

BIOCONVERSION OF PETROLEUM HYDROCARBONS IN SOIL USING APPLE FILTER CAKE

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ABSTRACT

The aim of this study was to investigate the feasibility of using apple filter cake, a fruit-processing waste to enhance the bioremediation of petroleum contaminated soil. A rotating barrel system was used to study the bioconversion of the xenobiotic compound by natural occurring microbial population. The soil had been accidentally polluted with a total petroleum hydrocarbon concentration of 41,000 ppm. Although this global value was maintained during the process, microbial intervention was evidenced through transformation of the petroleum fractions. Thus, fractions that represent a risk for the environment (GRO, Gasoline Range Organics i.e., C6 to C10-12; DRO, Diesel Range Organics i.e., C8-12 to C24-26 and RRO, Residual Range Organics i.e., C25 to C35) were significantly reduced, from 2.95% to 1.39%. On the contrary, heavier weight fraction from C35 plus other organics increased in value from 1.15% to 3.00%. The noticeable diminution of low molecular weight hydrocarbons content and hence environmental risk by the process plus the improvement of the physical characteristics of the soil, are promising results with regard to future application at large scale.

Key-words: apple filter cake, bioconversion, bioremediation, petroleum contaminated soil.

INTRODUCTION

Oil and oil distillate spills are frequent contamination events that affect vast land areas, causing damages to the ecology and the economy of rural regions. Thus, the development of ecological, compatible and inexpensive technologies aimed to remediate hydrocarbon contamination, becomes a priority. These techniques have to be designed and tested thoroughly before applying them to a field problem. Usually, hydrocarbon-contaminated soils lack organic matter of botanical origin and have low microbial activity. The addition of a fruit-processing waste can be beneficial since it represents a source of co-substrates, nutrients and microorganisms and ameliorates the structure and water-retaining capacity of the soil in favour of aerobic conditions. The incorporation of exogenous microorganisms might accelerate the degradation of contaminants in soil (27). Aerobic metabolism generates heat, resulting in significant temperature increases caused by changes in the microbial population and physiology in the mixture. Temperature affects not only the physiological reaction

rates and population dynamics of microbes, but also most of the physicochemical characteristics of the environment (2,3).

The selection of waste substrates involves the difficulty of finding a residue with the right balance of nutrients to support optimal growth and production (21). For the experiment of this study, we proposed the utilization of apple filter cake for the bioremediation of petroleum contaminated soil because it is an industrial waste of the impacted zone. Although this material may have other industrial uses, large amounts are generated, what makes it easily available. Transportation distances from apple manufactory to soil treatment areas are short and this lowers its cost. Even though addition of organic material to contaminated soil is not a new concept for improving its remediation (20,22), the use of local waste materials in Argentina has not often been tested before. Since the use of local available organic wastes appears to be a promising cheap alternative to accelerate TPH decomposition in contaminated soil, the success of this approach is not only important locally, but it could be applied in other regions and with the use of other wastes. The aim of this study was thus to investigate the feasibility of

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employing apple filter cake to enhance the bioremediation of a soil accidentally polluted with petroleum using a rotating barrel system. This preliminary test would help to determine the operational requirements of the system in order to attain target end-point concentrations and its practical application on large-scale treatment.

MATERIALS AND METHODS

Experimental Design

A rotating barrel of 200 litres capacity was specially constructed for this experiment. A 0.5 mm (pore size) net set at the bottom of the barrel defined a compartment for possible leachates collection added with a purge valve. The composition of the mixture consisted of 70 kg of contaminated soil and 30 kg of apple filter cake. The soil was loamy sand with a residual concentration of 41,000 ppm of TPH (Total Petroleum Hydrocarbons). It was air dried and sieved with a 2 mm grid. Hydrocarbon (HC) fractions extractable with dichloromethane from dry soil were: GRO: 0.13% (Gasoline Range Organics; i.e., C6 to C10-12); DRO: 2.59% (Diesel Range Organics; i.e., C8-12 to C24-26); RRO: 1.38% (Residual Range Organics; comprising 0.23% from C25 up to C35 and 1.15% compounds of more than C35 and other organics). Aromatic compounds as benzene, toluene, ethylbenzene, xylene (BTEX), trimethylbenzene, methylethylbenzene, propylbenzene and naphthalene (aromatics up to C10) were determined individually. All of them were below the detection limit of 0.01 ppm. The content of volatile compounds shorter than C10 was 20 ppm.

Chemical analysis indicated that total nitrogen content of the soil was 300 ppm, phosphorous content was 6.2 ppm, 6.58% (wt/wt) of moisture, saturation point 35% and pH = 7.2. The concentration of heavy metals was under the guideline values (13).

The apple filter cake was obtained from a cider factory of Tunuyán, Mendoza, Argentina. Its humidity content was 85%. The initial pH value of 4 was modified by the addition of 3.33% (wt./wt.) of Ca(OH)₂ to reach a value of pH = 7. To estimate the HC-like contribution to the mixture made by the apple filter cake, it was treated identically as the soil for TPH extraction. The extractable compounds with dichloromethane from the dry material expressed as hydrocarbon fractions were: GRO: 0.00%; DRO: 16.00%; RRO: 43.00% and 41.00% of C35 and other organic compounds.

The extractable compounds with dichloromethane from the mixture expressed as TPH were 40,000 ppm (4% wt/wt.). The expression as hydrocarbon fractions was: GRO: 0.04%; DRO: 1.97%; RRO: 0.32% C25 up to C35 and 1.67% C35 and other organic compounds.

BTEX and hydrocarbons shorter than C10 were under detection limit of 0.01 ppm. An initial C:N:P ratio of 100:5:1 was selected based on previous studies (9). For this purpose, 12g of

urea dissolved in distilled water were added in three fractions every ten days during the first 30 days. The fractionated addition of urea was done to avoid partial loss of nitrogen and fungicidal effects of ammonia gas product of the microorganisms urease conversion over urea (11,12). Control reactor consisted of 70 kg of 100% contaminated soil placed in a bin. The aeration by rotation of the barrel was daily during the first 45 days of treatment to assure oxygen provision and then on days 75, 90 and 210. Subsequently, the mixture was allowed to mature without rotation up to day 360.

Systems and environmental temperature, humidity content, pH and *Aerobic hydrocarbon degrader microorganisms* were measured every two days up to day 14, on a weekly regime up to day 45 and then on days 135, 210 and 360 of the process.

Petroleum hydrocarbon analysis were performed on samples of the initial components individually and on both systems taken on days 0, 45, 75, 90, 210 and 360 of the process.

An average sample of 200 g was obtained by mixing five aliquots of approximately 40 g extracted from different points of the reactor. Collected and mixed, samples were dried at room temperature for 24 h and then sieved (2 mm diameter openings). All analytical determinations were performed in duplicate.

ANALYTICAL METHODS

For every sample, a 10 cc aliquot was mixed with 50 ml of distilled water for five minutes before the pH was measured with an electrode. Approximately 10g of sample was placed in a preweighed porcelain capsule and dried at 105°C for 24h. The capsule with sample was weighed before and after drying to obtain the percent humidity mass. Distilled water was stirred with soil and brought to saturation. Subsequently, the saturation point was determined as percent humidity mass. Total nitrogen was determined following a micro-Kjeldahl analysis (5) with minor modification for soils. Absorbed phosphorous was determined according to Bray & Kurtz (8). Total HC were extracted with dichloromethane by Soxhlet to conduct further detailed chemical analyses. A 20g sample was mixed with 5 g Na₂SO₄ and 10 g MgSO₄. Once all the residual moisture had been absorbed by the MgSO₄, the mixture was pulverized and placed in a paper extraction thimble. The top of the thimble was filled with glass wool. The HC were extracted at a rate of 20 cycles h⁻¹ for 4.5 h. Fifteen grams of silica gel were added to the extraction solvent in a stoppered container and was mixed continuously for 10 min. The solvent was then filtered through glass wool, placed in a clean pre-weighed porcelain capsule and evaporated. The capsule was dried at room temperature for 24 h and weighed to obtain the mass of total extract by gravimetry (GR). A simulated distillation of the extract was performed with the aim of determining the amount of residue at 320°C. This fraction is comprised of those compounds that remain in the chromatographic injector at

320°C. Subsequently, gas-liquid chromatography analyses with flame ionization detection GC-FID were performed using a Clarus 500 Perkin Elmer chromatograph fitted with a moderately polar 30 m column (Perkin Elmer). The column temperature was programmed at an initial temperature of 45°C for 5 min, then increased at 10°C min⁻¹ to 300°C and held there for 40 min. Carlo Erba brand calibrators were used as markers for linear hydrocarbons from C6 to C20. ASTM D7169-5 norm (7) was followed as reference.

BTEX and benzene isomers were determined using a Clarus 500 Perkin Elmer gas-chromatograph equipped with a mass selective detector (GC/MS) and automatic Headspace sampler. A 30 m moderately capillary column HP5 type was used working isothermally at 50°C. Specific ions were used to quantify the aromatic compounds up to C9. EPA Method 8260 B SW846 norm (18) was followed as reference.

Culturable hydrocarbon degrading bacteria were enumerated by standard plate technique with appropriate mineral media (12) using 10% (vol/vol.) diesel fuel as the sole carbon source.

RESULTS AND DISCUSSION

Evolution of the operational variables of the system

The control of temperature was of great concern for our experiment, considering it is a fundamental parameter for control of the activity of the biological process.

Fig. 1 shows the temperature variation of the mixture soil-apple filter cake inside the reactor and room temperature during the length of the experiment. An important difference can be appreciated during the first 23 days, which correlated with a change in the physical structure of the mixture, due to the fast degradation of apple filter cake. A strategy of temperature control between 40°C and 45°C would seem to be appropriate for the bioremediation of PAH-contaminated wastes since it allows for the maximum microbial diversity to be expressed for both,

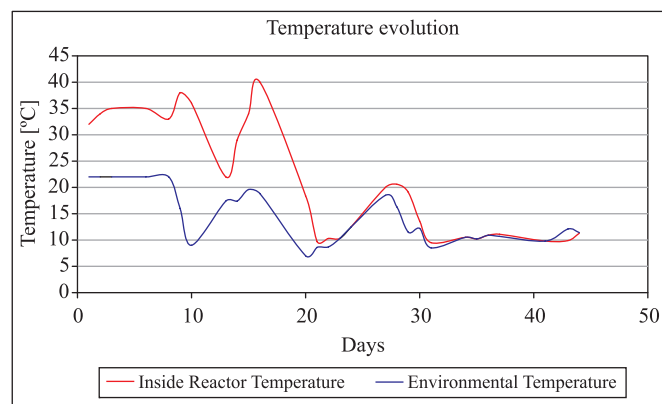


Figure 1. Inside reactor and environmental temperature evolution during the biological process.

bacteria, actinomycetes, and fungi (4). On this base and considering the increase in the microbial population, we can accept the temperature range reached of 32 °C to 36 °C for eleven days as acceptable for the benefit of our objectives.

Fig. 2 shows that in spite of the changes of temperature, the hydrocarbon degrader microbial population stayed in the order of 10⁸ cfu g⁻¹ soil representing an extension of the active phase of the process up to day 40. On day 210 descended to the order of 10⁶ cfu g⁻¹ soil, which is still a significant quantity of active HC degrader biomass.

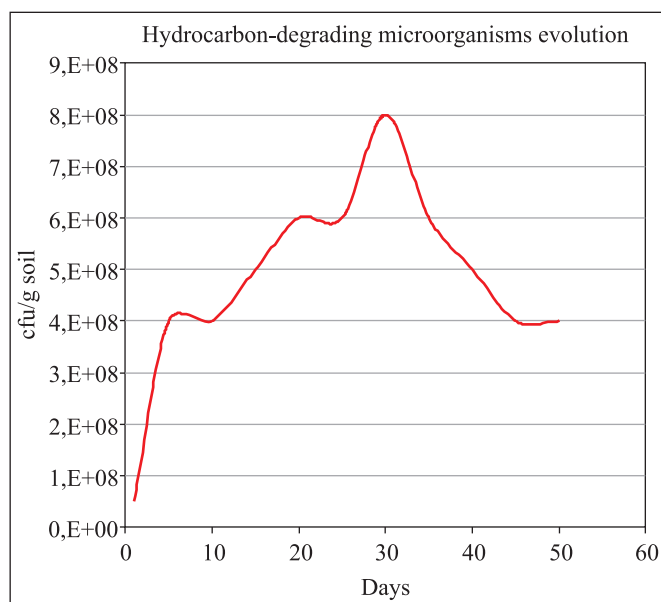


Figure 2. Numbers of Hydrocarbon-degrading microorganisms during treatment.

It is known that the content of cellulose, hemicellulose, lignin and proteins in crop residues influences their own initial decomposition rate. In our system, the apple filter cake exerted a positive effect that increased the microbial population by two orders of magnitude. The reasons of this phenomenon could be that apple filter cake acts as a nutrient supplier and physical conditioner contributing to increased oxygen diffusion and mineral nutrient availability, as well as carbon source and mechanical support surface for bacterial adsorption. A noticeable change in soil structure, aspect and odour, alike a garden soil was observed after treatment compared to control soil. The improvement of soil physicochemical characteristics probably accelerated microbial adaptation and selection and provided additional microorganisms, which contributed to metabolise or maybe cometabolise contaminant hydrocarbons. Furthermore, the contribution of organic matter to soil is generally accepted as a fundamental factor of fertility because its contribution with

essential nutrients and improvement of physical conditions (10,24). The decrease of the biological parameters after day 40 could probably be attributed to a reduction in nutrients and carbon source bioavailability, to hydrocarbons adsorption to apple filter cake material and soil or to toxic effects produced by residual petroleum fractions (1,16,19).

Moisture content of the mixture remained between 16.28% and 17.12% up to day 45, which corresponds to approximately 45% to 50% saturation. The saturation point of the soil was determined to be approximately 35% moisture by weight of soil. This is consistent with the literature which suggests optimum moisture content for biodegradation of hydrocarbons in soil of approximately 40 to 48 percent of soil saturation (23,25). Nevertheless, our data differed from the majority of previous studies that recommended the maintenance from 30% to 80% (3,9,15,19). The difference in optimal moisture content among these studies may be attributed mainly to the scale of work and thus the environmental conditions prevailing in the reactors. Fresh apple filter cake had a moisture level of 86% and the resultant level in the composting mixture with soil, was enough to avoid compaction and leachates formation. Thus, this moisture range let to the maintenance of aerobic conditions. Besides, with the course of the process, apple filter cake physical characteristics changed becoming a good bulking agent, what further improved the aeration of the process.

Hydrocarbon mineralization is favoured by near neutral pH values and where it has been shifted away by manmade changes, biodegradability is likely to be impaired (17). The addition of Ca(OH)₂ was efficient to maintain pH values between 7.2 and 7.6 during the complete process, providing a suitable media for microbial growth.

HYDROCARBON ANALYSIS

In the experimental reactor a slight difference among the total hydrocarbon content along the process (41,000 ppm in the initial contaminated soil and control reactor, 39,000 ppm for the mixture at day 75 and 42,000 ppm at the end of the process) was

observed, which could be attributed to sampling and analytical variations. Under these considerations, 40,000 ppm is considered constant during the process (Table 1).

A diminution of the shorter and the appearance of longer chain compounds can be observed. This phenomenon is also evident in the physical aspect of the Soxhlet extracts. Those obtained at initial stages of the process showed aspect and behavior of oil while the extracts obtained at later stages demonstrated a proportional increase in chain length behaving as a hard wax.

Based on these results a transformation of low molecular weight (GRO and DRO) into heavier paraffin molecules is demonstrated. Apple filter cake has light paraffin content of low molecular weight and an important paraffin content of high molecular weight (greater than C28). These paraffins contributed by apple filter cake to the mixture are of botanical origin and with the evolution of the process, its concentration increases to expenses of the natural humification process and of the fractions GRO and DRO that practically disappear. This phenomenon of residues formation could be interpreted as a result of the microbial activity by incorporation of metabolites into the organic soil matrix. With this hypothesis we reinforce that of Kästner *et al.* (14) who worked with (¹⁴C) anthracene and demonstrated that the microbial activity produces covalently bound metabolites that become integral parts of humic matter which cannot be further identified.

Low molecular weight hydrocarbons are the main health risk factors in petroleum hydrocarbon mixtures (6). In this experiment, BTEX and other light hydrocarbons up to C10 were under detection limits before and after the process. To evaluate the performance of the process, the analysis was focused on lighter paraffin chromatographic fractions, what showed a diminution of hydrocarbons from 2.95% (GRO, DRO and RRO up to C35) to 1.39% in 90 days of treatment and to 1.16% after the maturation phase. The noticeable diminution of low molecular weight hydrocarbons content by the composting process encourages us to consider the technique as a valuable tool for bioremediation.

Table 1. Composition of chromatographic fractions of dichloromethane extractable hydrocarbons.

Components	Dry soil Day 0	Dry mixture Day 45	Dry mixture Day 90	Dry mixture Day 90	Dry mixture Day 90	Control reactor Day 360
GRO(%)	0.13	0.04	0.02	0.01	0.01	0.12
DRO(%)	2.59	1.97	0.96	0.63	0.35	2.59
RRO up to C35 (%)	0.23	0.32	1.16	0.75	0.80	0.25
RRO sup to C35 and others (%)	1.15	1.67	1.86	2.51	3.00	1.14

GRO, Gasoline Range Organics i.e., C6 to C10-12; DRO, Diesel Range Organics i.e., C8-12 to C24-26 and RRO, Residual Range Organics i.e., C25 to C35; Chromatographic fractions are expressed in weight percentage on the gravimetric determination of dichloromethane extractable compounds.

The success of the composting process depends not only on decreasing chemical concentrations, but also on the soil's suitability to support the normal soil fauna and flora (14). In this sense, ecotoxicity tests on the product would be relevant to prove the productivity of the soil after the bioremediation treatment.

As a bioremediation alternative, the utilization of apple filter cake was successful considering that the hydrocarbon fractions of environmental concern were noticeably reduced. Thus, its use for amelioration of hydrocarbon contaminants promotes soil sustainability and re-use.

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RESUMO

Bioconversão de hidrocarbonetos de petróleo no solo pelo uso de bagaço de maçã

O objetivo deste estudo foi investigar a viabilidade de aplicação de bagaço de maçã, um resíduo do processamento de frutas, para melhorar a biorremediação de solo contaminado com petróleo. Para estudar a bioconversão de compostos xenobióticos pela população microbiana naturalmente presente empregou-se um sistema de barril rotativo. O solo havia sido acidentalmente contaminado com um total de hidrocarbonetos de petróleo na concentração de 41.000 ppm. Embora esse valor tenha se mantido durante o processo, a intervenção microbiana ficou evidenciada através da transformação de frações do petróleo. Assim, as frações de risco para o meio ambiente (GRO, Gasoline Range Organics, i.e., C6 a C10-12; DRO, Diesel Range Organics, i.e. C8-12 a C24-26 e RRO, Residual Range Organics, i.e. C25 a C35) foram significativamente reduzidas de 2,95% para 1,39%. Por outro lado, frações mais pesadas, acima de C35, e outros compostos orgânicos aumentaram de 1,15% para 3,00%. A diminuição notável do conteúdo de hidrocarbonetos de baixo peso molecular e conseqüentemente do risco ambiental por esse processo, além da melhoria das características físicas do solo, são resultados promissores para uma futura aplicação em grande escala.

Palavras-chave: bagaço de maçã, bioconversão, biorremediação, solo contaminado com petróleo

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