



Biodegradability of soil water soluble organic carbon extracted from seven different soils

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Abstract

Water soluble organic carbon (WSOC) is considered the most mobile and reactive soil carbon source and its characterization is an important issue for soil ecology study. A biodegradability test was set up to study WSOC extracted from 7 soils differently managed. WSOC was extracted from soil with water (soil/water ratio of 1:2, W/V) for 30 min, and then tested for biodegradability by a liquid state respirometric test. Result obtained confirmed the finding that WSOC biodegradability depended on the both land use and management practice. These results suggested the biodegradability test as suitable method to characterize WSOC, and provided useful information to soil fertility.

Key words: biodegradability test; cumulated oxygen uptake; water extractable organic carbon; water soluble organic carbon

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Introduction

Water soluble organic carbon (WSOC) accounts only for a small portion of the total organic carbon in soil (Metting, 1993). Nevertheless WSOC is considered the most mobile and reactive organic carbon fraction, thereby can control a number of physical, chemical and biological processes in both aquatic and terrestrial environments (Marschner and Kalbitz, 2003). Technically, WSOC can be obtained through many chemical and physical separation processes such as water extraction, carried out under different conditions (e.g., different temperatures, extraction volumes, extraction time, etc.) (Schnabel *et al.*, 2002; Zsolnay, 2003; Jones and Willet, 2006).

These conditions can significantly influence the yield of the soluble organic fraction. Only a fraction of the whole WSOC (the fraction dissolved in interstitial water pores) is actually extracted as part of WSOC remains linked to the mineral soil fraction (Tao and Lin, 2000). This fraction of WSOC is called the water extractable organic carbon (WEOC) (Zsolnay, 2003). As WSOC is the most important carbon source for soil microorganisms (Metting, 1993; Schnabel *et al.*, 2002; Marschner and Kalbitz, 2003) both the quantitative and qualitative aspects of this fraction are very important for soil ecosystem studies (Schnabel *et al.*, 2002; Gregorich *et al.*, 2003; Kalbitz *et al.*, 2003; Bolan *et al.*, 2004; Embacher *et al.*, 2007).

Tao and Lin (2000), by studying three soils, proposed a new approach to quantify WSOC content. This procedure,

called multiple solid-water ratio procedure, allows for the indirect determination of the WSOC, using different soil/water ratios. Qualitative aspects of WSOC are related to the availability of WSOC to microbes (Marschner and Kalbitz, 2003; Bolan *et al.*, 2004). Typically, the microbial availability of WSOC is directly estimated by measuring WSOC disappearance due to microbial utilization (Embacher *et al.*, 2007) or by measuring O₂ uptake or CO₂ evolution during biodegradation under standardized conditions (Marschner and Kalbitz, 2003).

Recently, D'Imporzano and Adani (2007) described a liquid-state respirometric test to detect the availability of dissolved organic carbon in the compost soluble fractions. The method, performed under optimized and standardized conditions, enables for the definition of a biodegradability index expressed as the cumulative oxygen uptake measured during a 20 h test (COUR₂₀). The objective of this work was to assess the biodegradability of the WSOC extracted from different soils by respirometric test.

1 Materials and methods

1.1 Soil sampling and laboratory analyses

Since WSOC biodegradability depends on land use and management practices (Chantigny, 2003), seven different soils as described below were considered: two forest soils (beech soil-FB-Humic Cambisol, FAO classification and a spruce soil-FP Humic Cambisol, FAO classification), two contaminated soils (with heavy metal CHM and hydrocarbon-CHC) from industrial activity, two

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agricultural soils amended with sewage sludge (AS-Eutric Gleysol, FAO classification and compost AC-Calcaric Cambisol, FAO classification) and one artificial soil (ART-quarzifer sand 88% (W/W) plus clay 9% (W/W) plus compost, 3% (W/W)) (Adani *et al.*, 2006). Soils were air-dried, sieved at 20 mm and then grinded to 2 mm. Then soils were characterized for total organic carbon (TOC) and total nitrogen (TN) contents, cation exchange capacity (CEC), texture (clay, silt, sand content) and pH using routine analyses (Faithfull, 2002) (Table 1). In addition, the contaminated soils CHM and CHC were characterized for heavy metal (Pb, Ni, Zn, Cu, Cr) and hydrocarbon contents according to Margesin and Schinner (2005) (Table 2).

1.2 Water extracted soil organic carbon (WEOC) extraction procedure

1.2.1 Preliminary test to define the operative conditions of extraction of WEOC from soil

Preliminary tests for WEOC extraction were carried out to determine both optimal soil/water ratio and time of extraction. All extraction tests were carried out at room temperature (20°C) using the most common ratios and extraction time reported in literature (Jones and Willen, 2006; Embacher *et al.*, 2007).

One hundred gram of each soil was treated with deionized water using different soil/water ratios (1:1, 1:2, 1:5, W/V) for 30 min, respectively, at room temperature under agitation (130 times/min) in a Dubnoff bath. After the extraction, samples were centrifuged at 6500 r/min for 15 min. Then, supernatants were filtrated with a 0.45- μ m Millipore membrane (Advantec MFS, Pleasanton, CA) and WEOC was quantified by organic carbon determination as reported previously (ISO, 2002).

One hundred gram of each soil was extracted with deionized water at ratio of 1:2 (W/V) for 30, 60, and 120 min, respectively at room temperature under agitation (130 times/min) in a Dubnoff bath. HgCl (0.5‰, g/mL) was added as bacteriostatic solution to avoid both hydrolysis and biodegradation (D'Imporzano and Adani, 2007). Then, samples were treated and WEOC was quantified.

WSOC was determined using the multiple solid-water ratio procedure (Tao and Lin, 2000). In brief, a weighed soil sample (W_s , g) was extracted using three different water volumes (V_w , L) to obtain three different soil-water ratios (1:1, 1:2, and 1:5, W/V), for 30 min at room temperature under agitation (130 times/min) in a Dubnoff bath. The samples were treated and WEOC was quantified.

Experimental data of W_s , V_w , and WEOC obtained

Table 2 Heavy metal and hydrocarbon contents for contaminated soils (mg/kg)

contaminant	CHM	CHC
Pb	1621 \pm 153	652 \pm 183
Ni	572 \pm 104	124 \pm 27
Zn	4530 \pm 334	973 \pm 87
Cu	3907 \pm 87	270 \pm 3
Cr	307 \pm 29	485 \pm 21
C < 12 ^a	63 \pm 8	5640 \pm 348
C > 12 ^b	195 \pm 11	325 \pm 25

Data are expressed as mean \pm standard deviation; ^a hydrocarbon with less than 12 carbons; ^b hydrocarbon with more than 12 carbons.

with different ratios allowed for the determination of both WSOC (mg/g) and sorption constant (k_s) by Eqs. (1)–(3) (Tao and Lin, 2000).

$$\text{WEOC} = \frac{\text{WSOC} \times W_s}{W_s k_s + V_w} \quad (1)$$

$$\text{WSOC} = \frac{\text{WEOC} (W_s k_s + V_w)}{W_s} \quad (2)$$

$$k_s = \frac{\text{WSOC} \times W_s - V_w \times \text{WEOC}}{\text{WEOC} \times W_s} \quad (3)$$

1.3 Setting of the biodegradation test of WEOC

1.3.1 Preparation of WEOC solutions

One hundred gram of soil was extracted with 200 mL of deionized water (soil/water ration of 1:2, W/V) at 20°C for 30 min under agitation (130 times/min) in a Dubnoff bath. After the extraction, samples were centrifuged for 15 min at 6500 r/min. Then supernatants were filtered with 0.45 μ m Millipore membrane (Advantec MFS, Pleasanton, CA) and the obtained solution was brought to a final volume of 200 mL with deionized water in which the biodegradability test was performed.

1.3.2 Assessment of analytical condition for biodegradability test

Biodegradability tests were performed by measuring the liquid oxygen uptake rate (OUR) of the microorganisms under standardized condition (D'Imporzano and Adani, 2007). In brief, OUR was measured under liquid condition for 20 h with a soil: water ratio of 1:2 (W/V). During the test, standard conditions were maintained to assure optimum microbial activity and reaction rates. In particular, to assure no limiting growing condition with regard to pH and nutrients occurred, aqueous extracts (200 mL) were set in a flask and the following solutions were added: 4.8 mL of phosphate buffer solution (KH₂PO₄ 0.062 mol/L,

Table 1 Properties of the soil studied

Property	FP	FB	CHM	CHC	AC	AS	ART
pH	4.55 \pm 0.03	6.16 \pm 0.07	8.25 \pm 0.05	8.31 \pm 0.04	8.24 \pm 0.03	7.04 \pm 0.05	7.72 \pm 0.02
Clay (g/kg dw)	82.9 \pm 4.3	1.5 \pm 0.2	49.3 \pm 6.7	4.8 \pm 0.3	497.9 \pm 63.2	67.2 \pm 10.6	90
Silt (g/kg dw)	35.9 \pm 2.2	96.6 \pm 7.8	107.8 \pm 11.0	61.6 \pm 6.8	430.1 \pm 103.7	536.3 \pm 88.0	0
Sand (g/kg dw)	881.2 \pm 34.9	901.8 \pm 45.1	842.9 \pm 22.7	933.5 \pm 34.1	71.9 \pm 14.4	396.5 \pm 32.3	880
TOC (g/kg dw)	122.4 \pm 1.2	162.7 \pm 0.6	25.1 \pm 0.7	14.4 \pm 0.1	10.9 \pm 0.1	11.0 \pm 0.2	10.4 \pm 0.0
TN (g/kg dw)	7.8 \pm 0.2	12.3 \pm 0.3	0.9 \pm 0.1	0.5 \pm 0.1	1.5 \pm 0.1	1.6 \pm 0.2	1.1 \pm 0.1
CEC (cmol(+)/kg)	26.18 \pm 0.08	51.25 \pm 4.39	3.64 \pm 0.62	4.46 \pm 0.88	18.19 \pm 1.42	12.75 \pm 0.52	11.23 \pm 0.38

Data are expressed as mean \pm standard deviation. CEC: cation exchange capacity.

K₂HPO₄ 0.125 mol/L, Na₂HPO₄·7H₂O 0.125 mol/L; pH 7.2), and 2 mL of nutritive solution (CaCl₂ 0.25 mol/L, FeCl₃ 0.9 mmol/L, and MgSO₄ 0.09 mol/L), following the standard method for BOD₅ test procedures (APHA, 1992). The solution was kept under agitation by a magnetic stirrer performing intermittent aeration: 20 min on and 30 min off. OUR was calculated by measuring the slope of the decrease of the oxygen concentration in the solution in the absence of aeration (Lasaridi and Stentiford, 1998). The degree of biodegradability was reported as the cumulative oxygen consumption during the 20 h test period (COUR₂₀) (mg O₂/(g WEOC·20 h)), and was calculated by Eq. (4):

$$\text{COUR}_{20} = \frac{V}{m_d \times \text{WEOC}} \int_{t=0}^{20} |S_t| \times dt \quad (4)$$

where, S_t (mg O₂/(L·h)) is the slope of the decrease of the oxygen concentration in the solution at time t of 20 h; V (L) is volume of the solution, m_d (g) is the weight of the dry soil, WEOC (% dw) is the water extractable organic carbon content of soil (Lasaridi and Stentiford, 1998).

2 Results

2.1 Water extractable organic carbon extraction

2.1.1 Soil/water ratio

The highest extraction ratio (1:1, W/V) used provided high WEOC concentration (mg/L) in the solution. As soil/water ratio decreased, WEOC content also decreased (Table 3). Nevertheless, the total WEOC extracted (mg/g soil dw) (Table 3) was not statistically different for soils CHM, CHC, AS, and ART obtained with different soil/water ratio. On the contrary, soils FP, FB, and

AC showed the highest total WEOC content at higher soil/water ratios (Table 3). This was more evident for the 1:5 soil/water ratio, and it was still statistically different at 1:1 ratio. Furthermore, WEOC of soil FB was statistically significant only at 1:5 ratio with respect to 1:2 ratio. As 1:5 ratio was determined to be an excessive dilution of the soluble carbon which would have compromised the biodegradability test (weak signal) (data not reported), the 1:2 soil/water ratio extraction was considered for the following experiments (Section 2.3).

2.1.2 Extraction time

The soils analysed showed different trends of WEOC content when different extraction times were used (Table 4). In particular, two different trends were observed: (1) extraction time did not affect WEOC yields for FB, CHM, CHC, AC, and AS soils; (2) for soils FP and ART, as extraction time increased, WEOC yield decreased. For the latter soils, a biological degradation of WEOC can be excluded as HgCl was added to the solution to prevent this.

2.2 Determination of WSOC in soil

WSOC content (Table 5) tends to be proportional to the TOC soil content suggesting that the WSOC production and concentration are determined primarily by the amount of organic carbon present in the soil (Guggenberg *et al.*, 1994; Møller *et al.*, 1999; Chantigny, 2003; Zsolnay, 2003). The good correlation found for WSOC vs. TOC confirmed this conclusion ($r = 0.89$; $P < 0.05$).

However, soil ART had a similar TOC content to the other mineral soils resulting in a high WSOC content. This anomaly can be explained by the fact that soil ART was an artificial soil amended with compost resulting in

Table 3 WEOC yield by different extraction ratios at room temperature for 30 min

Soil	Soil:water	WEOC (mg/L)	WEOC (mg/g soil dw)
FP	1:1	735.64 ± 63.46 c	0.731 ± 0.063 a
	1:2	389.53 ± 3.06 b	0.771 ± 0.006 ab
	1:5	184.59 ± 5.10 a	0.914 ± 0.025 b
FB	1:1	663.33 ± 10.54 c	0.790 ± 0.013 a
	1:2	395.02 ± 3.51 b	0.790 ± 0.007 a
	1:5	189.92 ± 1.56 a	0.909 ± 0.008 b
CHM	1:1	59.53 ± 1.98 c	0.060 ± 0.002 a
	1:2	30.23 ± 0.07 b	0.060 ± 0.006 a
	1:5	12.30 ± 0.10 a	0.061 ± 0.000 a
CHC	1:1	63.72 ± 1.58 c	0.064 ± 0.002 a
	1:2	35.54 ± 4.41 b	0.071 ± 0.009 a
	1:5	12.30 ± 1.58 a	0.061 ± 0.008 a
AC	1:1	92.06 ± 1.84 c	0.092 ± 0.002 a
	1:2	53.38 ± 3.95 b	0.116 ± 0.008 ab
	1:5	20.82 ± 1.34 a	0.141 ± 0.010 b
AS	1:1	58.52 ± 8.08 c	0.058 ± 0.008 a
	1:2	34.45 ± 2.35 b	0.070 ± 0.005 a
	1:5	15.52 ± 0.12 a	0.078 ± 0.000 a
ART	1:1	485.26 ± 5.43 c	0.486 ± 0.005 a
	1:2	238.67 ± 17.37 b	0.433 ± 0.034 a
	1:5	117.78 ± 17.92 a	0.451 ± 0.005 a

Data are expressed as mean ± standard deviation. Values of the same column for the same soil followed by different letters are statistically different ($P < 0.05$ Tukey test).

Table 4 WEOC yield obtained on soil/water ratio of 1:2 and different extraction time at room temperature

Soil	Extraction time (min)	WEOC (mg/L)
FP	30	383.65 ± 2.09 b
	60	52.72 ± 18.89 a
	120	34.73 ± 7.15 a
FB	30	420.07 ± 6.92 a
	60	440.71 ± 3.55 a
	120	420.17 ± 3.19 a
CHM	30	34.94 ± 1.49 a
	60	30.09 ± 1.78 a
	120	26.51 ± 2.09 a
CHC	30	35.44 ± 2.06 a
	60	33.27 ± 1.97 a
	120	30.48 ± 2.41 a
AC	30	45.65 ± 0.00 a
	60	44.67 ± 0.65 a
	120	45.90 ± 0.80 a
AS	30	33.37 ± 1.94 a
	60	30.88 ± 2.78 a
	120	35.70 ± 1.11 a
ART	30	248.22 ± 4.84 b
	60	255.37 ± 5.24 b
	120	95.79 ± 2.78 a

Data are expressed as mean ± standard deviation. Values of the same column for the same soil followed by different letters are statistically different ($P < 0.05$ Tukey test).

Table 5 WEOC experimental data and derived WSOC and k_s

Soil	WEOC (mg/g soil dw)	WSOC (mg/g soil dw)	k_s^* (L/g)	k_s (L/g)
FP	0.771 ± 0.006 a	0.945 ± 0.116 a	0.000381 ± 0.000293	0.016**
FB	0.790 ± 0.007 a	1.049 ± 0.163 b	-0.000542 ± 0.000402	ND
CHM	0.060 ± 0.006 a	0.050 ± 0.010 a	0.000192 ± 0.000465	ND
CHC	0.071 ± 0.009 a	0.066 ± 0.013 a	-0.000580 ± -0.000025	ND
AC	0.116 ± 0.008 a	0.121 ± 0.033 a	0.000118 ± 0.000063	ND
AS	0.070 ± 0.005 a	0.084 ± 0.008 a	0.000435 ± 0.00002	ND
ART	0.433 ± 0.034 a	0.499 ± 0.101 a	0.000192 ± 0.000465	0.003***
Wetland soil (S1)		2.858***	ND	0.130***
A horizon of upland soil (S2)		0.571***	ND	0.030***
AB horizon of upland soil (S3)		0.242***	ND	0.012***
River bottom sediment (S4)		0.247***	ND	0.005***

Data are expressed as mean ± standard deviation; values of the same line followed by different letters are statistically different ($P < 0.05$, Tukey test); * k_s was calculated after 30 min of extraction; ** k_s was calculated after 120 min of extraction; *** WSOC and k_s were calculated after 24 h of extraction (Tao and Lin, 2000); ND: not determined.

a high water soluble C availability. The exclusion of this soil considerably increased the correlation coefficient ($r = 0.98$; $P < 0.05$).

2.3 Water extractable organic carbon: biodegradability degree

Respirometric tests conducted for 20 h produced a typical trend as reported in Fig. 1.

OUR showed a microbial growth-associated kinetic in the first part of the curve (Stenström *et al.*, 2001). After reaching a peak, the OUR decreased quickly and became almost constant (D'Imporzano and Adani, 2007) (Fig. 1). The OUR cumulated for 20 h (COUR₂₀) represents the biodegradability degree for WEOC extracted from all soils (Table 6). From a quantitative point of view COUR₂₀ (reported as mg O₂/(g soil dw·20 h)) reflected TOC content as confirmed by their correlation coefficient ($r = 0.88$, $P < 0.05$), i.e., a higher TOC contents mean a higher COUR₂₀.

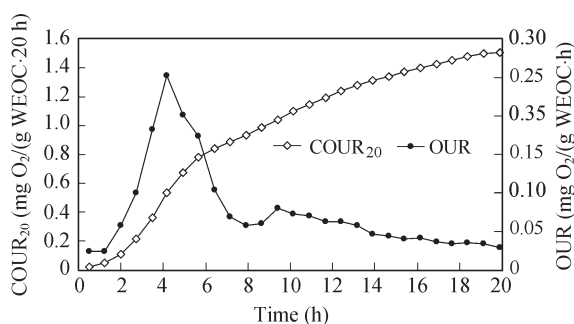


Fig. 1 Typical trend of oxygen uptake rate (OUR) and of cumulative OUR (COUR₂₀) during the biodegradation test: soil FP.

Table 6 Biodegradability degree determined by respirometric test

Soil	COUR ₂₀ (mg O ₂ /(g soil dw·20 h))	COUR ₂₀ (mg O ₂ /(g WEOC·20 h))
FP	90 ± 12 f	1.52 ± 0.04 b
FB	146 ± 6 g	2.16 ± 0.05 c
CHM	2 ± 1 b	6.75 ± 1.28 e
CHC	0 ± 0 a	0 ± 0 a
AC	9 ± 1 d	6.92 ± 0.66 e
AS	3 ± 0 c	7.19 ± 0.53 e
ART	74 ± 14 e	3.74 ± 0.66 d

Data are expressed as mean ± standard deviation. Values of the same column followed by different letters are statistically different ($P < 0.05$, Tukey test).

3 Discussion

WEOC represents the extracted part of WSOC as part of this fraction remains linked to the mineral soil fraction (Tao and Lin, 2000). On the other hand, the WSOC can be estimated starting from WEOC (Table 4) (Tao and Lin, 2000). Result obtained showed that WEOC was obtained from soil, after water-extraction for 30 min adopting a soil/water ratio of 1:2 (W/V). On the other hand, for soil FB, WEOC was not completely extracted and a higher soil/water ratio was necessary. As 1:5 ratio was determined to be an excessive dilution of the soluble carbon which would have compromised the successive biodegradability test (weak signal) (data not reported), the 1:2 soil/water ratio extraction was kept, also, for this soil. We are aware that this choice could underestimate 15% of the total WEOC content for soil FB. It should be considered in the future to test more soil/water ratios.

WSOC data calculated using Eqs. (1)–(3), were very similar to those of WEOC (Table 5). As consequence of that the sorption constants (k_s) calculated for soils studied (Eq. (5)) were close to zero (Table 5). As k_s represents the ratio between the soluble C adsorbed on soil particles (WSOC – WEOC) and the soluble C in water (WEOC) (Tao and Lin, 2000), k_s close to 0 (Table 5) indicates that WSOC was completely extracted.

$$k_s = \frac{\text{WSOC} - \text{WEOC}}{\text{WEOC}} \quad (5)$$

The k_s values calculated were two to three magnitudes lower than those reported by Tao and Lin (2000) (Table 5). Tao and Lin (2000) calculated k_s at the equilibrium after 24 h of extraction, therefore using longer extraction time than those used in the present study (30 min). It is likely that longer extraction time determined sorption phenomena on soil particles and the resulting the increase in k_s values (Gjetterman, 2005; Gjetterman *et al.*, 2007). Results obtained for soils FP and ART seemed to confirm this hypothesis as lower WEOC was recovered as longer extraction-time was used.

In order to check these effects, k_s was re-calculated for soils FP and ART using Eq. (3) and the data were obtained after 120 min of extraction. We assumed that the WSOC equals to the WEOC obtained after 30 min of extraction, because k_s was close to zero (Table 5). We also computed

the experimental WEOC data obtained after 120 min of extraction (Table 5). The k_s increased (Table 5) for both soils becoming similar (of the same order of magnitude) to the data reported by Tao and Lin (2000). Therefore, we can conclude that the use of short extraction times allows all water soluble C to remain in solution preventing sorption phenomena. As a consequence, the WSOC calculated by Eq. (2) was similar to WEOC (Table 5). The extraction of the WSOC (with the exception of soil FB) is very useful as it allowed us to perform the biodegradability test on the total amount of water soluble C, getting a more reliable result.

Biodegradability test confirm the finding that WSOC biodegradability depends on the both land use, e.g., higher biodegradability for forest soils than for agricultural soils, and management practises, e.g., higher biodegradability for soil treated with compost than for soil treated with sludge, and lower biodegradability for contaminated soils than for agricultural soils (Chantigny, 2003) (Table 6).

More interesting was the effect of the soil composition on the degree of biodegradability (COUR₂₀ as mg O₂/(g soil dw·20 h)). First of all, COUR₂₀ of forestal WSOC was lower than agricultural and artificial soils because of the different plant residues on soil (Chantigny, 2003). Litter from tree canopy forest (e.g., FP and FB soils) contains more recalcitrant molecules (lignin, tannins, and phenols) than agricultural crop residues (Kuiters and Sarink, 1986; Kuiters and Denneman, 1987; Chantigny, 2003). The latter has a higher content of labile molecules (e.g., carbohydrates and aminoacids) that can be easily degraded by microorganisms (Delprat *et al.*, 1997; Leinweber *et al.*, 2001; Kalbitz *et al.*, 2003).

Forest soils (FP and FB) showed differences in the WSOC biodegradability as well (Table 6). Several authors have reported a higher content of recalcitrant fraction (e.g., phenolic acid, hydrophobic aromatic compounds) for pinetree litter (FP) than for hardwood forest litter (FB) (Kuiters and Sarink, 1986; Kuiters and Denneman, 1987) which contains more easily degradable, hydrophilic, low molecular weight compounds (e.g., sugars, amino acids and aliphatic acids) (Kiikkilä *et al.*, 2006).

Finally, WSOC biodegradability of contaminated soils was also different. The CHM soil showed a biodegradability value similar to the agricultural soil AS. This result indicated that heavy metal did not affect microbial activity probably because high soil pH kept heavy metal in an insoluble forms (Table 1). However, the HCH soil did not show any respirometric activity as a consequence of the presence of organic contaminants (Table 2) that probably inhibited microbial activity.

4 Conclusions

Measuring the liquid oxygen uptake rate (OUR) of the microorganisms to degrade WSOC under standardized condition have been shown to be useful as biodegradability test. Results obtained showed that biodegradability is affected by the total WSOC content in addition to soil management, land use and the presence of contaminants.

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