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Influence of plant residues' increment on the recovery of soil impacted by oil sludge

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***Abstract*— The bioremediation of contaminated soil with oil may be incremented with the incorporation of residual plant materials to increase the degradation of the contamination components. The improvements of the soil characteristics may include physical changes, such as better water retention and soil aeration, and nutritional changes, as additional carbon or nitrogen sources to the microorganisms present on the soil, thus, the improvement of contaminants degradation. This study analyses the influence of plant residues used for the recovery of a soil impacted with oil sludge. The evaluation consists of the indirect evaluation of organic compounds degradation by quantifying the accumulated CO2 released from the soil (soil respiration). Sugarcane bagasse (SCB) and the residues of the leguminous forest tree Mimosa caesalpiniifolia, also called sabiá (LL), were used alone or combined in different concentrations (per 50 g contaminated soil, 1 or 2g of SCB, 0.5 or 1g of LL) to evaluate the soil respiration during 13 weeks of soil treatment. When the plant residues were used alone, SCB resulted in a more significant soil respiration than LL, regardless the concentrations used. The combination of both plant residues was more effective than the use of the residues alone, mainly when the highest concentration of SB was applied. The lignin content was lower in SCB than in LL, which may have facilitated the microorganisms' growth on the former residue. The nitrogen content of LL is 10 times higher than in SCB, which may have been used as nitrogen source on the assays where SCB and LL were combined. This study suggests that the treatment of a landfarming sample of contaminated soil with plant materials residues are beneficial to the soil recovery.**

***Index Terms*— Biodegradation, contaminated soil recovery, plants residue materials, soil respiration.**

I. INTRODUCTION

The inappropriate disposition of residues produced from the oil industry, associated to the frequent spill of crude oil and derivatives are highly responsible for the increasing contamination of the environment [1], [2]. In the case of accidental soil contamination from petrol, different techniques are available for decreasing the effects of such undesirable event. There is an increasing interest of applying microorganism to act as bioagents in the regeneration of the damaged places, as they are considered of simple execution and relatively low-cost context, the use of bioremediation for restoring the soil which has been contaminated with hydrocarbons from crude or refined oil is a technique that allows the application of some microorganisms' capacity of degradation those compounds [3].

In order to implement a bioremediation technique on a contaminated soil with oil sludge, the degree of decontamination during the treatment may be evaluated by direct or indirect methods. An example of the former is the determination of the total petroleum hydrocarbons (TPH) content throughout the bioremediation period [4]. The latter may be done by monitoring of the CO_2 released by the microorganisms during the process of the biodeterioration of TPH. The measurements of CO_2 may be an indicative of the metabolic performance of the microorganisms which are already present in the soil [5].

Different plant residues may be used for improving the soil regenerated by biodeterioration since they may promote an efficient cycling and nutrient mobilization, acting also as a biostimulation for the microorganisms and contributing for the water draining and oxygen diffusion in the soil [6], among others advantages [7]. Sugarcane bagasse is an abundant plant residue in Brazil produced in the sugarcane industry, and it has been used for favoring the biodegradation of THP from contaminated soil [8]–[11]. The plant scientifically named *Mimosa caesalpiniifolia* (commonly named *sabiá*), which is a leguminous forest tree, has its roots biologically associated with nitrogen fixing bacteria and mycorrhizal fungi. Compared to the non-leguminous plants, it has a high content of nutrients in the plant composition, mainly nitrogen and phosphorus sources [12]. The residue from the *sabiá* tree, which is all the plant material that falls from the tree to the surface of the soil in the forest, may be also used as a complement to the bioremediation process.

The combination of those two plant residues, associated to the THP deterioration promoted by the microorganisms presented in the soil, may be an efficient and low-cost method of recovering contaminated soil. The addition of plant material residues may also reduce the costs of fertilization, which is a biostimulation technique often used in bioremediation processes [7], [13].

This study has the objective of evaluating the influence of sugarcane bagasse and the *sabiá* residues on the recovery of a real sample of soil impacted by oil sludge treated with bioremediation and evaluated by accumulated CO_2 released.

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II. Materials and Methods

A. Origin of the contaminated soil used

The soil samples were originated from a landfarming unit composed of three cells of 5,000 m^2 each, which receive oily sludge from the refinery waste treatment station at regular intervals. The five samples of soil were collected of a soil layer from 0 to 20 cm (height). They were homogenized manually and quartered to obtain a single and representative sample.

B. THP analysis

The TPH content in soil sample and in the samples from treatments was quantified as described by [4]. The soil was characterized with 32,251.0 mg TPH kg^{-1}soil.

C. Plant materials residues used

It was used two types of plant materials residues in this study: sugarcane bagasse (SCB) and residues of the *sabiá* tree, named here as leaf litterfall (LL). The SCB was obtained from the discharge of sugarcane juice extraction process, while the LL was harvested from the surface of a 15 years old leguminous forest of *Mimosa caesalpiniifolia*, at a regenerated area located in Conceição de Macabu city, Rio de Janeiro, Brazil (23°35' S-23°50' S; 46°45' W-47°15' W).

D. Chemical characterization of SBC and LL

The nitrogen (N) content from the plant residues was analyzed by the Kjeldahl method, and the organic carbon (C) content was determined by potassium dichromate-acid oxidation as described in [14]. The SCB used contained (in %w): 39.46 organic C, 0.1473 organic N, 6.96 lignin, 0.383 polyphenols. The LL used contained (in %w): 36.361 organic C, 1.3841 organic N, 51.08 lignin, 0.496 polyphenols.

E. Experiment location

The experiments were carried out on a laboratory located at Universidade Federal do Rio de Janeiro, 22°51'S and 43°13'W, with an average temperature of 26 ± 2°C. According to the Köppen climate classification system, the region is classified as humid subtropical zone while a dry megathermal subunit is identified by the model of Thornthwaite.

F. Soil bioremediation combined with plant residues

Different assays were performed to compare the soil recovery when plant residues are incorporated to the contaminated soil at different concentrations. The 8 assays were as follows (per 50 g of contaminated soil): 1 g SCB; 2 g SCB; 0.5 g LL; 1 g LL; 1 g SCB and 0.5 g LL; 1 g SCB and 1 g LL; 2 g SCB and 0.5 g LL; 2 g SCB and 1 g LL. Literature presents that the concentrations of plant materials to contribute to the treatment of contaminated soil by oil vary from 2 to 12% w/w [7]–[10]. However, in this study, LL was also tested at 1%.

G. Accumulated CO_2 released analysis of the treated soil

The mixture of soil and the plant(s) residue(s) were done in a cylindrical glass flask, which were placed in a plastic container sealed with PVC film avoid the contact of external CO_2.

The quantification of the accumulated respiration of the soil (meaning the liberation of CO_2 from the soil) was done by a modified technique described by [5], [12]. In this study, it was used plastic containers (in triplicate) instead of percolators, as illustrated on Fig 1. The accumulated CO_2 released throughout the experiment was quantified on the first week, and then at equal intervals of 7 days between each analysis until the 7th week, which was analyzed at the 9th and 13th weeks. The result of accumulated CO_2 quantified was presented as the accumulation of the gas released throughout the soil treatment.

H. Plant residues as C or N sources for microorganism

In other to acknowledge whether the plant residue was used as carbon and nitrogen sources by the microorganisms from the soil, an analysis was done with a mineral medium containing the plant materials and inoculated with a microorganism suspension obtained from the contaminated soil without any treatment.

It was used Bushnell Haas (BH) mineral medium, and its composition was modified according to the experiment of C or N sources used by the microorganisms. For the analysis of whether SCB or LL was used as carbon sources, 5g of one of the plant residues was added to the mineral medium (respectively media named as BH/SCB and BH/LL). For the analysis of whether LL is used as nitrogen source, NH_4NO_3 was not added to the original composition of BH medium and instead it was added 0.5 g of glucose (as carbon source) and 5 g of LL (medium named BH/N). The plant residues quantity was chosen considering that they are composed by 2% of total carbon and that a culture medium contains 1-2% of C.

The microorganism inoculum (I) was prepared with 20 g of the treated soil placed in a 500 mL capacity Erlenmeyer flask containing 180 mL of saline solution (0.85% w/v NaCl). The flask was maintained in agitated shaker for 30 minutes at 120 rpm and 26 ± 2°C. The suspension of microorganisms and soil particles was filtered with whatman filter and 20 mL of the filtered was used as inoculum in a 500 mL capacity Erlenmeyer flask containing 80 mL of the modified BH medium. Two forms of control analysis were done: the biotic control contained plant material with the addition of inoculum (medium BH + I); the abiotic control contained the material added of the biocide $AgNO_3$ (medium BH + I + $AgNO_3$).

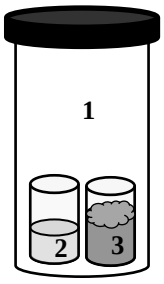

Fig. 1 Sealed plastic container (1) with NaOH (2) and with the assay of soil treatment (3).

The modified BH media were used for the quantification of the microorganisms after 30 days of cultivation. The pour plate count technique was used for determining culturable total heterotrophic bacteria (THB) and total fungi (TF) enumeration. The quantification of hydrocarbon-degrading microorganisms (HDM) was performed using the three-tube most probable number (MNP) technique, according to [15]. The inoculated Petri dishes or the tubes were incubated at 30°C for 10 days.

III. Results and Discussion

The contaminated soil used in this study presented a high content of TPH, presented on the previous session, considering the values presented on the New Dutch List (Dutch Target and Intervention Values 2000). That demonstrates the need to adopt decontamination measures to succeed in the deactivation of the landfarming areas, especially whether the region is surrounded by houses.

The results on Fig. 2 show that the CO_2 was gradually released during the incubation period for each assay. Considering the control assay, which was observed the lowest accumulated CO_2 release (905.06 mg CO_2 kg^{-1} soil), it is evident that the use of plant residues is an efficient method for stimulating the soil respiration, but with different effect. The profile of accumulated CO_2 release of each assay of soil treatment shows that an average of 57% of the accumulated CO_2 release occurred until the 4th week, while 88% of CO_2 was released by the 9th week (Fig. 2). Similar results were observed by [5], [16], [17], who reported higher accumulated CO_2 release by the third week of incubation and attributed this result to decomposition of the labile organic fractions.

The decrease of organic compounds, represented by the accumulated release of CO_2, throughout the soil treatment may suggest an increasing concentration of microorganisms present in the soil, thus, a greater decomposition of pollutants compounds, such as TPH.

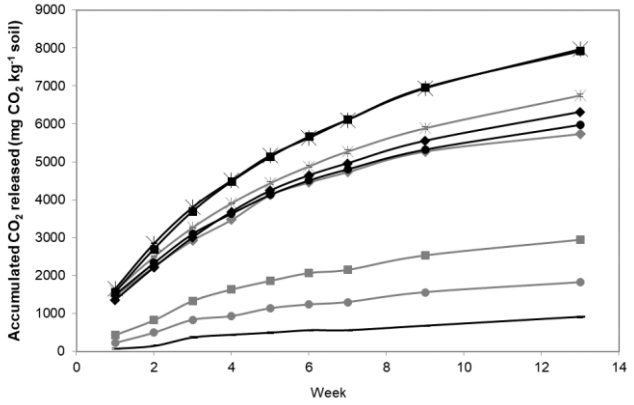

Fig. 2 Accumulated values of CO_2 released during the bioremediation of a contaminated soil with oil sludge implemented with residual plant materials: sugarcane bagasse (SCB) and *sabiá* tree leaf litterfall (LL). The initial values of the plant residues added to the soil were (per 50 g of contaminated soil): 1 g SCB (*gray diamond*); 2 g SCB (*gray star*); 0.5 g LL (*gray circle*); 1 g LL (*gray square*); 1 g SCB and 0.5 g LL (*black circle*); 1 g SCB and 1 g LL (*black diamond*); 2 g SCB and 0.5 g LL (*black square*); 2 g SCB and 1 g LL (*black star*). The standard deviations were less than 5% for each analysis.

For the assays which contained each plant residue separately (Fig. 2), it is observed that by the 13th week, the soil that contained only LL had the less significant accumulated CO_2 release, regardless the concentration applied (2380.71 mg CO_2 kg^{-1} soil – mean of the results of 0.5 or 1 g of LL), while SCB alone was responsible for 2.6 times more accumulated CO_2 release than LL (6244.74 mg CO_2 kg^{-1} soil – mean of the results of 1 or 2 g of SCB). When used alone, the use of the higher quantities of either SCB or LL promoted greater accumulated CO_2 release.

The combinations of the plant residues (Fig. 2) were, overall, more effective than the treatments with each residue alone. The two assays with the highest quantity of SCB (2g of SCB) had equivalent and the highest accumulated CO_2 released throughout the treatment, regardless the content of LL used (0.5 or 1 g LL), with a mean of 7942.49 mg CO_2 kg^{-1} soil. The assays with the lowest quantity of SCB (1g SCB) with each concentration of LL were less effective on the accumulated CO_2 release than with 2 g SCB alone, but higher than 1 g SCB alone. The benefits of the soil treatment with SCB or LL showed to have a more significant effect when they were applied together. The SCB and LL may have promoted two distinct effects on the improvement on the soil treatment, regarding nutritional and physical factors.

As nutritional source, SCB may have provided sucrose to the microorganisms as the fibers probably contained residues of sugarcane juice extraction process, and sucrose is an easily assimilable C source. Moreover, the lignin content from SCB was low compared with the values from LL (section D, Material and Methods), which probably facilitated access to the cellulose fraction of the SCB compared with LL [18]. Meanwhile, LL was used as nutrients source for the microorganisms, mainly N. The LL, which contains higher content of N than SCB (section D, Material and Methods), thus, a lower relation C/N (respectively 26 and 267.9), may have stimulated the growth of microorganisms which consume C sources from THP present on the soil as oil sludge.

Changes on the physical characteristics of the soil may have occurred on the soil with the use of SCB or LL, with an additional effect when the residues were used together. The plant residues possibly had an effect of structuring material for the soil, thus, promoting a more appropriated aeration and humidity of the soil [19]. Since SCB was presented in greater quantity than LL (respectively 1g or 2 g SCB, and 0.5 g and 1 g LL), the structuring material factor may have been more effective with SCB.

The results of soil respiration in this study were more significant than the results presented by [1], [5], [12]. However, those studies presented treatments of soils from plantations of sugarcane, eucalypt and *sabiá* tree respectively, therefore, they did not contain THP. The THP present on the soil used in this study may have been used as carbon source for the microorganisms [7], as the oil contaminant is composed by different concentration of more or less recalcitrant forms of hydrocarbons (C source), besides the effects of the plant residues.

The highest values of respiration of a soil incremented with plant materials may be attributed to the decomposition of more easily oxidizable C sources portions, to the high levels of N content, and to the low content of lignin and polyphenols present on the plant materials [20], [21]. Those characteristics, associated to intrinsic factors of the soil as mentioned previously, may result in a faster decomposition of the organic compounds on the soil than with the treatment without the increment of plant materials [20], [21].

The corroboration of the use of the plant residues as C or N sources by the microorganisms is presented on Table I. The populations of THB, TF and HDM had the lowest concentrations on the medium without plant residues (assay 1) compared to the other assays, except for the abiotic assays (assays 2 and 8). It is observed that the growth of the three types of microorganisms were equivalent for the assays with or without the addition of inoculum from the soil, represented by the HDM values that are equivalent (assays 3 and 4) or higher (assays 5 and 6) when SCB or LL were added. This

suggests that the microorganisms presented on the plant residues were also important for incrementing the biodegradation capacity. The assay 7 shows that the microorganisms were able to use the LL as N source, since that plant residue was the only source of that nutrient, and it has high content of N (section D, Material and Methods).

Table I also shows that SCB and LL promotes growth of different types of microorganisms. The microorganisms' growth was more significant on the media with SCB (assays 3 and 4) than with LL (assays 5 and 6), corroborating the results of soil respiration (Fig. 2). However, the medium with LL was more efficient on the stimulation of HDM growth (assays 5, 6 and 7), possibly due to the higher N content and other nutrients from that plant material compared to SCB. This plant residue, on the other hand, was a better source of C, possibly mainly due to the sucrose residues on the fiber, as mentioned previously, which highly stimulated the proliferation of THB and TF. That, coupled with the fact that both can act as structuring materials, indicates the possible beneficial effect of the combination of LL and SCB to obtain the degradation of HTP, and consequently in soil recovery. A

Table I. Microorganisms types – total heterotrophic bacteria (THB), total fungi (TF) and hydrocarbon-degrading microorganisms (HDM) – quantified on mineral medium Bushnell Haas (BH) modified with SCB or LL.

Day	Assay	Modified BH medium	THB	TF	HDM
			--- CFU g^{-1} soil ---		NMP g^{-1} soil
0	0	BH + I[a]	$1.1x10^4$	0	$4.5x10^3$
30	1	BH + I	$1.2x10^7$	0	$1.1x10^{10}$
30	2	BH + I + A[b]	0	0	0
30	3	BH/SCB[c]+ I	$>10^{13}$	$>10^7$	$1.4x10^{10}$
30	4	BH/SCB	$>10^{13}$	$>10^7$	$1.1x10^{10}$
30	5	BH/LL[d] + I	$2.3x10^{13}$	$3.5x10^4$	$1.1x10^{13}$
30	6	BH/LL	$2.6x10^{13}$	$3.3x10^4$	$4.5x10^{12}$
30	7	BH/N[e] + I	$9.9x10^{10}$	$8.2x10^6$	$2.5x10^{12}$
30	8	BH/N + A	0	0	0

[a] Inoculum; [b] AgNO3; [c] BH with SCB; [d] BH medium with LL; [e] BH medium without NH_3NO_4, with glucose and LL.

direct analysis bioremediation could demonstrate more precisely the effect of the plants residue materials acting on the soil.

IV. Conclusion

The highest values of accumulated CO_2 release were obtained with both SCB and LL added to the contaminated soil, mainly when the greatest quantity of SCB was used. Both SCB and LL are source of C and structuring material, and LL is source of N for the soil treatment. THB and TF growth were higher in medium with SCB than LL, while HDM had better growth in LL than SCB medium. This study suggests that the use of SCB and LL on contaminated soil with oil sludge may be beneficial to stimulate the degradation of organic compounds, represented by the accumulated release of CO_2.

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