Soil Organic Matter and its Stability in Aerobic and Anaerobic Conditions

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Abstract: In eight samples of organic and organomineral surface horizons we studied the stability of soil organic matter in aerobic and anaerobic conditions expressed by the rate constant of its biochemical oxidation, total biochemical oxygen demand, substrate production of methane and degradability in anaerobic conditions. In the eight very different samples no relationship was found between aerobic and anaerobic stability of their organic matter; nor was the expected relationship between total biochemical oxygen demand and "active carbon" C_{hws} proved. Methods of determination are described.

Keywords: soils; organic matter; stability; aerobic and anaerobic conditions

Soil organic matter (SOM) is mostly described only by the value C_{ox} although it is generally known that C_{ox} also expresses primary organic matter of low ion exchange capacity and high tendency of mineralisation as humified organic matter with just opposite characteristics (Kolář & Kužel 1999). Therefore it is a progressive approach if researchers give other characteristics of soil organic matter: degree of humification $D_H = C_{ox\,HA} + C_{ox\,FA}/C_{ox} \times 100$, HA:FA ratio, "active carbon" C_{hws}, C_{cws} (water-soluble C-matters at 20°C). Very appreciable is the separation of humic acid fractions by gel chromatography and the expression of percentage proportions of fractions with average relative molecular weight – it is to note that low-molecular humic acids have chemical reactions identical to those of fulvic acids but completely different from high-molecular HA. The colour quotient $Q_{4/6}$ is very easy to determine but it is rather a problematic variable, similarly like the interpretation of IR spectra of humic acids that may seem clear and simple only to researchers with smaller knowledge of IR spectroscopy, in spite of the fact that this method has been applied several times recently (Capriel *et al.* 1995; Capriel 1997).

The humified part of SOM can be described satisfactorily by ion exchange reactions. A description of the reactive, decomposable part of primary organic matter is more problematic. It is a material for mineralisation and a source of energy for soil microorganisms; it is also necessary to mention its function of an active reagent in the system of chemical and biochemical reactions in soils. The

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efforts to describe it were aimed at determination of the water-soluble portion of soil organic matter in water or salt solutions of various concentrations and temperatures (Schulz 1990; Körschens *et al*. 1990; Weigel *et al*. 1998). In many papers this portion of SOM is described by the level of its stability against oxidation, using e.g. differently modified respirometric tests. At the University of South Bohemia in České Budějovice we worked on the development of a method to determine the kinetics of mineralisation of SOM degradable part and we tried to express the stability of this fraction by the value of the rate constant of its biochemical oxidation (Kolář *et al*. 2003); we wanted to reduce the labour and time consumption of experimental works by the use of expeditious but relatively more costly photometric tests of Merck company (Kolář *et al*. 2005a). In order to simplify experimental works and to cut their costs we evaluated the lability of SOM degradable part by the ratio of biochemical oxygen demand to total chemical oxygen demand (BOD_5:CHOD), which is used for the evaluation of the level of organic matter stability in hydrochemical analytics (Štindl *et al*. 2005). But this method was not found suitable for SOM evaluation because the values of BOD_5 were too low for SOM and the relative experimental error was too high.

The stability of any organic matter, i.e. also of SOM, is somewhat different in aerobic conditions when mineralisation takes place, and in anaerobic conditions when anaerobic digestion takes place with production of biogas, in which methane is a dominant component. We proposed a method to evaluate organic matter degradability in anaerobic conditions for the needs of anaerobic digestion and biogas production (Kolář *et al*. 2005b).

The same amount of oxygen as for the oxidation of an original substrate is consumed for total oxidation of methane, the product of organic matter decomposition under anaerobic conditions.

Average oxidation number (AOXN) of carbon with the limit values +4 for CO_2 and –4 for CH_4 is the measure of an oxidation degree of organic matter. The lower its value, the higher the methane yield. It is proportionate to the theoretical chemical oxygen demand CHOD for the amount of organic carbon:

$$AOXN = 4 - 1.5\ CHOD/C_{org}$$

Obviously, CHOD of organic substrate = CHOD of methane. But the actual yield of methane is lower because CHOD also involves the biologically undegradable part of CHOD and a part of substrate CHOD is consumed for the growth of new biomass.

The theoretical yield of methane, expressed as the weight amount of CH_4 per unit weight of substrate, is calculated from the relation:

$$Y_{CH_4\ theor} = 0.25\ CHOD \qquad (g/g)$$

or as the methane volume per unit weight of substrate under standard conditions, i.e. 0°C and pressure 101.3 kPa:

$$Y_{CH_4\ theor} = 0.35\ CHOD \qquad (l/g)$$

Straka *et al.* (2003) published conversion coefficients of the units of CH_4 and CHOD:

1 mole CH_4	2 moles O_2; 64 g CHOD; 22.4 l
1 g CHOD	0.25 g CH_4; 0.35 l CH_4
1 g CH_4	4 g CHOD; 1.4 l
1 l CH_4	2.857 CHOD

In the course of anaerobic decomposition of organic matters not only CH_4 but also CO_2, i.e. biogas, is produced. The theoretical concentration of CH_4 in produced biogas is calculated from this formula:

$$CH_4 = 18.75\ CHOD/C_{org} \qquad (\%)$$

But the actual concentration of CH_4 in biogas is different – CO_2 is dissolved and bound in the liquid phase of fermenting mixture while CH_4 is not practically soluble in water. Nitrogen and sulphur compounds in organic matter decrease the quantity of electrons for the production of methane, reducing its yield. To adjust the methane yield it is necessary to subtract from total CHOD the substrate CHOD consumed for the reduction of nitrogen and sulphur:

$$Y_{CH_4\ theor} = 0.25\ [CHOD - (CHOD)_N - (CHOD)_S]\ (g/g)$$

or

$$Y_{CH_4\ theor} = 0.35\ [CHOD - (CHOD)_N - (CHOD)_S]\ (l/g)$$

where:

$(CHOD)_N - 2.86\ (N_{NO_2} + N_{NO_3})$ (g O_2)

$(CHOD)_S - 2\ (S_{total})$ (g O_2)

To determine the anaerobic degradability of organic matters the tested material can be defined by

its content of C_{org} or by its specific CHOD in g/g. Degradability is expressed as the amount of C_{org} transformed into biogas out of the total amount of C_{org} and/or out of the total weight amount of the tested sample. The conditions of degradability test are set down by the standard ISO CD 11734. The standard defines conditions for the ultimate anaerobic degradation, i.e. such degradation that the resultant products of degradation will be CH_4, CO_2, salts and new biomass. A source of the inoculum, i.e. functional anaerobic biomass, is either sludge of anaerobic stabilisation tanks or anaerobic digesters with suspension biomass. However, the inoculum must have the lowest possible own production of gas (endogenous production).

The substrate production of biogas or CH_4 (V_{BPS}, V_{CH_4S}) is calculated by subtracting the volume of endogenous production from the volume of produced biogas or CH_4.

This substrate production indicates anaerobic degradability of an organic matter sample compared to the theoretical substrate production that equals the theoretical yield of methane

$$Y_{CH_4\ theor} = 0.35\ CHOD \qquad (l/g)$$

that is designated for real conditions (101.3 kPa, 35°C) as theoretical substrate production of TV_{CH_4S}:

$$TV_{CH_4S} = 0.31\ CHOD \qquad (l/g)$$

Then anaerobic degradability of organic matter D_{CHOD} is given by the relation:

$$D_{CHOD} = V_{CH_4S}/TV_{CH_4S} \times 100 \qquad (\%)$$

If we use an apparatus in which it is problematic to determine biogas composition, the evaluation can be done according to C_{org}. Degradability is computed from the ratio of carbon in the gaseous phase C_g at the end of the test to organic carbon of substrate C_s at the beginning of the test. Because 1 mole CO_2 and 1 mole CH_4 contain 12 g of carbon, the carbon content in grams of C_g in the given volume of biogas is expressed by the relation:

$$C_g = 12\ p\ V_{BPS}/RT$$

where:

p – pressure (Pa)

V_{BPS} – biogas production of substrate (m^3)

R – gas constant 8.134 J/mol °K

T – temperature (°K)

(For the conversion from Pa to millibars and from m^3 to litres the conversion coefficient is 0.1).

Degradability D_c is given by the relation:

$$D_c = C_g/C_s \times 100 \qquad (\%)$$

This expression of degradability bears an error as a result of neglecting biomass formation and due to the amount of CH_4 and mainly of CO_2 dissolved in the liquid phase. It is possible to get a more exact value by adding the increment of inorganic carbon at the end of the test to C_g.

MATERIAL AND METHODS

Measurements in aerobic conditions

In 2003 we proposed and tested a method to evaluate the kinetics of mineralisation of a degradable portion of soil organic matter by the vacuum measurement of biochemical oxygen demand (BOD) of soil suspensions in an Oxi Top Control system of WTW Merck Company, designed for the hydrochemical analysis of organically polluted waters (Kolář *et al.* 2003). The measurements will provide BOD at the particular days of incubations; this data will be used to determine total limit BOD_t and to calculate the rate constant K of biochemical oxidation of soil organic matters (per 24 h) as the measure of stability of these matters. The dilution method (Horáková *et al.* 1989) is a traditional method of measuring BOD and also rate constants. This method was applied to determine the stability of soil organic matters but its time and labour consumption was too high. Therefore we introduced the Oxi Top Control method based on measurements of the vacuum in flasks equipped with heads with displays of infrared interface facilitating the communication with OC 100 and/or OC 110 controller; documentation is carried out by ACHAT OC programme run in a PC, formerly through a TD 100 thermoprinter. The measuring heads will store up to 360 data records in their memory that can be represented graphically by the controller; it is also possible to measure through the glass or plastic door of the flask thermostat on shaking boards.

The rate of biochemical oxidation of organic matters as the reaction of degree 1 is proportionate to the residual concentration of matters that have not been oxidised yet:

$dy/dt = K_1 (L - y) = K_1 L_r$

where:
L – total BOD
y – BOD at time t
L_r – residual BOD
k_1, K_1 – rate constants

By the integration of this relation from 0 to t we get the equation:

$L_r = L \times e^{-K_1 t} = L \times 10^{-k_1 t}$

In general for BOD at time t it holds good:

$y = L (1 - 10^{-k_1 t})$

where:
y – BOD at time t
L – BOD_{total}
k_1 – rate constant (24 h^{-1})

The rate constant k_1 may have markedly different values. For pure glucose k_1 = 0.87 per day and for peptone k_1 = 0.74 per day. Organic matters in municipal sewage water have k_1 = 0.1–0.19 per day, soil organic matters k_1 = 0.1–0.01 per day, exceptionally 0.001. (The extremely low values of rate constants of SOM biochemical oxidation are caused by the unusually fast oxidation of available and very labile organic matters by the variegated and numerous soil microflora; when the soil samples are taken, there remains only a slowly hydrolysing residue of labile organic matters and so their stability is incomparably higher than e.g. in organic matters of sewage water).

Our working procedure is identical with the method of measurement recommended by the manufacturer according to the Proposal for German Uniform Procedures DEV 46, Bulletin – H 55, which was also published in a handbook of BOD (on CD-ROM) of WTW Merck Company. Fresh samples of the original moisture content (dry matter was determined parallelly at 105°C) were only mechanically disintegrated in distilled water, filtered through a 1mm sieve, and a soil suspension was prepared of such a concentration that the assumed value of BOD would correspond to 30–50% of CHOD and in an undiluted form it would correspond to BOD 0–40 mg O_2/l. Using the dilution factors 2, 5, 10, 20, 50 to 100 in the apparatus it is possible to measure BOD up to 4000 mg O_2/l. The pH value of soil suspension being lower than pH = 6, the suspension was neutralised with 0.1M NaOH to pH = 6. To promote the activity of microorganisms and to suppress nitrification the BOD nutrient mixture with allylthiourea of MERCK Company, cat. No. 100688, was added. We inoculated with an addition of 2 vol. % of aerobic inoculum from an aerobic digester with soil. The inhibition of nitrification can also be done with the nitrification inhibitor NTH 600, which is a part of the kit. Sample incubation was carried out in an Oxi Top Box thermostat at 20°C. The settlement of soil suspensions in Oxi Top IS 6 sample flasks is prevented by agitating trays IS 6.

Measurements in anaerobic conditions

We used an Oxi Top Control AN 12 Merck apparatus that differed from the apparatus for work in aerobic conditions in fermentation flasks with two side tubes and different pressure measuring heads of these flasks. The methodical procedure was described in detail in a preceding paper (Kolář *et al.* 2005b). The sample amount was 80–100 mg/l of organic carbon, the inoculum concentration was 3 g/l.

For the computation we used the equation of state:

$n = p \times V/RT$

where:
n – number of gas moles
V – volume (ml)
p – pressure (hPa)
T – temperature (°K)
R – gas constant 8.134 J/mol °K

and we calculated CO_2 and CH_4 moles in the gaseous phase of fermentation flasks:

$n_{CO_2 \text{ g } CH_4} = (\Delta p \times V_g/RT) \times 10^{-4}$

where:
$\Delta p = p_1 - p_0$
p_0 = the initial pressure

The fermentation at 35°C and continuous agitating of flasks in the thermostat takes 60 days, the pressure range of measuring heads is 500–1350 kPa and the time interval of the measuring of pressure changes is 4.5 min. Anaerobic fermentation is terminated by an injection of 1 ml of 19% HCl with syringe through the flask rubber stopper into the substrate. The acidification will displace CO_2 from the liquid phase of the fermentation flask.

The process is terminated after 4 h. The number of CO_2 moles is calculated from the liquid phase:

$$n_{CO_2\,l} = \{[p_2\,(V_g - V_{HCl}) - p_1 \times V_g]/RT\} \times 10^{-4}$$

It is followed by an injection of 1 ml of 30% KOH into the rubber tank in the second tube of fermentation flask. After 24 h the sorption of CO_2 from the liquid phase of the flask is terminated and from the pressure drop in the flask the total number of CO_2 moles in the gaseous and liquid phase is calculated:

$$n_{CO_2\,l,\,CO_2\,g} = \{[p_3\,(V_g - V_{HCl} - V_{KOH}) - p_2\,(V_g - V_{HCl})]/RT\} \times 10^{-4}$$

where:

Δp – difference in pressures (hPa)
V_g – volume of the gas space of fermentation flask (ml)
p_1 – pressure of gases before HCl application (hPa)
p_2 – pressure of gases before KOH application (hPa)
p_3 – pressure of gases after KOH application (hPa)
R – gas constant = 8.134 J/mol °K
T – absolute temperature = 273.15 + X °C
V_{HCl} – volume of added HCl (ml)
V_{KOH} – volume of added KOH (ml)

From the results it is easy to calculate the number of CO_2 moles in the gaseous phase and by subtraction from $n_{CO_2\,g\,CH_4}$ the number of moles of produced methane:

$$n_{CH_4} = (n_{CO_2\,g\,CH_4} + n_{CO_2\,l}) - n_{CO_2\,l\,CO_2\,g}$$

The total number of moles of gases of transported carbon:

$$n_{CO_2\,g\,CH_4} + n_{CO_2\,l} = n_{total}$$

Baumann's solution A + B in deionised water of pH = 7.0 (Süssmuth *et al.* 1999) is used as a liquid medium:

A: (in 1 000 ml H_2O)	B: (in 1 000 ml H_2O)
5.44 g KH_2PO_4	2.19 g $CaCl_2 \times 6\ H_2O$
6.97 g K_2HPO_4	2.03 g $MgCl_2 \times 6\ H_2O$
10.70 g NH_4Cl	0.4 g $FeCl_2 \times 4\ H_2O$
	6.3 mg $MnCl_2$
	1.0 mg $ZnCl_2$
	0.6 mg $CuCl_2$
	0.2 mg $Na_2MoO_4 \times 2\ H_2O$
	12.2 mg $Co(NO_3)_2 \times 6\ H_2O$
	1.0 mg $NiCl_2 \times 6\ H_2O$
	1.0 mg Na_2SeO_3

A standard addition of inoculum accounts for about 0.3% by volume (aqueous sludge from the anaerobic tank of digester). Instead of Baumann's solution it is possible to use ready-made nutrient salt of MERCK Company for this apparatus.

For our study we used SOM of organic and organomineral surface diagnostic horizons according to Němeček (2001) with the content of C_{ox} > 12–18% by weight. Eight samples were diagnosed in this way:

Sample	Horizon	Horizon specification
1	Horizons of the forest floor of forest soils, anhydrogenous	(Fermentation) amphigenous horizon of detritus F_a
2	Horizons of the forest floor of forest soils, anhydrogenous	Residual horizon of mull H_r
3	Horizons of the forest floor of forest soils, hydrogenous	Fibric horizon O_f
4	Horizons of the forest floor of forest soils, hydrogenous	Humus horizon O_h
5	Horizons of the forest floor of forest soils, peaty	Mesic horizon T_m
6	Horizons of the forest floor of forest soils, peaty	Sapric horizon T_s
7	Organomineral surface hydrogenous horizon, humic	Peatified (anmoor) A_t
8	Organomineral surface cultural horizon, humic	Turfy A_d

RESULTS AND DISCUSSION

The values in Table 1 show that in spite of high C_{ox} the degree of humification is in general very low in all samples, correlating with relatively low values of the ion exchange capacity *T*, which exceptionally in samples of T_s, A_t and A_d reaches the values typical of medium-heavy soils although they were taken from humus horizons. The low bulk density indicates that these are mostly undecomposed organic materials that form the essential part of these samples. The diagnostic traits were less marked in some samples, and so it was difficult to determine an organic horizon exactly. But we did not exclude these samples because this problem did not influence the objective of the study. The quality of organic matter of these samples was sufficiently defined by data in Table 1.

Table 2 shows the values of active carbon C_{hws} (Körschens *et al.* 1990), i.e. carbon of water-soluble C-matters at the boiling temperature, and the values of CHOD in the used suspensions of samples as the measured values of C_{ox} cannot be used because the character of oxidation is similar but it differs according to dilution, acid concentration and oxidation time (Horáková 1989). Table 2 also documents the value of total BOD_t, i.e. over 20 days of incubation. It corresponds to the biochemically oxidisable part of sample SOM. Obviously, these values measured in the same suspensions are very low compared to CHOD. The ratio of BOD : CHOD in easily degradable matters is usually 0.5–0.8; if it is below 0.1, these matters are hardly degradable. The level of their stability in relation to oxidation, and/or their lability, is given by the value of the rate constant of such biochemical oxidation. The results show that the value of C_{hws} does not correspond to the value of BOD_t, and that it is far from corresponding to the values of the rate constants of biochemical oxidation although both variables, C_{hws} and BOD_t, are mainly determined by water-soluble C-matters. But

Table 1. Characteristics of the samples of organic and organomineral surface horizons (oxidisable carbon C_{ox}, degree of humification D_H, ion exchange capacity *T* and reduced bulk density O_r)

$D_H = C_{ox\ HA+FA}/C_{ox\ total} \times 100$

Sample	C_{ox} (%)	D_H (%)	*T* (mgekv/kg)	O_r (g/cm^3)
F_a	16.4	1.5	68	0.55
H_r	17.8	4.8	75	0.71
O_f	12.7	3.5	51	0.48
O_h	15.1	11.1	97	0.69
T_m	18.4	8.4	110	0.12
T_s	20.1	14.2	148	0.35
A_t	9.8	9.7	143	1.04
A_d	11.0	15.2	172	0.92

Table 2. Content of active carbon C_{hws} in the samples of organic and organomineral surface horizons, chemical oxygen demand CHOD and total biochemical oxygen demand BOD_t, BOD_t:CHOD ratio and rate constant *K* of biochemical oxidation

	F_a	H_r	O_f	O_h	T_m	T_s	A_t	A_d
C_{hws} (g/1000 g)	0.72	0.43	0.25	0.29	0.51	0.30	0.18	0.44
CHOD (g O_2/g DM)	1.40	1.16	1.28	0.85	1.20	1.01	0.96	1.22
BOD_t (g O_2/g DM)	0.23	0.29	0.16	0.17	0.30	0.20	0.16	0.31
BOD_t:CHOD	0.16	0.25	0.12	0.20	0.25	0.20	0.17	0.25
K (24 h)	0.0352	0.0224	0.0135	0.0018	0.0090	0.0107	0.0282	0.0495

Table 3. Theoretical substrate production of methane TV_{CH_4S}, substrate production of methane C_{CH_4S}, anaerobic degradability of organic matter D_{CHOD} in samples of organic and organomineral surface horizons. Calculated values $(D_{CHOD})_{mol}$ and difference $D_{CHOD} - (D_{CHOD})_{mol}$

	F_a	H_r	O_f	O_h	T_m	T_s	A_t	A_d
TV_{CH_4S} (l/g) (101.3 kPa, 35°C)	0.43	0.36	0.40	0.26	0.37	0.31	0.30	0.38
V_{CH_4S} (l/g)	0.0206	0.0074	0.0087	0.0031	0.0064	0.0019	0.0043	0.0028
D_{CHOD} (%)	4.81	2.05	2.17	1.20	1.74	0.60	1.45	0.75
$(D_{CHOD})_{mol}$	4.80	2.03	2.14	1.18	1.75	0.59	1.43	0.75
$D_{CHOD} - (D_{CHOD})_{mol}$	0.01	0.02	0.03	0.02	0.03	0.01	0.02	0.00

the kinetics of their oxidation is so different that it dominates the common material basis of both analytical determinations. In BOD_t hydrolases of the inoculum and of newly formed microbial biomass are probably active in a very different way.

The correlation analysis at $n = 16$ and on a significance level $\alpha = 0.05$ was done for the mathematical and statistical evaluation of the relationship between two variables, i.e. average values of BOD_t and active carbon C_{hws}, and of the values determining the aerobic and anaerobic stability of soil organic matter, i.e. means of the rate constant of biochemical oxidation K and anaerobic degradability D_{CHOD}. The critical value α_{crit} is still too high to prove a statistically significant correlation. Therefore the results are not presented, and we will try to prove the correlation of these variables after we have collected a higher number of data pairs (Eckschlager *et al*. 1980).

Table 3 shows the results of degradability of organic matter of samples in anaerobic conditions. It documents theoretical substrate production of methane TV_{CH_4S} derived from CHOD and substrate production of methane V_{CH_4S} calculated from the measured production of CH_4 in the test apparatus Oxi Top Control AN 12 Merck in accordance with ISO CD 11734 after subtraction of endogenous production of CH_4 by the inoculum added to test flasks. Anaerobic degradability of organic matter of samples of organic and organomineral horizons is expressed in % as D_{CHOD}. So we transferred our investigation to comfortable work with CHOD, but as mentioned above in the theoretical part, even if the Oxi Top Control AN 12 Merck apparatus is not available, it is possible to determine organic matter degradability in anaerobic conditions, though less exactly, from the ratio of carbon in the produced volume of biogas in an alternative apparatus to the organic carbon content of the sample at the beginning of the test. Because in these alternative apparatuses it is not usually possible to determine the numbers of CO_2 moles in the gaseous and liquid phase and numbers of CH_4 moles, and of these values the total number of moles of transformed carbon, we work only with the substrate production of biogas V_{BPS}. The results are only approximate, and it is necessary to decide whether the orientation values are acceptable with regard to considerable acceleration of work.

The last value $(D_{CHOD})_{mol}$ in Table 3 is the value that was not derived from the methane volume at 101.3 kPa and 35°C but from the calculated numbers of produced CH_4 moles converted to CHOD. The difference between this value and D_{CHOD} shows the existence of some differences but the error is negligible.

The values in Tables 2 and 3 document an important fact that there is no relationship between aerobic and anaerobic degradability of SOM of organic and organomineral soil horizons, which we did not assume at the beginning of our study.

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