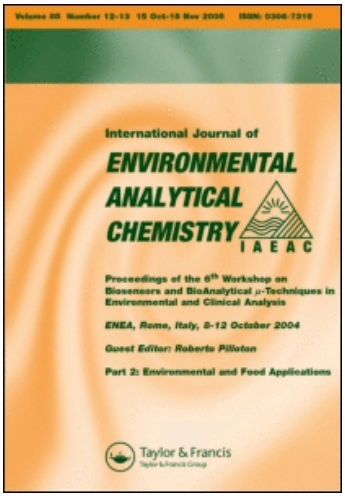

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Studies of biodegradability of certain oils in forest soil as determined by the respirometric BOD OxiTop method

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Studies of biodegradability of certain oils in forest soil as determined by the respirometric BOD OxiTop method

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The suitability of the respirometric BOD OxiTop method for biodegradation studies of different oils in soil was assessed. Different forestry chain oils and wood-preservative oils were used as model substances. Experiments were carried out on different types of Finnish forest soils. The results of these experiments are in good agreement with our earlier results of oil-biodegradation experiments in water. The BOD OxiTop method proved to be a highly suitable analysis method for biodegradation studies of oils in soil.

Keywords: Biodegradation; Wood preservative; Chain oil; Soil; BOD OxiTop method

1. Introduction

Different types of oils are used on a large scale in forested areas, in the form of machine hydraulic oils, motor oils, chain oils or oils used to preserve wood-based structures. Thus, forest soil and groundwater beneath the soil layer are almost continuously threatened by oil contamination. Soil type and especially its permeability have a significant impact on the effects of oils on the forest environment. If soil is highly permeable, the possibility of oil endangering groundwater is extremely significant, because biodegradation reactions most likely do not have enough time to proceed to a great extent. On the other hand, if oil does not pass through the soil layer very quickly, microbial populations have a greater probability of adapting to oil biodegradation. Effective biodegradation in the soil layer above groundwater diminishes the risk of the groundwater becoming contaminated with oil hydrocarbons. We have studied oil biodegradation in groundwater, and more generally in water, by means of standardized tests in our earlier studies [1–3]. So, there is an obvious need to widen this research area to biodegradation studies in soil.

Biodegradation studies of oils and petroleum products in soil, as well as the wide area of bioremediation of oil-contaminated sites, are quite common subjects in recent

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literature. The methods used to evaluate oil biodegradation include different respirometric techniques, chromatographic methods and radiological methods, where organic carbon is spiked with ^{14}C, and this isotope is detected in degradation products [4–14].

The BOD OxiTop method, which proved to be highly reliable in biodegradation measurements of oils in a water medium, was chosen as the measurement technique in our experiments. As far as we know, the BOD OxiTop method has not been used in biodegradation measurements of oils in soil. So, the first aim of this study was to determine if the respirometric BOD OxiTop method is suitable for biodegradation measurements of different oils in soil. The oils selected for these experiments are exactly the same forestry chain oils and wood-preservative oils which we have formerly studied in water [2, 3]. So, this is an obvious continuation study of our former studies, and it helps to enhance our understanding of the behaviour of these oils when they are spilled in a forest environment.

2. Experimental

2.1 *Studied oils*

The oils used in our experiments were from two categories: forestry chain oils and wood preservative oils. The forestry chain oils were all commercial products of different manufacturers. The oils were different bio-oils (tall oils and rapeseed oils). The wood-preservative oils were either commercial products (linseed oil and creosote oil) or substances from a project at our university which is implementing environmentally sound wood preservation using tall oil-based substances. The experimental substances included a wide range of different tall oil-based substances (e.g. fatty acids, esters and iron salts). Some promising wood-preservation substances were selected for our experiments. In both categories studied here, biodegradation must not begin during use of the oils, but only when they are spilled in the environment. This is especially important with chain oils, which are discharged directly into the environment during use.

2.2 *Soil samples and their analyses*

The soil samples used as measurement media were gathered from different forests in the small Finnish municipality of Alavieska in August 2003. The soils were sampled near the surface. One forest area had been fertilized with NPK fertilizer earlier. Soil type, pH, conductivity and the amount of major nutrients (Ca, P, K and Mg) in the soil were determined at Suomen Ympäristöpalvelu Oy, a laboratory specializing in soil fertility studies of agricultural land. The concentration of nitrogen was not determined because in Finland, it is seldom included in the fertility analyses of agricultural soil. However, the concentration of soluble nitrogen in the soil is an important parameter when biodegradation potentials are evaluated, and thus it will be determined in future studies.

pH and conductivity were measured from a 1 : 2.5 (v/v) soil–water suspension with a Consort C 831 pH/EC analyser. Concentrations of exchangeable calcium, potassium, magnesium and easily soluble phosphorus were determined from a 1 : 10 (v/v) extract of a mixture of 0.5 M acetic acid–0.5 M ammonium acetate. Phosphorus was determined with a spectrophotometric molybdenum blue method using a Foss-Tecator

5000 FIA Star Flow Injection Analyser. Calcium, potassium, and magnesium were measured with a Perkin Elmer AAnalyst 700 FAAS instrument. The bulk density of the soil was measured with a standardized test apparatus with a measuring cylinder of 1 L and a weight of 650 g (8 g cm^{-2}) [15]. Soil moisture was determined by drying the soil in a heating chamber at a temperature of 105°C for 2 h. To remove larger particles, the soil samples were sieved through a 4 mm sieve before the biodegradation experiments.

2.3 *Instrumentation of biodegradation measurements*

The soil was placed in a MG 1.0 bottle (WTW, Weilheim, Germany) for soil measurements with a BOD OxiTop measuring device (WTW). The studied oils were added to the soil in concentrations of about 1000 mg kg^{-1}, which is one recommended limit value for oil contamination in Finland, but which depends greatly on soil type and will be evaluated on a case basis. A 50 mL beaker filled with a 1 M sodium hydroxide solution was placed on the holder. Sodium hydroxide absorbs any carbon dioxide produced. Solid NaOH pellets, used in water measurements, cannot be used here because they may absorb moisture from the soil, too. The bottles were sealed tightly, and OxiTop C measurement heads (WTW) were screwed onto the bottles. The BOD OxiTop instrument follows the diminishing pressure in the bottle. Because oxygen in the gas phase is consumed and the produced carbon dioxide is readily absorbed by sodium hydroxide, the diminished pressure can be used to evaluate the biodegradation of the samples. The OxiTop bottles were held in an incubation cabinet at a temperature of 20.0 ± 0.2°C. The measurements were carried out for 14 days. A relatively short measurement period was chosen because the method was chosen to be tested with a great variety of samples in a moderately short period. An incubation time of 28 days is often used in biodegradation tests, and it is also recommended by the OECD guidelines [16]. On the other hand, reaching the saturation levels in the biodegradation reactions of the studied oils would need even longer incubation times as our previous water experiments show [2, 3]. A blank test was always conducted to determine soil respiration. The carbon content of the oils was determined with a Perkin Elmer 2400 Series II CHNS/O analyser (so-called Dumas method) based on combustion of the samples and determination of carbon as carbon dioxide. The carbon content had to be known in order to calculate the degree of biodegradation.

2.4 *Calculations*

First of all, the volume of the soil must be calculated. When the weight of the soil (m_{soil} (g)) and its bulk density (d_{soil} (g L^{-1})) are known, the volume of the soil (V_{soil} (L)) can be calculated using equation (1):

$$V_{soil} = m_{soil}/d_{soil.} \tag{1}$$

Using the calculated volume of the soil, the free gas volume (V_{fr} (L)) of the measurement bottle can be calculated using equation (2), where V_{bottle} is the volume of the bottle (0.96 L), V_{vessel} is the characteristic volume of the absorption vessel (0.01 L), and V_{NaOH} is the characteristic volume of the absorption agent (0.05 L):

$$V_{fr} = V_{bottle} - V_{vessel} - V_{NaOH} - V_{soil}. \tag{2}$$

The BOD OxiTop instrument gives the change in pressure (Δp) in hPa, which has to be converted to $\mathrm{J\,L^{-1}}$ for the calculations. Δp can be converted to the amount of consumed oxygen (Δm (g)) using equation (3), where M_{O_2} is the molar mass of oxygen ($32\,\mathrm{g\,mol^{-1}}$), R_{O_2} is the specific gas constant for oxygen ($8.301\,\mathrm{J\,mol^{-1}\,K^{-1}}$), and T is the measuring temperature (293.15 K):

$$\Delta m = \Delta p \cdot V_{\mathrm{fr}} \cdot M_{O_2}/(R_{O_2} \cdot T). \tag{3}$$

To be able to monitor only the biodegradation of the oil, soil respiration is excluded by subtracting the Δm value of the soil from the Δm value of the soil/oil mixture. The biological oxygen demand of the oil is calculated from the Δm value of the oil (Δm_{oil} (g)) and given in equation (4), where m_{sample} is the weighed amount of oil used in the experiment:

$$\mathrm{BOD\ (g/g)} = \Delta m_{\mathrm{oil}}/m_{\mathrm{sample}}. \tag{4}$$

Theoretical oxygen demand (ThOD (g/g)) can be calculated when the carbon content of the sample is known:

$$\mathrm{ThOD\ (g/g)} = [m_C \cdot (M_{O_2}/M_C)]/m_{\mathrm{sample}}. \tag{5}$$

m_C is the mass of carbon, and M_C is the molar mass of carbon. For example, the hydrogen and oxygen contents also affect the ThOD value. Two hydrogen atoms consume one oxygen atom from the gas phase, whereas one carbon atom consumes two oxygen atoms. On the other hand, one oxygen atom in the molecule to be biodegraded diminishes the oxygen demand from the gas phase with one oxygen atom. So, the influence of these atoms on the ThOD value is partially opposite and not very significant when compared with carbon, which is the main component in the oil samples. The studied substances are mixtures for which no molecule formula can be given. So, every elemental concentration should be determined. The hydrogen content could be easily determined with our elemental analyser (Dumas method) but the determination of oxygen cannot be carried out by this particular device. So, we decided to calculate the ThOD value using only the carbon content, and thus at least all our results of the degrees of biodegradation are fully comparable with each other.

The degree of biodegradation as a percentage can be calculated using equation (6):

$$\mathrm{Degree\ of\ biodegradation} = (\mathrm{BOD/ThOD}) \cdot 100\%. \tag{6}$$

3. Results and discussion

3.1 *Properties of the soils used*

Four different soils were used as a measurement medium. Certain properties of these soils are given in table 1.

Soils 1–3d were gathered in August 2003. Soils 3s and 3d were sampled from the same place; 3s from the surface and 3d from deeper. As can be seen from table 1, soil 3s was

Table 1. Soil properties.

Sample	Soil type	Organic matter (%)	pH	Conductivity ($cS\,cm^{-1}$)	Ca ($mg\,L^{-1}$)	P ($mg\,L^{-1}$)	K ($mg\,L^{-1}$)	Mg ($mg\,L^{-1}$)	Ca/Mg
Soil 1	Mud	–	4.1	3.4	127	1.1	22	26	4.88
Soil 2	Fine sand	4–8	4.4	1.4	101	1.3	34	36	2.81
Soil 3s	Fine sandy till	12–20	4.5	3.5	307	3.4	66	148	2.07
Soil 3d	Fine sandy till	<4	4.7	1.5	101	1.5	21	83	1.22

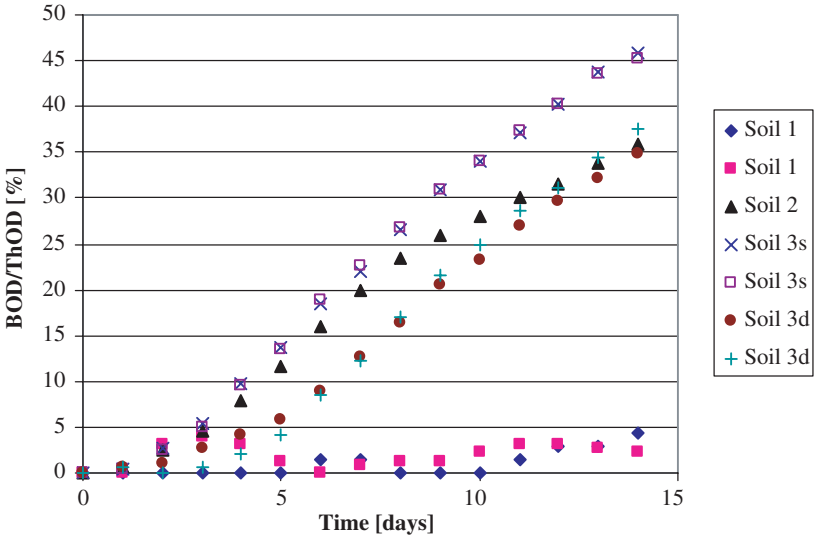

Figure 1. BOD/ThOD value *vs.* time for tall oil fatty acid (TOFA) in different soils.

most fertile, because the forest area had been fertilized with NPK fertilizer earlier. Soil 3s also had the most organic matter. The same soil, when sampled from a deeper layer (3d), had significantly lower amounts of the basic nutrients and organic matter. All the soils except soil 1 were categorized as coarse mineral soils. All the soils were moderately acidic, but the observed acidities are excellent for forest soils. The conductivity of some soils (1 and 3s) was rather high.

3.2 *Biodegradation of tall oil fatty acid (TOFA) in different soils*

Because the suitability of this method in determining biodegradation of oils was evaluated, the biodegradation of a typical oil sample was determined in the collected soil samples. Tall oil fatty acid (TOFA) was chosen to be the model oil in this particular case. It was formerly observed to be moderately biodegradable in groundwater and in the standard conditions of OECD 301 F; after 28 days, the degrees of biodegradation were 12.9 and 56.9%, respectively [3]. Its biodegradation was monitored in soils 1–3d for 14 days. The behaviour of TOFA in different soils is shown in figure 1.

It is apparent from figure 1 that TOFA biodegraded most effectively in soil 3s; the degree of biodegradation was over 45% after 14 days. The obvious reason for this observation is that soil 3s had no nutrient limitations. Microbes in soil 3s also

seemed to adapt quickly to biodegrade tall oil fatty acid. Based on the results obtained in this test, soil 3s was used in biodegradation tests hereafter in order to facilitate the quickest possible biodegradation reactions of oils within these soils. Soils 2 and 3d would also have been moderately good media for measuring the biodegradation of oils; the degrees of biodegradation of TOFA varied between 34.9 and 37.5% after 14 days. On the other hand, the biodegradation of TOFA was quite poor in soil 1. This is probably due to nutrient limitation. However, it should be noted that reaching the saturation levels of biodegradation of TOFA in any of these soils would require a longer incubation time. Replicate tests of the biodegradation of TOFA in different soils revealed that the precision of the BOD OxiTop method is rather good (see figure 1).

3.3 *Biodegradation of typical model substances in the selected soil*

To further prove that the selected soil (3s, see table 1) was highly acceptable as a measurement medium of oil biodegradation, the degree of biodegradation of a couple of typical, quickly biodegrading model substances was determined in this particular soil. The substances were sodium acetate and sodium benzoate. The BOD/ThOD value should be over 60% in 10 days, when the measurement medium is not nutrient- or microbially limited. The biodegradation of the model substances in soil 3s is shown in figure 2.

Sodium acetate and sodium benzoate both reached 60% in 10 days, and after 14 days their degrees of biodegradation were 89.9 and 85%, respectively. Hence, the selected soil 3s seems to very suitable for evaluating the biodegradation of different oils, as already seen in the tests with tall oil fatty acid. Neither nutrient nor microbial limitations were observed. However, it should be noted that these results are valid only in this particular soil. These substances could have been used also to validate the other soils if they were chosen for routine use in biodegradation experiments.

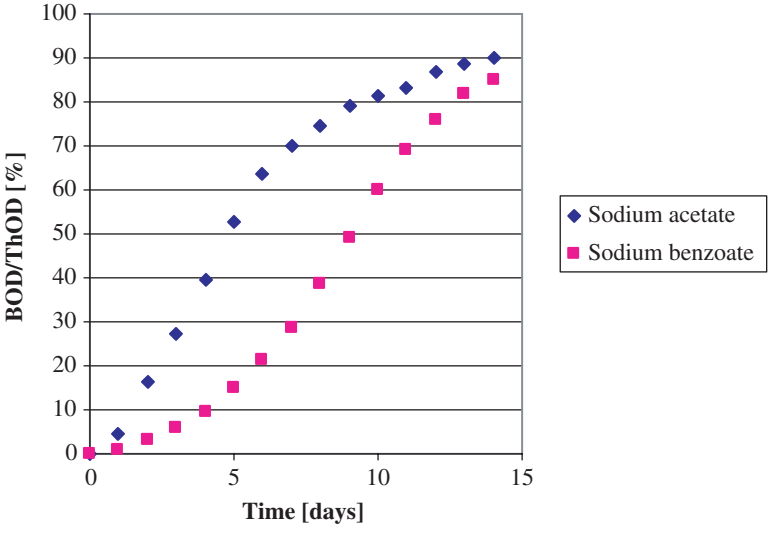

Figure 2. BOD/ThOD value *vs.* time for certain rapidly biodegrading substances in soil 3s.

Here, the main interest was to clarify the suitability of the soils in oil biodegradation (see figure 1).

3.4 *Biodegradation of certain wood preservatives in the selected soil*

The biodegradation of wood preservatives was formerly studied in groundwater and in the standard conditions of OECD 301 F in water [3]. Tall oil-based wood preservatives, as well as linseed oil, were moderately biodegradable in groundwater. In standard conditions, their biodegradation was better. Creosote oil, which has been widely used in wood preservation, had no aptitude for biodegradation in groundwater, but in standard tests it had a BOD/ThOD value of 24.9% after 28 days [3]. Some of the wood preservatives were experimental substances, which were being studied in a R&D project. The biodegradation behaviour of these wood preservatives in soil 3s is presented in figure 3.

Linseed oil reached a moderate BOD/ThOD value of 42.9% after 14 days, but the experimental substances reached even higher values. All of the experimental substances studied here are derivatives of tall oil fatty acid (TOFA). Fe(III)/TOFA is a mixture containing 20% Fe(III) salt of TOFA and 80% pure TOFA. Mn(II)/TOFA is a similar mixture of Mn(II) salt of TOFA and TOFA. NPG ester of TOFA is formed from neopentylglycol and TOFA. More information on these substances can be found in our earlier article [3]. Fe(III)/TOFA seems to be the fastest biodegrading substance here, but it should be noted that some of the oxygen consumption here may be due to oxidation of Fe(II) to Fe(III), thus causing a higher BOD/ThOD value. However, oxygen consumption in the oxidation of iron and in biodegradation reactions could not be separated, and both of them are handled as biodegradation in this study. Mn(II)/TOFA reaches almost the same value as Fe(III)/TOFA. On the other hand, esterification of TOFA produced almost the same BOD/ThOD value observed here when compared with pure TOFA. Creosote oil also biodegrades moderately in this soil, but it has already reached a stable phase with a BOD/ThOD value of 25%, and most likely it will not biodegrade significantly better in longer-lasting measurements. However, as biodegradation of the other wood preservatives is still effective after 14 days, longer incubation time would produce even higher values of degrees of biodegradation.

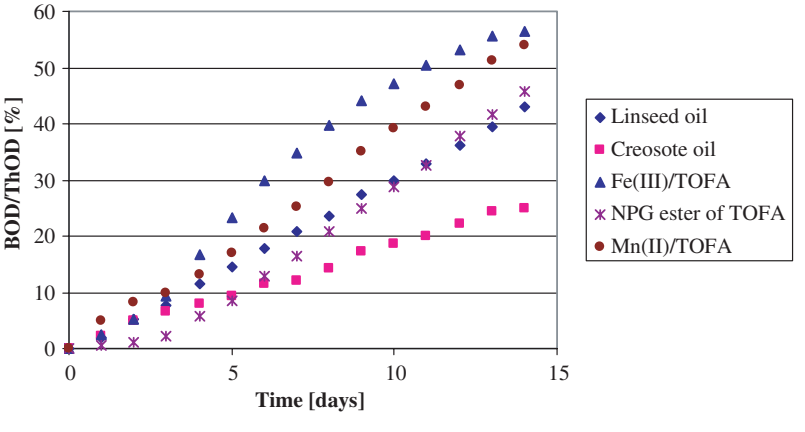

Figure 3. BOD/ThOD value *vs.* time for some wood preservatives in soil 3s.

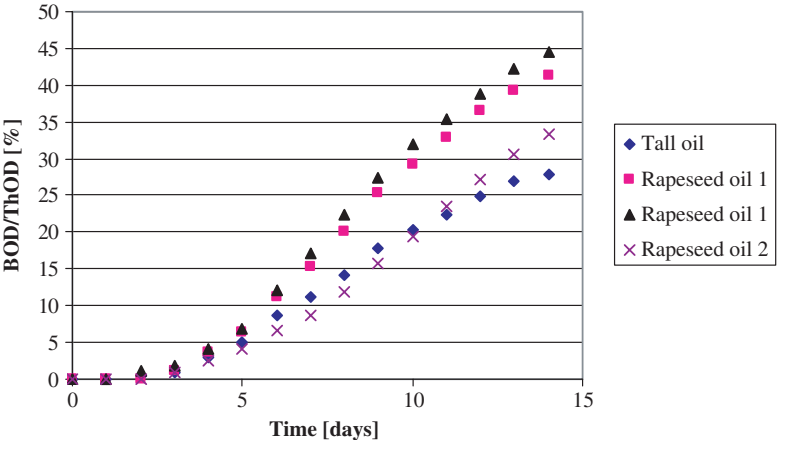

Figure 4. BOD/ThOD value *vs.* time for some chain oils in soil 3s.

3.5 *Biodegradation of forestry chain oils in the selected soil*

Forestry chain oils were studied earlier in groundwater and in conditions described by OECD 301 F in water [2]. For a comparison and clarity, tall oil = tall oil 1, rapeseed oil 1 = rapeseed oil 3 and rapeseed oil 2 = rapeseed oil 2 in the earlier publication [2]. The degrees of biodegradation of these chain oils over 14 days are presented in figure 4.

Rapeseed oils have been observed to biodegrade slightly better than tall oils in groundwater and in standard conditions in water. A similar observation can also be made from the experiments carried out in soil. The BOD/ThOD values over 14 days of rapeseed oils varied between 33.3 and 44.5% in this soil, while tall oil had a BOD/ThOD value of 27.8% during the same time. However, a longer incubation period will produce higher values of BOD/ThOD, because none of these oils reached a stable phase of biodegradation in 14 days. Replication tests of rapeseed oil 1 gave quite similar results (the BOD/ThOD values were 41.4 and 44.5% after 14 days) in this particular soil; hence, the precision of the method is rather good. Of course, replication tests give more precise results in water, which is a more homogenous measurement medium [2].

4. Conclusions

The suitability of the respirometric BOD OxiTop method in determining biodegradability of different oils in forest soil was evaluated. Soil 3s proved to have the most potential to biodegrade oils of the studied forest soils when tested with tall oil fatty acid. This selected soil was further validated with sodium acetate and sodium benzoate. Their BOD/ThOD values exceeded the 60% limit value in 10 days. Thus, biodegradation reactions will not be nutrient- or microbially limited in the soil 3s. In addition, certain wood preservatives and forestry chain oils were biodegraded in the selected soil 3s. Linseed oil and certain experimental wood preservatives biodegraded moderately in this soil over a short period of 14 days, and their biodegradation was still effective. On the other hand, creosote oil reached a stable

value of 25% in 14 days. The studied forestry chain oils also biodegraded moderately in this soil.

The BOD OxiTop method proved to be a suitable method for evaluating the biodegradability of oils in soil. Pressure measurements were accurate, and the precision of the whole method also seemed to be good enough for quantitative work. Tests carried out with real oil samples supported earlier observations of the biodegradation of these oils in water. Longer measurement periods of biodegradation and effective evaluation of drifting processes of these oils in soils would be beneficial in order to enhance our knowledge of them even more in the future.

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