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Influence of bacteria inoculum and organic concentration on the biodegradation of soil conditioning agents in aqueous solutions

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ABSTRACT: Earth Pressure Balance (EPB) technology is currently the most widely used technique in mechanized tunnelling with Tunnel Boring Machines (TBMs) and particularly in urban environments, because of several advantages, as the possibility to excavate in different geological conditions and effectively controlling the induced effects on the pre-existing structures. EPB technology requires the continuous injection of chemicals during the advancement: this may induce the accumulation of xenobiotic compounds in the excavated debris. The present work aims at studying the role of several parameter, as dosage of conditioning agents and environmental conditions, in the biodegradation process through the development of an experimental activity involving the use of commercial soil conditioning agents. Results provide elements useful to improve the knowledge of the interaction phenomena between the soil and the chemicals, the management of excavated soil and the design of the environmental monitoring activity.

1 INTRODUCTION

In mechanized tunnelling with TBM-EPB technology the management of the soil conditioning process plays a key role in the success of the excavation; different conditioning parameters are constantly monitored and modified step by step to accommodate the variability of the conditions that the TBM faces.

Nature and chemical composition of the commercial products used during the excavation and the management of several conditioning parameters determine the amount of single compounds injected into the soil and, consequently, their environmental impact.

In recent times is visible in Europe an increase in the attention reserved to the theme of the reuse of non-renewable natural resources and of course the management of large volumes of soil produced by the tunnels and deep excavations is fully integrated into this virtuous path.

The desire to reuse as much as possible the soil produced by the excavation and the increase in excavation performance (and consequently the quantity of chemicals injected) have led to the need to develop studies of the interaction between these chemicals and the soil. These studies are based on two main elements: the environmental impact of the compounds present in the commercial products and the biodegradation process; from the combination of these two independent factors depends the possibility and the methods to safely reuse of the soil.

In-depth studies and specific laboratory activities can be carried out to analyze the environmental impact of a specific chemical substance. These activities are conceptually developed by administering to a series of target micro-organisms increasing doses of the chemical involved and monitoring a series of parameters such as mortality and growth.

The biological degradability or "biodegradability" is the process for which the organic compounds (as surfactants present in the foaming agents injected during the excavation) are reduced as simple molecules by microorganisms. In particular, the development of the biodegradation process in aerobic conditions may lead to the complete oxidation (mineralization) of

the organic substance (with the formation of CO_2 and H_2O) and, where nitrogenous species or compounds rich in phosphorus are present, also to the formation of nitrates and phosphates which are used as fertilizers for the growth of algae and other organisms. In particular case, the biodegradation could also be partial, leading to the formation of simpler organic compounds. In anaerobic conditions, the main product is biogas, made of methane and other reduced gaseous compounds [Garg, 2017].

As can be easily understood, the study of biodegradation is influenced by numerous factors, some of which are related to the characteristics and the dosage of the chemical products, while others related to the environmental conditions in which the biodegradation process takes place.

For the description of the biodegradation process different parameters are required: the Biochemical Oxygen Demand (BOD) that identifies the amount of oxygen necessary for the biological stabilization of the organic compounds, the Chemical Oxygen Demand (COD) that identifies the oxygen required for the chemical oxidation of organic compounds and the organic carbon content in a medium, defined by Total Organic Carbon (TOC) parameter.

Respirometric tests are performed for the determination of BOD value on time; if the oxidable compounds present in the medium are all biodegradable the initial COD value can be consider as possible BOD ultimate value. In any case, all three parameters are required for a complete investigation of the biodegradation process.

A joint research activity between Astaldi and Sapienza University of Rome to study the biodegradation of the most commonly used chemicals has been developed, and in the present work several laboratory biodegradation tests performed using selected conditioning agents are reported.

The presented results will allow to deep understand the effect of the chemical composition and dosage of the commercial product as well as the environmental conditions on the biodegradation process.

Results provide elements useful to improve the knowledge of the interaction phenomena between the soil and the chemicals during the biodegradation process. In particular, the main factors affecting the biodegradation process of the chemicals used in tunnel excavation with TBM-EPB were highlighted, thus allowing to share some suggestions on the most effective methodologies for laboratory tests and, finally, to offer some interesting insights for constructive discussions and future studies.

2 EXPERIMENTAL PROCEDURE

Different solutions were prepared by the dilution of chemicals in distilled water. The set-up of the system used for the biodegradation tests was developed by using synthetic solutions containing a biodegradable compound such as sodium acetate at 1 g/L [Kuokkanen et al., 2004]. The biodegradation of sodium acetate solution was compared with the biodegradation of a solution containing fumaric acid at the same concentration and at the same organic carbon content.

Biodegradation tests of conditioning agents, identified by capital letters A, B and C, were performed by preparing different solutions at different dosages of foam agents (0.10% and 0.20%).

For the BOD tests, a volume of 400 mL of synthetic solution was filled in a Closed Bottle system with the addition of a mix of salts according to the procedure 301D OCDE/OECD [APAT-IRSA/CNR] and 2 mL of a microorganism source. In case of specific microorganisms, *Bacillus Clausii* (BACT1) was used as bacterial inoculum while in the other tests the inoculum source derives from a soil humus (BACT2), obtained washing in water a proper soil amount (100 g per 1 L of water) and then acclimating in batch aerated reactor the supernatant before using [APAT-IRSA/CNR].

BOD value at 5, 10 and 28 days were recorded and to complete the evaluation of the biodegradation rate TOC (Shimadzu TOC-L CSH/CSN analyzer) and COD were measured according to the standard procedures.

The removal efficiency of TOC that suggests the mineralization of the organic carbon into CO_2 and H_2O was calculated as $R(\%) = 100(TOC_0 - TOC_f)/TOC_0$, where TOC_0 identify the initial value of the specific parameter and TOC_f the final value of the same.

The initial TOC and COD value are summarized in Table 1 reported below.

Table 1. Total Organic Carbon and Chemical Oxygen Demand of foam agent solutions at different initial concentration.

	TOC [mg/L]		COD [mg/L]	
Product	0.10%	0.20%	0.10%	0.20%
A	140.55	297.33	160.23	310.42
B	67.94	134.32	78.17	175.16
C	75.60	146.02	78.35	147.30

All tests were conducted at room temperature and neutral pH conditions (the pH was measured using a Crison GLP 421).

3 RESULTS AND DISCUSSION

3.1 *Set-up test*

The biodegradation curves of sodium acetate and fumaric acid as standard solutions are reported in Figure 1 where the biodegradability is represented by the ratio between BOD value at time t and the initial value of COD.

In Figure 1 it is possible to observe a classical BOD curve trend: it is known that sodium acetate is an easy biodegradable compound and its biodegradation curve showed high BOD values according to the literature [Baker et al., 2000]. When fumaric acid is used at the same concentration of 1 g/L the BOD values recorded were lower that the corresponding value of sodium acetate solution. This because of fumaric acid TOC content was higher than sodium acetate: both are linear organic molecules but the carbon content of fumaric acid is higher than that of sodium acetate and required more time for its removal as a consequence of a possible inhibitory effect by excess of substrate. At the same TOC initial concentration, the BOD value of a solution of 0.71 g/L of fumaric acid increased but the BOD curve maintained a lower profile than those of sodium acetate. This confirm the hypothesis of excess substrate and the possibility to appreciate these differences with known biodegradable matrices make the system ideal for our purpose. Considering the commercial conditioning agents, no details in term of chemical composition are

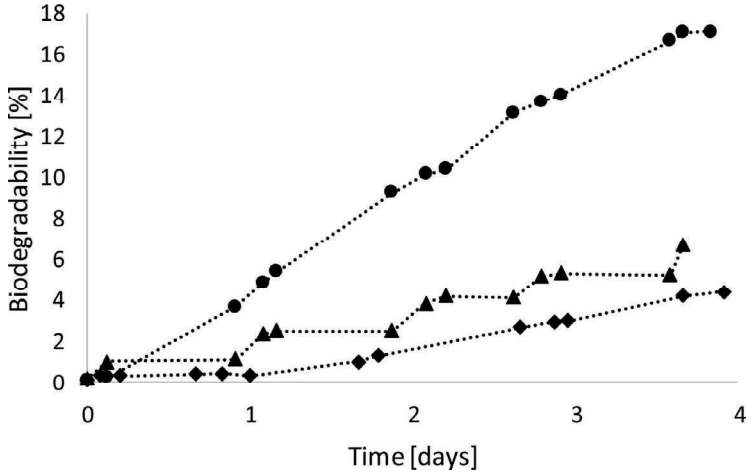

Figure 1. BOD curves for Sodium Acetate and Fumaric Acid solutions. Conditions: (●) 1 g/L sodium acetate solution, (▲) 0.71 g/L and (♦) 1 g/L of Fumaric Acid solutions.

known and only the overall index (TOC and COD) were defined (Table 1). However, the system allows to detect similarities and/or differences with the above reported completely biodegradable substance. The performed preliminary tests were carried 5 times for each chemical compound, showing a remarkable reproducibility of the tests with a percentage of 5% as deviation standard.

3.2 *Biodegradation: effect of conditioning agents initial concentration*

Three soil conditioning agent (A, B and C) solutions at 0.20% as concentration were filled in the Closed Bottles system and the results about the BOD trends up to 28 days are reported in Figure 2.

How it is possible to observe in Figure 2, all BOD curves described similar behavior: after a delay of about 3 days, the BOD value exhibited during the test with A solution reached the same value of B and C. This delay suggested that A is a mixture characterized by a slower kinetic of biodegradation in comparison with B and C, but after 28 days the BOD values recorded were similar: 74.00 mgO_2/L, 64.00 mgO_2/L and 62.00 mgO_2/L for A, B and C respectively.

In case of complete biodegradation of the oxidable compounds for each solution the BOD ultimate has to be close to the COD initial value (Table 1). After 28 days only a 23.84%, 36.54% and 42.10% of the oxidable compounds respectively for A, B and C was removed in comparison to their initial COD values and an additional time is required to appreciate high removals. In case of TOC removal, as reported in Figure 3, the 18.07%, 43.21% and 57.36% for A, B and C was measured. With a summary of these results C and B solutions can be considered more ready biodegradable mixtures in comparison to A.

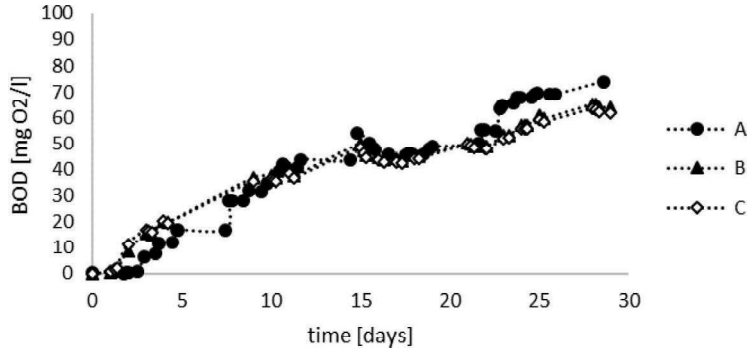

Figure 2. BOD curve for A, B and C solutions at 0.20% as concentration.

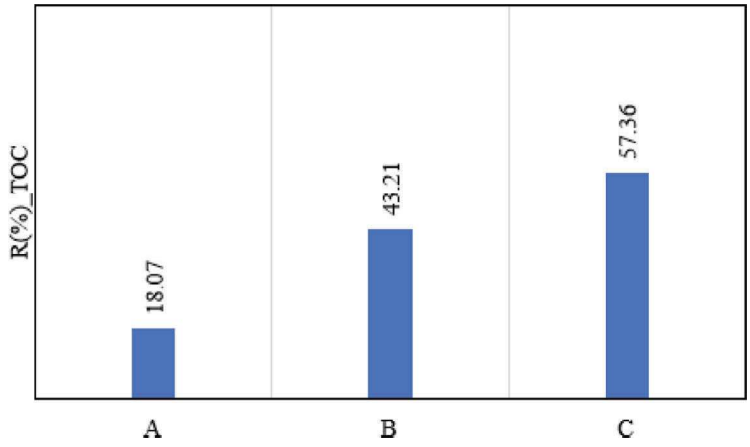

Figure 3. TOC/TOC_0 value for mixtures A, B and C after 28 days of biodegradation process.

To investigate if a possible inhibitory effect by excess substrate might be a cause of the results previously reported similar tests were conducted with the same mixtures at lower concentration by providing a dilution with a dosage of 0.10%. After 10 days the BOD values of 91.50 mgO_2/L, 101.00 mgO_2/L and 112.67 mgO_2/L for A, B and C respectively were recorded.

The high concentration acted as inhibitor of biodegradation process and for this reason a general delay of the process was observed in Figure 2. The same effect on TOC removal was observed and a 59.95%, 81.33% and 78.99% for A, B and C was calculated. Considering the ratio between BOD ultimate and initial COD it was possible to observe that in case of B and C solution this ratio, after 10 days was up to 1. This result suggested the presence of compounds that can be oxidized only through a biological process and were not chemically oxidable such as nitrogenous compounds by nitrification process [Fenchel et al., 1977].

In conclusion, all mixture showed good biodegradability as reported among the final remarks of several similar studies [Barra Caracciolo et al., 2017] but the high organic carbon content of A, defined by the TOC initial value, resulted in a slow biodegradation process. Conversely, B and C showed a faster biodegradation and this result gives the possibility to detect after 10 days the presence of possible non-carbonaceous and not chemically oxidable compounds.

The initial concentration of conditioning agent in the soil sample is important as directly related with the time in case of preliminary study devoted to establish the biodegradation behavior of chemicals adopted during the excavation process.

3.3 *Biodegradation: effect of bacteria inoculum*

As previously assessed, the biodegradation process of a single chemical product may be affected by the environmental conditions in which the conditioned soil is placed. To quantify the difference in the biodegradation curves, BOD tests at 0.20% of soil conditioning agent's concentration were repeated by using soil bacteria source (BACT2) taken directly from a soil sample and consequently containing several different bacteria families frequent in natural environments. The results are reported in Figure 4.

The use of a mixed consortium enhanced the biodegradability and high values of BOD after 28 days were recorded: in this case, no delay was detected and the BOD curve of mixture A reached higher values than B and C. The inhibitory effect by excess substrate was overcome by changing the kind of inoculum.

In order to define the advantages on using a mixed consortium, close to the real conditions on site, it is possible to compare the BOD value after 10 days recorded in test with *Bacillus Clausii* (BACT1) and with the mixed consortia (BACT2). The results are reported in Table 2.

The adoption of a mixed culture enhanced the speed of biodegradation process: the differences in term of BOD were extremely different even at 10 days (one order of magnitude). This time can be considered sufficient to evaluate if the biodegradation occurs and the oxidation degree directly

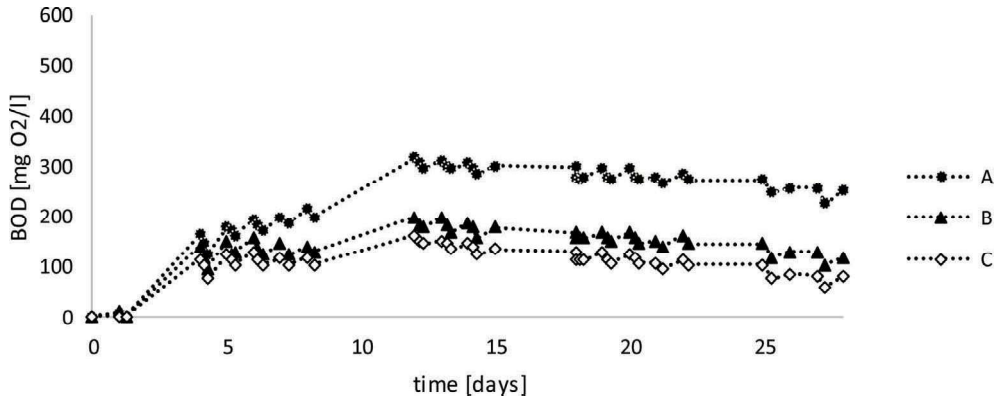

Figure 4. BOD curve for A, B and C solutions at 0.20% as concentration with BACT2 as inoculum.

Table 2. BOD removal after 10 days with a mixed inoculum at 0.2% foam agent dosage.

	BOD_{10} [mgO_2/L]	
Foaming Agent	BACT1	BACT2
A	32.90	316.00
B	32.99	198.00
C	36.73	123.00

by using a mixed inoculum. Indeed, the development of test with a real inoculum is correlated directly with the excavation site and is a description of a real biological process, but the use of a specific inoculum guarantees the achievement of repeatable results, fundamental during a comparative study.

4 CONCLUSIONS

The present study is an investigation about the biodegradability of soil conditioning agent solutions used in TBM-EPB technology. Experimental tests at different dosages and bacteria sources were performed.

Results allow to deep understanding the key role played, from one hand by the chemical composition and dosages of the foaming agents and, on the other, the role played by the environmental conditions in which the biodegradation process take place.

Particularly, the BOD test is able to detect the difference in the biodegradation of different products at the same dosage, underlining the importance of the chemical composition in the biodegradation time. Increasing the dosage of the injected chemical can be induced an inhibition effect in the microorganism responsible for the biodegradation of the product and finally leading to a higher biodegradation time.

Finally, the kind and composition of the bacteria inoculum selected for the laboratory test and, on site, the microorganism consortium and the environmental conditions may have a fundamental impact in the biodegradation process.

These factors, all affecting the biodegradation process, should be seriously taken into consideration in the experimental activities realized to foreseen the features of the excavation debris during time and finally to design the management of the excavated soil.

Even if the surfactants mainly present in the foaming products used in the tunnel excavation with TBM-EPB are well known and studied, there are still missing information on the environmental impact and on the effects on the biodegradation process of several additives and polymers often added to the chemical formulation or directly injected during the excavation

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