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Reuse of polycyclic aromatic hydrocarbons (PAHs) contaminated soil washing effluent by bioaugmentation/biostimulation process

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ABSTRACT

The reuse feasibility of the bioremediated effluent of the combination of sequential soil washing followed by biological treatment for removal of phenanthrene (PHE) from contaminated soils was investigated for several soil washing. In the first stage a removal efficiency of 74.4% ± 3.5% was obtained under the optimum conditions of: surfactant (Tween 80) concentration of 5000 mg/L, liquid/soil ratio of 30 v/w, humic acid concentration of 2.31 mg/L (6.93%), and washing time of 2 h. In the second stage complete biodegradation of PHE from soil washing effluent was achieved by inoculating enriched bacterial consortium ($OD_{600nm} = 1$) within 7 days. Reuse possibility of biologically treated solution for the next cycles of soil washing process was conducted by adjusting the surfactant concentration. The findings displayed that reusability of the biologically recycled solution was maintained up to 7th cycle, reaching a removal efficiency of >99% and >97% in artificial and real contaminated soils respectively, indicating the effectiveness of reused surfactant solution.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic pollutants composed of two or more benzene rings in a linear, angular, or cluster structure [1,2]. These compounds are widely occurring in the environment as a result of incomplete combustion of fossil fuels, oil refinement and leakages, and gas and coal production [3,4]. PAHs have potential mutagenicity and carcinogenicity and pose serious threats to human health [5,6]. These widespread compounds are strongly adsorbed by soil, mainly due to its particular properties such as low volatility, low aqueous solubility, and high octanol–water partition coefficients (K_{ow}). The hydrophobicity of PAHs makes them very persistence in soil, requiring a long-lasting bioremediation process [3]. Soil washing using surfactant solution is a promising technology for rapid desorption of PAHs, resulting in speeding up the reclamation process in the liquid media [3,7]. Surfactants enhance solubilization of hydrophobic organic compounds (HOCs) by decreasing the interfacial tension between the pollutant and water and accumulating them into the hydrophobic cores of the surfactant micelles [4,8]. In general, non-ionic surfactants have higher solubilization capacity and are more cost-effective and biodegradable than cationic and anionic surfactants, being used widely in the soil washing processes to remediate PAHs-contaminated soils [7,9].

Performance of the soil washing process is affected by several factors. Studies have revealed that PAHs desorption enhances by increasing the surfactant concentration [10,11]. However, there are few studies reporting decreased desorption of PAHs after a certain concentration of the surfactant [3,8]. The optimum concentration of surfactant is important due to economic and surfactant may cause colloid mobilization and clogging of the soil pores [12]. On the other hand, the residual surfactants in the treated soil may lead to imbalanced biological activities and toxic effects on indigenous microorganisms. Liquid/soil ratio is also another influencing factor in the soil washing process [10,13]. Peng et al. (2011) reported that PAHs desorption increased with increasing liquid/soil ratios [10]. In a system with lower liquid/soil ratios, greater amounts of the surfactant are needed to achieve the same level of reduction in the surface and interfacial tension [14]. It is important that the increasing of which one has more effect on the desorption efficiency, surfactant concentration or liquid/soil ratio. However, on a real scale, higher volume of liquid will require more water and more energy use. Studies have reported the increment of PAHs desorption rate due to the coexistence of dissolved organic matter

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(DOM) and surfactants [15,16]. Conte et al. (2005) reported that humic acid (HA) has the same desorption efficiency for PAHs as do synthetic surfactants [17]. However, a number of studies have reported that binding of PAHs to HA cause the contaminant to become more resistant to desorption [18,19]. In natural soils, the OM content of the soil is in the range of 3–6%, so its effect in the soil washing system needs to be investigated. Reaction time is also another important factor influencing the PAHs desorption in the soil washing method. A few studies have reported that 24 h is required to reach the PAHs desorption equilibrium, but equilibrium times of 8, 3 and 1 h have also been reported [10,13,16,20].

Therefore, considering the existing controversies regarding the factors involved in the soil washing process it is essential to evaluate the influential parameters in this process and to optimize them in order to shorten the required time and perform a cost-effective process.

The effluent of soil washing must subsequently be treated before being discharged into the environment. Moreover, the treated soil washing effluent may be reusable for frequent soil washing. This benefit makes the application of the soil washing process more practical on an industrial scale. Several technologies have been used so far to remove PAHs from the soil washing solution [3,4,7,21,22]. Among these technologies bioremediation is a more practical, economical, and environmentally friendly strategy for this purpose, due to complete mineralization of PAHs with minimum costs and energy use [23]. However, due to high level of microbial density the reusability of bioremediated effluent for soil washing is important. In the present work, we aim to evaluate the impact of influential factors, including surfactant concentration, liquid/soil ratio, organic matter, and washing time on the desorption efficiency of phenanthrene (PHE), as a representative of PAHs, using a nonionic surfactant, i.e. Tween 80. Box-Behnken design using response surface method (RSM) was applied to determine the optimum conditions and represent the impact rate of each independent variable and the interactions among them. The present work also aims to investigate the biodegradation of PHE from the soil washing effluent using bacterial consortium isolated from petroleum-contaminated soil. We also sought to investigate the effect of frequent soil washing with the recycled effluent on PHE desorption.

2. Materials and methods

2.1. Chemicals

PHE, a three-ring PAH, which has been listed among the US EPA priority pollutants, was purchased from Merck Company with purity of >98%. Humic Acid and Tween 80 were obtained from Fluka. Mercuric chloride was supplied from BDH. Acetone, NaCl, mineral salt medium (MSM) including K_2HPO_4, KH_2PO_4, NH_4NO_3, Nutrient Agar, and High-performance liquid chromatography (HPLC) grade acetonitrile (99.9%) were also purchased from Merck Company.

2.2. Stage I: Soil washing experiments

2.2.1. Surfactant solubilization experiments

In order to investigate the effectiveness of Tween 80 in soil washing process, solubilization capacity of the surfactant for PHE had to be considered. Batch experiments were conducted to determine the solubility enhancement of PHE by Tween 80. Twenty mL of a series of surfactant concentrations were placed in a 50 mL Erlenmeyer flask. Then, PHE was added to each flask slightly more than the required amount to saturate the solution. The flasks were put on a shaker (180 rpm, 20 ± 1 °C) for 24 h. After that, the samples were centrifuged (Hettich D7200) at 5000 rpm for 20 min to completely separate the undissolved solute. Afterwards, an appropriate volume of the supernatant was carefully withdrawn and filtered through 0.45 μm PTFE Syringe filter and then injected to HPLC (Cecil 7400) to determine the PHE concentration [11].

2.2.2. Optimization of soil washing process

Clean soil was collected from a depth of 10–20 cm below the ground surface, Tehran, Iran. It was air-dried at room temperature and passed through a 2-mm sieve. The soil contained 27% clay and silt, 60% sand, and 13% gravel. The soil organic matter content was 4.2% and the pH and electrical conductivity (EC) of the soil were 8 and 2.174 mmhos/cm, respectively. Four grams of the dry soil was placed into each 250-mL Erlenmeyer flask as non-continuous reactors. Then, they were spiked with PHE dissolved in acetone to have a final PHE concentration of 500 mg/kg [3].

In order to measure PHE desorption from contaminated soil, Tween 80 aided soil washing tests were conducted in batch experiments.

The Box–Behnken statistical design was used to evaluate the effect of the main influential factors and their interactions on the PHE removal efficiency in the soil washing process and to statistically optimize the independent variables. In the present work, the impacts of 4 factors on PHE desorption in 3 different levels were investigated. These factors were: (A) surfactant concentration, (B) liquid/soil ratio, (C) Humic Acid concentration, and (D) washing time; the 3 coded levels were: High (+1), middle (0), and low (−1) (Table 2).

Blank runs were also done without surfactant and by considering other factors in the above mentioned levels.

All the aqueous solutions contained 0.01 M NaCl to keep a constant ionic strength and 0.001 M $HgCl_2$ as an inhibitor for bacterial growth [15]. The samples were shaken (180 rpm, 20 ± 1 °C) for different times and the aqueous PHE concentrations were analyzed by HPLC. Each experimental run was conducted in triplicate and the results were reported with 95% confidence level. The Design Expert V.7, (Stat-Ease, USA) statistical software and Excel were used to design and analyze the samples.

2.3. Stage II: Bioremediation of the soil washing effluent

2.3.1. Toxicity impact assessment of Tween 80 on bacterial consortium growth

In order to have the best condition of bioremediation, the toxic effect of Tween 80 on the growth of the bacterial consortium needs to be assessed. The consortium was included of *Bacillus sporogenes*, *Bacillus licheniformis*, *Capnocytophaga ochracea* (presumably), *Acinetobacter sporogenes*, and *Staphylococcus xylosus*, isolated from a petroleum-contaminated soil in our previous study [24]. To have the necessary nutrients, mineral salt medium (MSM), consisting of 6.3 g/L K_2HPO_4, 1.8 g/L KH_2PO_4, 1 g/L NH_4NO_3, was prepared and sterilized in an autoclave. Then, 100 mL of the sterile MSM was poured into 250-mL Erlenmeyer flasks; afterwards, Tween 80 was added to each flask at concentrations of 500, 1000, 2500, and 5000 mg/L (Table 3). Then they were inoculated with bacterial consortium in an optical density of 1 at 600 nm using CECIL UV/Vis spectrophotometer (model 7100). A control sample, consisting of the sterile MSM and without the surfactant, was also used. Then, all the samples and the control were incubated for 24 h in a shaker incubator (30 °C, 180 rpm). The toxic effect of Tween 80 on the bacterial consortium was estimated based on the difference between the initial and the final bacterial populations [2].

2.3.2. Biodegradation of PHE from the soil washing effluent

The effluent of the PHE contaminated soil washing from the optimal operation conditions, after settling the soil particles was centrifuged at 5000 rpm for 20 min and filtered to thoroughly