Journal of Thermal Analysis and Calorimetry, Vol. 88 (2007) 2, 373–375

EVALUATION OF OILY SOIL BIODEGRADABILITY BY MEANS OF THERMOANALYTICAL METHODS

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The oil content of an artificial polluted soil was estimated at different time period of a biodegradation process by means of thermogravimetry under non-isothermal conditions and in oxidative atmosphere. The model pollutant was hexadecane and for biodegradation a *Pseudomonas aeruginosa* culture was used. The intensity of the biodegradation process was correlated with the number of Colony Formation Units. In this study *Pseudomonas aeruginosa* was used, because these species are not pathogenic and easy to maintain in time and is resistant to hexadecane.

Keywords: *biodegradation, hexadecane, Pseudomonas aeruginosa, thermogravimetry*

Introduction

Substantial quantities of crude oil are transported in the world, resulting a releasing of significant quantities of oil into environment [1] with serious pollution and economic problems. Particularly the soil pollution during exploitation, transport and storage causes serious disturbances, and the demand to a softy and versatile oil decontamination procedure is obvious.

It is known that under certain conditions some living microorganisms like bacteria, yeasts, molds and filamentous fungi can alter and/or metabolize various classes of compounds present in oil, a set of processes collectively called oil biodegradation. The rate of oil biodegradation in the subsurface appears to be limited by available nutrients and not by the carbon source [2, 3].

The aim of the present work is to evaluate the biodegradation of an artificial polluted soil by means of the thermoanalytical technique. Thermogravimetry under non-isothermal conditions and in oxidative atmosphere is a versatile method to determine the organic oxidable components of a sample. Rapid determination of pollutant content of an oily soil is a recent encouraging example [4, 5].

Experimental

Artificially polluted soil

The soil was a chernozem (with high concentration of humus). As model for the oil (as pollutant), chemically pure hexadecane was used. In each case the concentration of the pollutant is determined by thermogravimetry.

Bacteria cultures

The stock culture was *Pseudomonas aeruginosa* from the 'Cantacuzino' Institute for Microbiology Bucureşti.

Sample preparation

Eight soil samples were used. One sample without hexadecane, is considered as blank sample. The other seven samples with 10 mass% of hexadecane were distributed as follows: one without bacteria was the standard, respectively the remaining six samples, were inoculated 2 mL of bacteria cultures in each 5.5 g of polluted soil [6, 7]. The inoculated soil samples were put into a thermostat at 37°C for certain days, after which the number of Colony formation units (CFU) and the mass loss by thermogravimetry (TG) were determined.

The experimental setup is summarized in Table 1.

Thermogravimetry

TG data were obtained on a MOM Q 1000 Derivatograph in static air atmosphere and Al_2O_3 crucible. Because in this work the TG data are used only for the determination of the total mass loss, one heating rate, i.e. 10°C min^{-1} was used till 500°C.

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1388–6150/$20.00

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Table 1 Experimental conditions of prepared samples

Sample symbol	blank	1	2	3	4	5	6	7
Polluted samples (10 mass% of pure hexadecan)	–	+	+	+	+	+	+	+
Inoculated samples (2 mL bacteria culture to 5.5 g of polluted samples)	–	–	+	+	+	+	+	+
Time of biodegradation/day	–	–	0	4	8	12	16	20

Colony formation units (CFU)

After the established time for the biodegradation process (Table 1), from the assigned sample approx. 1–4 mg was introduced into a test-tube with 10 mL meat sauce. This tube was thermostated 24 h at 37°C and after that a successive 1:10 dilution with culture media was performed. This diluted solution, in a series of 1:10, 1:100…1:10^9 was inoculated in Petri dishes, 1 mL of solution into agar media at 45°C. The Petri dishes were also thermostated for 24 h at 37°C and then, the numbers of colonies were counted under magnificator [8]. For precise results, two Petri dishes for each dilution were used, and that dilution was taken for measurement which present 20–30 colonies per dish (Fig. 1).

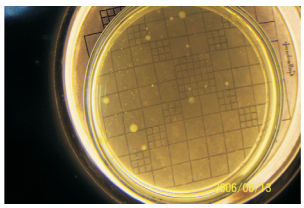

Fig. 1 Image of a Petri dish ready for CFU counting

Results and discussion

In Table 2 the obtained data are collected. The mass loss of the blank sample is due to the oxidation of the organic component of the soil (humus). The mass loss of the standard sample is composed of humus (blank value) and the added pollutant, so that the pollutant concentration for all samples is 24–8.8~15.2%. At sample no. 2 the higher value of Δm is due to supplementary added water during inoculation. A smaller Δm value for the other samples is a sign of a partial loss of hexadecane.

The beginning of the biodegradation process is considered at zero day, for sample no. 2. Biodegradation under aerobic conditions can be expressed by a general equation (Eq. (1)):

$$C_xH_y+(x+y/4)O_2 \rightarrow xCO_2+y/2H_2O \quad (1)$$

The small molecules (products of the biodegradation process) leave the soil during incubation, so that the difference between the mass loss after D days, Δm_D, and the mass loss for zero day, Δm_0, is a quantitative measure of the biodegradation. This can be expressed as:

$$\Delta(\Delta m)=\Delta m_0-\Delta m_D \quad (2)$$

Taking into consideration also the time Δt=D-0 necessary to attain the value $\Delta(\Delta m)$, an expression similar to a reaction rate, i.e.:

$$r=\frac{\Delta(\Delta m)}{\Delta t} \quad (3)$$

was obtained.

The dependence of r *vs*. CFU is depicted in Fig. 2, using the experimental data in Table 2.

From Fig. 2 it can be noticed an approximately linear dependence between the rate of biodegradation and the CFU value. The decrease of the CFU values with the increasing time of biodegradation is due to a diminishing of bacterial nutrient, i.e. the polluting substance. This observation is of a special practical importance: in order to maintain a high biodegradation potential in a soil inoculated with bacteria, a continuous flow of food (non-pollutant organics like methanol) is necessary.

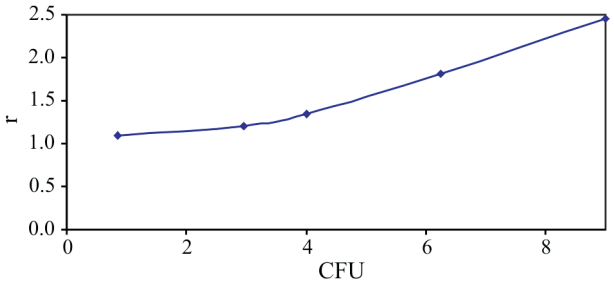

Fig. 2 Relationship of r *vs*. CFU

Table 2 Mass losses and microbiological test of the samples

Sample No.	blank	1	2	3	4	5	6	7
Mass loss, Δm/%	8.8	24.0	35.0	25.2	20.5	18.8	15.8	13.0
CFU, 10^6	0	0	350	9.0	6.25	4.0	2.95	0.85

Conclusions

Thermogravimetry under non-isothermal conditions and in oxidative atmosphere seems to be a versatile method for quantitative determination of organic components in a soil sample. Therefore, this method is also recommended for evaluation the process of biodegradation of an oily soil.

By a very simple TG data processing a quantitative relationship between the biodegradation rate and the number of colony formation units was established. Because the pollutant is the bacterial food, in the absence of pollution with hydrocarbons, a carbon source of non-polluting organics is necessary to maintain the biodegradation ability.

Acknowledgements

This work was supported by the Grant MENER 633 of the Romanian Ministry of Education and Research.

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DOI: 10.1007/s10973-006-8014-3