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BIOMECC Process for Mechanochemical Biodegradation of Polycyclic Aromatic Hydrocarbons in Marine Sediments

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Abstract

BIOMECC, a new two-stage process based on a short mechanochemical (MC) pre-treatment followed by biological treatment with purposely selected bacteria (*Novosphingobium pentaromativorans*), was investigated to detoxify Taranto's harbour (S. Italy) marine sediments contaminated by polycyclic aromatic hydrocarbons (PAHs) and classified as hazardous waste, according the Italian law (Legislative Decree 152/2006). The experimental results, aimed at determining the overall efficiency of the BIOMECC treatment, indicated that PAHs degradation may be achieved in a very effective (97 %) and fast (<14 days) manner, yielding a non-toxic waste largely reusable as building material. In particular the MC pre-treatment allowed bigger degradation of high MW PAHs; because of their larger aromatic structure, after collisions between sediment particles and milling bodies these congeners may generate more stable free radicals with respect to low MW ones and the probability of their formation is higher, hence they are MC degraded to larger extent. GC-MS analysis of MC degradation products of benzo(a)pyrene (BaP), spiked on clean sediment (taken from Taranto's harbour too), suggest that the first probable step is an almost complete destruction of BaP rings and the formation of oxidized heavy molecules with residual aromaticity. Then the breakdown of this kind of compounds and the formation of small radicals give origin to many volatile organic molecules which, through an accretion process, form heavier compounds up to graphitization.

Key words: PAHs, marine sediment, mechanochemical treatment, biological treatment

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of over 300 biorecalcitrant cumulable organic compounds containing ≥ 2 fused aromatic rings, generated by various industrial preparations, natural phenomena or anthropogenic processes and occurring ubiquitously *outdoor* and, more often, *indoor* [1–4]. Their health and environmental adverse effects are widely known, as benzo(a)pyrene and others PAHs are classified *certainly* (Group 1), *probably* (Group 2) or *possibly* (Group 3) carcinogenic for humans by IARC since 2008 [5] and listed among *priority pollutants* [6]. Their detrimental effects on flora, fauna and humans ($\approx 80\%$ by ingestion, $\approx 18\%$ inhalation) depend mainly on physical and chemical characteristics of PAH mole-

cules, with higher MW usually associated with higher toxicity and longer environmental persistence [7].

Integrated remediation technologies appear the best way forward to reclaim soil and waste contaminated by PAHs [8]. Major advantage of the biological approach (bioremediation) is its potential for *in situ* or *on site* application, usually requiring, however, extremely long duration [9]. Chemical oxidation and physical treatments (photocatalysis, electrokinetics, thermal degradation *etc.*) are fast, although often entailing high costs and toxic by-products [8].

Mechanochemistry (MC), a well known technique dealing with chemical reactions improved by the action of mechanical forces [10], has been recently proposed also for environmental problems with promising results [11].

TABLE 1

Characteristics of Taranto's harbour sediments at the "hot spots" A, B and C

Parameters (mg/kg)	Samples		
	A (non hazardous)	B (hazardous)	C (non hazardous)
Silt (%)	59±2	56±2	39±4
Clay (%)	14±4	27±2	24±6
Humidity (%)	43±1	43±1	42±2
pH	8.90±0.11	8.90±0.04	9.10±0.14
Arsenic	84±7	74±7	36±14
Organic substance	41 900±10 600	25 900±6100	34 500±4600
HHC (Heavy Hydrocarbons, with C > 12)	1280±760	2360±220	2050±540
Total PCBs	2.0±0.3	1.6±0.7	0.6±0.4
Total PAHs	835±270	1220±580	400±300
Benz[a]anthracene ^{2B}	60±25	95±52	21±27
Benzo[a]pyrene ¹	45±21	75±40	19±19
Benzo[b]fluoranthene ^{2B}	27±18	52±21	13±10
Benzo[g,h,i]perylene ³	32±14	51±22	19±9
Benzo[k]fluoranthene ^{2B}	74±60	159±72	56±12
Dibenz[a,h]anthracene ^{2A}	8±4	13±5	6±1
Indeno[1,2,3-cd]pyrene ^{2B}	69±5	76±2	79±7
Pyrene ³	65±23	98±54	21±32

Note. Bold characters indicate concentrations exceeding the limits of col. B, Annex 5 to Italian L.D. 152/06.

1. ^{2A}, ^{2B}, ³ Indicates *certainly*, *probably* and *possibly* carcinogenic for humans according to IARC classification.

Following the Port Development Master Plan of Taranto, third Italian industrial harbour, huge amounts of marine sediments must be dredged, largely contaminated by PAHs and eventually classified *hazardous waste* according to Italian legislation (L.D. No. 152/06). In order to avoid the unsustainable economic and ecological burden of their safe landfill disposal, a

3-yrs R&D project was undertaken aiming to detoxify these sediments *on site*.

The paper summarizes the results of a new biophysic process (BIOME¹), based on MC treatment followed by aerobic biological degradation with a selected bacterium.

¹Patent application pending.

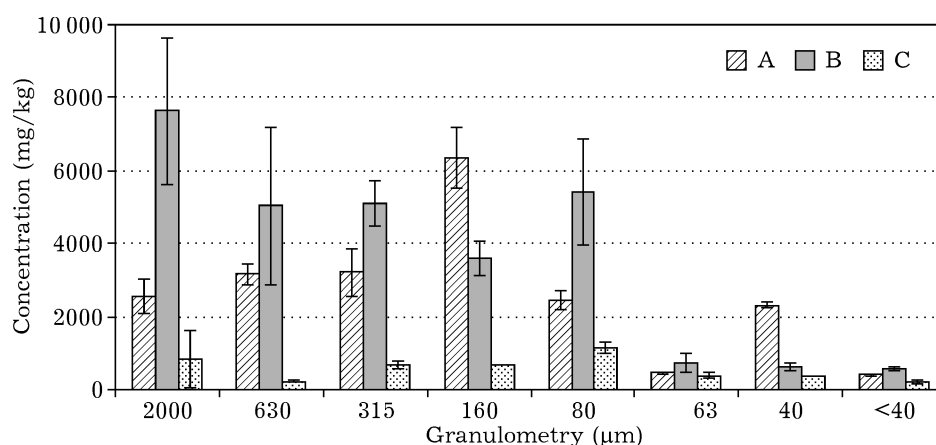


Fig. 1. PAHs total concentration in different granulometric fractions of the "hot spot" samples A, B and C.

TABLE 2

Analytical methods employed in determination of contaminants

Contaminants	Operation	Method	Reagents
PAH	Ultrasonic extraction	U.S.EPA 3550C	Acetone/Hexane = 1 : 1
	Clean-up by GPC	U.S.EPA 3640A	Cyclohexane/Dichloromethane = 7 : 3
	Quantification by HPLC	U.S.EPA 8310	Water, Acetonitrile
HHC	Ultrasonic extraction	U.S.EPA 3550C	Acetone/Hexane = 1 : 1
	Clean-up, quantification by GC-FID	U.S.EPA 3620C	Florisil
BaP degradation by-products	Ultrasonic extraction	U.S.EPA 3550C	Acetone/Hexane = 1 : 1
	Quantification by GC-MS	U.S.EPA 8270C	

MATERIALS AND METHODS

Taranto's harbour sediments

Representative sediment samples (60 kg each) dredged in triplicate from 3 "hot spots" [12] were characterized (Table 1). Microbiological tests (*fecal Streptococci*, *Salmonella* spp., total coliforms, *Staphylococci* and *Escherichia coli*) were negative, contrary to the ecotoxicological ones (*Vibrio fisheri*, *Skeletonema costatum*, *Acartia clausi*) with chemical contaminants often exceeding Italian legislation on hazardous and non hazardous waste (see Table 1). Although showing appreciable variation, the characteristics of the three "hot" samples, with high occurrence of heavy metals ($\leq 25\ 000$ mg/kg Fe), organic substance ($\leq 40\ 000$ mg/kg), organotin compounds (≤ 350 mg/kg), HHCs (≤ 2500 mg/kg) and PAHs (≤ 1250 mg/kg), occasional PCBs (≤ 2 mg/kg) and ecotoxicological threatening, represented the average contamination of local marine sediments [12, 13]. Wet vibro-sieving showed that most samples (80 % of A and B; 60 % of C) pass 40 μm sieve, indicating the silt-clay nature of Taranto's sediments, while PAH composition suggests their source from coke-oven processes and/or combustion of heavy oils [14]. PAHs total concentration was larger in coarser (sand, $\text{Ø} > 60\ \mu\text{m}$) than in finer grain sizes (silt and clay) (Fig. 1). According to Wilichowski [15], PAHs form large tar particulate ($\text{Ø} > 100\ \mu\text{m}$) that tend to build-up in the coarser fractions.

Contaminants analysis

PAHs and HHCs were determined according to U.S.EPA [16] (Table 2) using Sigma-Aldrich (the USA) solvents and reagents.

Microorganism and culture media

Biodegradation was carried out with *Novosphingobium pentaromativorans* US6-1 bacterium (DSMZ, Braunschweig, D), capable of using high MW PAHs (in particular BaP) as unique source of C and energy [17, 18], grown with 2 culture broths:

1) *Trypticase Soy Broth* (TSB) rich medium, prepared from a lyophilized commercial mixture (Biolife, I) and used to store the bacterium on Petri plates at 4 °C;

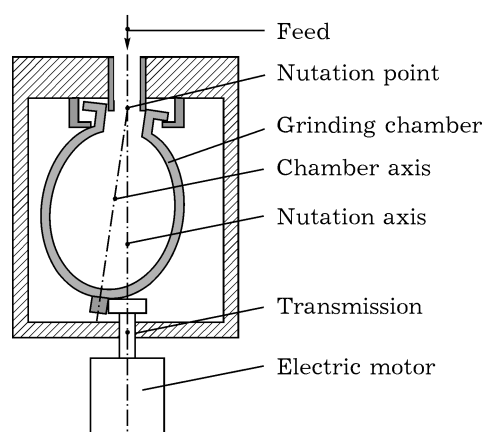


Fig. 2. Picture and scheme of the high energy nutational mill Hicom 15.

TABLE 3

Technical characteristics and operating conditions of the MC pre-treatment

Technical characteristics of Hicom 15 high-energy mill	
Jar net volume and material	5.3 L, Stainless Steel AISI 304
Milling balls diameter and material	Ø 10 mm, Stainless Steel AISI 420C
Operating conditions	
Milling (steel balls)-to-milled (sediment) mass ratio	10 : 1
Jar filling ratio	30 % v/v
Milling rotation speed	1000 rpm

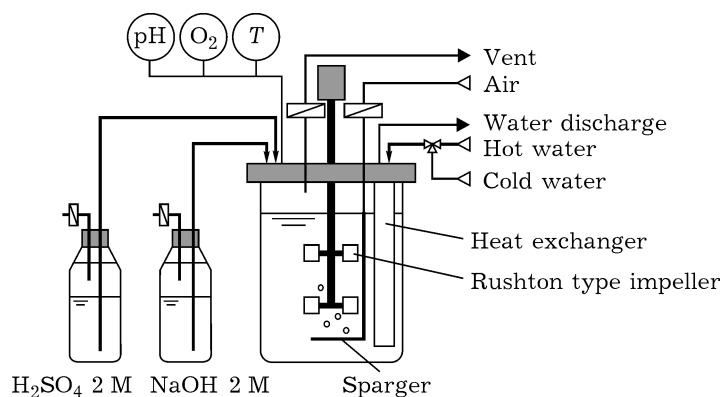


Fig. 3. Picture and flow sheet of the bioreactor Esedra plus 3.5.

2) M9 minimal medium [19], added with 62.5 mg/L yeast extract to help initial growth phase. BaP (Carlo Erba Reagents, I) was added to M9 medium to prepare inocula.

BIOMECC tests

Each sediment sample was pretreated by MC treatment, followed by biological treatment into bioreactor. MC treatment was carried out with the high energy nutational mill Hicom 15 (Hicom Technologies, Pinkeba, Australia), where the geometrical axis of the jar is inclined with respect to rotation axis, generating a nutational movement (Fig. 2).

Sediment sample (64.7 g) was slurried with tap water (10 % w/v) and mechanochemically treated for 10 min under the conditions reported in Table 3.

Biological degradation was carried out in a 3.5 L bioreactor (Esedra plus 3.5, Solaris Biotechnol., I) (Fig. 3) stirred at 500 rpm and aerated with sterile air (flow rate 1 L/min). Temperature was maintained at 30 °C, pH 6.8 and

oxygen saturation near 100 %. The bioreactor, filled with pre-mechanochemically treated and slurried sediment (200 g dry matter) and its aqueous solution

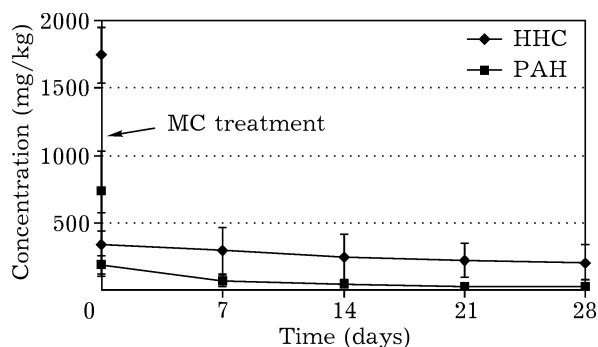


Fig. 4. Average contaminant degradation in “hot spot” samples A, B and C with the BIOMECC treatment.

TABLE 4

Average degradation results of contaminant classes in the 3 hot spots (see Fig. 4)

Contaminant classes	MC step	BIO step	Total
HHCs	(80±11) %	(7±6) %	(88±7) %
PAHs	(74±1) %	(22±2) %	(96±1) %

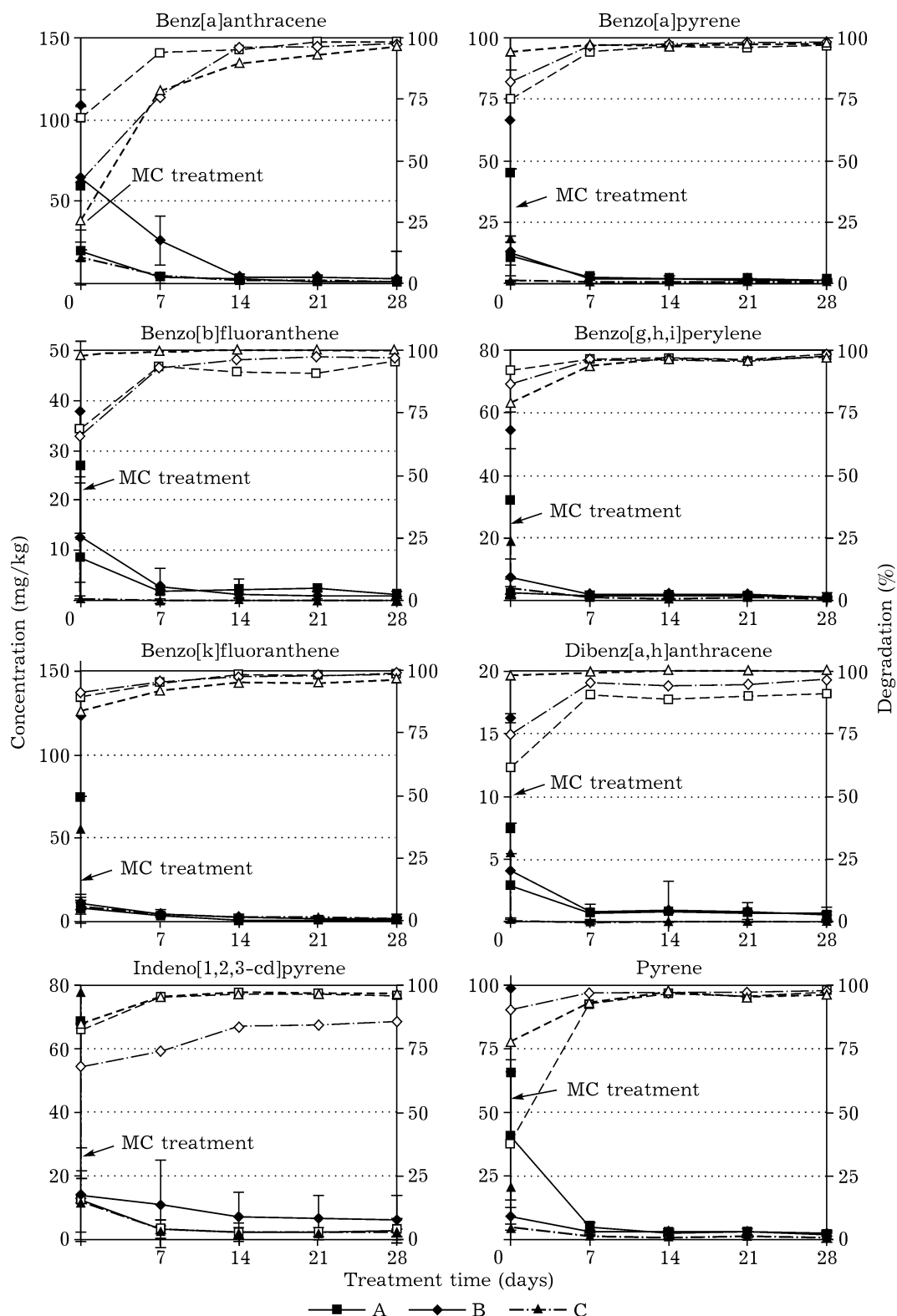


Fig. 5. Absolute and percent (dashed lines) degradation of PAHs in spots A, B and C.

(2300 mL), was added with 3.8 g (1.5 % w/w) of Humic Acids (Fluka, I) and M9 medium to 2880 mL final volume. Then the slurry was inoculated with

3 % v/v culture of *N. pentaromativorans* grown with 1 g/L of BaP in M9 medium. Small slurry aliquots (10 mL) were withdrawn every 7th day, added with

TABLE 5

Average degradation results of single contaminants in the 3 "hot spots" (see Fig. 5)

Single contaminants	MC step, %	BIO step, %	Total, %
Benz[a]anthracene	45±21	53±20	97±41
Benzo[a]pyrene	84±10	14±10	97±20
Benzo[b]fluoranthene	77±18	20±16	98±34
Benzo[g,h,i]perylene	86±6	12±7	98±13
Benzo[k]fluoranthene	88±4	10±3	98±7
Dibenz[a,h]anthracene	78±18	18±14	96±32
Indeno[1,2,3c,d]pyrene	78±9	15±3	93±12
Pyrene	69±28	28±28	97±56

0.5 mL trichloroacetic acid 2 M to stop bacterial activity and centrifuged; the pellet was analyzed for PAHs and HHCs.

For sake of simplicity, the experimental data hereinafter refer to PAH and THC (Total Hydrocarbons) only, the contaminants of major interest in the present investigation. Further information and all detailed analytical and experimental results obtained during this 3-yrs extensive investigation are available by request [20].

Benzo[a]pyrene mechanochemical degradation tests

A preliminary test to understand MC degradation mechanism of BaP was carried out in dry conditions with BaP-spiked clean sediment taken from nearby Mar Grande having similar physical characteristics to the "hot spot" samples utilized in this study. According to U.S.EPA [21], aliquots of this sediment were spiked with BaP to a final concentration of 65 mg/kg. The contaminated sediment sample (647.2 g) was dry-ultramilled (see Table 3); every minute

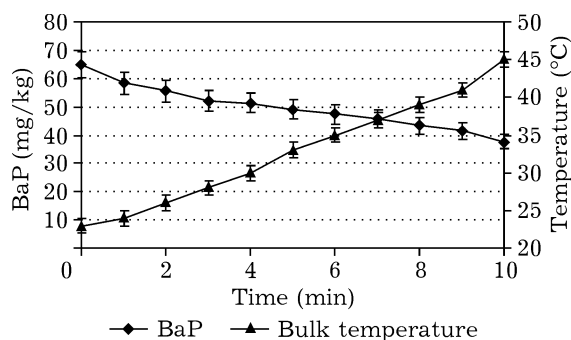


Fig. 6. Concentration and temperature variation during dry MC treatment of BaP-spiked sediments.

small aliquots were withdrawn and analyzed for BaP concentration and identification of degradation products by GC-MS analysis.

RESULTS AND DISCUSSION

Contaminants degradation

Figure 4 and Table 4 shows the experimental results of HHC and PAH degradation by the BIOMECH process on sediment samples dredged from A, B and C "hot spots" of Taranto harbour.

It can be seen that almost complete degradation of HHCs (88 %) and PAHs (96 %) was obtained on all sediment samples in 4 weeks. This astonishing result was due mainly to the

TABLE 6

By-products deriving from dry MC treatment of artificially BaP-spiked sediments

By-products	Time of existence (min)
Diisopropyl ether	1, 2, 3, 4
Methylcyclopentane	1, 2, 3, 4
5-Methyl-2-hexene	1, 2, 3, 4
Cyclopentanone	1, 2, 3, 4
Acetic acid	1, 2, 3, 4
Acetyloxyacetic acid	1, 2, 3, 4
4-Hydroxy-4-methyl-2-pentanone	1, 2, 3, 4
4-Penten-2-one	1, 2, 3, 4, 5, 6
3-Methyl-3-hexen-2-ol	1, 2, 3, 4, 5, 6
2,3-Dimethyl-2-pentene	1, 2, 3, 4, 5, 6
Unidentified compound A	1, 2, 3, 4, 6, 7, 8, 9, 10
Unidentified compound B	1, 2, 3, 4, 6, 7, 8, 9, 10

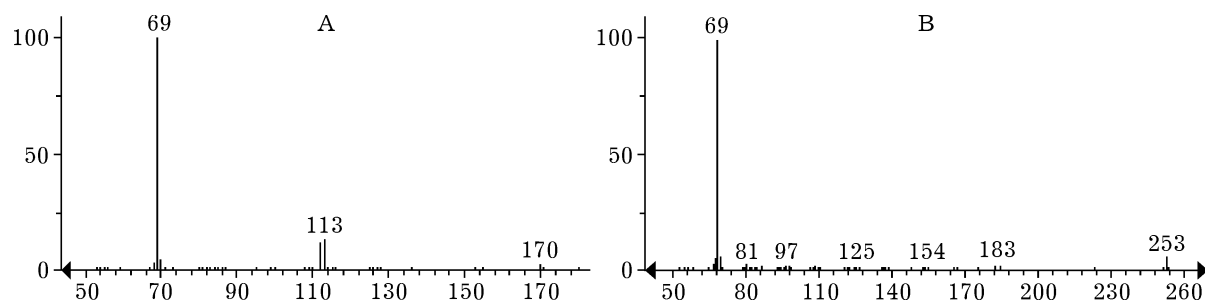


Fig. 7. Mass spectrograms of the unidentified compounds A and B.

MC pretreatment (81 and 74 % for HHC and PAH), complemented by 7 and 22 % biodegradation respectively, usually requiring years to achieve such overall yield [22].

Similar performances occurred for each PAH, without significant differences among samples neither from the 3 “hot spots” nor for the high-MW (acknowledgedly most biorecalcitrant) PAHs, as detailed in Fig. 5 and Table 5.

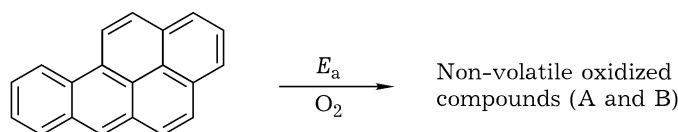
PAHs mechanochemical degradation

Currently, very little is known about the mechanism of PAH degradation by MC, except for the role of aerial oxygen in their oxidation during ball milling [23]. An interesting aspect is the effect of organic matter, whose

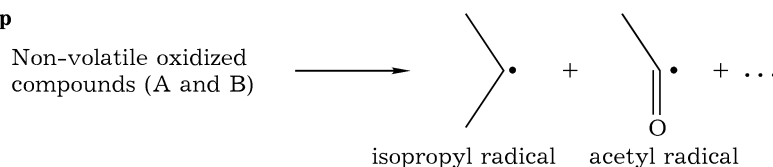
presence can act as radical scavenger so to decrease PAH degradation yield [24]; on the other hand, organic matter binds PAH, who tends to concentrate at the surface of sediment particle and to interact closely with its inorganic part, preferentially treated by the MC treatment. It is known that chemically reactive radical centers capable to transform organic compounds form at the surface under the mechanical destruction of crystalline quartz [25, 26]. This helps to explain why MC pretreatment permitted higher molecular mass PAHs to undergo larger overall degradation, in spite of their higher biorecalcitrance.

Figure 6 shows the variation of BaP concentration and temperature during the dry MC treatment of artificially contaminated sediments.

First step



Second step



Subsequent steps

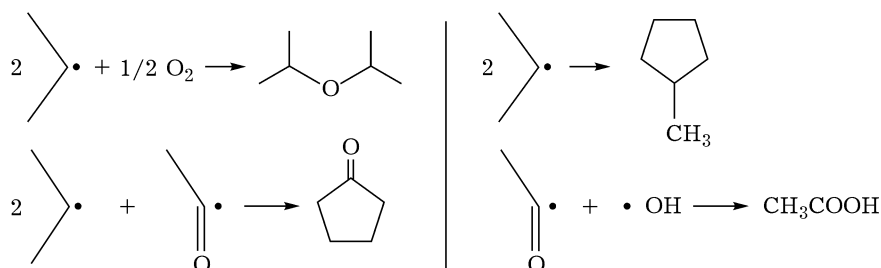


Fig.8. Possible steps of BaP degradation during MC pre-treatment.

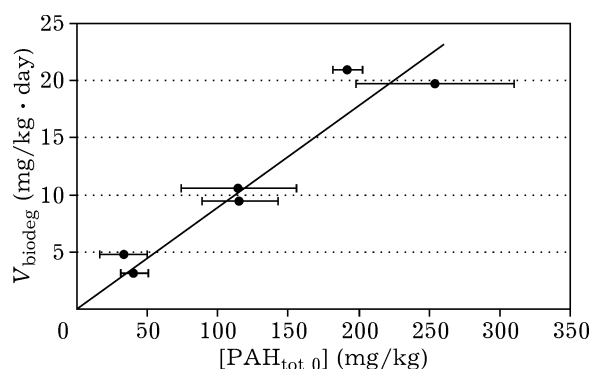


Fig. 9. Total PAHs biodegradation by the BIOMECC process according to Michaelis Menten rate model.

Table 6 reports the by-products identified by GC-MS, none of which is toxic. Two unidentified molecules (A and B) formed first among them. Their spectrum (Fig. 7) miss the typical high peak of aromatic compounds like PAHs, suggesting that A and B are not PAH derivatives and may be heavy (probably branched) and oxidized (with oxygenated functional groups) hydrocarbons.

On these premises, Fig. 8 shows a possible mechanism of BaP degradation by MC treatment in the conditions investigated. The first step is the almost complete destruction of BaP molecule through radical attack to the rings and the formation of A and B molecules with residual aromaticity (with double bonds) and oxidized (with carbonyl or OH groups), likely deriving from recombination of radicals produced by MC treatment. The second step is the breakdown of A and B to form small radicals, "building blocks" of volatile compounds in the subsequent steps. All compounds volatilize from the sediment surface with temperature increase. Heavier molecules are probably generated by an accretion process which, in case of long ultra-milling, would bring to graphitization [11, 27].

Kinetic information

Experimental data of total PAHs biodegradation were analyzed by the well-known Michaelis Menten reaction rate model of enzymatic reactions:

$$V_{\text{biodeg}} = V_{\text{max}} [\text{PAH}_{\text{tot } 0}] / (K_{\text{MM}} + [\text{PAH}_{\text{tot } 0}]) \quad (1)$$
 where V_{max} is maximum value of PAH biodegradation rate (V_{biodeg} , mg/(kg·day)); $[\text{PAH}_{\text{tot } 0}]$

is concentration of total PAHs after MC treatment (mg/kg); K_{MM} = Michaelis Menten constant (mg/kg).

Figure 9 shows the initial part (where $[\text{PAH}_{\text{tot } 0}]$ is very low), almost linear, of the MM plot.

Curve-fitting of experimental data by eq. (1) allowed to estimate MM parameters of the biological treatment (bottleneck of the BIOMECC treatment) as $V_{\text{max}} = 43$ mg/(kg·day) and $K_{\text{MM}} = 366$ mg/kg in the experimental conditions investigated. Being $[\text{PAH}_{\text{tot } 0}]$ in sediment after MC treatment (≤ 250 mg/kg, see Fig. 4) lower than K_{MM} , $V_{\text{biodeg}} \approx 1/2V_{\text{max}}$ achievable in those conditions. As shown in Fig. 1, preliminary sediment sieving permits to select more contaminated granulometric fractions (*i. e.*, with higher $[\text{PAH}_{\text{tot } 0}]$) in order to allow for faster PAHs biodegradation by *N. pentaromativorans*.

CONCLUSIONS

A new process able to provide fast and efficient degradation of toxic waste has been developed, called BIOMECC, based on a quick mechanochemical treatment followed by conventional aerobic biological oxidation. The BIOMECC treatment has been successfully applied to biorecalcitrant organic pollutants like PAHs and HHCs in marine sediments with excellent performance (97 % overall degradation for PAHs, 82 % for HHC in 1 month) with no formation of toxic by-products.

The MC pre-treatment made it possible to degrade more effectively the most bio-recalcitrant congeners, in agreement with the complex reaction mechanism postulated.

The increase of reaction rate with contaminant concentration suggests to proceed through a preliminary sediment sieving in order to select the more contaminated fraction sizes.

Pilot plant experiments are now planned to obtain basic information for engineering the BIOMECC process as well as to design continuous reaction equipment and optimize reaction conditions at large scale.

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