A method of measuring the degree of organic matter degradability

Metoda k měření stupně rozložitelnosti organické hmoty

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ABSTRACT

A method has been proposed to determine the degree of degradability of any organic material based on the kinetics of its hydrolysis in H_2SO_4 of the concentration 11.5 mol.l^{-1} at a temperature of 108°C. The result and the measure of degradability degree are the rate constant of hydrolytic reaction and the carbon percentage of unhydrolyzable residue in total carbon of the sample.

Keywords: degradability of organic matter, hydrolysis kinetics, rate constant

ABSTRAKT

Je navržena metoda k stanovení stupně rozložitelnosti jakékoliv organické hmoty založená na kinetice její hydrolýzy v H_2SO_4 koncentrace 11,5 mol.l^{-1} při teplotě 108°C. Výsledkem a měřítkem stupně rozložitelnosti je rychlostní konstanta hydrolytické reakce a procentický podíl uhlíku nezhydrolyzovatelného zbytku u celkového uhlíku vzorku.

Klíčová slova: rozložitelnost organické hmoty, kinetika hydrolýzy, rychlostní konstanta

DETAILED ABSTRACT

Rozložitelnost organické hmoty biomasy jakéhokoliv původu v přírodě je dána rychlostí enzymatické hydrolýzy polysacharidu celulózy hydrolytickými enzymy mikroorganismů. Tato hydrolýza je brzděná a někdy téměř znemožněna strukturou daného druhu celulózy a to stabilizací intermolekulárními vodíkovými vazbami mezi glukózovými jednotkami v sousedních “listech”, tj. rovinných útvarech z paralelně seřazených celulózových vláken, čili mikrofibril. Tyto vodíkové vazby existují mezi kyslíky pyranosových cyklů a hydroxyskupinami C-6. Další stabilizací proti hydrolýze jsou asociace mikrofibril nevazebnými interakcemi a kovalentními vazbami s dalšími strukturními polysacharidy, s hemicelulosami a pektinem, ale také s proteiny a hlavně s ligninem.

Brzdící efekt těchto faktorů na rychlost hydrolýzy celulózy je stejnosměrný I pro chemickou kyselou či alkalickou hydrolýzu, i když v detailech velikosti účinku jsou pochopitelné rozdíly. Proto je možné při studio rozložitelnosti organické hmoty

biomasy, která je analogem rozložitelnosti její celulózy, nahradit obtížně standardizovatelné biochemické metody enzymatické hydrolýzy. Pro mnoho výhod dává řada autorů přednost hydrolýze kyselé. Rovira, Vallejo (2002, 2007) používají k hydrolýze roztoky H_2SO_4, různou dobu a teplotu hydrolýzy a organickou hmotu podle výsledku hydrolýzy dělí na 3 frakce, labilní, semilabilní, stabilní. Podobně postupuje i Shirato, Yokozawa (2006).

Jiní autoři se snaží zjistit stupeň rozložitelnosti celulózy biomasy nikoliv z průběhu hydrolýzy, ale z odolnosti k oxidaci (Blaire et al. 1995, Tirol-Padre, Ladha 2004, Chan et al. 2001). Oxidují roztoky $KMnO_4$ nebo $K_2Cr_2O_7$ v prostředí H_2SO_4.

Naše metoda, kterou předkládáme v této práci, patří mezi metody kyselé hydrolýzy. Jsme přesvědčeni, že metody oxidační mají horší reprodukovatelnost výsledků, než metody hydrolytické. Existující a vyhovující metody kyselé hydrolýzy (Rovira, Vallejo 2002, 2007, Shirato, Yokozawa 2006) jsou však pracné a zdlouhavé a také pracovně náročné. Pokusili jsme se metodu kyselé hydrolýzy organické hmoty (celulózy biomasy) zjednodušit tím, že jsme použili jen jedinou koncentraci H_2SO_4 (11,5 $mol.l^{-1}$) a jen jedinou teplotu reakce (105°C). Zjednodušení postupu je možné proto, že současně sledujeme kinetiku reakce jako reakce I. řádu a výsledkem je rychlostní konstanta hydrolýzy. Jejím násobením koncentrací lze získat údaj o okamžité rychlosti reakce. Stabilní podíl celulózy, který nepodlehl podmínkám hydrolýzy při naší metodě, stanovíme jako procentický podíl uhlíku z celkového uhlíku vzorku před hydrolýzou.

Srovnáním tab. 1 a tab. 2 je zřejmé, že rozložitelnost organické hmoty šesti různých vzorků biomasy (kukuřice – zrno, bříza – listy, borovice – jehličí, dub – dřevo větví, lněné semeno, Miscantus chimensis – listy) podle původní složité metody Rovira, Vallejo (2003, 2007) a Shirato, Yokozawa (2006) a naší zrychlené a zjednodušené metody v této práci je téměř shodná a v některých případech je rychlostní konstanta citlivějším ukazatelem, než obsah uhlíku v jednotlivých frakcích labilní, semilabilní a stabilní organické hmoty původních hydrolytických metod.

INTRODUCTION

The degree of degradability of given organic matter is a matter of interest in many fields of technology: compost producers demand easily degradable organic matter in order to achieve a desirable temperature in composted material, pedologists are interested in this value from the aspect of a sufficient source of energy for the soil micro-edaphon, animal nutritionists are concerned with limit digestibility of feeds, waste processing companies focus on a possibility and rate of organic waste degradability during aerobic or anaerobic processes, biogas producers aim at the same process at a biogas station.

Three methods can basically be used for assessment of organic matter degradability: based on resistance to oxidation or biochemical variant, resistance to anaerobic decomposition in the process of anaerobic digestion and resistance during acid or alkaline hydrolysis according to Rovira and Vallejo (2002), 2007); further, organic matter degradability is estimated from hydrolyzability in a H_2SO_4 solution of the concentration 1 – 2.5 $mol.l^{-1}$ at a temperature of 100 – 105° C for 0.5 – 12 hours into three fractions: labile, semi-labile and stable ones. A modification of this method was described by Shirato et Yokozawa (2006). Organic matter lability according to resistance to oxidation was studied by Tirol-Padre et Ladha (2004) in a solution of neutral $KMnO_4$ at a concentration of 33 $\mu mol.l^{-1}$, in a $K_2Cr_2O_7$ solution and H_2SO_4

concentration of 6 + 9 +12 mol.l^{-1} (Chan et al. 001). The method using oxidation in three $KMnO_4$ solutions of various concentrations for the partition of organic matter according to degradability, i.e. according to lability, is also interesting (Blair et al. 1995). Many authors employed a combination of hydrolytic and oxidation methods (Zhang et al. 2006, Juany et al. 2006, Soon et al. 2007), others evaluated organic matter degradability from the aspect of physical properties, e.g. carbon content in the fraction of particulate organic matter (Marriot et al. 2006) or from the aspect of biological stability, e.g. from carbon of basal respiration, microbial biomass carbon, carbon of amino sugars, mineralizable carbon and nitrogen from the rate of a change in O/N – alkyl carbon to alkyl carbon and its hydrophobic character (Kögel-Knabcher et al. 1992, Baldock et al. 2004, 2007, John et al. 2005, Rethemeyer et al. 2005). Some authors studied only the most stable fraction of organic matter represented by carbon compounds soluble in cold water (C_{cws}), hot water (C_{hws}) or in solutions of different salts (Körschens et al. 1990).

Tirol-Padre et Ladha (2004) were the first to investigate the reaction kinetics of organic matter degradation using the chemical oxidation method. Organic matter lability was assessed by means of the rate constant of oxidation process K_{chem}. However, the range of K_{chem} of various similar organic materials was small and its value did not correspond with the intensity of transformation of e.g. soil organic matter to such an extent as it did with the percentage of oxidizable carbon in $KMnO_4$ solution in the total carbon content of a sample (Kolář et al. 2009). This is the reason why these authors used a biochemical method for the investigation of oxidation kinetics and measured the rate of a decrease in biochemical oxygen demand (BOD) in an Oxi Top Control Merck vacuum system designed for BOD measurement. The rate constant K_{bio} was able to reflect the intensity of soil organic matter transformation more appropriately (Kolář et al. 2011).

Disadvantages of all the above-mentioned methods for determination of the degree of degradability of any organic matter are high labour consumption, high time consumption to perform analyses and also higher requirements for the analyst's working skills in more complicated methods while a disadvantage of simpler methods is limited information on degradability of tested matter. We wanted to contribute to a solution of this problem by comparing the kinetics of biochemical oxidation based on K_{bio} determination with determination of anaerobic degradability D_{COD} (Kolář et al. 2006) when a patent was granted to this method (Kolář et al. 2011).

Further research revealed that hydrolytic methods are more suitable for routine practice than oxidation methods and that measurement of the kinetics of a hydrolytic process provides deeper knowledge of the character of degradability of any organic matter. These results are the object of the present paper.

MATERIAL AND METHODS

The rate of hydrolysis of organic materials as the first-order reaction is proportional to the concentration of organic matter not hydrolyzed yet:

$$\frac{dy}{dt} = K(L - y) = K.L_z$$

where: L = total organic matter
y = hydrolyzed proportion of organic matter at time t
L_z = remaining (unreacted) organic matter at time t
K = rate constant

The integration from 0 to t of this relation results in the following equation:

$$L_z = L.e^{-Kt} = L.10^{-kt}$$

In general, it holds good for hydrolyzed organic matter at time t:

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

where:	y	= hydrolyzed proportion of organic matter at time t	[% C]
	L	= total organic matter	[% C]
	K	= rate constant	[24 hrs]

A sample of finely ground organic matter of total weight 3 g in dry matter with determined content of total organic carbon C_{org} is hydrolyzed with a solution of 70% H_2SO_4 (11.5 mol^{-1}) of density 1.612 (d 20°/4°) in an open vessel at a temperature of 105°C. The volume of the acid is 200 ml, stirring is done with an electromagnetic stirrer with a metal stir bar in a ceramic case. During the first half an hour, particular samples of 2-ml volume are taken in ten-minute intervals always from the same depth in a hydrolyzation vessel using an automated pipette connected with aspirator, then the sampling interval is one hour. Eight samples are taken in total, hence the total time of hydrolysis is 5.5 hours. Particular samples are taken into centrifugation test-tubes; centrifugation is performed after turbidity settles out, the supernatant is decanted into a sample tube labelled with sampling No., and 3 ml of distilled water are added to the solid residue in the centrifugation test-tube, all is stirred, centrifuged again and the supernatant is added to the first supernatant into the sample tube. Samples (1 – 8) collected in the sample tube are diluted with distilled water to the total volume of 20 ml and the content of organic carbon is determined in them. After termination of hydrolysis, undissolved unhydrolyzed organic matter from the hydrolyzation vessel is let settle out. The clear solution above the sediment is decanted (if it is not clear, centrifugation is necessary) and the organic sediment with the remaining solution is dried on an evaporating dish in an oven at a temperature of 105° C. It is necessary to determine its dry weight and C_{org} content. The percentage of this carbon in the total carbon of 3 g sample is stable C_{stab} and its quantity is a significant value for the character of organic matter degradability. Another characteristic value for degradability of the given organic matter is the rate constant

of a hydrolyzable portion of this organic matter. It is determined by analyses of particular samples in sample tubes 1 – 8.

The time of taking samples from the beginning of hydrolysis is plotted on semi-logarithmic paper on the x-axis while the remaining concentration of organic matter expressed as % of residual carbon is plotted on the y-axis (logarithms). To the experimental points is fitted a straight line, the slope of which is given by the tangent of angle α, i.e. by the ratio of lengths of the opposite side to the adjacent side of the drawn rectangle. It holds good that:

$$tg_{\alpha} = \frac{k}{2.303}$$

and:

$$k = 2.303 \frac{length\ A}{length\ B}$$

To determine carbon, any routine method can be used, e.g. ISO 10694, ISO 14235, and also ashing methods on a SKALAR analyser during elemental analysis, etc. Very similar is the use of TOC-V analyser manufactured by Shimadzu Co., Tokyo, Japan, for the analysis of solutions of particular samples and NC-analyser Sumigraph NC-900, Sumika Chemical Analysis Service, Ltd., Osaka, Japan, for the dry sample of the residue after hydrolysis.

For a comparison of the results it is possible to use maize grains that hydrolytically differ only little in the particular varieties and hybrids – perhaps due to the high content of their characteristic starch.

The above-described method was applied to describe the degradability of organic matter of maize grains, birch leaves, pine needles, oak branch wood, meadow hay and leaves of the ornamental grass *Miscanthus chinensis*. The results were compared with the results of Rovira et Vallejo's (2002) conventional hydrolytic method, as modified by Shirato et Yokozawa (2006). Mathematical and statistical processing of results was done according to Dean and Dixon for several-element sets (Eckschlager et al. 1980).

RESULTS AND DISCUSSION

Table 1. Degradability of organic materials expressed by the percentage of carbon of labile (LP1), semi-labile (LP2) and residual (RP) fractions in total carbon of the sample according to Rovira et Vallejo's (2002) method as modified by Shirato et Yokozawa (2006). The reliability interval of the mean for α = 0.05 was calculated from R range

Tabulka 1. Rozložitelnost organických hmot vyjádřená procentickým podílem uhlíku ve frakci labilní (LP1), semilabilní (LP2) a residuální (RP) z celkového uhlíku vzorku podle metody Rovira et Vallejo (2002) v úpravě Shirato et Yokozawa (2006). Interval spolehlivosti průměru pro α = 0,05 vypočítán z rozpětí R

Organic matter	% C in C_{tot} of fractions		
	LP1	LP2	RP
Maize – grain	61 ± 4	15 ± 1	24 ± 2
Birch – leaves (Betula verrucosa)	22 ± 2	23 ± 2	55 ± 4
Pine – needles (Pinus)	10 ± 1	23 ± 2	67 ± 5
Oak – branch wood (Quercus alba)	8 ± 1	24 ± 1	68 ± 6
Meadow hay	38 ± 2	25 ± 1	37 ± 4
Miscanthus – leaves (Miscanthus chinensis)	20 ± 1	43 ± 3	37 ± 3

Table 2. Degradability of organic materials expressed by the percentage of carbon of the stable fraction (resistant to hydrolysis) in total carbon and by the rate constant of acid hydrolysis in given conditions

Tabulka 2. Rozložitelnost organických hmot vyjádřená procentickým podílem uhlíku stabilní frakce (odolné hydrolýze) z celkového uhlíku a rychlostní konstantou kyselé hydrolýzy v daných podmínkách).

	% C in C_{tot} of stable fraction	Rate constant of hydrolysis k [hrs]
Maize – grain	26 ± 3	1.26 ± 0.21
Birch – leaves	60 ± 7	0.19 ± 0.03
Pine – needles	71 ± 7	0.08 ± 0.01
Oak – branch wood	75 ± 9	0.02 ± 0.00
Meadow hay	40 ± 5	0.72 ± 0.01
Miscanthus – leaves	45 ± 5	0.44 ± 0.04

If the results of conventional hydrolytic Rovira et Vallejo's (2002) method in Tab. 1 are evaluated, the order of the six tested organic materials by increasing stability to hydrolysis will be as follows:

maize < meadow hay < birch leaves < Miscanthus leaves < pine needles < oak wood

However, this order represents only a sequence with the decreasing content of substances very sensitive to hydrolysis: saccharides, starch polysaccharides, hemicelluloses and other low-molecular compounds. If fractions LP1 and LP2 are added up, we get a group of compounds that are somewhat less capable of rapid hydrolysis but under the effect of enzymes of hydrolytic microorganisms they are also hydrolyzable more or less slowly. If stability is compared on the basis of the sum of

LP1 and LP2 fractions, the order of organic matter by increasing stability will be different:

maize < meadow hay + Miscanthus leaves < birch leaves < pine needles + oak wood

Obviously, there is no difference in hydrolyzability between meadow hay and Miscanthus leaves any more; similarly, there is no difference between pine needles and oak wood.

If the organic materials are evaluated in the same way by non-hydrolyzability using the method described in Tab. 2, and if the stable fraction is considered, the order will be as follows:

maize < meadow hay < Miscanthus leaves < birch leaves < pine needles < oak wood

The order is almost identical to that obtained by Rovira et Vallejo's (2002) method, only with a small difference: the positions of Miscanthus leaves and birch leaves have been interchanged.

CONCLUSION

If the values of hydrolysis rate constants are examined, the interval of found values is obviously much wider than the interval of carbon percentages in the stable fraction of the newly proposed method and of carbon in all fractions according to Rovira et Vallejo's method. It is particularly evident in the difference in the organic matter of pine needles and oak wood between which there is such a minute difference in carbon fractions that it is hardly identifiable at the given reliability interval of the mean at $\alpha = 0.05$. The rate constants of hydrolysis of these two organic materials are quite significantly different at the determined reliability interval of the mean.

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