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Short communication:

Degradation rates of aged petroleum hydrocarbons are likely to be mass transfer dependent in the field.

Katarina Björklöf*, Jani Salminen, Pirjo Sainio, Kirsten Jørgensen

Finnish Environment Institute, P.O. Box 140, FI-00251 Helsinki, Finland

*Corresponding author.

Dr. Katarina Björklöf, Senior Researcher

Finnish Environment Institute (SYKE)

Research Programme for Contaminants and Risks

Hakuninmaantie 6, FIN-00430 Helsinki, FINLAND

Tel. +358-400148596

Fax. +358-9-40300890

E-mail: katarina.bjorklof@ymparisto.fi

Running head: Mass transfer dependent degradation rates

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Summary

Evidence for on site biodegradation may be difficult to provide at heterogeneous sites without additional experiments in controlled laboratory conditions. In this study, microbial activities measured as CO_2 and CH_4 production were compared *in situ*, in intact soil cores and in bottle microcosms containing sieved soils. In addition, biodegradation rates were determined by measuring the decrease in petroleum hydrocarbon concentrations at 7 °C in aerobic and anaerobic conditions. Elevated concentrations of CO_2 and CH_4 in the soil gas phase indicated that both the aerobic and anaerobic microbial activity potentials were high at the contaminated site. Aerobic and anaerobic microbial degradation rates in laboratory experiments of petroleum hydrocarbons were highest in soils from the most contaminated point and degradation in the aerobic and anaerobic microcosms was linear throughout the incubation, indicating mass transfer dependent degradation. Different results for microbial activity measurements were obtained in laboratory studies depending on pretreatment and size of the sample, even when the environmental conditions were mimicked. These differences may be related to differences in the gas exchange rates as well as in changes in the bioavailability of the contaminant in different analyses. When predicting by modeling the behavior of an aged contaminant it is relevant to adapt the models in use to correspond to conditions relevant at the contaminated sites. The variables used in the models should be based on data from the site and on experiments performed using the original aged contaminant without any additions.

Introduction

Monitored natural attenuation is a remediation method that relies on naturally occurring biodegradation processes that decreases concentrations of contaminating substances in the environment over time. When monitored natural attenuation is used as a remediation strategy, it has to be demonstrated that the degradation processes are taking place (USEPA 1999). Degradation data are also needed in modelling-based impact assessments (Rügner et al. 2006). Demonstrating *in situ* biodegradation of contaminants is, however, often challenging, especially at heterogeneous sites where representative time series demonstrating decrease in contaminant concentrations are difficult to obtain. Therefore, experiments in controlled laboratory conditions may have to be performed. Microbiological degrada-

tion rates have been determined for several specific compounds, often using microbial cultures, laboratory microcosms or with mixed cultures in the field. The degradation kinetics for any compound in any specific habitat varies depending on the prevailing microbial community as well as on highly variable environmental factors such as temperature, pH, soil moisture, other C sources and presence of inhibiting compounds (Alexander 1994; Moreels et al. 2004) as wells as on the properties of the compound in question and the age of the contamination (Hazinger and Alexander, 1995). For this reason, degradation rates reported in literature may not reflect the degradation rates at a specific site and therefore the degradation rates at contaminated sites should always be investigated on a case by case basis (Rügner et al. 2006).

In this study, we used both field and laboratory methods to determine the degradation rates of petroleum hydrocarbons (PHC) under aerobic and anaerobic conditions in unsaturated soil with an aged mixed contamination at an old landfill. The impact of soil sampling and handling on rates of microbial activity was also investigated.

Material and methods

The site and microcosm studies. Samples were taken from an abandoned landfill in Southern Finland where oily wastes have been dumped some 30 years ago. The study area is known to be heavily contaminated with lightweight fuel and lubrication oils in both the saturated and unsaturated zone and partly with heavy metals in the top soil (Salminen et al. 2004). The composition of soil gases in the unsaturated zone on the site was measured from soil gas monitoring wells that had been installed previously (Salminen et al. 2004). Soil concentrations of O_2, CO_2 and CH_4 were monitored from the wells twice a year using a Dräger Multiwarn II multigas analyzer (Drägerwerk, Lübeck, Germany). Concentrations of methane were confirmed in the lab (see below) from gas samples taken from the gas monitoring wells. Soil samples for microcosm studies were obtained by excavation from two or three depths from three different excavation pits in 2004 (SM1; hot spot area; SM2; low contamination area, SM3; moderately contaminated area). Samples were taken from both the aerobic and anerobic zones. Each sample was sieved in the field through an 8-mm sieve and dry weights and ignition losses (SFS 3008, 1990) were measured. Subsamples of 10-g (aerobic tests) or 30-g (anaero-

bic tests) were distributed into parallel 116 ml serum bottles or into 500-g samples in 1 l bottles. Bottles from the anoxic zone were immediately flushed with N_2 in the field to achieve anaerobic conditions. No nutrients or substrates were added to the soils. The bottles were sealed airtight immediately after filling. The PHC degradation rates were estimated from bottle microcosms by analyzing the reduction of PHC concentration in soil samples during a 4 months (aerobic tests) or 11 months (anaerobic tests) incubation period at 7 °C in the dark. Triplicate bottles were sacrificed for PHC analysis (ISO 16703, 2004) at regular intervals. The sum of all hydrocarbons between C_{10} and C_{40} are reported. Production rates of CO_2 and CH_4 were measured regularly from two replicate bottles. Evaluation of CO_2 was measured by IR-spectrometry with a carbon analyzer (Easy Quant, Lammi, Finland) (Laine and Jørgensen 1997) or a Shimadzu TOC-5000 (Shimadzu, Deutschland, Gmbh). Methane production was determined with an HP5890 series II gas chromatograph equipped with a FID and a Hayesep Q column (Salminen et al. 2004).

Comparisons of microbial activity in intact soil cores and bottle experiments. Six steel cylinders were designed for taking intact soil core samples. The cylinders were 30 cm long and 15 cm in diameter. The lids were designed to produce an airtight seal for the cylinders. Each cylinder had holes plugged with butyl rubber stoppers for subsequent gas sampling. Samples were taken from two excavation pits in 2005 (SM30; hot spot area and SM31; moderately contaminated area) at the depth of the oil plume (SM30; 1.9 m and SM31; 1.5 m). Three parallel intact soil cores (I, II, III) were obtained from each pit by pushing the cylinder into the soil profile and digging out the cylinders after filling. The lids of the cylinders were closed immediately and the holes for gas samplings were plugged. Cylinders were incubated in the laboratory at 7 ^{o}C after flushing the soil cores with air for 30 min. Carbon dioxide production was measured 6 times for the first 24 h and for the next three days once a day. To be able to compare the CO_2 production in intact soil cores and soils samples in bottle experiments containing sieved soil, the cylinders were later opened, the soil removed, sieved, and transferred to bottles and incubated as above for repeated CO_2 measurements. This way the CO_2 production in exactly the same soil sample was compared before and after the sieving procedure.

Results

Soil gas conditions *in situ*

Concentrations of O_2, CO_2 and CH_4 in gas monitoring wells were determined in spring and autumn during 2004 and 2005. The measured values were very similar throughout this period (Table 1). At the hot spot, O_2 concentrations were reduced in unsaturated conditions at 0.4-0.7 m below the soil surface. The CO_2 concentrations were correspondingly elevated when O_2 was reduced. Methane was detected at the hot spot and at depths below 2 m in the moderately contaminated zone where PHC concentrations exceeded 2 000 mg kg^{-1}. This indicated that a large part of the unsaturated soil zone above the ground water table was anoxic. In the clean areas close to the contaminated site, O_2 concentrations were close to atmospheric levels even at depths of several meters (Table 1). In clean soils and in soils with PHC concentrations below 2 000 mg kg^{-1}, no CH_4 was detected.

Soil gas production and PHC degradation rates in bottle microcosms

Microbial activities and degradation rates of PHC were determined by studying gas production and disappearance of PHC at 7 °C in bottle microcosms from the same samples. Degradation of PHC C_{10}-C_{40} in the aerobic and anaerobic microcosms was linear over the whole incubation period (Figure 1). Both the aerobic and anaerobic microbial degradation rates were highest at the most contaminated points and the degradation rates roughly depended on the initial mineral oil concentrations (Figure 1 and Table 2). Degradation rates were approximately two times faster in aerobic conditions compared to anaerobic conditions (see aerobically and anaerobically incubated SM3 1.6-1.9 m, Figure 1). The degradations rates showed no relationship with either soil depth or organic matter content. Percentage degradation rates varied between 0.2 % per day and 0.4 % per day resulting in 30-40 % removal of the PHC in bottle microcosms. Both CO_2 and CH_4 production in aerobic and anaerobic bottle microcosms from the three points also corresponded with the initial PHC concentrations but not with soil depth or organic matter content (Table 2). In bottles containing 500 g soil samples, the aerobic CO_2 production rate was 20 % lower and the CH_4 production rate was 26 % lower compared to bottles containing only 10 g or 30 g of soil (Table 2).

The impact of samples treatment on the degradations rates observed in the bottle experiments above was studied by comparing the microbial activity, measured as CO_2 production rates of the same soil samples first in soil cores and thereafter after sieving the soil, in bottle experiments. The first part was done by measuring CO_2 production from steel cylinders containing intact soil cores. Three parallel cylinders were analyzed from two different sampling sites, SM30 and SM31 (Table 3). The dry weight contents of the soils were 92.4 % and 80.1 %, respectively, and the air filled space in the cylinders were 22.5 % and 6.8 %, respectively. When post-aeration CO_2 production were measured over 24 hours from 2 cylinders the CO_2 production rates were 0.1 µg $(g\ dw \times h)^{-1}$ (Table 3). The cylinders became anaerobic after 24 hours incubation and the CO_2 production rates dropped to 5 times lower compared to the aerobic situation. After the soil samples had been removed from the cylinders, sieved and bottled, the CO_2 production rate of the soils in aerobic conditions in bottles were 11 times higher compared to the aerated soil cores. The CO_2 production rates were lower in soil cores from SM31 than in soil cores from SM30. In two out of three of the SM31 soil cores, CH_4 production was also observed (SM31II 330 µg g^1 h^{-1} and SM31III 68 µg g^{-1} h^{-1}).

DISCUSSION

The contaminated site under investigation is 30 years old. The presence of very high PHC concentrations indicates that either the degradation processes have not been very efficient or that the contamination source has not been removed and a free phase of PHC is still present at the site. Earlier studies on the site show that genes responsible for aerobic PHC degradations are found at the site (Tuomi et al. 2004) and that the PHC disappear over time (Salminen et al. 2004) but no exact degradation rates have been reported so far.

The high concentrations of CO_2 and CH_4 in the soil gas phase indicated that both aerobic and anaerobic microbial activity potentials were high at this site. Both the microbial activity measurements and degradation rates confirm earlier observations from the site (Salminen et al. 2004) that there is considerable microbial activity potential also in the anaerobic conditions prevailing at depths below 1.5 m in the unsaturated zone. The dominating anaerobic processes are iron reduction, fermen-

tation and methanogenesis (Salminen et al. 2006). It should be considered however, that all microbial activity at this site is not simply linked to degradation of PHC, as other organic matter is present. Soil gas compositions is the result of very complex interactions (Scanlon et al. 2000) and gas diffusion in the microhabitats relevant for microbes is often considered limited (Stotzky 1997). Therefore the soil gas composition may represent a saturated or steady state condition, where microbial activities are not optimal. For this reason, soil gases may not provide evidence for continuously ongoing microbial activity. To obtain quantitative information about PHC degradation rates on the site, laboratory experiments were carried out. These were designed to interfere as little as possible with the original conditions; care was taken to study the whole mixed aged contamination, which may metabolized very differently to single compounds, to use ambient incubation temperatures of 7 °C and not to add nutrients to the soils.

The degradation rates measured in bottle experiments in closely environmental mimicking conditions were relatively high and the degradation rates in anaerobic conditions were even as high as half of the aerobic rates. Oxygen is expected to penetrate many contaminated soils for up to several meters at degradation rates of 2.5-10 mg of PHC per kg of soil per day (Huesemann and Truex 1996). In this work, the degradation rates under non-limiting O_2 conditions were greater than 10 mg of PHC per kg of soil per day and therefore it is not surprising that anaerobic conditions were found relatively close to the soil surface. Microbial activities, measured at 7 °C, were high both in aerobic and anaerobic conditions. Others have also shown that biodegradation potentials may be considerable at low temperatures (Whyte et al. 2001).

Degradation rates measured as PHC disappearance rates were linear during the whole experiment. This indicates that the microbial communities responsible for PHC degradation processes at the site has reached the maximum capacity for this habitat and that the community may be limited by some substrate or growth factor (Alexander 1994). At this site, the community is likely to grow on C compounds that have low water solubility. The linearity of degradation curves indicate that the C compound in the aqueous solution have been totally consumed (Alexander 1994) and the degradation

rate is dependent on the dissolution rate of contaminants to the soil water phase. Microbes in soils are located on the moist surface of soil particles or in the water film between these particles. Their metabolism is often restricted due to both unfavorable conditions and reduced bioavailability of substrates (Stotzky 1997). In soil slurries, the degradation of hydrocarbons has been shown to be limited by microbial factors during early phases of degradation and by mass-transfer later in the process (Huesemann et al. 2004). Dissociation of organic contaminants in saturated conditions, which reflects their bioavailability is also a lengthy process (Eberhardt and Grathwohl 2002).

When comparing microbial activity measurements as CO_2 production rates it was shown that different results may be obtained in laboratory studies depending on pretreatment and the size of the sample, even when the environmental conditions were mimicked carefully. Comparison of microbial activity measurements based on CO_2 production rates between sieved soils and intact soil cores indicated that microbial activities in sieved soil samples were overestimated. A likely explanation for the enhanced microbial activities observed in bottle experiments containing sieved soils may be enhanced gas exchange and release of tightly bound compounds from the soil surface, resulting in improved contacts between microbes and contaminant. The nutrient conditions in the soil was however still limiting the exponential growth of microbes. The microbial activities were on the other hand reduced in soil samples incubated as larger batches of 500 g in comparison of 10 g or 30 g soil batches. These results are in agreement with earlier reports showing that small-scale laboratory experiments overestimated breakdown rates measured in field conditions (Aichberger et al. 2005). This observation may also be explained by considering lower mass transfer rates and/or soil gas exchange capacity in the larger sample. The difficulty to thoroughly mimic the environmental conditions of the microhabitats in laboratory experiments should be kept in mind when interpreting results from laboratory experiments.

The time frames estimated for complete degradation of PHC present at the site based on the observed PHC degradation rates are less than 20 years for aerobic degradation and less than 40 years for anaerobic degradation. Intact soil cores have been suggested to be good tools for determination of

kinetic parameters in environmentally realistic conditions (Moyer et al. 1996). If extrapolating the difference in microbial activities between soil cores and sieved soils in bottles to the degradation rates, the time frames for the clean up process would increase considerably. The true time frame is therefore difficult to estimate. Rügner et al. (2006) recently suggested that no categorical time frame for clean up should be included in the monitored natural attenuation concept in the EU.

When predicting by modeling the behavior of an aged contaminant it is relevant to adapt the models in use to correspond to conditions relevant at the contaminated sites. The variables used in the models should be based on data from the site and on experiments performed using the original aged contaminant without any additions. This study indicates that the time frame needed for natural attenuation processes will be difficult to estimate accurately and emphasis should therefore be placed on the demonstration on immobility of the contamination and on risk reduction.

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Figure 1. Degradation rates of petroleum hydrocarbons in aerobic and anaerobic bottles

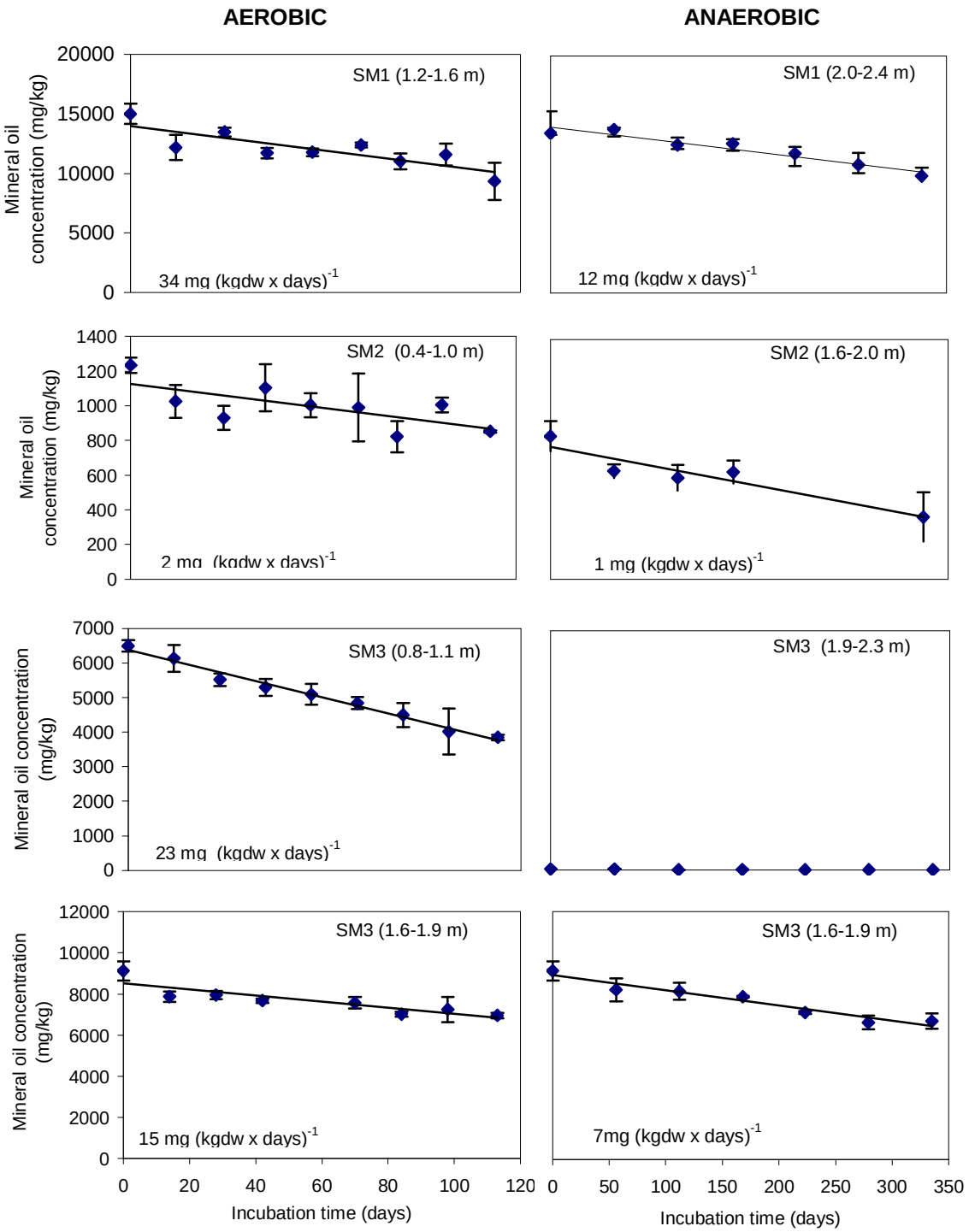

Table 1. Average composition of soil gases at the investigated site. Soil gases were measured from soil gas tubes situated in areas with different amount of oil contamination. Averages are calculated for spring (measured on 12.5.2004 and 10.5.2005) and for autumn (measured on 17.9.2004 and 6.10.2005). SD, standard deviation.

		O_2 (%)				CO_2 (%)				CH_4 (%)			
	Depth	**Spring**		**Autumn**		**Spring**		**Autumn**		**Spring**		**Autumn**	
	(m)	**mean**	**sd**	**mean**	**sd**	**mean**	**sd**	**mean**	**sd**	**mean**	**sd**	**mean**	**sd**
Hot spot:	**0.4**	15.2	2.7	13.3	1.4	6.7	1.9	8.6	0.2	0.0	0.0	0.0	0.0
	0.7	13.7	4.2	10.3	2.3	8.9	2.2	11.5	0.5	0.0	0.0	0.0	0.0
	3.4	1.0	0.7	0.4	0.0	17.8	0.7	19.6	0.9	16.3	3.2	16.0	8.5
	4.8	0.4	0.1	0.3	0.1	17.9	1.0	19.5	1.3	15.8	3.1	17.4	6.9
Moderate contamination:	**0.6**	20.6	0.5	19.8	0.5	0.5	0.0	1.0	0.1	0.0	0.0	0.0	0.0
	2.3	1.9	1.0	0.4	0.1	11.3	2.7	16.5	0.4	7.3	5.3	4.9	6.9
Clean:	**0.8**	20.9	0.6	20.2	0.0	0.2	0.0	0.5	0.0	0.0	0.0	0.0	0.0
	2.2	19.3	0.4	18.5	0.3	2.1	0.1	3.7	0.8	0.0	0.0	0.0	0.0
	4.5	18.7	0.2	17.7	0.4	2.2	0.0	4.5	0.7	0.0	0.0	0.0	0.0

Table 2. The initial petroleum hydrocarbon (PHC) concentrations, the organic matter content, the petroleum hydrocarbon degradation rates and CO_2 and CH_4 production rates in bottle microcosms containing sieved soil from different depths from three sampling points.

Sample name	Depth	PHC conc. (mg x $kgdw^{-1}$)	Org. matter (%)	PHC degrad. rate [mg (kgdw x $days)^{-1}$]	CO_2 prod. [µg (gdw x h) $^{-1}$]		CH_4 prod. [ng (gdw x h) $^{-1}$]	
Aerobic					**10 g**	**500 g**	**30 g**	**500 g**
SM1	1.2-1.6	15000	4.0	34.3	1.4	0.27	nd*	nd
SM3	1.6-1.9	9120	0.6	14.8	0.3	nd	nd	nd
SM3	0.8-1.1	6490	0.7	23.0	0.8	nd	nd	nd
SM2	0.4-1.0	1230	7.4	2.3	0.2	0.04	nd	nd
Anaerobic								
SM1	2.0-2.4	13470	1.7	11.6	nd	0.08	76.4	19
SM3	1.6-1.9	9120	0.6	6.8	nd	nd	0.0	nd
SM2	1.6-2.0	830	6.4	1.2	nd	nd	2.6	nd
SM3	1.9-2.3	<50	0.5	nd	nd	nd	0.0	nd

*nd, not determined

Table 3. Comparison of microbial acitivity measured as CO_2 production in intact soil cores and in the corresponding sieved soil samples in bottle microcosms. Three parallel cylinders from two different points were investigated.The mean from 2 different sampling ports per cylinder are reported.

	CO_2 production [µg (g dw x h) $^{-1}$]		
Sample name	**Aerated soil cores (1-24 h)***	**Aerated Soil cores (2-4 days)****	**Aerobic bottles**
SM30 I	nd	0.002	1.0
II	0.1	0.02	1.1
III	0.1	0.02	1.1
SM31 I	nd	0.001	1.0
II	nd	< 0.001	0.5
III	nd	0.002	1.4

*The CO_2 production of the soil was measured 6 times during 24 hours.

**Anaerobic conditions prevailed in the cylinders after one day.The CO_2 production of the soil was measured between day 2 and 4 in anaerobic conditions.