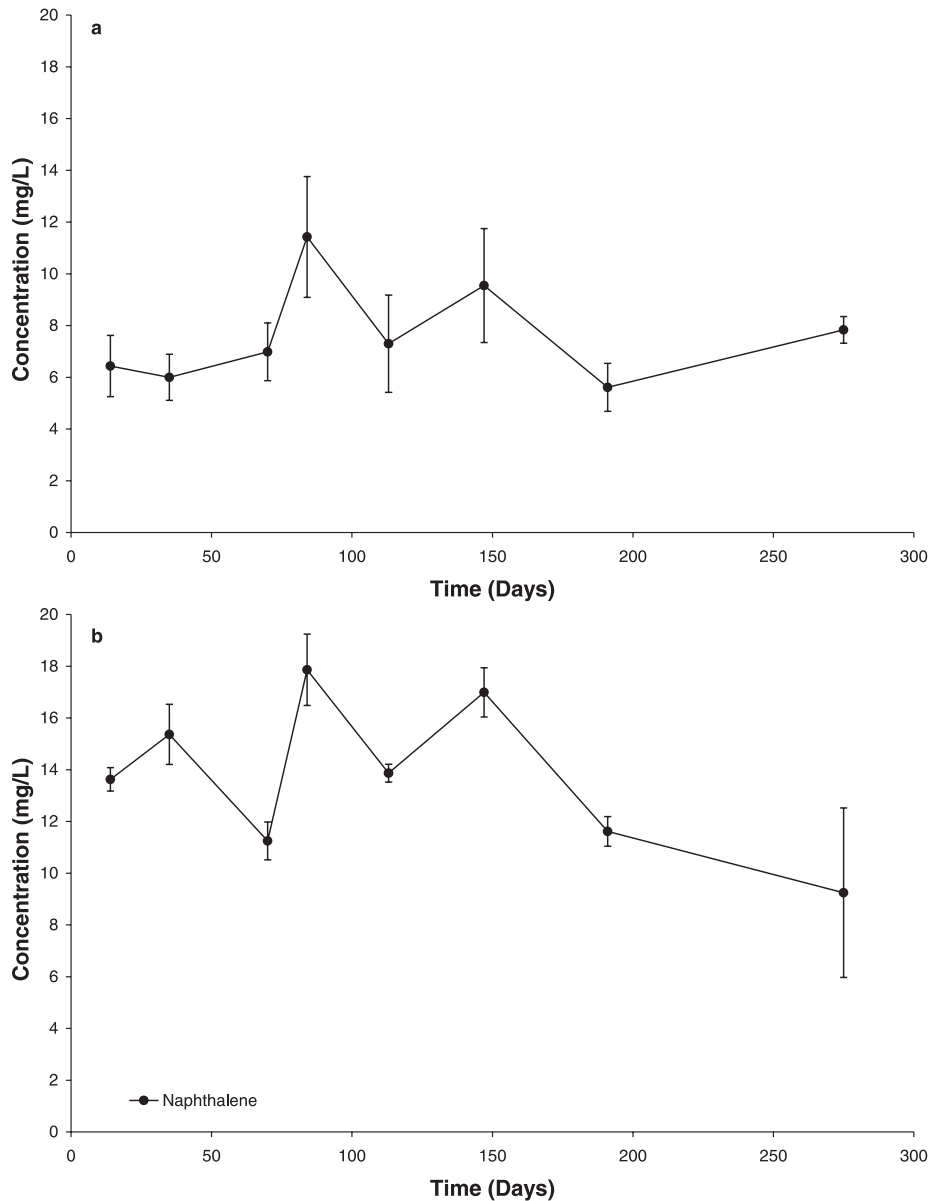
Fig. 2. Recovered PAH concentrations for the serum bottles experiment. (a) Denitrifying condition, (b) Sulfate reducing condition. All PAH amounts are referred to dry weight of the pumice, expressed in mg/kg, and normalized against initial amount. The data points indicate the average of two measurements (sample and replicate), and the bars indicate the range between them.



zero shear experiment. The control reactor was not seeded. The mixing was provided by a pair of plastic paddles attached to a stainless steel shaft that was driven magnetically by a com-

pressed air rotor. All three reactors were sealed and kept at 36 °C inside a temperature-controlled chamber. A fourth reactor was set as an anaerobic digester that provided enough biogas to keep

Fig. 3. Concentration of naphthalene in aqueous phase for the serum bottles experiment. (a) Denitrifying condition. (b) Sulfate reducing condition. The data points indicate the average of two measurements (sample and replicate), and the bars indicate the range between them.



the other reactors under anaerobic conditions; the biogas was transported to the CBRs by plastic tubing. Each CBR was connected to a tip meter that served both for gas measurement and as a hydraulic seal.

For every sampling event, approximately 10 mL of the suspension were drawn from each reactor. The samples were transferred to a previously weighted 10 mL glass vial and centrifuged, and aliquots from the liquid were taken, filtered through a 0.45 μm filter and used for the determination of dissolved COD, electron acceptor concentration, and PAH in solution. From the remaining wet solid, an aliquot was taken for determination of solid phase COD, and the weight of the residual solid and vial was then recorded. This residual (approx. 1 g)

solid was extracted to determine PAH removal. After extraction, the solids were dried and their weight recorded in order to determine water content.

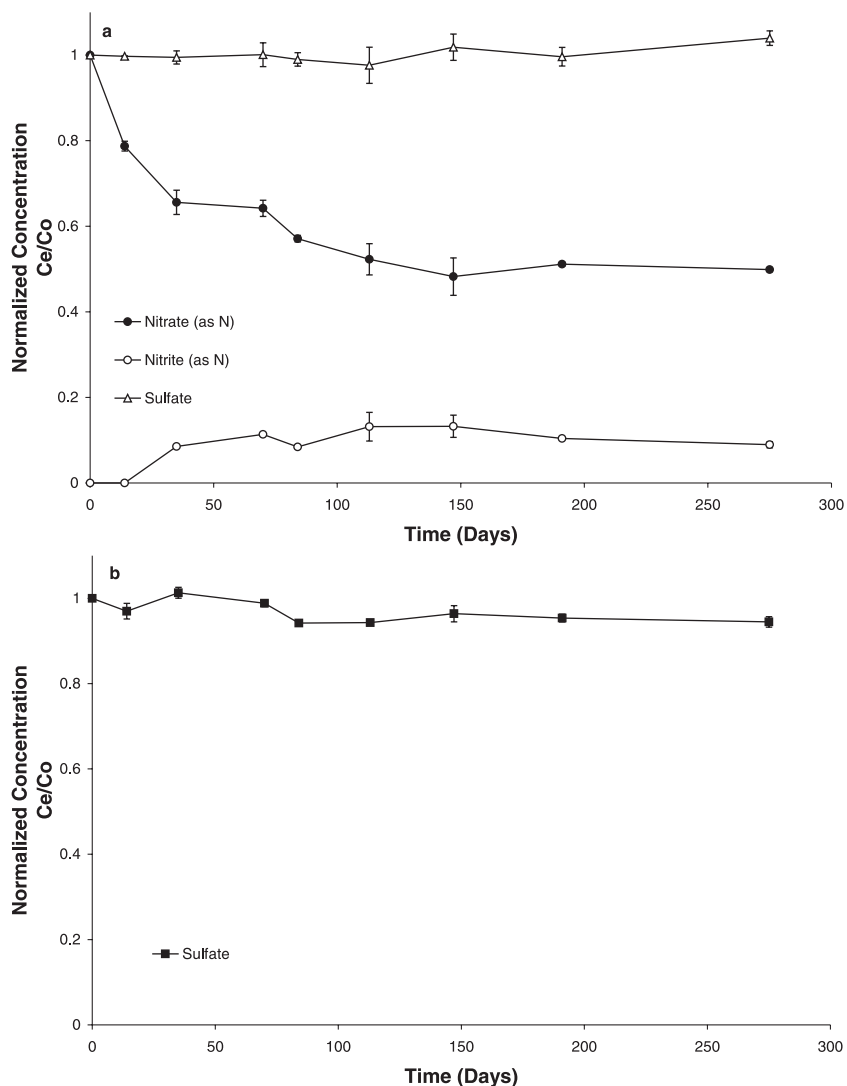
Analytical methods

Liquid phase

Dissolved COD

Two millilitres of filtered sample were added to a 10 mL digestion vial containing 4 mL of COD digestion solution. The COD was determined spectrophotometrically using the HACH HR Closed Reflux Method, which is equivalent to method No. 5220D of the Standard Methods for the Examination of Water and Wastewater (AWWA et al. 1995)

Fig. 4. Evolution of the electron acceptors for the serum bottles experiment. (a) Denitrifying condition. (b) Sulfate reducing condition. The data points indicate the average of two measurements (sample and replicate), and the bars indicate the range between them.



Electron acceptor concentration

All samples taken to determine electron acceptor concentration were filtered and diluted 50 times. One millilitre of the diluted sample was drawn from the dilution flask and its concentration determined by ion chromatography (Dionex DX-120) following method No. 4110 of the Standard Methods for the Examination of Water and Wastewater (AWWA et al. 1995)

PAH concentrations

Two millilitres aliquots of the filtered samples were taken to determine the aqueous concentrations of PAH by means of a liquid-liquid extraction, with hexane as the extraction solvent in a 2:1 solvent to water ratio. Before the addition of solvent, a known amount of 2-fluorobiphenyl was added as surrogate. The samples were tumbled for 36 h and an aliquot of the solvent phase was taken for analysis by gas chromatography with a flame ionization detector (GC-FID; GC Agilent 6890, Supelco column DB5 L = 30 m, $T_o = 50\text{ }^\circ\text{C}$, $10\text{ }^\circ\text{C}/\text{min}$, $T_f = 300\text{ }^\circ\text{C}$, hold 4 min.). The recovery of the surrogate after

extraction was always within the acceptable range (70–120 %). o-dichlorobenzene was utilized for all cases as the internal standard; the GC was calibrated using a series of solutions for all four PAHs simultaneously. The response factors were all within the acceptable range.

Solid phase

Solid COD

The solid COD was determined in a similar way as the dissolved COD. A small amount of solid was weighed and introduced into the digestion vial. Two millilitres of deionized water were added (to complete the required volume) and mixed thoroughly. After that, the same procedure as for the dissolved COD was followed.

PAH amount

After weighing the wet sample, a known amount of 2-fluorobiphenyl surrogate was added to the wet solid. Two millilitres of acetone were then added and mixed thoroughly

Fig. 5. Recovered PAH concentrations for the CBR experiment. (a) Denitrifying condition. (b) Sulfate reducing condition.

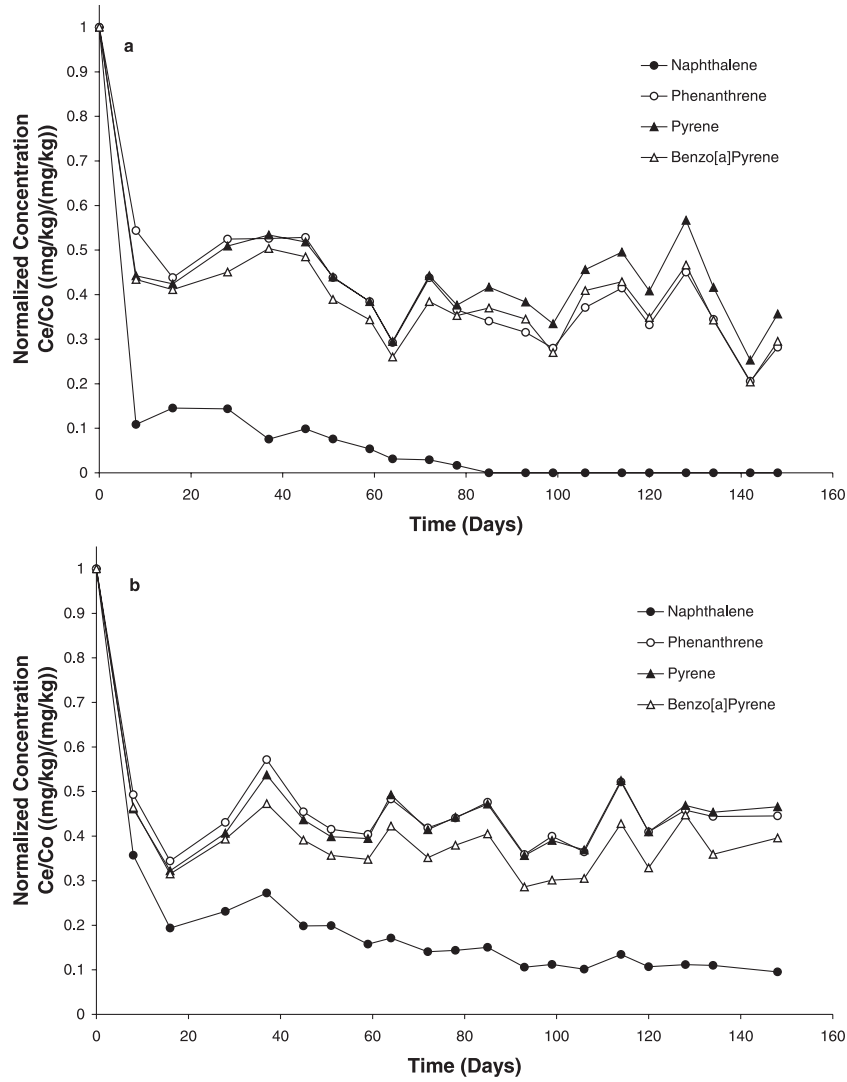


Fig. 6. Evolution of dissolved naphthalene. CBR experiment.

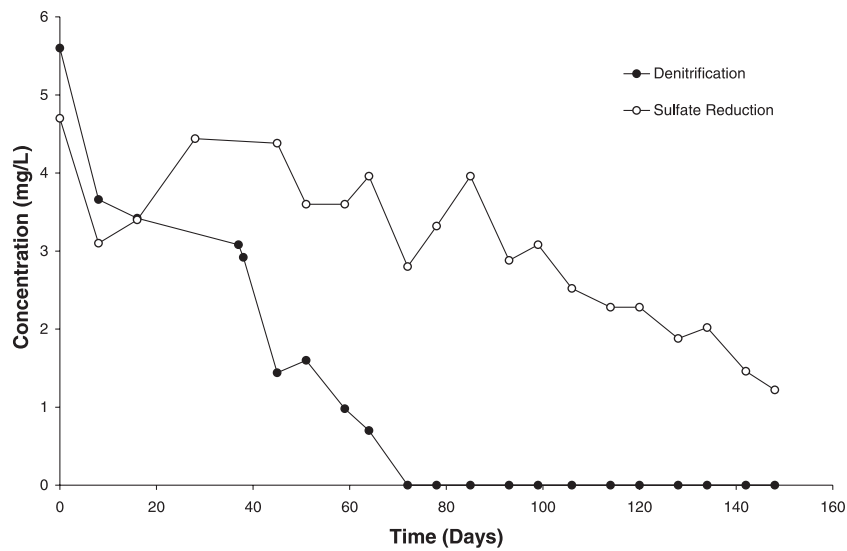
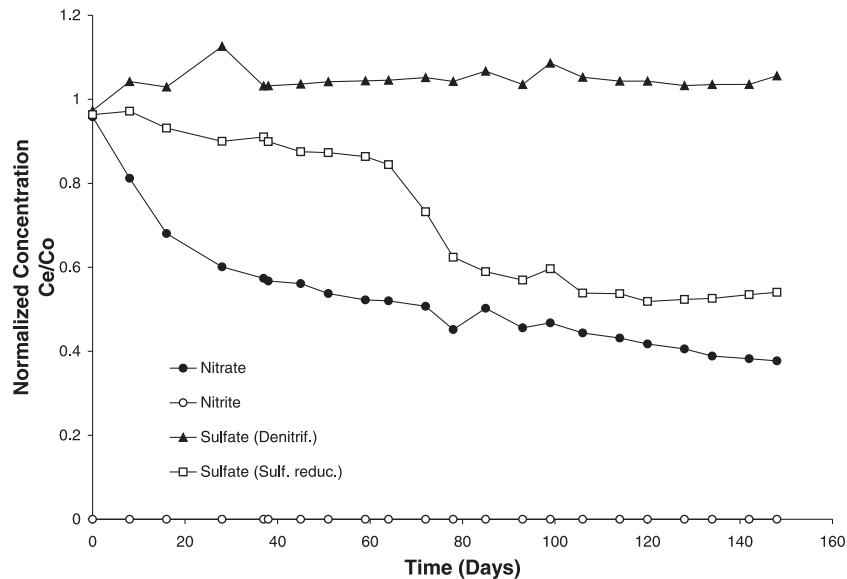


Fig. 7. Electron acceptor utilization observed for the CBR experiment. Concentrations normalized against initial condition.**Fig. 8.** Picture showing the accumulation of PAHs on the glass wall of CBRs.

Accumulation of PAH on the glass surface

in order to trap the water, and 4 mL of hexane were added as the extraction solvent; the solvent to solid ratio was close to 4:1. The samples were tumbled for 36 h and an aliquot of the hexane was taken for PAH determination using GC-FID in the same way as for the liquid phase.

Results and discussion

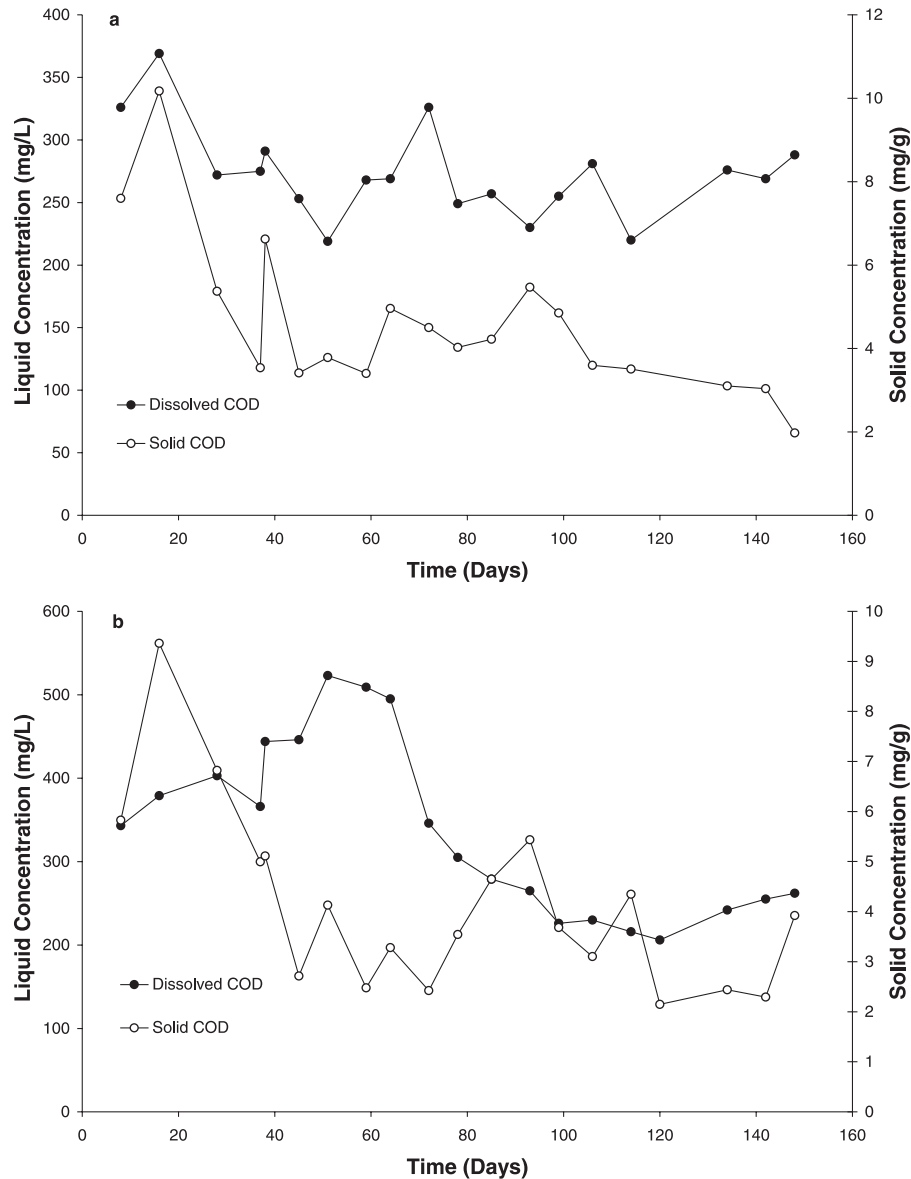
Figures 2a and 2b show the recovery of the four PAHs obtained with stagnant conditions under denitrifying and sulfate reducing conditions, respectively. It can be seen that naphthalene is the only PAH to show a different behavior as compared to the others. Naphthalene had a removal efficiency of 82% under denitrifying conditions. It showed an efficiency of 69% removal in the sulfate reducing experiment, but sulfate reduction did not start during the length of the experiment. The tendency to recover lesser amounts of PAH as time goes by (all other PAH were removed from 55 to 60%) can be explained by a strong

adsorption onto the organic matter present, also known as aging (Hatzinger and Alexander 1995; Quantin et al. 2005).

The difference in the recovery for naphthalene can be explained by the fact that it is the only PAH of the group with a significant water solubility (~ 30 mg/L) and the highest Henry's constant ($4.5 \text{ atm L mol}^{-1}$), implying a significantly higher mass transfer potential between solid to liquid phase and from the liquid phase into the headspace of the bottle until equilibrium is reached. However, under denitrifying conditions, this phenomenon not only happened faster, but also to a larger extent. Comparing the concentrations of naphthalene in solution for both cases, it can be observed that under denitrification the dissolved naphthalene concentration is always less than that measured for the sulfate reducing experiment (Figs. 3a and 3b). Utilization of naphthalene as a source of carbon by the denitrifiers seems like a plausible explanation.

Figures 4a and 4b show the amount of electron acceptor utilization for denitrification and sulfate reduction experiments,

Fig. 9. Evolution of the dissolved and solid COD for the CBR experiment. (a) Denitrifying condition. (b) Sulfate reduction condition.



respectively. In the case of denitrification, it can be observed that around one fourth of the nitrate consumed was converted into nitrite. An increase in sulfate concentration would have indicated the presence of sulfur utilizing denitrifiers (Flere and Zhang 1999; Koenig and Liu 2001; Lampe and Zhang 1996), however, no increase in the concentration of sulfate can be observed; therefore, sulfide dependent autotrophic denitrification can be ruled out as the main nitrate consumption process. Hydrogenotrophic denitrification might also be occurring (Chang et al. 1999), but cannot explain all the nitrate consumption.

Figures 5a and 5b show the normalized recovered concentration of the four PAHs from the CBR experiments under denitrifying and sulfate reducing conditions, respectively. In this experiment, as with the serum bottles, naphthalene shows a significantly different behavior as compared with the other PAHs; moreover, under denitrifying conditions, naphthalene is shown to be removed completely after 85 d, whereas under sulfate

reducing conditions naphthalene is still present (removal efficiency = 91%) and removal is slowly decreasing, with the data closely correlated with a first order decay ($r^2 = 0.85$) and a rate constant value $k = 9.8 \times 10^{-3} \text{ d}^{-1}$ with respect to the solids concentration. In a similar way as for the serum bottles case, (see Fig. 6), naphthalene was the only PAH found in solution, although, for this case, the initial concentrations were very similar between the two conditions. Under denitrification, naphthalene could no longer be detected after 70 d, whereas under sulfate reducing conditions the concentrations remained more or less stable until day 64 where sulfidogenesis began (see Fig. 7). From that point on, naphthalene showed a steady reduction in concentration ($k = 1.06 \times 10^{-2} \text{ d}^{-1}$, $r^2 = 0.80$, with respect to liquid concentration). A point worth noting is that the denitrification rate is identical regardless of the hydrodynamic condition, corroborating the idea of endogenous decay under denitrification. It is interesting to point out that, under denitri-

fication, phenanthrene shows a slight tendency for removal as compared with the heavier compounds, $k = 4.3 \times 10^{-3} \text{ d}^{-1}$, with respect to solids concentration, whereas the other compounds show no discernable tendency at all.

It is also important to note that the recovered concentrations for the PAHs are below 50% of the initial amount. A plausible explanation can be drawn from the fact that the PAHs were only coated on the surface of the pumice; in the CBRs, the solids were subjected to high shear, thus producing a lot of abrasion between the particles. This abrasion could have easily knocked the PAH crystals off the surface, and given their high hydrophobicity, they could have gone into suspension and accumulated on the glass surface of the reactor within the small headspace, making a sort of cake just above the liquid surface (See Fig. 8).

Figures 9a and 9b show the evolution of the COD, both dissolved and solid phase, under denitrification and sulfate reduction, respectively. A distinctive fermentation phase was observed for the sulfate reducing reactor. The increase, and later consumption, of the dissolved COD correlates very well with the electron acceptor utilization.

Conclusions

As a general conclusion, it can be said that most of the removal of the PAHs was probably caused by adsorption onto the organic matter (biomass) and subsequent aging, and that only naphthalene showed a significant difference as compared with the other three (85% and 69% removal efficiency for denitrification and sulfate reduction, respectively, under no shear; and 100% and 91% for denitrification and sulfate reduction, respectively, under high shear, as compared with 55 to 60% removed for all the others under all conditions). Biological activity could be responsible for the difference, especially in the CBRs.

The mixture of PAHs did not show any toxic effect on the biomass, either for denitrifiers or sulfate reducers. However the PAHs were not used as their main carbon source either.

The hydrodynamic condition had a significant impact on the development of the systems, and its effects were much more pronounced in the case of sulfate reduction. The hydrodynamic condition was shown to be significant in terms of PAH recovery also, given the effect of abrasion between the pumice particles and the fate of the PAH within the CBR reactor vessels.

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