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Categorical Multifactor Design to Evaluate Different Operative Parameters During the Bioremediation of Hydrocarbon-Polluted Soil in Early Experimental Stages

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A nome del Gruppo Italiano di Ingegneria Chimica dell'Università, GRICU, vi porgiamo un cordiale benvenuto al Convegno Nazionale:

GRICU 2022: Centralità dell'Ingegneria Chimica in un Mondo che cambia

in svolgimento dal 3 al 6 Luglio 2022 nell'isola di ISCHIA.

Siamo lieti di aprire con voi GRICU2022 e di condividere con voi l'atmosfera unica di questo evento, che offre oltre ad un programma molto interessante anche l'opportunità di scambiare idee con colleghi di tutti i settori e condividere la presentazione delle ultime innovazioni chiave all'interno dell'ingegneria chimica.

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L'organizzazione di questo Convegno Internazionale è stata possibile grazie all'aiuto e alla cura di molti colleghi: ringraziamo il contributo degli Autori dei manuscritti e dei Membri dei Comitati.

A nome dei Comitati del Convegno vi auguriamo giornate stimolanti e produttive, nonché un affascinante soggiorno nell'isola di ISCHIA.

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Categorical Multifactor Design to Evaluate Different Operative Parameters During the Bioremediation of Hydrocarbon-Polluted Soil in Early Experimental Stages.

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1.Introduction

Pollution by hydrocarbons is associated with different industrial activities which may harm the environment and human health Shahryar [1]. It is generally accepted that this kind of pollution has an anthropogenic origin, often due to accidental spills from production units and transport pipelines, leakages from storage facilities and underground tanks, mining, human activities in the production, transportation, and improper or illegal behaviors in waste treatment and disposal. These contaminants can be accumulated in soil due to their low degradation rates, affecting the physical, physiological, and biochemical properties of this valuable resource [2]. The bioremediation of soils polluted with hydrocarbons demonstrated to be a simple and cheap technique, even if it needs a long time [3]. The current paper shows the application of statistical analysis, based on two factors involved in the biological process at several levels. We focus on the Design of Experiments to determine the number and kind of experimental runs, whereas the use of the categorical factors has not been widely exploited up to now. This method is especially useful to analyze factors with levels constituted by categories and define the interaction effects. Categories or labels built from the combination of interest predetermined variables should be useful especially in the early stages of bioremediation projects, where the effects of multiple ideas are tested at the same time. Particularly, we focused on the statistical analysis of 1) experimental runs carried out at laboratory scale (test M, in microcosm), on soil polluted with diesel oil, and 2) bench scale runs (test B, in biopile), on refinery oil sludge mixed with industrial or agricultural biodegradable wastes. Finally, the main purpose was to identify the factor's significance in both the tests and their potential interactions against the different response variables, by applying ANOVA.

2. Methods

Two categorical multifactor arrays were designed, randomized, and performed. In both cases, the effects of two factors on the interest response variables concentration were assessed. For each study case, the experimental runs are described, namely: i) Microcosms test (test M), based on a small amount of polluted soil (200 g); ii) Bench test (test B), based on a larger polluted mass (around 38 kg).

For both tests, a 6-levels categorical factor called “Treatment” was defined. In test M, the treatments Mi (i= 1, 2…6), corresponding to a combination of values water content (WC%) and carbon to nitrogen ratio (C/N). These combinations are labeled: M1(8 WC%-120 C/N); M2 (8 WC%-180 C/N); M3 (12 WC%-120 C/N); M4 (12 WC%-180 C/N); M5 (15 WC%-120 C/N); M6 (15 WC%-180 C/N). On the other hand, the categorical factor levels in test B, Bi, correspond to the different treatments of the biopiles technique. Each run had a supplement of a specific organic waste as an additive bulking agent in the mixtures of soil and hydrocarbons. The levels were labeled as follows: B1 (sugarcane bagasse); B2 (sugarcane filter cake); B3 (sawdust); B4 (coffee pulp); B5 (beef manure); B6 (Thalassia testudinum residues). Likewise, in both the tests (M and B), the other factor was the “Time”, defined at 5 levels Tmi (i=0, 15, 70, 112, 131). and Tbj (j= 0, 60, 90, 150, 240) respectively. Each level corresponded to the time when the residual TPH concentration was monitored after the run started. This second factor was chosen under the hypothesis that the different treatments could have interactions during the bioremediation.

The Total Petroleum Hydrocarbon (TPH) concentration was the response variable in both tests. In the M test the TPH was measured in sample extracts achieved from each microcosm. The extraction was done by the EPA method 3546 (moisture 15-30% b.w.), based on microwave heating, as described in a previous paper [2]. Then, each extract was analyzed twice by the EPA method 8015 to measure the TPH concentration. On the other hand, the TPH in the B test was measured with samples collected from each biopile. The TPH

concentration was determined by adapting the EPA Method 1664 to the biopile system. The analysis was based on the TPH extraction from solid by an organic solvent and evaluation of the TPH mass by gravimetry. This method results reliable because the solid sample was over 5 g and the TPH concentration over 0.3% by weight.

Both experiments has combined all the levels of the factors (6x5). For M test, since two replicas were carried out, a total of 60 runs were available, achieving 30 degrees of freedom (Df). The statistical parameters of the DOE were considered statistically consistent with the strengths in the analytical determination method of the TPH concentration. On the other hand, for the B test a total of 120 runs due to 4 replicas of the experimental array. This ensured 90 degrees of freedom, which were considered statistically robust, compensating for the weakness of the gravimetric determination method if compared to the gas chromatographic one. All the analyses were performed using a confidence level of 95% employing the professional software STATGRAPHICS Centurion v. 16.1.18. Moreover, the assumptions for the model were corroborated. Table 1 offers the synthesized information for both tests.

Table 1. Operative conditions for the studied tests.

Operative conditions	Microcosms (Test M)	Biopiles (Test B)
Response variable	TPH	TPH
Factor 1-Treatments (6 categorical levels)	M1(8 WC%-120 C/N) M2 (8 WC%-180 C/N) M3 (12 WC%-120 C/N) M4 (12 WC%-180 C/N) M5 (15 WC%-120 C/N) M6 (15 WC%-180 C/N)	B1 (sugarcane bagasse) B2 (sugarcane filter cake) B3 (sawdust) B4 (coffee pulp) B5 (beef manure) B6 (*Thalassia testudinum*)
Factor 2-Time (5 levels)	0 15 days 70 days 112 days 131 days	0 60 days 90 days 150 days 240 days
Mass	200 g	38 kg
Pollutant	Diesel oil	Oily Sludge
Water content (WC%)	8% 12% 15%	20%
Carbon to nitrogen ratio (C/N)	60 120 180 300	10
Kind of soil	Sandy soil	Sandy soil
pH	6-8	6-8

3. Results and discussion

Since the categorical multifactor was used under the hypothesis that different interaction between factors could influence the removal of the hydrocarbons, it is of interest to know which combination of treatment and time would ensure better results for any process period. Figure 1 shows the interactions plots of both factors in each experiments.

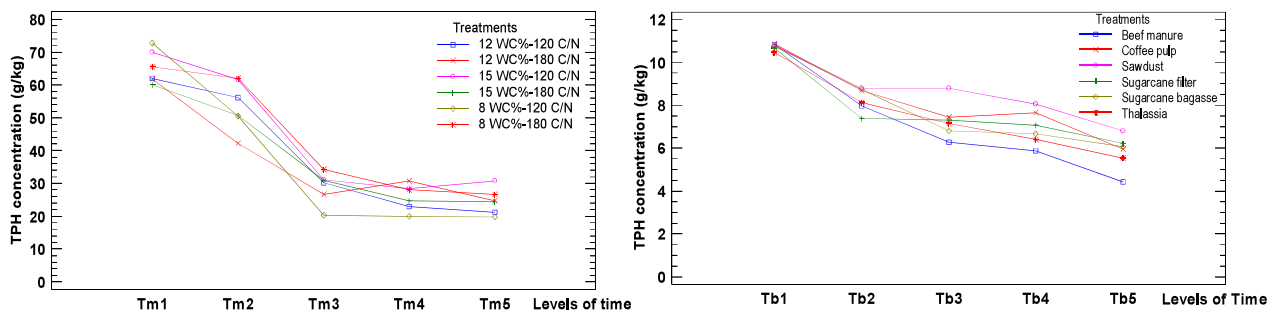

Figure 1. Interaction plot for the factors. a) test M b) Test B.

The lines in Figure 1 on both the left (a) and the right one (b) represents the interaction effect between the significative factors obtained in the Analysis of Variance (P-value= 0.000 for test M; P-value= 0.0012 for test B) and indicates that during the process, some treatments alternated their order of degradation.

Specifically, in test M, the Treatment M1 (8 WC%-120 C/N) had the lowest TPH concentration at each level of time after the first 15 days. Likewise, after 71 days M3 (12 WC%-120 C/N) was ranked second with lower TPH concentration, suggesting not only the best degradation of the treatments with the less C/N ratio but also the influence of water content in the first period of the run. These findings are consistent with the previous ones modelled by Raffa et al. [2] using an RSM model, confirming the accurateness of the categorical multifactor design. After the third month, the TPH concentration decreasing was negligible for all treatments. This could represent a crucial issue to estimate the times for the in situ exploitation of the technology.

In test B, in all treatments, the TPH concentration decreased until 240 days, with removal percentages between 40% and 60%. The treatments order rank was B5, B6, B2, B1, B4, B3. Specifically, Treatment B5 (beef manure) was the one that reported the lowest TPH concentrations at 240 days, keeping this trend after 60 days. The means obtained for B5 presented marked statistical differences with the rest of the treatment. In contrast, B3 (sawdust) presented the worst TPH removal, keeping this trend after the 60 days. Despite the rank order, the TPH means of B6, B2 and B1 do not present significative differences among them for a 95% confidence level. Furthermore, the differences between the levels in the factor Time evidence the evolution in the degradation process.

4. Conclusions

The categorical multifactor array based on categorical factors assessed at different levels demonstrated its applicability to studies done in different operative conditions. Particularly, we focused on a laboratory experiment and bench-scale test to evaluate the reliability of the proposed statistical method in testing the performance of biostimulation processes of contaminated soil, but the application is not limited to this kind of experiment or scales.

The structured method implemented in this study enables the identification of the best candidate in microcosms polluted with diesel oil (combinations of water content and carbon to nitrogen ratio were assumed as treatments). Specifically, the treatment M1 (8 WC%-120 C/N) showed the best diesel oil removal. In the experimental runs on biopiles to bioremediate oily sludge supplementing different renewable organic wastes as treatments, the treatment B5 (biopile with beef manure as a bulking agent) showed the best hydrocarbons removal.

References

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