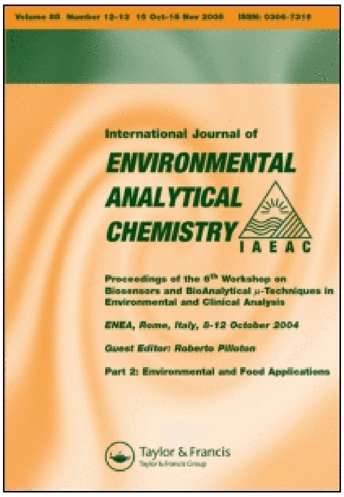

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Suitability of the respirometric BOD oxitop method for determining the biodegradability of oils in ground water using forestry hydraulic oils as model compounds

Toivo Kuokkanen[a]; Pekka VÄhÄoja[a]; Ilkka VÄlimÄki[a]; Risto Lauhanen[a]
[a] Toivo Kuokkanen Pekka VÄhÄoja Ilkka VÄlimÄki Risto Lauhanen Department of Chemistry University of Oulu P.O. Box 3000 FIN-90014 Oulu Finland Suomen YmpÄristÖpalvelu Oy Kiilakiventie 1 FIN-90250 Oulu Finland SeinÄjoki Polytechnic School of Forestry Tuomarniementie 55 FIN-63700 ÄhtÄri Finland .

To cite this Article Kuokkanen, Toivo , VÄhÄoja, Pekka , VÄlimÄki, Ilkka and Lauhanen, Risto(2004) 'Suitability of the respirometric BOD oxitop method for determining the biodegradability of oils in ground water using forestry hydraulic oils as model compounds', International Journal of Environmental Analytical Chemistry, 84: 9, 677 — 689

To link to this Article: DOI: 10.1080/03067310410001688435

URL: http://dx.doi.org/10.1080/03067310410001688435

Intern. J. Environ. Anal. Chem.
Vol. 84, No. 9, pp. 677–689, 10 August 2004

SUITABILITY OF THE RESPIROMETRIC BOD OXITOP METHOD FOR DETERMINING THE BIODEGRADABILITY OF OILS IN GROUND WATER USING FORESTRY HYDRAULIC OILS AS MODEL COMPOUNDS

TOIVO KUOKKANEN[a,*], PEKKA VÄHÄOJA[a],
ILKKA VÄLIMÄKI[b] and RISTO LAUHANEN[c]

[a]*Department of Chemistry, University of Oulu, P.O. Box 3000, FIN-90014 Oulu, Finland;*
[b]*Suomen Ympäristöpalvelu Oy, Kiilakiventie 1, FIN-90250 Oulu, Finland;*
[c]*Seinäjoki Polytechnic, School of Forestry, Tuomarniementie 55, FIN-63700 Ähtäri, Finland*

(Received 21 May 2003; In final form 9 February 2004)

Different forestry hydraulic oils were used as model compounds for evaluating the applicability of the respirometric BOD Oxitop method in following the progress of biodegradation of oils in ground water. It is obvious that the biodegradability values of substances depend on the measurement medium used. In this study the same ground water was used in all the ground water experiments. It was observed that the biodegradability of hydraulic oils is dependent on the oil type, being faster for bio oils than for mineral oils. The rate of biodegradation of used hydraulic oils was slower than that of corresponding new oils. All the experiments show that the respirometric BOD Oxitop method is a good and reasonable method for monitoring the biodegradability of oils in water, and the method is independent of the biology and composition of the measurement medium. No changes to the equipment are necessary if conditions are changed. Because the method needs only one sample for measurement, and monitoring of the pressure in the closed bottles is automatic, biodegradability is determined easily and quickly. The precision of the results studied was good. By contrast, traditional measurement of biodegradability using IR, for example, is time consuming, requiring several samples and a considerable amount of poisonous extraction solvent (CCl_4). Nevertheless, studies describing the biodegradability of oils using the respirometric BOD Oxitop method have not been published previously.

Keywords: Forestry oil; Ground water; Biodegradability; BOD Oxitop method

INTRODUCTION

Most of the forests in Finland have been certificated, i.e., wood procurement is based on sustainable forestry, and thus environmental issues are also very important in the

---

*Corresponding author. Fax: +358-8-5531603. E-mail: toivo.kuokkanen@oulu.fi

ISSN 0306-7319 print: ISSN 1029-0397 online
DOI: 10.1080/03067310410001688435

field of modern forestry. The proportion of mechanical forest operations was over 90% in 2001. There were about 1800 harvesters and 2000 forwarders working in Finnish forests in February 2001. Commercial domestic round wood removal was 53.2 million cubic meters in Finland in 2001 [1]. Protection of forest soil and ground water areas against contamination by oil hydrocarbons is an important issue today and there has also been a demand for the use of biodegradable oils in forest machines by some organizations. On the other hand, the possible harmful effects on forest machines of the biodegradable oils studied have been discussed [2].

It is surprising that the environmental effects of forestry oils on forest soil and ground water are not widely studied, even though there are significant possibilities of causing environmental accidents while working and from the storage of oils. The biodegradability and environmental effects of forestry oils (mineral, tall and rapeseed oils) have been studied in Finnish forest soils in our earlier investigations over recent years. It has been shown that bio oils biodegrade more rapidly than mineral oils in forest soil [2]. There is also an obvious need to study the behavior of forestry oils in ground water. In our earlier studies the progress of the biodegradation of various oils in soil and ground water has been followed using the FTIR spectroscopic method (SFS 3010 standard in Finland [3], modified for soil samples). However, this method for determining the progress of the biodegradation of oils is slow, expensive, requires very poisonous extraction solvent (CCl_4) and contains problems in sampling. An alternative method for following biodegradation of oils in water could be a measurement of total organic carbon within the sample.

However, studies of the applicability of the persulphate/UV TOC analysis method to the quantitative analysis of forestry oils and to following the progress of their biodegradation, using different chain oils (tall, rapeseed and mineral oils) as model compounds, showed that the $K_2S_2O_8$/UV oxidation method used is not sufficient to oxidize chain oils completely. The oxidation efficiency changed from about 46% measured for tall oil to about 25% observed for rapeseed chain oil. The addition of Triton X-100 surfactant at a concentration of up to 2% (w/w) was observed to increase the oxidation efficiency, e.g., to 75% for tall oil. The observations can be explained by assuming that the emulsions are more homogenous and stable in the presence of a surfactant [4].

The aim of our studies is to find good methods for monitoring the biodegradability of different oils. Using different types of forestry hydraulic oils as model compounds the aim of this study was to evaluate the applicability of the respirometric biological oxygen demand (BOD) Oxitop method in following the progress of oil biodegradation in ground water. Our concern was not to find the best conditions for biodegradation of oils but to make observations in an environment that is threatened by oil pollution in everyday life. Other, minor, aims were to find out the differences between oil types and to test the BOD Oxitop method in conditions described in OECD 301 F standard. As far as we know, the use of this method to study the biodegradability of oils has not been reported earlier. Previously the respirometric BOD Oxitop method has been used to study the biological oxygen demands of waste water and polymers, among others [5–7]. Reuschenbach *et al.* have proven in a comparison test between the Sapromat and Oxitop methods that the Oxitop method is a reliable method for evaluating the biodegradability of chemicals [8].

EXPERIMENTAL

Theory of the Measurement

The respirometric BOD Oxitop method is based on very accurate automatic pressure measurement in a closed bottle. When organic matter biodegrades, it demands a certain amount of oxygen. When oxygen is consumed, pressure falls. At the same time carbon dioxide is produced, but in this method it is absorbed onto solid sodium hydroxide pellets, as described in Eq. (1), so it does not affect the measured pressure.

$$\mathrm{CO_2(g) + 2NaOH(s) \rightarrow Na_2CO_3(s) + H_2O(l)} \qquad (1)$$

The measurement is fully automated and the instrument calculates BOD in mg/L using the ideal gas law modified for conditions in a closed bottle. Equation (2) is valid if phase equilibrium between the gas and liquid phases can be facilitated. Phase equilibrium can be reached when there is constant stirring in the bottle, continuous and fast gas exchange and also slow biodegradation reactions as the main reactions.

$$\mathrm{BOD[mg/L]} = M(\mathrm{O_2})/RT_\mathrm{m} \times [(V_\mathrm{tot} - V_\mathrm{l})/V_\mathrm{l} + \alpha T_\mathrm{m}/T_0] \times \Delta p(\mathrm{O_2}) \qquad (2)$$

$M(\mathrm{O_2})$ is the molecular weight of oxygen (32 000 mg/mol), R is the gas constant (83.144 L hPa mol^{-1} K^{-1}), T_m is the measuring temperature (K), T_0 is 273.15 K, V_tot is the bottle volume (mL), V_l is the liquid phase volume (mL), α is the Bunsen absorption coefficient (0.03103) and $\Delta p(\mathrm{O_2})$ is the difference in partial oxygen pressure (hPa).

At the beginning of the measurement it must be decided which measurement range will be used. That determines the amount of water used. For example, when a measurement scale of 0–80 mg/L is used, 365 mL of water is needed. The measurement is also more accurate when a narrower scale is used. The measurement time can be anything between 30 min and 99 days. Twenty-eight days is often used as a measurement time in biodegradation measurements and is recommended by OECD experts [9].

There are various parameters that affect biodegradation processes. The most important is the amount and quality of the microbes within the medium (usually soil or water). Other affecting parameters are temperature, oxygen concentration, moisture, mineral content and pH value. The measurement temperature in our simulations was 20.0 ± 0.2°C. The chosen medium was ground water, because pure drinking water is one of our most important natural resources, and oils can contaminate water, especially in ground water areas and other nearby aquifers.

OECD divides biodegradability tests into three categories: ready biodegradability tests, inherent biodegradability tests and simulation tests. Ready biodegradability tests have conditions which provide limited opportunity for biodegradation. In tests of inherent biodegradability, conditions can be set to optimal. Simulation tests provide information on the biodegradability of substances under particular environmental conditions. Pass levels for ready biodegradability are 60% of ThOD and for inherent primary biodegradability, 20% [9]. But as said before, the main interests of this study were the biodegradation processes in natural aquifers and the possible threats that oils can cause to our drinking water, e.g., cases of oil spillage. As stated in the results section, ground water from the Paavola water treatment station was chosen as the measurement medium.

Oils Examined in This Study

This study concentrates on certain new and used forestry hydraulic oils. Used oil samples were taken from oil changes of forestry machines. Some of them were bio oils and some were traditional mineral oils. The bio oils studied were not vegetable oils, but were mostly sold as easily biodegradable oils. They were probably synthetic esters. The chemical composition of the oils was not determined. They were manufactured by well-known oil-refining companies. The intention was to clarify the effect of the oil type (bio oil/mineral oil) and oil usage on the degree of biodegradation.

Measurement of Oil Biodegradation Using the Respirometric BOD Oxitop Method

Ground Water as Measurement Medium

One hundred mg/L solutions of the studied oils were prepared. The weighed oil masses were needed later in calculating the degree of biodegradation. The volume of the solution depended on the measurement scale chosen. If the water that is used contains nitrogen and the oxygen consumption of carbon is to be followed, nitrification must be prevented with a small amount of *n*-allylthiourea. Triton X-100 surfactant can be added to the water to homogenize and stabilize the sample [4,10]. However, in our ground water simulations, neither a nitrification inhibitor nor an emulsifier was used. Every time a new batch of ground water was taken into use, a blank test containing the same water as in the real samples was done. The bottles were sealed with a rubber sleeve, which contained NaOH pellets (p.a.) for carbon dioxide absorption. The measuring probes of the Oxitop bottles were screwed onto the bottles and the samples were stabilized in the incubation cabinet (temperature $20.0 \pm 0.2°C$) six hours before the measurement was started. The measurement lasts as long as programmed by the user, and the pressure changes in the gas phase are measured automatically. The narrower the measurement scale, the shorter is the time between the pressure measurements.

Conditions Described by OECD

Measurements were done in nutrient solution applying the OECD 301 F standard. Nutrient solution consisted of KH_2PO_4, K_2HPO_4, $NaHPO_4 \cdot 2H_2O$, NH_4Cl, $CaCl_2$, $MgSO_4 \cdot 7H_2O$ and $FeCl_3 \cdot 6H_2O$ in concentrations given in the OECD 301 F standard. Oil concentrations were between 150 and 200 mg/L. An extra microbe source was added in the form of waste water. Nitrification was prevented with allylthiourea, because waste water may include nitrogen. A measurement region of 0–200 mg/L BOD was used in most cases. The BOD values of samples were detected for 28 days [9].

Abiotic Degradation

For abiotic control, the samples (oil concentration 80–140 mg/L in distilled water) were heated in an autoclave for 15 min at a temperature of over 100°C and at a pressure of 1.5 bar to kill all microbes within the sample. Treated samples were cooled to room temperature overnight before measurement was started. BOD values were collected for 28 days. The results show the influence of abiotic degradation, which is mainly due to hydrolysis. Photolysis can be prevented by using dark bottles.

Calculations

The WTW BOD Oxitop instrument gives the BOD value in milligrams per liter. It is often more useful to use the amount of consumed oxygen in milligrams per milligram of the sample, because it takes into consideration the mass of the sample. This is used later in the form BOD [mg/mg]. The conversion of BOD [mg/L] to BOD [mg/mg] is given in Eq. (3).

$$\text{BOD [mg/mg]} = (\text{BOD [mg/L]} \times \text{liquid phase volume [L]})/\text{sample mass [mg]} \quad (3)$$

The degree of biodegradation is calculated as follows. Firstly, the carbon content of the sample must be known. The carbon content of the samples was measured with a Perkin-Elmer 2400 series II CHNS/O analyzer. The principle of this measurement is combustion, and the total amounts of carbon, hydrogen and nitrogen are determined as simple gases (CO_2, H_2O, N_2). Potassium dichromate oxidation can also be used, but it is time-consuming, requires poisonous and highly acidic reagents, and the oxidation reactions can sometimes be inappropriate. Using the carbon content of the sample, the theoretical oxygen consumption of the sample (ThOD) can be calculated using Eqs. (4)–(6).

$$m(\mathrm{C}) = \text{Carbon content [\%]} \times \text{sample mass [mg]}/100 \quad (4)$$

$$m(\mathrm{O_2}) = m(\mathrm{C}) \times [M(\mathrm{O_2})/M(\mathrm{C})] \quad (5)$$

$$\text{ThOD} = m(\mathrm{O_2})/\text{sample mass [mg]} \quad (6)$$

In Eqs. (4)–(6) $m(\mathrm{C})$ is the mass of carbon [mg], $m(\mathrm{O_2})$ is oxygen consumption [mg], $M(\mathrm{O_2})$ is the molecular weight of oxygen (32.00 g/mol) and $M(\mathrm{C})$ is the molecular weight of carbon (12.01 g/mol).

The degree of biodegradation is given as the ratio of BOD and ThOD [Eq. (7)]:

$$\text{Degree of biodegradation [\%]} = \text{BOD/ThOD} \times 100\% \quad (7)$$

RESULTS AND DISCUSSION

Biological Oxygen Demands of Different Waters

Because the effect of the chosen water on the biodegradation of oils is obvious, the oxygen consumption of water taken from different sources was tested first. The waters studied were ultra-pure water, Oulu municipal water, Liminka River water and ground water from Paavola. Liminka and Paavola are both located near the city of Oulu.

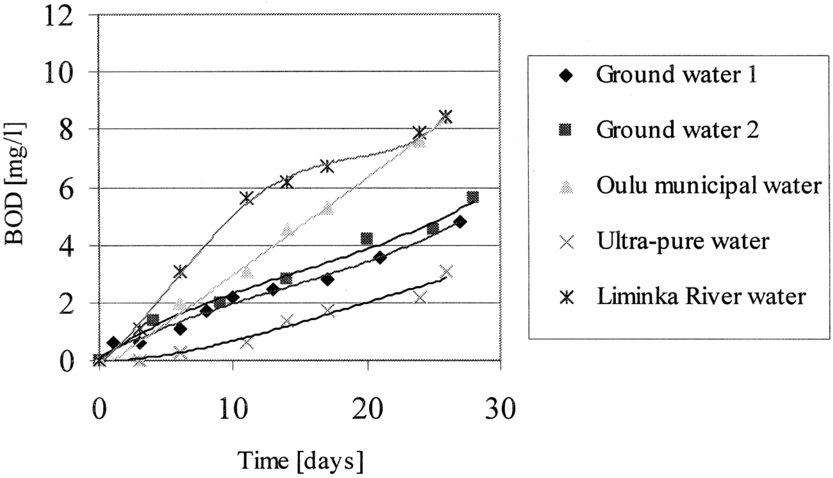

FIGURE 1 Oxygen consumption of different waters.

TABLE I Water analysis data for filtered water from the Paavola water treatment station

Property of water	*Result*	*Unit*
Fecal coliform bacteria (44°C)	< 1	CFU/100 mL
Coliform bacteria (37°C)	< 1	CFU/100 mL
Heterotrophic bacteria (37°C)	< 1	CFU/mL
Conductivity (22 ± 2°C)	14.7	mS/m
pH (22 ± 2°C)	7.5	
Color	15	mgPt/L
Appearance	Bright	
Alkalinity	1.4	mmol/L
CO_2	7.8	mg/L
$KMnO_4$-count	9.9	mg/L
Hardness	0.10	mmol/L
SO_4^{2-}	2.7	mg/L
NO_3^-	0.09	mg/L
NO_2^-	< 0.01	mg/L
NH_4^+	0.02	mg/L
Al	0.01	mg/L
Cu	0.01	mg/L
Mn	0.05	mg/L
Fe	0.44	mg/L
Zn	< 0.01	mg/L

As can be seen from Fig. 1, oxygen consumption differs slightly between the studied waters. As expected, the municipal water and the river water demand a little more oxygen, and the least carbon-containing ultra-pure water consumes the least oxygen. The ground water samples seem to consume almost the same amounts of oxygen. Because forestry machines often work on potential ground water areas, ground water was chosen as the measurement medium. The chosen ground water is supplied by the Paavola water treatment station, and some water analysis data of this ground water are presented in Table I. It can be seen that the carbon, microbe and mineral concentrations of the Paavola ground water are relatively small.

The Biological Oxygen Demand of Ground Water

BOD determinations of oils using the respirometric BOD Oxitop method and the same ground water supplied by the Paavola water treatment plant have been carried out for a couple of years. The BOD value of ground water after 28 days usually varies around $5.8 \pm 2.5\,\mathrm{mg/L}\,(n = 13)$. The BOD values of ground water vary a little from batch to batch from natural causes such as different amounts of carbon, minerals and bacteria, but the variations seem to be quite small. It is also worth noting that when the same batch of water is used, the BOD values vary only minimally. This means that ground water used as a measurement medium is quite homogenous and constant from batch to batch. However, when actual biodegradation measurements of oils are done, the effect of water on oxygen consumption must be subtracted from the result, i.e., a blank test always needs to be carried out when a new batch of ground water is taken into use. When the aqueous medium is changed, let us say from ground water to river water, the measured results are different. So, it is necessary to use the same water in every simulation and the reason is obvious: every aquifer contains its own carbon, microbe and mineral concentrations.

Effect of Oil Type on Biodegradation and the Behavior of the Model Substance

We wanted to know if it is possible to use the respirometric BOD Oxitop method to simulate variations between the biodegradability values of different types of oils in a ground water medium. Five different kinds of bio oils and two mineral oils were tested. They were all products of well-known oil companies, but our research group wants to emphasize that this study is *not* a commercial study financed by oil companies or a comparison study between oils of different producers. The carbon content of all the studied oils is presented in Table II. The biodegradation of the mentioned new oils is presented as BOD/ThOD percentages as a function of time in Fig. 2.

Sodium acetate, which should be a quickly biodegrading substance, was used as a reference/model substance. It should reach a BOD/ThOD value of 60% in 10 days in suitable conditions [9]. It can be seen from Fig. 2 that the degree of biodegradation of sodium acetate after 28 days was as low as 7.3% in the ground water used. A replication test gave a slightly smaller value (BOD/ThOD was 5% after 28 days) for the biodegradability of sodium acetate. However, some of the oils seem to biodegrade more easily than sodium acetate in ground water conditions. One reason for this unexpected observation could be that the carbon of the sodium acetate is difficult to biodegrade without minerals, or the ground water studied does not contain suitable microbes for the degradation reactions of sodium acetate. We wanted to clarify this with the help

TABLE II Carbon content of new and used forestry hydraulic oils

Sample	*Carbon content* (%)
Bio oil 1, new/used	78.16/77.71
Bio oil 2, new	77.74
Bio oil 3, new/used	77.23/76.48
Bio oil 4, new	76.48
Bio oil 5, new	76.79
Mineral oil 1, new/used	86.09/85.02
Mineral oil 2, new/used	85.31/84.38

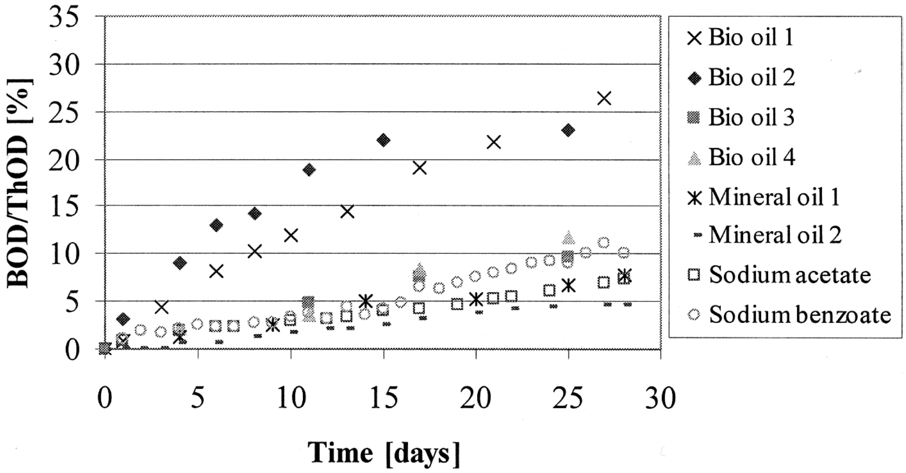

FIGURE 2 Plots of BOD/ThOD *vs.* time for the studied new hydraulic oils and reference substances.

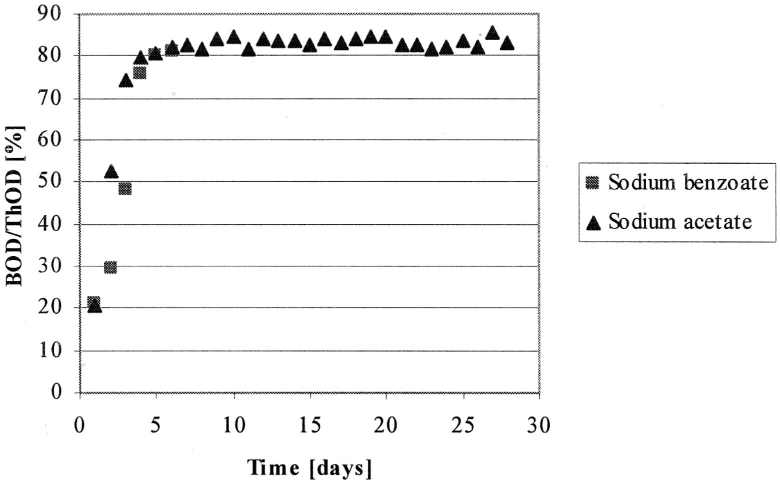

FIGURE 3 Behavior of some common reference substances in conditions including extra minerals and inoculum.

of a few tests. The behavior of sodium acetate in the ready biodegradability tests, where extra microbes and nutrients were used, is presented in Fig. 3.

Sodium acetate reaches a degree of biodegradation of over 80% after only five days in the conditions described by OECD 301 F [9]. So, it seems that the only reason for slow biodegradation of sodium acetate in ground water must be connected with the lack of microbes and minerals. The degradation of acetate shifts the pH to alkaline values, so the biodegradation tests should be done in well-buffered solutions. Because our main interest was to observe the differences between biodegradation of different oils in real ground water conditions, the ground water was used as such. Because almost all the studied oils biodegraded faster than sodium acetate, it seems that sodium acetate is not a good model of a rapidly biodegrading substance when the measurement medium does not contain enough minerals. However, microbes in the ground water seem to be efficient enough at least to degrade sodium acetate when there are enough minerals present. The degree of biodegradation of sodium acetate was more than 60% after five days in ground water to which the extra minerals described in OECD 301 F [9] were added. But if effective degradation is necessary, extra microbes may still be needed when other substances are degraded. Based on the earlier results another reference substance was also sought to test in ground water conditions. Sodium benzoate was chosen as the alternative model substance. The behavior of sodium benzoate can be seen in Fig. 3. It biodegraded at a similar rate to sodium acetate in the conditions recommended by OECD. However, the degree of biodegradation of sodium benzoate in ground water (see Fig. 2) is only slightly better (10.1%) than for sodium acetate (7.3%).

As can be seen from Fig. 2, the mineral oils biodegrade slowly in ground water, but bio oils 3 and 4 do not biodegrade significantly better. However, bio oils 1 and 2 seem to biodegrade considerably faster. The degree of biodegradation of bio oils 1 and 2 after 28 days in ground water is over 20%, which could be an indication of a potentially degrading substance in ground water. A direct comparison of these results with the results of tests done under optimal conditions is difficult because reference substances behave so differently in ground water than in the mineral- and microbe-containing medium.

Effect of the Use of Oil on Biodegradation

One purpose of this study was to clarify how the use of oils affects the degree of biodegradation of oils, if at all. Information on the behavior of used oils in ground water is important, because the majority of oil spillages occur during usage of oil, and the biodegradability data of new oils cannot necessarily be applied. The degrees of biodegradation of some new and corresponding used bio and mineral oils taken from oil changes are presented in Fig. 4.

As can be seen from Fig. 4, all the used hydraulic oils studied biodegrade somewhat more slowly than the corresponding new ones, and the change in the biodegradation rate of the bio oils seems to be significant. Used bio oil 3 even seems to biodegrade as slowly as mineral oil 1 in ground water. However, it must be noticed that when the conditions are changed, another kind of result can be observed. But, based on this study, used bio oil 3 cannot be claimed to be easily biodegradable in a ground water environment, when even mineral oil biodegrades at an equal rate.

Fast biodegradation of oils can be seen as a good property of oil when oil is accidentally spilled into the environment, but sometimes rapidly biodegradable oils can produce harmful degradation products due to imperfect biodegradation [11]. On the other hand, oils should not begin to biodegrade in machines in the presence of moisture, for example. Our results indicate that used oils biodegrade more slowly than corresponding new ones, and with bio oils the changes in biodegradability rates are quite large. No certain explanation for this can be given, but some assumptions can be made, although verifying these assumptions requires more research. One reason for the observation could be that during use some more easily degradable components within oil degrade (e.g., biodegradation, oxidation) or volatile substances evaporate, and when the biodegradation of used oil is compared with the biodegradation of the

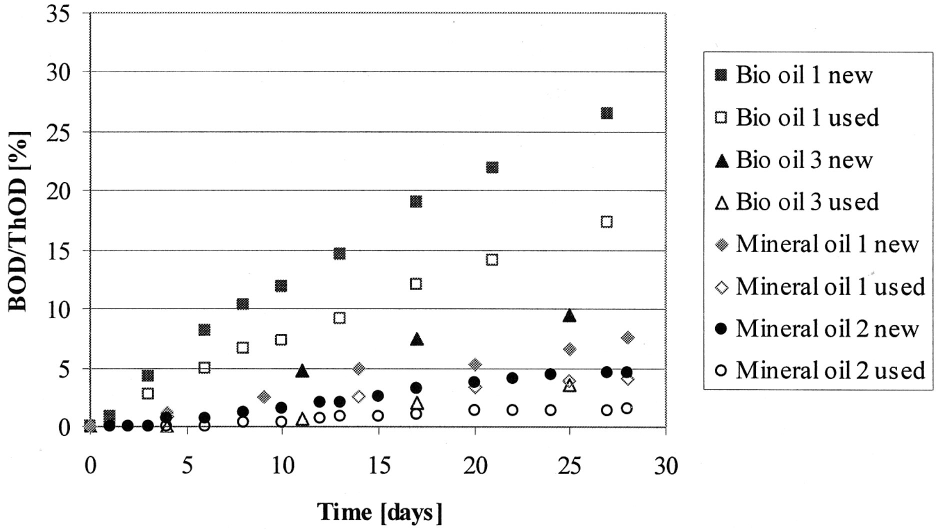

FIGURE 4 Plots of BOD/ThOD *vs.* time for new and corresponding used (taken from oil changes) hydraulic oils.

corresponding new oil, the observed rate is slower. Another reason could be that certain metals (such as zinc and nickel) within oils can catalyze the degradation of oil during use [12]. However, this is not a realistic assumption with the hydraulic oils studied here, because the metal concentrations of these oils were very small (Fe < 2 mg/L; Cr, Cu, Pb < 1 mg/L and Cd, Ni, V < 0.1 mg/L). The metal concentrations were determined using the ICP-OES technique [13].

Tests of Certain Bio Oils in the Conditions Recommended by OECD

Although the main goal of this study was to follow the biodegradation of oils in a ground water environment, some additional experiments were done in the more optimal conditions described by OECD 301 F [9]. These tests were done to prove the capability of the BOD Oxitop method to measure biological oxygen demands of oils in different conditions. The oils chosen for these tests were all bio oils previously observed to be somewhat biodegradable in ground water. The degrees of biodegradation of these bio oils in conditions described by OECD are shown in Fig. 5.

Bio oil 5 biodegraded most rapidly and bio oil 1 slowest; the degrees of biodegradation were 89.1 and 25.6% respectively. The behavior of bio oil 5 was expected, because the value of its degree of biodegradation was definitely the greatest (see Fig. 6) in the pure ground water environment. Bio oil 1 gave a surprising result, because when compared with the results in the ground water environment, the degrees of biodegradation were almost the same after 28 days. No simple explanation for this observation can be given. The microbes of both environments seem to be efficient enough to biodegrade certain components of bio oil 1. Bio oils 2–4 are all moderately biodegradable in the biodegradability tests in additional medium- and microbe-containing medium and the change of conditions from ground water to more optimal seems to double or even triple the degrees of biodegradation.

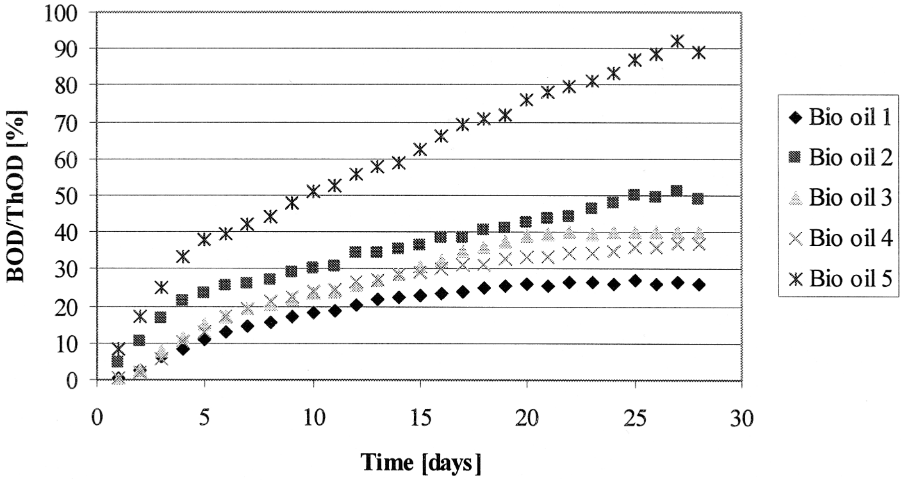

FIGURE 5 Biodegradability tests of certain new bio oils in the extra mineral- and microbe-containing medium described by OECD 301 F.

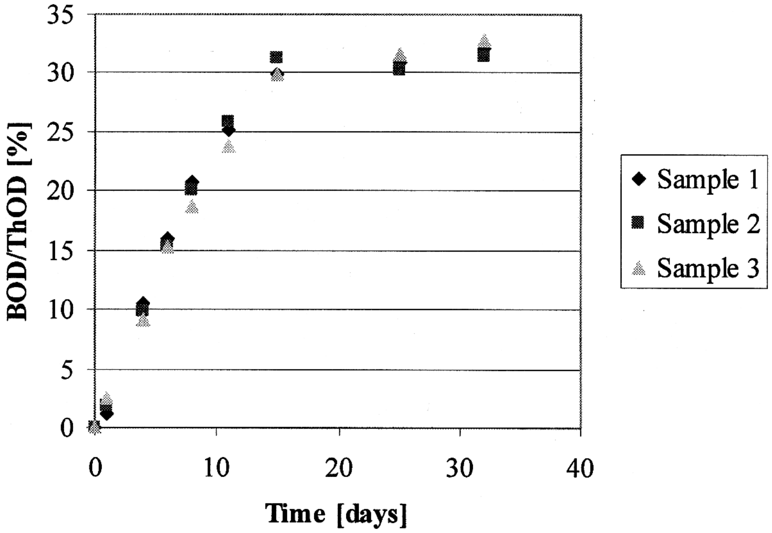

FIGURE 6 Plots of BOD/ThOD values *vs*. time for bio oil 5 in precision test ($n = 3$) in ground water.

Abiotic Degradation of Certain Oils

Especially with quickly biodegrading substances, the influence of abiotic degradation (hydrolysis, photolysis) can be significant. Abiotic controls were performed with bio oils 1–5. Results are given in Table III.

As can be seen from Table III, abiotic degradation is rather minor with the oils studied. The main part of the degradation process is more likely to be due to biodegradation. Of course, in some cases abiotic degradation can be much more significant.

TABLE III Abiotic degradation of certain new bio oils in 28 days

Sample	*BOD/ThOD* [%]
Bio oil 1	2.5
Bio oil 2	1.8
Bio oil 3	2.6
Bio oil 4	1.4
Bio oil 5	2.4

Precision of the Respirometric BOD Oxitop Method

One of the most important parameters of a new method of analysis is its precision. The precision of the BOD Oxitop method in determining the biodegradability of oils was studied by determining the BOD values of three different samples of the same bio oil (bio oil 5) using the BOD Oxitop method. The BOD/ThOD values calculated for these three samples are given in Fig. 6.

As can be seen from Fig. 6, the precision of the BOD Oxitop method is excellent. The calculated BOD/ThOD percentages from the measured BOD values are almost the same with all three determinations (average 32.08 ± 0.34 after 32 days, RSD is 1.06%). The excellent precision of this method is due to various reasons, including accurate pressure measurements, no sample-taking errors, accurate temperature control, relatively homogenous ground water and so on. Based on these results, it can be said that the precision of the BOD Oxitop method is good enough for it to be used for analytical determinations of the BOD value of oils. However, replications are always worth measuring from time to time. What is still important to notice is that bio oil 5 biodegrades quite effectively (BOD/ThOD > 30% after 28 days) in a ground water environment.

CONCLUSIONS

The BOD Oxitop measurement method is precise and handy for determining BOD. Until now the published information on BOD Oxitop use in biodegradability studies is limited [5–6,8]. However, our results show that this method is also capable of measuring the BODs of oils in chosen aqueous media, such as ground water. The biology and composition of the chosen medium naturally affect the results. When different inocula are used, results will vary from each other. When the carbon content of oil is also known, the BOD values measured with the BOD Oxitop method can be used in calculations of the degrees of biodegradation of various substances. Because the method is fully automated, biodegradability measurement can be done fairly easily. The results of these determinations are widely applicable when the environmental effects of oils are studied, for example in accident situations. This kind of research is important because the physical and chemical properties of oils can change during use. So the biodegradability values of new oils cannot necessarily be applied when the effects of used oils on the environment are estimated. The truth of this can be seen from our results; used hydraulic oils biodegrade significantly more slowly in ground water than the corresponding new oils. The reasons for these observations should be clarified in the future.

It should still be noted that this study is just the beginning of a longer research series. The BOD Oxitop method will be used more widely in biodegradability studies of different oils, both in ground water and in soil. Also some additional experiments with longer time periods should be done in the future.

Acknowledgements

The authors would like to thank Suomen Ympäristöpalvelu Oy for the opportunity to use their WTW BOD Oxitop instrument and the personnel of the trace element laboratory of the Department of Chemistry at the University of Oulu for metal determinations with ICP-OES. Professor Paavo Perämäki, M.Sc. (Chem.) Sauli Vuoti, and Mrs. Reetta Kolppanen are also greatly acknowledged for their help during this study. Pekka Vähäoja would like to thank the Tauno Tönning Foundation and the Academy of Finland for financial support.

References

[1] *Finnish Statistical Yearbook of Forestry*, pp. 189–192. Agriculture, forestry and fishery, SVT:45, Finnish Forest Research Institute, Helsinki (2002).
[2] R. Lauhanen, R. Kolppanen, S. Takalo, T. Kuokkanen, H. Kola and I. Välimäki, In: *Proceedings of the International Scientific Conference – Forest and Wood Technology vs. Environment*, pp. 203–206. Brno, Czech Republic (20–22 November 2000).
[3] SFS 3010: Determination of oil and grease in water, IR-method. Finnish Standards Association (1980).
[4] H. Kola, T. Kuokkanen, I. Välimäki, P. Perämäki and R. Lauhanen, *Intern. J. Environ. Anal. Chem.*, **83**, 157–165 (2003).
[5] F. Conzelmann, *Melliand Textilberichte*, **77**, 700–702 (1996).
[6] R. Mittenzwey, T. Seidenstuecker, H.-G. Fritz and R. Suessmuth, *Starch/Staerke*, **50**, 438–443 (1998).
[7] WTW BOD Oxitop operating instruction sheet.
[8] P. Reuschenbach, U. Pagga and U. Strotmann, *Wat. Res.*, **37**, 1571–1582 (2003).
[9] *OECD Guidelines for the Testing of Chemicals* (Section 3: Degradation and accumulation, Test 301: Ready biodegradability). Updated Guideline, adopted 17.7.1992 (OECD, France).
[10] J. Hoffmann, I. Řezníčèková, S. Vaňòková and J. Kupec, *Intern. Biodeterioration Biodegradation*, **39**, 327–332 (1997).
[11] M. Alexander, *Introduction to Soil Microbiology* (Wiley, New York, 1977), 2nd ed., pp. 203–222.
[12] A.U.C. Maduako, G.C. Ofunne and C.M. Ojinnaka, *Tribology Intern.*, **29**, 153–160 (1996).
[13] T. Kuokkanen, P. Perämäki, I. Välimäki and H. Rönkkömäki, *Intern. J. Environ. Anal. Chem.*, **81**, 89–100 (2001).