

Effects of different operating conditions on sediment slurry sequencing batch reactors treating marine port sediments contaminated by PAHs

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Abstract. Due to intensive anthropic activities, marine port sediments are often contaminated by organic pollutants like polycyclic aromatic hydrocarbons (PAHs), which represent a significant environmental threat. In this study, two sediment slurry sequencing batch reactors (SS-SBRs) were used to biologically degrade a mixture of PAHs (namely fluorene, phenanthrene, fluoranthene and pyrene) from marine sediments dredged from Cagliari (Italy) and El Kantaoui (Tunisia) ports. To enhance PAHs removal by biostimulation, nutrients were added in both sediments, acetate was used as co-substrate (El Kantaoui, SS-SBR1), and saponins were used as surfactants (Cagliari, SS-SBR2). Moreover, different levels of contamination (up to 200 mg_{PAHtot}/kg_{dw} in SS-SBR1; up to 400 mg_{PAHtot}/kg_{dw} in SS-SBR2) and solid to liquid ratios (S/L, up to 0.2 in SS-SBR1; up to 0.1 in SS-SBR2) were applied. As to SS-SBR1, the highest average removal efficiencies (99-100% and 98-100% for 3- and 4-ring PAHs, respectively) were achieved when the level of contamination and S/L were set at 200 mg_{PAHtot}/kg_{dw} and 0.1, 150 mg_{PAHtot}/kg_{dw} and 0.13, and 150 mg_{PAHtot}/kg_{dw} and 0.20, respectively; as to SS-SBR2, very high average removal efficiencies (99÷100% and 98÷100% for 3- and 4-ring PAHs, respectively) were observed during the whole experimental campaign. Such results are promising and provide a useful background for testing other important operating parameters (*e.g.*, the volumetric exchange ratio).

Keywords: biostimulation, marine sediments, polycyclic aromatic hydrocarbons, SBR, sediment slurry

1. Introduction

Proper management of dredged marine port sediments constitutes a serious environmental issue, since large amounts of organic and inorganic

pollutants may be released during dredging operations (Bortone and Palumbo, 2007). Among organic pollutants, polycyclic aromatic hydrocarbons (PAHs) represent a large class of highly recalcitrant and persistent compounds, mainly produced by anthropogenic activities, that can easily accumulate in sediments; due to their toxic, mutagenic and/or potential carcinogenic properties, PAHs represent a potential hazard for both aquatic environment and human health (Bamforth and Singleton, 2005). As to PAHs-contaminated sediments, biodegradation by algae, bacteria and fungi is the major removal process, which is strongly influenced by PAHs chemical structure, sediment physical and morphological characteristics, 'ageing' of contamination, and environmental factors such as pH, temperature, oxygen and nutrients content (Bamforth and Singleton, 2005). Aerobic degradation has been extensively investigated for the bioremediation of PAH-contaminated sediments; although anaerobic biodegradation of PAHs occurs at a slower rate, the removal of 2- and 3-ring PAHs under methanogenic, iron-reducing and sulfate-reducing conditions has been also reported (Wick *et al.*, 2011). Bioremediation of PAHs-contaminated sediments can be enhanced by stimulating indigenous microbial metabolism through the supply of a bulking agent such as wood chips and/or nutrients (optimal C:N:P ratio is between 100:15:3 and 120:10:1), as well as by inoculating microorganisms with known PAHs degradation ability (*i.e.*, bioaugmentation). Biodegradable surfactants may be added to enhance PAHs bioavailability, and simultaneously promote biomass growth (Erby *et al.*, 2014). Among sediment treatment technologies, *ex situ* bioslurry reactors are characterized by system simplicity and optimal process control, with high PAHs degradation rates (Chiavola *et al.*, 2010; Dean-Ross, 2005). In such reactors, the contaminated sediment is treated as an aqueous suspension, and pneumatic or mechanical

mixing is provided in order to maximize mass-transfer rates and favor the contact between microorganisms, pollutants and nutrients (Robles-González *et al.*, 2008). Despite their interesting characteristics, only few studies investigated the application of sediment slurry sequencing batch reactors (SS-SBRs) to the treatment of PAHs-contaminated sediments: Giordano *et al.* (2005) investigated the PAHs removal from contaminated lagoon sediments with different hydraulic retention times, and adding an external carbon source; Chiavola *et al.* (2010) successfully treated artificially contaminated river sediments using different influent organic loads, feed compositions and hydraulic retention times.

In this study, two SS-SBRs were used to bioremediate PAHs-contaminated marine sediments from Cagliari (Italy) and El-Kantaoui (Tunisia) ports. Different PAHs concentrations and solid to liquid ratios (S/L) were tested in each reactor, in order to determine the optimal operating conditions and maximize process performance. The Italian guidelines for specific sediment reuse or disposal (ICRAM, 2007) were used as the target environmental quality standards for both sediments.

2. Materials and methods

2.1 Marine sediments

Sediments were collected from Cagliari (Sardinia, Italy) and El Kantaoui (Tunisia) ports, sieved in order to eliminate particles greater than 2 mm in diameter, and stored at 4 °C in a dark container, in order to prevent the occurrence of photo- and bio-oxidation. Raw sediments from Cagliari and El-Kantaoui differed in terms of carbon content (4 and 10% w/w, respectively), as well in terms of silt-clay, sand and gravel composition (65/35/0 and 50/50/0 % w/w, respectively). A detailed characterization is provided in Erby *et al.* (2014).

2.2 SS-SBR configuration

Both SS-SBRs consisted of an amber Pyrex water-jacketed vessel with 5 L working volume (7.3 L total volume; height 32 cm; working height 22 cm; diameter 17 cm). Reactors were maintained under temperature-controlled conditions (25 °C) by a circulating water bath. Air was supplied through a porous stone placed at the reactors bottom and connected to an external aeration system (Schego Optimal). Siemens LOGO! 12/24 RCE was used for process timing. Main process parameters were controlled by M300-ISM transmitter (Mettler Toledo): oxygen was monitored through an O₂ sensor (Mettler Toledo, InPro 6850i) and maintained above 2 mg/L; pH was monitored by a pH sensor (Mettler Toledo, InPro 4260i) and maintained between 6.8 and

7.3 by adding acid (HCl 1.5 M) or base (NaOH 1.5M) solution.

2.3 PAHs degradation assessments

The reactors were operated in 5-day cycles, each cycle consisting of a feeding phase (5 minutes), a reaction phase (5 days), an effluent withdrawal phase (5 minutes). No settling phase was implemented, as described by Chiavola *et al.* (2010). Feed was provided manually, from the top of the reactor. Mixing (125 rpm) was provided during the reaction phase by a mechanical shaft mixer (IKA RW 20). The effluent was manually drawn from a port located at 19.8 cm from the bottom (the corresponding volumetric exchange ratio was 0.1). Before feeding, sieved sediments were spiked using an acetone-based stock solution prepared with equal amounts of 3-ring (fluorene, Flu; phenanthrene, Phe) and 4-ring PAHs (fluoranthene, Fla; pyrene, Pyr). Artificial seawater was produced by adding 35 g/L of sea salts (Sigma Aldrich) in distilled water, and used to prepare the sediment slurry with different solid to liquid ratios (S/L).

Initial operating conditions for both SS-SBRs were derived from a previous study (Erby *et al.*, 2014). In particular, nutrients (N, P) were added in both sediments to maintain the C:N:P ratio at 100:10:1; readily degradable sodium acetate (10x the feeding PAHs content, in terms of organic carbon) was added as co-substrate in El Kantaoui sediments (SS-SBR1); saponins were used as biodegradable surfactants (up to 1% of dry weight sediment content) in SS-SBR2 (Cagliari). Different PAHs concentrations (mg_{PAHtot}/kg_{dw}), S/L and volumetric organic loading rates (vOLR, mg_{PAHtot}/L·d) were applied to each reactor, as summarized in Table 1.

2.4 Analytical methods

For PAHs determination, samples were periodically collected and centrifuged at 4,000 rpm for 15 minutes; the solid phase was dried by adding diatomaceous earth (1:1 w/w) and exposed to accelerated solvent extraction (Dionex, ASE 150). Before analysis, the liquid extracts were dried by adding anhydrous sodium sulfate (Na₂SO₄), and filtered at 0.20 µm. Analysis were performed by HPLC (Dionex, P680) equipped with an UV detector at 220 nm and an Agilent Zorbax Eclipse PAH column. The compounds were eluted using a linear gradient (from 60/40 to 100/0 (v/v) acetonitrile/demineralized water) over 10 min at 1.5 ml/min. The detection limit of each PAH was 0.01 ppm.

PAHs removal efficiency (E, %) was calculated using the inlet concentration (C_{IN}, µg/g) and the effluent concentration (day 5, C_{OUT}, µg/g), as $E=100 \cdot (C_{IN}-C_{OUT})/C_{IN}$.

Table 1. Experimental phases of SS-SBR1 (El Kantaoui) and SS-SBR2 (Cagliari)

Reactor	SS-SBR1					SS-SBR2		
	Phase	A	B ^(*)	C	D	E	A	B
Total PAHs concentration [mg _{PAHtot} /kg _{dw}]	200	100	150	150	200	200	400	200
S/L	0.1	0.1	0.13	0.2	0.2	0.1	0.05	0.1
vOLR [mg _{PAHtot} /(L·d)]	0.4	0.2	0.4	0.6	0.8	0.4	0.4	0.4
Cycles	1÷7	8÷13	14÷26	27÷37	38÷57	1÷13	14÷29	30÷57

(*) = sediment was spiked only with fluorene and phenanthrene

3. Results and discussion

3.1 PAHs removal in SS-SBR1

Although total PAHs removal efficiency was very high during the whole experimental campaign (98.5±3.0%), the best process performance was achieved during Phase A (99.3±0.2%; cycles #1-4), Phases C and D (99.4±0.2% and 99.4±0.1%, respectively). With few exceptions, SS-SBR1 was able to satisfy the ICRAM limits (0.144, 0.544, 1.494, and 1.398 µg/g for Flu, Phe, FlA and Pyr, respectively). As shown in Fig. 1, initial high PAHs removal efficiencies (99.3±0.2%) dropped down to 93 and 71% for 3- and 4-ring PAHs, respectively, as an accumulation of 4-ring PAHs occurred in the second half of Phase A (starting from cycle #5), due to a failure in the aeration system. As a consequence, no compounds complied with ICRAM limits.

In order to restore process performance, the vOLR was reduced by decreasing total PAHs concentration to 100 mg_{PAHtot}/kg_{dw} (Phase B), using the same operating strategy described by Chiavola *et al.* (2010). Only 3-ring PAHs were spiked and, as a consequence, residual concentrations of 4-ring PAHs (which accumulated into SS-SBR1 during Phase A) decreased below ICRAM limits. Coherently, total PAHs removal efficiency increased up to 99.8±0.1%, indicating complete process recovery.

During Phase C, all PAHs were spiked again (total PAHs concentration was 150 mg_{PAHtot}/kg_{dw}), and the vOLR was set at 0.4 mg_{PAHtot}/L·d (the same as Phase A) by increasing S/L from 0.1 to 0.13. High total PAHs removal efficiency was achieved (99.4±0.2%) and all compounds complied with ICRAM limits, despite some negligible fluctuations in Flu and Phe removal efficiency observed in cycles #24-26.

In Phase D, S/L was set to 0.2 in order to increase the applied vOLR (from 0.4 to 0.6 mg_{PAHtot}/L·d), without changing total PAHs concentration. Some fluctuations in Flu and Phe removal efficiencies were observed also at the beginning of Phase D (until cycle #31), and Phe removal efficiency slightly decreased at the end of the Phase (cycles #36 and #37). Despite the higher vOLR, no decrease in process performance occurred (the total PAHs removal efficiency was 99.4±0.1%), and PAHs residual concentrations were

below the ICRAM limits during the whole experimental Phase.

During Phase E, total PAHs concentration was increased to 200 mg_{PAHtot}/kg_{dw} (same as Phase A), resulting in a vOLR of 0.8 mg_{PAHtot}/L·d. Although total PAHs removal efficiency was still high (98.7±1.0%), significant fluctuations in pyrene removal (between 85 and 98%) were observed in the second half of Phase E (cycles #47-57), indicating a less stable process behavior (Fig. 1). As a consequence, ICRAM limit for pyrene was exceeded. A similar toxic effect due to high PAHs concentrations was previously observed by Yuan *et al.* (2000). Since total PAHs concentration was the same as in Phase A (which run smoothly until a system malfunction occurred, as previously described), such unstable behavior may be rather ascribed to a delayed effect of the high vOLR applied in Phase E.

3.2 PAHs removal in SS-SBR2

As to SS-SBR2, the vOLR was always kept at 0.4 mg_{PAHtot}/L·d. Working conditions were the same in Phases A and C (200 mg_{PAHtot}/kg_{dw} and S/L=0.1), whereas in Phase B the total PAHs concentration was doubled (400 mg_{PAHtot}/kg_{dw}) and the solid to liquid ratio was halved (0.1). The total PAHs removal efficiency was above 98% during the whole experimental campaign (Fig. 2), although ICRAM limits were always respected only in Phase B. During Phase A, Flu limit was exceeded twice (cycles #8 and #11), when some slight fluctuations in removal efficiencies were observed.

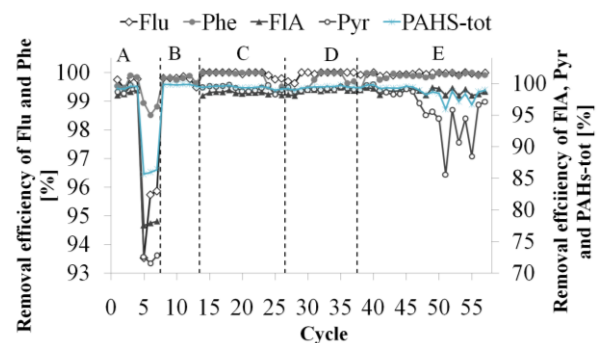


Figure 1. Removal efficiency of PAHs during each Phase (SS-SBR1)

The highest total PAHs removal efficiency was achieved during Phase B ($99.7 \pm 0.1\%$), when the highest total PAHs concentration was applied; however, FIA and Pyr removal efficiencies gradually decreased from cycle #22 onward (Fig. 2), likely due to the delayed effect of the higher (double) total PAHs concentration, compared with Phases A and C. Despite such negative trend, no ICRAM limit was exceeded.

The subsequent reduction of total PAHs concentration (Phase C) did not stabilize the process, and fluctuations in FIA and Pyr removal efficiencies (between 96 and 100%) were observed (from cycle #35 to #57). However, only the ICRAM limit for Flu was exceeded in the second half of Phase C (from cycle #48 to cycle #56).

3.3 Degradation profiles in SS-SBRs

PAHs degradation profiles in both reactors showed (Fig. 3 and 4) that 3-ring PAHs were degraded completely, and faster than 4-ring PAHs, in agreement with the results achieved by Chiavola *et al.* (2010) and Giordano *et al.* (2005). As an exception, incomplete 3-ring PAHs removal was observed in SS-SBR1 during Phase A (Fig. 3b), due to a system malfunction; Fig. 3c clearly shows the enhancement of PAHs depletion achieved during Phase B; as FIA and Pyr were spiked again in Phase C, an increase in FIA removal rate was observed (Fig. 2d); despite the increased $vOLR$, the degradation profiles of each PAH in Phase D had steep slopes and showed low residual levels at the end of the cycle (Fig. 3e), confirming the excellent process performance achieved; as a confirmation of process instability in the last part of Phase E, Pyr degradation profile determined on cycle #50 (Fig. 3f) showed a very low degradation rate with a corresponding high residual Pyr concentration ($C/C_0 > 35\%$).

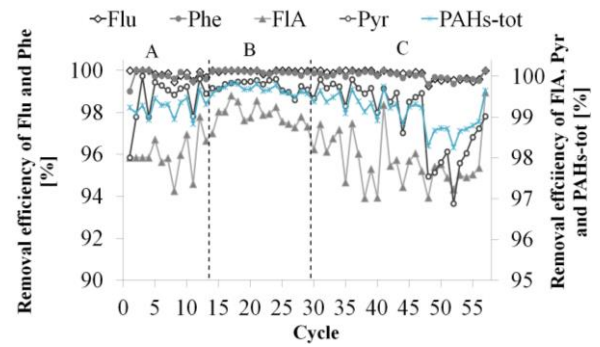


Figure 2. Removal efficiency of PAHs during each Phase (SS-SBR2)

As to SS-SBR2, FIA removal was faster in Phase A (Fig. 4a, cycle #9), than in B (Fig. 4b, cycle #21), and C (Fig. 4c, cycle #47) confirming the extremely good process performance observed during the Phase. The lowest Pyr maximum removal rates were determined during Phase C, as indicated by the slopes of the steepest part of Pyr concentration profiles, in agreement with the pronounced fluctuations in removal efficiency observed during this Phase (from cycle #35 to #57), and previously described.

4. Conclusions

In this study, two SS-SBRs were run with different operating conditions to bioremediate PAHs-contaminated marine sediments from El-Kantaoui and Cagliari ports. Although high PAHs removal efficiencies were achieved in both reactors, ICRAM limits for specific sediment reuse or disposal were not always satisfied. As to SS-SBR1 the best results were achieved at $vOLR$ up to $0.6 \text{ mg}_{\text{PAH}_{\text{tot}}}/\text{L}\cdot\text{d}$ (Phases A, first half, C and D), when ICRAM requirements were always satisfied. In particular, as the $vOLR$ was further increased to $0.8 \text{ mg}_{\text{PAH}_{\text{tot}}}/\text{L}\cdot\text{d}$ (Phase E),

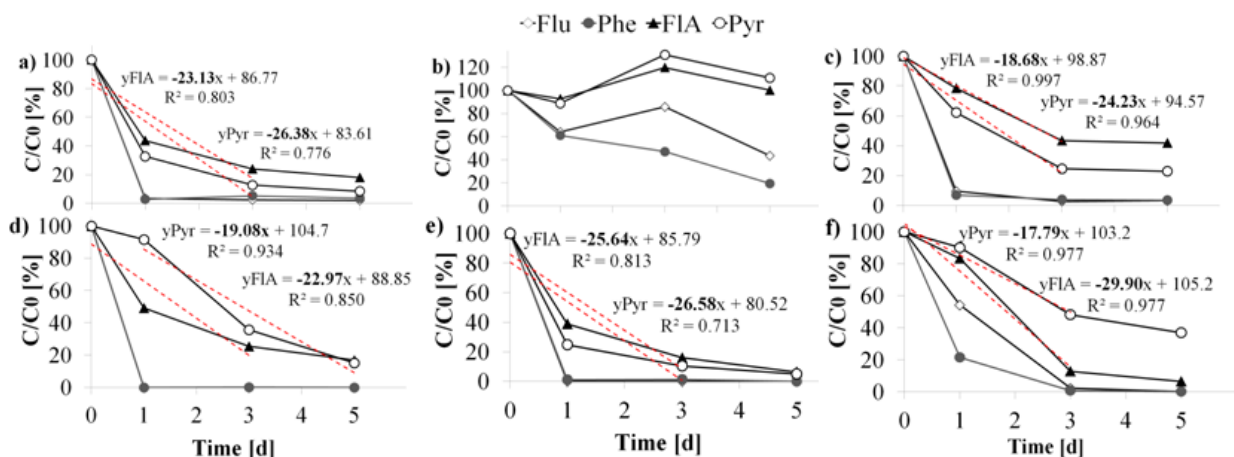


Figure 3. Degradation profiles of PAH compounds for SS-SBR-1 in a) cycle #1 and b) cycle #6 (Phase A), c) cycle #9 (Phase B), d) cycle #21 (Phase C), e) cycle #33 (Phase D), and f) cycle #50 (Phase E); C_0 is the PAHs concentration at day 0 ($\mu\text{g/g}$). Dashed red line: linear regression

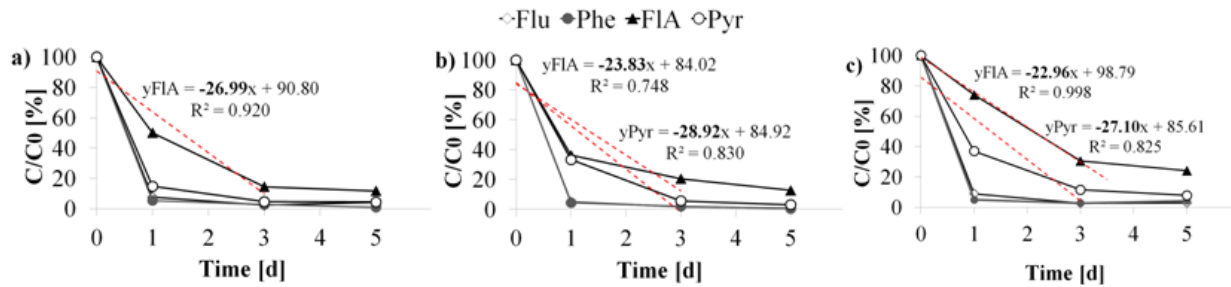


Figure 4. Degradation profile of PAH compounds for SS-SBR-2 in a) cycle #9 (Phase A), b) cycle #21 (Phase B), and c) cycle #47 (Phase C); C_0 is the PAHs concentration at day 0 ($\mu\text{g/g}$). Dashed red line: linear regression

a clear worsening of overall process performance was observed.

As to SS-SBR2, the best results were achieved during Phase A, when high total PAHs removal efficiencies were observed and ICRAM limits were always satisfied (with the exception of Flu on cycles #8 and #11). Although the limits were always met during Phase B, the progressive decrease in FlA and Pyr removal efficiencies (from cycle #22 onward) indicated a delayed, negative effect of the higher total PAHs concentration applied. Restoring the initial operating conditions in Phase C was not enough to restore process stability, and ICRAM limit for Flu was exceeded in the second half of the Phase (from cycle #48 to cycle #56).

Interestingly, very good process performances were achieved in both SS-SBRs when the same total PAHs concentration ($200 \text{ mg}_{\text{PAHtot}}/\text{kg}_{\text{dw}}$), S/L (0.1) and vOLR ($0.4 \text{ mg}_{\text{PAHtot}}/\text{L}\cdot\text{d}$) were applied, despite the significant differences in sediments characteristics and initial operating conditions.

Such promising results deserve further investigation, in order to evaluate the effects of other important process parameters on process performance.

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