Bioaugmentation process for PAHs contaminated soil remediation through microbial inocula from anaerobic treatment of lignocellulosic substrate

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Abstract
In the present work, an anaerobic bioremediation treatment was investigated for reclamation of polycyclic aromatic hydrocarbons (PAHs) contaminated soil. The PAHs contaminated soil was artificially prepared and seven different contamination conditions were tested. In particular, four soils were contaminated solely by naphthalene (A), anthracene (B), pyrene (C) and benzo[a]pyrene (D), respectively, whereas, three soils were contaminated by benzo[a]pyrene coupled with one of the other investigated PAHs (i.e. A+D, B+D, and C+D tests). Such conditions were tested in order to study the possible degradation kinetic for the single involved PAH (with aromatic rings ranging from 2 to 5) as well as for PAHs mixed with a 5-aromatic rings contaminant (i.e. benzo[a]pyrene). The investigated treatment was carried out in bioaugmented condition through two microbial inocula obtained from anaerobic digestion tests on lignocellulosic substrate. In more detail, the two inocula were differently enriched through experiments characterized by sequential re-inoculation on new substrate, for its subsequent treatment, every 24 h and 96 h, respectively. The present study focused on the PAHs degradation efficiency and pathways, and microbiological abundance characterization, thus providing a comprehensive and interdisciplinary view to assess the feasibility of the suggested treatment in the field of PAHs contaminated soil remediation.
Keywords: Polycyclic aromatic hydrocarbons, Contaminated soil reclamation, Bioaugmentation, Anaerobic bioremediation

1. Introduction

Polycyclic aromatic hydrocarbon (PAH) soil contamination, from both natural and anthropogenic sources, represents a concerning environmental issue. In fact, the high PAHs hydrophobicity leads to adsorption phenomena on the organic matter of solid particles causing a risk to human and ecological health (Tang et al., 2005). According to this, several approaches have been implemented for the remediation of PAHs contaminated soils. For instance, bioremediation techniques have been widely investigated by testing several operational conditions such as various temperature values, different nutrients and/or carbon source addition, aerobic or anaerobic condition, involvement of indigenous or allochthonous microbial/fungal community (Lukić et al., 2017). In particular, a growing specific scientific interest is focused on the involvement of anaerobic condition as well as inoculation of ligninolytic species due to their capability to produce feasible enzymes for PAHs oxidation.

On this basis, the aim of the present work has been to thoroughly investigate the PAHs anaerobic biodegradation efficiency in an artificially contaminated soil through two specific inocula differently enriched from the anaerobic digestion treatment of lignocellulosic substrate. Accordingly, analysis on PAHs degradation trend and microbiological community were carried out in order to provide a wide overview of all the aspects concerning the investigated process.

2. Materials and Methods

2.1. Artificial contaminated soil preparation

Artificial soil samples contamination was carried out according to the OECD guidelines (OECD/OCDE 317, 2010) by preparing seven different contamination conditions. In particular, fours soil samples were contaminated solely by naphthalene (A), anthracene (B), pyrene (C) and benzo[a]pyrene (D), respectively, whereas, three soil samples were contaminated by benzo[a]pyrene coupled with one of the other investigated PAHs (i.e. A+D, B+D, and C+D tests). Then, initial PAH concentration in the spiked samples was almost equal to 34 mg kg^{-1} for soils with single PAH

contamination while 17 mg kg^{-1} for each PAH in the multi-contaminated soil samples.

2.2. Batch bioremediation tests

Glass reactors with a total working volume of 12 ml were used in each test and three different configurations were investigated for each contamination condition (i.e. control test and two bioaugmentation tests).

Table 1. Experimental set-up of bioremediation tests

Test	Composition
Control	Contaminated soil (6 g)+0.1M phosphate buffer solution (12 ml)
Bioaugmentation	Contaminated soil (6 g)+0.1M phosphate buffer solution (11.7 ml)+enriched inocula (0.3 ml)

The bioaugmentation tests were carried out by involving two different inocula both enriched through seven times sequential re-inoculation on new lignocellulosic substrate (wheat straw) during the anaerobic digestion treatment. In particular, the two inocula were re-inoculated on new substrate every 24 h (I-24) and 96 h (I-96), respectively. Test compositions were reported in Table 1.

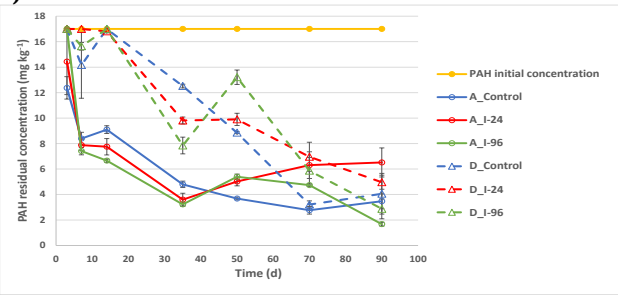
3. Results and Discussions

In general, slight higher PAH removal yield was observed for all single contamination conditions in I-24 bioaugmentation test (84%, 65% and 52% for A, B and D, respectively) compared to I-96 bioaugmentation test (68%, 54% and 45% for A, B and D, respectively) and control test (71%, 56% and 38% for A, B and D, respectively) after 90 d. On the contrary, for samples contaminated with pyrene, the use of I-24 resulted in the lowest removal yield (12%) compared to the I-96 bioaugmentation and control tests (46% and 34%, respectively) after 90 d. Accounting for the multi-contamination conditions, it was generally observed a higher removal yield for each contaminant in both bioaugmentation and control tests compared to the experiments characterized by single PAH contamination. However, in this case, the I-24 inocula displayed lower efficiency compared to the I-96 one. In fact, considering the A+D contamination condition as example (Figure 1a), I-24 bioaugmentation test showed a final removal yield of almost 81% and 85% for A and D respectively. Both values were lower if compared to I-96 bioaugmentation test (95% and 92% for A and D, respectively) and control test (90% and 88% for A and D, respectively). Finally, the cell density values at 90 treatment days of the control and bioaugmentation tests for A, D and A+D contamination conditions were reported in Figure 1b. The results suggest that data on the bacterial abundance could provide significant information about the inocula acclimatization capability. In fact, both the inocula proved to be able to overcome the toxic effect of A and D, considering that the control test generally showed 10-fold lower bacterial abundance values.

4. Conclusions

In the present work, results showed a noteworthy efficacy in terms of PAHs removal yield achieved for all the contamination conditions, thus assessing for the two involved inocula feasible applicability. Indeed, deeper insight of this study could be provided from more detailed microbiological and statistical analysis respectively useful to specifically characterize the involved microbial communities and evaluate the correlation among all the key-factors of the process.

a)



b)

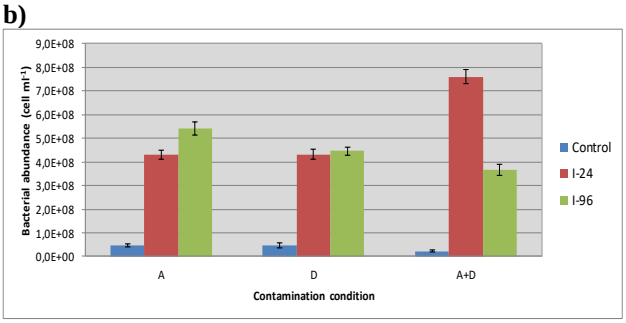


Figure 1. PAHs residual concentration in bioaugmentation and control tests for soil sample contaminated by A+D (**a**) and cell density values after 90 treatment days for soil samples contaminated by A, D and A+D (**b**)

References

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