

The use of Spectroquant Merck BOD photometric test to evaluate the stability of organic matters in soil

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ABSTRACT

We proposed a new method to determine the rate constant of biochemical oxidation of soil organic matters that makes it possible to evaluate their stability by Spectroquant Merck BOD photometric tests; if smaller samplings are used and individual determinations are performed, it can replace the method of BOD vacuum measuring by an Oxi Top Control Merck system. The time and labour consumption of this vacuum method is sometimes criticised, but it has demonstrable work advantages for a large series of samples and so it is still recommended. The two methods are identical as for the correctness and coincidence of results and they do not differ by either lower or higher results and therefore we recommend the new method with photometric tests for smaller series of samples. For the high time and labour consumption we must warn against the use of traditional BOD dilution method with volumetric determination of oxygen for the determination of rate constants.

Keywords: soils; organic matters; analysis; lability of organic matters; photometric method; comparison of methods

In 2003 we proposed a method to evaluate the kinetics of mineralisation of the decomposable fraction of soil organic matter that can be applied to a more detailed description of the lability and/or stability of organic matter fractions in the soil, particularly "active organic carbon" (Kubát et al. 1999) expressed as hot water extractable carbon C_{hws} (Schulz 1990, Körschens et al. 1990, Weigel et al. 1998), or to evaluate the stability of non-fractionated soil organic matter by the method of vacuum measurement of biochemical oxygen demand (BOD) of soil suspensions in an Oxi Top Control system of WTW Merck Company that was designed for hydrochemical analyses of organically contaminated waters (Kolář et al. 2003). These measurements will provide BOD data on the particular days of incubation; total limit BOD_t and the rate constant k of biochemical oxidation of soil organic matters with the dimension (per 24 hour) (Kolář and Kužel 2003) as a measure of stability of these matters can be calculated from these data.

Dilution method (Horáková et al. 1989) is a traditional method of measuring BOD and rate constants. We applied this method to determine the stability of soil organic matters, but it was too time, and labour consuming for extensive

determinations (Kolář et al. 2002). Therefore we introduced the Oxi Top Control method based on measurements of vacuum in flasks with heads with displays of the infrared interface facilitating communication with controller OC 100 and/or OC 110; the PC program ACHAT OC, formerly through a connection with thermoprinter TD 100, carries out the documentation. The measuring head stores up to 360 data records in its memory that can be represented graphically by the controller; it is possible to measure through the glass or plastic door of the flask thermostat directly on shaking boards. We were enchanted by the application of this method. Nevertheless, critical remarks of especially the users that do not analyse any large series of samples were made concerning the head tightness, complicated evaluation in the combination controller – PC, losses and failure rate.

This is the reason why we proposed an identically rapid, feasible and relatively cheap method for the same purpose that is to be used for these limited series of samples and is based on the use of Spectroquant Merck photometric tests. These are sophisticated testing kits of photometric tests equipped with integrated dosers and combinations of substances that have mostly been dosed previously. The results of both methods were

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compared in this paper by mathematical and statistical methods.

MATERIAL AND METHODS

Two collection-testing samples of soils were used for the determinations: one sample with a higher content of organic matters, the other with a lower content and higher stability of these matters. These two different samples were analysed by a newly proposed method of photometric tests Spectroquant Merck BOD KT with the range of 0.5–3000 mg O₂/l (cat. no. 100687) that is based on a modification of the traditional Winkler's method. We employed BOD standard for the preparation of 10 l of standard solution (cat. no. 100718) and BOD nutrient mixture with allylthiourea for 12 l of nutrient solution (cat. no. 100688). For each determination at least 4 oxygen reaction vessels for BOD determination (cat. no. 114663) were necessary. CHOD test with the range of 10–150 or 15–300 mg O₂/l (cat. nos. 114540 and 114895) was used to determine the total amount of organic matters. These are cuvette, not reagent tests.

Measurements were carried out in a Spectroquant NOVA 60 photometer that works with 12 filters by the diode field technique (reference ray technique), is connected to a PC with the software Windows Multi Achat II for data processing and has the memory capacity for 1000 data blocks. It has an Auto Select function for differentiation of tests

according to the bar code and AQA (Analytical Quality Assurance) function.

The two tested samples of the soils were analysed by the previously described method OXI Top Control WTW Merck (Kolář et al. 2003).

The procedure of sample preparation and the conduct of the cultivation process were the same as described in the cited paper. Because the photometric measurements are not accessible if the sample turns turbid, just before the photometric measurement the sample was drawn with a syringe and before the transfer into the cuvette of the apparatus it was filtered under an N₂ screen using an Anotop MERCK membrane filter of porosity 0.45 µm directly onto the perpendicularly upwards set syringe until air bubbles were removed from the filter. The spectrophotometer has a built-in function of "turbidity correction".

The correctness of the analytic process is checked by AQA system on the basis of the auto-check of optical properties of the device (AQA 1) and of the whole system including the proper analysis (AQA 2) by help of the enclosed Memo Chip AQA and kits Spectroquant Combi Check 20, 50, 60, 80 (cat. nos. 114675, 114695, 114696, 114689 and 114738). Checks in AQA 1 mode can be carried out by the Photo Check kit for NOVA 60 photometer and some other photometers (cat. no. 114693).

The two methods were tested on two samplings of both testing soils – small sampling A (*n* = 5 samples) and large sampling B (*n* = 20 samples) (Eckschlager et al. 1980). The interval of reliability of the mean

Table 1. Characteristics of soil organic matters of collection soils S1 and S2

| | S1 | S2 |
|---|-------|-------|
| C _{org} (g/kg) | 29.6 | 23.6 |
| C _{hws} (g/kg) | 0.93 | 0.34 |
| C _{cws} (g/kg) | 0.24 | 0.08 |
| C _{inert} (g/kg) | 19.4 | 18.3 |
| C _{dec} (g/kg) | 10.2 | 5.3 |
| C _{dec} (g/kg) (Schulz) | 13.9 | 5.10 |
| Chemical oxygen demand of experimental soil suspension 5% (CHOD) (mg O ₂ /l) | 389.5 | 308.2 |
| Total biochemical oxygen demand of 5% experimental soil suspension (BOD _t) (mg O ₂ /l) | 128.5 | 61.6 |
| Five-day biochemical oxygen demand of 5% experimental soil suspension (BOD ₅) (mg O ₂ /l) | 89.9 | 18.5 |

C_{org} was determined by the method according to ISO 10694, C_{inert} from the content of particles below 6.3 µm according to Körschens (1980), C_{dec} (decomposable) from the difference between C_{org} and C_{inert}, C_{dec} (Schulz) from C_{hws} according to Schulz (1990) and Kolář et al. (2003), C_{cws} is cold-extract carbon analogical to C_{hws}

Table 2. Interval of reliability of the mean $L_{1,2}$ of the rate constant k of biochemical oxidation of organic matters (per 24 hours) for soils S1 and S2 and for samplings A ($n = 5$) and B ($n = 20$) determined by photometric method (P) and Oxi Top Control Merck method (O); the value $K_n = 0.51$ (for $\alpha = 0.05$), critical value $t_\alpha = 2.093$ (for $\alpha = 0.05$)

| | Method P | | | | Method O | | | |
|---------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| | sampling A | | sampling B | | sampling A | | sampling B | |
| | soil 1 | soil 2 |
| \bar{a} | 1.190 | 0.568 | 1.199 | 0.591 | 1.241 | 0.623 | 1.228 | 0.598 |
| R | 0.294 | 0.128 | 0.307 | 0.135 | 0.132 | 0.057 | 0.152 | 0.058 |
| $s_{\bar{a}}$ | 0.56 | 0.24 | 0.07 | 0.03 | 0.25 | 0.11 | 0.03 | 0.01 |
| $L_{1,2}$ | 1.190 ± 0.150 | 0.568 ± 0.065 | 1.199 ± 0.033 | 0.591 ± 0.014 | 1.241 ± 0.067 | 0.623 ± 0.029 | 1.228 ± 0.014 | 0.591 ± 0.005 |

for small sampling A was calculated according to Dean and Dixon:

$$L_{1,2} = \bar{a} \pm K_n \cdot R \quad \bar{a} = \text{arithmetical mean}$$

and for large sampling B according to W. Gosset (Student's t -distribution):

$$L_{1,2} = \bar{a} \pm s \cdot t_\alpha / \sqrt{n} \quad s = \text{standard deviation}$$

for significance level $\alpha = 0.05$.

We also tested the correctness of the results in a small sampling A by Lord's u_n -test and in sampling B by Student's t -test:

$$u_n = |\bar{a} - \xi| / R \cdot t = |\bar{a} - \xi| / s$$

by comparison with the critical values $u_{n\alpha}$ and t_α taken from Tables.

The coincidence of the results obtained by both methods was tested, i.e. statistical significance of the difference ($\bar{a}_A - \bar{a}_B$) in set A by Lord's u -test, in set B by Student's t -test for $n_A = n_B = n$ samples and by comparison with the critical values u_α and t_α taken from Tables.

To eliminate the influence of small efficiency of tests when the number of determinations is relatively low, Wald sequential analysis was used to determine systematic error by a comparison of the proposed photometric method with previously proposed Oxi Top Control Merck method, which we consider as a standard method in this case.

RESULTS AND DISCUSSION

Table 1 shows that soil S1 has a higher content of decomposable organic matters C_{dec} and the corresponding values of CHOD, BOD_{17} , BOD_{57} , C_{hws} and C_{cws} ; it also has a higher content of total C_{org} even though it need not be the rule.

Table 2 documents that S1 also has a nearly two-fold value of the rate constant k of biochemical oxidation; it indicates higher lability of its organic matters compared to the stability of organic matters in soil S2.

The correctness of the results of measuring values k by the photometric test compared to the previously proposed Oxi Top Control method is confirmed because the critical values at $\alpha = 0.05$ of mathematical and statistical evaluation were not exceeded as it is illustrated in Table 3; the same statement applies to the coincidence of the results (Table 4). In the small sampling ($n = 5$ samples) of soil S2 with a low value of rate constant k the value $u = 0.297$ seems to approach the critical value $u_\alpha = 0.306$. Therefore, in addition, we carried out sequential analysis and a sign test to compare the differences between both methods (Table 5).

The sign test indicates a difference between the results of the tested method and standard method by value + and value - and the ratio of the number of differences of a given sign to the total number of differences $p_{o+} = n^+/n$ and $p_{o-} = n^-/n$. In an ideal case if the determination is not loaded with a systematic error, $p_o = p_{o+} = p_{o-} = 0.5$. Because the pair of the results is a random sampling, we choose the upper and lower values p_A and p_B around the value 0.5, mostly $P_A = 0.25$ and $P_B = 0.75$. For $\alpha = \beta = 0.05$ we find the tabular values $a_1 = 3.7$ and $a_2 = 6.3$ and $b_1 = b_2 = 2.7$ for the construction of a sequential analysis

Table 3. Correctness of the results of determining the rate constant k of biochemical oxidation of organic matters for the same data as in Table 4; critical value for $\alpha = 0.05$ is $u_{n\alpha} = 0.507$ and $t_\alpha = 2.093$; the arithmetical mean \bar{a} of Oxi Top Control method was used as the basis of the value of rate constant of photometric method

| | Method P | | | |
|-------|------------|--------|------------|--------|
| | sampling A | | sampling B | |
| | soil 1 | soil 2 | soil 1 | soil 2 |
| ξ | 0.1241 | 0.623 | 1.228 | 0.598 |
| u_n | 0.173 | 0.429 | - | - |
| t | - | - | 0.414 | 0.233 |

Table 4. Coincidence of the results of determining the rate constant k of biochemical oxidation of organic matters for soils S1 and S2 and for samplings A ($n = 5$) and B ($n = 20$) by two methods P and O; critical values for $\alpha = 0.05$ are $u_\alpha = 0.306$ and $t_\alpha = 2.025$

| | Sampling A | | Sampling B | |
|---------------------------------|------------|--------|------------|--------|
| | soil 1 | soil 2 | soil 1 | soil 2 |
| $ \bar{a}_{FA} - \bar{a}_{OA} $ | 0.051 | 0.055 | - | - |
| $ \bar{a}_{FB} - \bar{a}_{OB} $ | - | - | 0.029 | 0.007 |
| $R_F + R_O$ | 0.426 | 0.185 | - | - |
| $s_F^2 + s_O^2$ | - | - | 0.006 | 0.001 |
| u | 0.120 | 0.297 | - | - |
| t | - | - | 1.641 | 0.953 |

diagram. If the broken line of plotted differences in the results intersects line l_4 , we can state with probability α that method F gives significantly lower results than method O , if it intersects line l_3 , it gives significantly higher results. If it intersects lines l_1 or l_2 and if it penetrates into the middle wedge-shaped part of the diagram, the method is equivalent to standard method.

Figure 1 shows that both methods for determination of the rate constant of biochemical oxidation in soil organic matters are fully equivalent.

Table 5. Differences in the pairs of determination of the rate constant k of biochemical oxidation in soil organic matters of soil 1 for sequential analysis (see Figure 1)

| Value k (per 24 hours) | | Difference | Sign |
|--------------------------|-----------------|------------|------|
| Photometric test | Oxi Top Control | | |
| 1.252 | 1.170 | 0.082 | + |
| 1.300 | 1.274 | 0.026 | + |
| 1.184 | 1.302 | 0.118 | - |
| 1.207 | 1.215 | 0.008 | - |
| 1.006 | 1.242 | 0.236 | - |
| 1.154 | 1.196 | 0.042 | - |
| 1.002 | 1.184 | 0.182 | - |
| 1.204 | 1.192 | 0.012 | + |
| 1.297 | 1.294 | 0.003 | + |
| 1.304 | 1.162 | 0.142 | + |
| 1.192 | 1.208 | 0.016 | - |
| 1.288 | 1.278 | 0.010 | + |
| 1.008 | 1.198 | 0.190 | - |
| 1.239 | 1.280 | 0.041 | - |
| 1.241 | 1.266 | 0.025 | - |
| 1.199 | 1.256 | 0.057 | - |

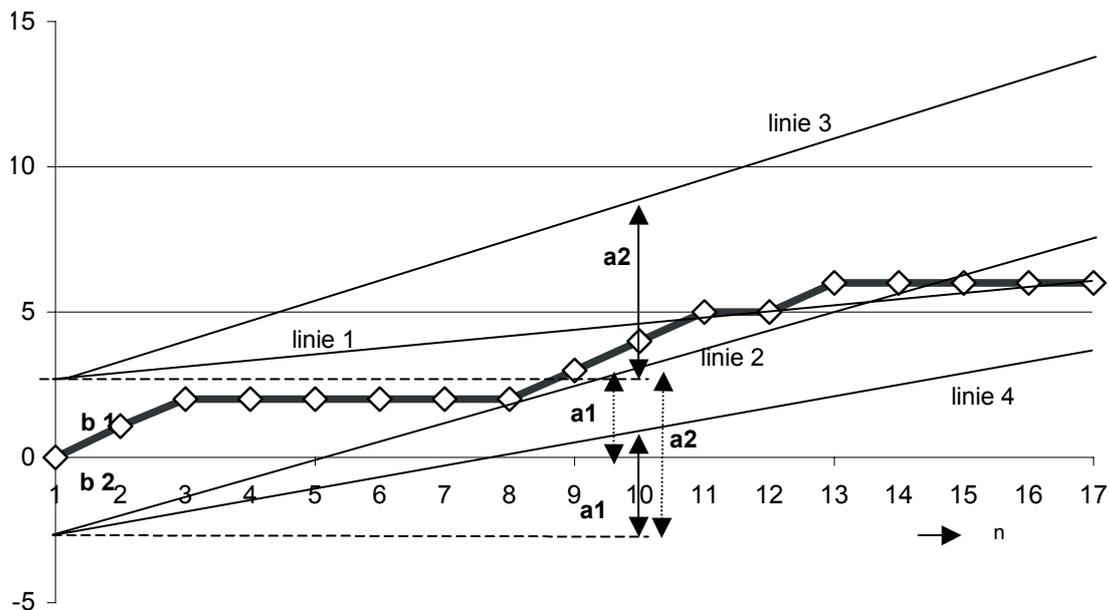


Figure 1. Testing of two analytical methods (P and O) for determination of the rate constant k of biochemical oxidation of soil organic matters by sequential analysis of soil S1 according to the sign of differences in the results if method O is taken as standard (Eckschlager et al. 1980); number of pairs of determination $n = 16$; the tabular values for $p_A = 0.25$ and $p_B = 0.75$ and $\alpha = 0.05$ are $a_1 = 3.7$, $a_2 = 6.3$ and $b_1 = b_2 = 2.7$

The newly proposed method to determine the rate constant of biochemical oxidation of soil organic matters, which can be used to evaluate the level of their stability by Spectroquant Merck BOD photometric tests, is fully comparable from the aspect of correctness and coincidence of results with the previously published method applicable for this purpose: this method of vacuum measuring of changes in BOD of soil suspensions by the Oxi Top Control Merck system, which we continue to recommend for work reasons for large series of samples.

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ABSTRAKT

Využití Spectroquant Merck BOD fotometrického testu k hodnocení stability organických látek v půdě

Byla navržena nová metoda stanovení rychlostní konstanty biochemické oxidace půdních organických látek, kterou lze hodnotit míru jejich stability, pomocí fotometrických testů Spectroquant Merck BOD, jež mohou při menších výběrech vzorků a při jednotlivých stanoveních nahradit podtlakovou metodu měření BOD systémem Oxi Top Control Merck. Tato podtlaková metoda je někdy kritizována pro náročnost a složitost, ale pro velké série vzorků má prokazatelné pracovní přednosti. Obě dvě metody jsou vhodné z hlediska správnosti a shodnosti výsledků a neliší se nižšími ani vyššími výslednými hodnotami, a proto doporučujeme novou metodu práce s fotometrickými testy pro menší série vzorků. Z časových a pracovních důvodů lze jen varovat před použitím klasické BOD zředovací metody s volumetrickým stanovením kyslíku k měření rychlostních konstant.

Klíčová slova: půdy; organické látky; analýza; labilita organických látek; fotometrická metoda; srovnání metod

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