Methods to evaluate substrate degradability in anaerobic digestion and biogas production

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ABSTRACT

Two methods developed by Prof. Dohányos and Doc. Zábranská from ICT in Prague (A) and Oxi Top Control AN 12 measuring system manufactured by MERCK Company (B), were used to determine the maximum yield of biogas and methane and the maximum rate of biogas and methane production per unit weight of biomass using buffered and macro- and micro-nutrient enriched grass biomass as a substrate. Statistical evaluation proved that the Oxi Top Control method did not provide significantly lower or higher results than the other method that is considered standard. Although the Oxi Top Control AN 12 method has a higher variance of measured values than the standard method, it can be recommended as a project and operation method for its work comfort and expeditiousness.

Keywords: anaerobic digestion; biogas; substrate; substrate degradability; methods of determination

The development of energy production from renewable sources is promising mainly for less-favoured areas (LFA) of the country where the utilisation of non-production functions of agriculture is assumed (Penk 2001). Anaerobic digestion with biogas production is one of these technologies (Schulz 1996). Perfect construction and technological feasibility projects are usually submitted but the evaluation of a substrate is mostly missing although a good method of evaluation developed by Prof. Dohányos and Doc. Zábranská from the Institute of Chemical Technology (ICT) is available (Straka et al. 2003). In the present paper the results obtained by this method were compared with the results of the measuring system Oxi Top Control AN 12, which is recommended by Merck Company with its device (Süssmuth et al. 1999).

The former method is based on the determination of anaerobic degradability of organic matters and anaerobic biomass activity (Archer et al. 1986, Dohányos and Zábranská 1988, Zábranská 1994, Ahring and Angelidaki 1997). Biogas production and microbial ecosystem activity are usually activity indicators; they are so called tests of methanogenic activity (TMA) (Zábranská et al. 1990). The determination of gaseous and dissolved hydrogen (Zábranská et al. 1985a), coenzyme F_{420} (Zábranská et al. 1985b) and dehydrogenase activity of anaerobic microorganisms (Zábranská and Dohányos 1987) provides valuable results. The tests of biogas production (tests of methane or biogas yield, tests of maximum rate of their production, test of maximum load of anaerobic biomass, tests of anaerobic biomass toxicity or adaptation and technological tests) provide substrate characteristics and facilitate the choice of suitable inoculum, type of anaerobic technology and estimation of technological parameters (Zábranská 1994).

MATERIAL AND METHODS

The Czech method developed by Zábranská and her colleagues from ICT in Prague is perfect as a whole but it is time and labour intensive and demands on instrumentation are very high. For the reasons of simplicity, accuracy and reliability the authors themselves recommend the use of TMA tests to solve the basic problems with the preparation of anaerobic digestion technology (Straka et al. 2003). Therefore we used their test for maximum biogas and methane yield, i.e. the amount of produced methane or biogas per unit weight of the examined substrate $Y_{CH4, S}$ and $Y_{BG, S}$ (l/g). The gas yield is calculated as a difference between the volumes of the total $V_{BG, T}$ and endogenous $V_{BG, e}$ production of biogas (reference test with biomass of inoculum, buffer and nutrients) divided by the

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initial amount of added substrate S that is expressed by the chemical oxygen demand COD:

$$Y_{BG} = (V_{BG, T} - V_{BG, e})/S = V_{BG, S}/S \ \ (l/g)$$

Analogically, methane yield is calculated from the substrate production of methane divided by the initial amount of added substrate.

Tests were performed in an experimental digester BN-PPS 3 (manufactured by the Italian company Bioindustrie Mantovane, with controller and control by 16-bit control programme and possible adjustment on a touch display; data outputs are dbf files compatible with MS Excel; the digester with PC 80 386 at 33 MHz has 32 digital and 16 analogue inputs and 8 analogue outputs with connection of sensors and auxiliary electrical devices) with an addition of phosphate buffer pH = 7 and standard addition of nutrient solution with macro- and micro-nutrients according to the requirements of the test; microbial biomass was used as an inoculum, cultivation temperature 35°C, air-dried and finely ground grass biomass as the tested substance. Its botanical composition is given in Table 1. The volume of gas production was measured after pressure equalisation inside the vessel with atmospheric pressure. Gas quality was determined by chromatographic analysis of the gaseous phase, and residual COD was determined after the test was terminated. The stirring of mixtures during the test was continuous.

Another TMA test was the test for the maximum rate of biogas or methane production $r_{X, BG, max}$ or $r_{X, CH_4, max}$ (l.g/h) per unit weight of biomass:

$$r_{X, BG, max} = r_{V, BG, max}/X \ (l/g.h) \ (BG, CH_4)$$

where X is the biomass concentration.

The maximum volume rate of gas production $r_{V, BG, max}$ is calculated as the slope of the tangent to the gas production curve in relation to time, divided by the volume of liquid phase in the area of maximum rate without substrate influence, using this equation:

$$r_{V, BG, max} = \Delta V_{BG} C/\Delta t.V_L \ (l/l.h)$$

Specific maximum rate $r_{X, CH4, max}$ is calculated by multiplying the term $r_{X, BG, max}$ by the volume fraction of methane in the biogas.

This test, which according to the authors of the method indicates the maximum possible activity of biomass under the given condition of the system, was carried out with a substrate (its composition is shown in Table 1) similarly like Oxi Top Control AN 12 Merck tests.

The Oxi Top measuring system consists of measuring pressure heads, controller, glass vessels and shaking plateau placed in a thermostat. Pressure heads have pressure sensors operating on the piezoelectric principle; they continually measure pressure variations in a digestion vessel. Measured data are stored in the memory of the measuring head. The stored data is transported wire-free to the operating memory of Oxi Top OC 110 controller where their filing and preliminary evaluation take place. There are two ways of communication between the controller and PC: by IR transfer or through RS 232 communication interface. ACHAT OC 32-bit programme, run under the operating system MS Windows, is used for the processing of measured data in PC. Data outputs are xls files (MS Excel).

This equation of state:

$$n = p.V/R.T$$

where: n = number of gas moles, V = volume (m^3), P = pressure (Pa), T = temperature (°K), is used to calculate CO_2 and CH_4 moles in the gaseous phase of digestion vessels:

$$n_{CO_2 g, CH_4} = (\Delta p \times V_g/R.T) \times 10^{-4}$$

Digestion at 35°C and continuous stirring of vessels in the thermostat takes place 21 days, the pressure range of measuring heads is 500–1350 hPa and the time interval of measuring pressure variations is 4.5 min.

Anaerobic digestion is terminated by the injection of 1 ml of 19% HCl into the substrate with a syringe through the rubber stopper of the vessel. As a result of acidification, CO_2 is displaced from the liquid phase of the digestion vessel. The process was terminated after 4 hours.

The number of CO_2 moles from the liquid phase is calculated:

$$N_{CO_2 l} = \{[p_z (V_g - V_{HCl}) - p_l.V_g]/R.T\} \times 10^{-4}$$

An amount of 1 ml of 30% KOH is injected into the rubber container in the neck of digestion vessel. CO_2 sorption from the gaseous phase of the vessel is terminated after 24 hours and the total number of CO_2 moles in gaseous and liquid phases can be calculated from a decrease in the pressure in the vessel:

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

where: Δp = pressure difference (hPa), V_g = volume of the gaseous space of digestion vessel (ml), p_1 = gas pressure before application of HCl (hPa), p_2 = gas pressure before application of KOH (hPa), p_3 = gas pressure after application of KOH (hPa), R = gas constant, 8.314 (J/mol.K°), T = absolute temperature, 273.15 + X°C, V_{HCl} = volume of added HCl (ml), V_{KOH} = volume of added KOH (ml)

Now 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:

$$n_{CH_4} = (n_{CO_2g,\,CH_4} + n_{CO_2\,l}) - n_{CO_2\,l,\,CO_2\,g}$$

Total number of moles of gases of transported carbon is as follows:

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

If carbon content in the original organic material and in the material after finished digestion is determined by a traditional method, the level of substrate degradability can be calculated from the value n_t, and the whole process can be checked on the basis of material C balance. This procedure is accelerated if the balance is determined from chemical oxygen demand (COD) for the oxidation of organic matters of well homogenised and disintegrated substrate in processing liquid, especially if rapid photometric tests of COD determination are used, e.g. Spectroquant COD KT tests manufactured by MERCK Company, Cat. No. 114 895 (15–300 mg/l O_2) and 114 691 (300–3500 mg/l O_2). For rough estimation, 1 mg of organic matters corresponds to 1.2 mg COD (Pitter 1981).

In this methodical conclusion part of this methodology was to note that all operations described in this paper are comparative, therefore it is necessary to observe a strict standardisation of conditions as for pH, macro- and micro-nutrient content and inoculum dose. Baumann's solution A + B in deionised water of pH = 7.0 was used as a liquid medium (Süssmuth et al. 1999):

A (per 1000 ml H_2O)	B (per 1000 ml H_2O)
5.44 g KH_2PO_4	2.19 g $CaCl_2.6\,H_2O$
6.97 g K_2HPO_4	2.03 g $MgCl_2.6\,H_2O$
10.70 g NH_4Cl	0.4 g $FeCl_2.4\,H_2O$
	6.3 mg $MnCl_2$
	1.0 mg $ZnCl_2$
	0.6 mg $CuCl_2$
	0.2 mg $Na_2MoO_4.2\,H_2O$
	12.2 mg $Co(NO_3)_2.6\,H_2O$
	1.0 mg $NiCl_2.6\,H_2O$
	1.0 mg Na_2SeO_3

The addition of standard inoculum was 10% by volume.

The maximum yield of biogas and methane (quantity of produced gases per unit weight of substrate) and the maximum rate of biogas and methane production per unit weight of biomass determined by the original method of Prof. Dohányos and Doc. Zábranská from ICT in Prague were evaluated by sequential statistical analysis based on so called sign test and by the calculation of mean $\bar{a}$, range R, standard deviation of the mean from the range s_R and reliability interval of the range mean r at $\alpha = 0.05$ according to Dean and Dixon (Eckschlager et al. 1980).

RESULTS AND DISCUSSION

For review, Table 1 shows the botanical composition of a grass biomass sample that was used to compare both methods: Doc. Zábranská's and Prof. Dohányos' traditional method (A) and Oxi Top Control AN 12 Merck method (B). Both methods basically differ in the determination of produced biogas volume: it is directly measured in method A while in method B it is calculated from pressure variations. Gas quality (CH_4 content in biogas) is also directly measured by chromatographic analysis of the gaseous phase in method A, in method B it is calculated from pressure variations that are influenced by CO_2 displacement from the liquid phase by the acid and by the strong lye sorption of CO_2 from the gaseous phase. It is to answer the question whether or not these influences implied the occurrence of errors are so tolerated that the work is comfortable, relatively easy analytics and automated measurements will still be applicable advantages when the problem of substrate degradability under anaerobic digestion is solved.

Tables 2 and 3 show mathematical and statistical evaluation of the maximum yield of biogas, the maximum yield of methane, the maximum rate of biogas production and the maximum rate of methane production if traditional methods A and method B (Oxi Top Control) are used. Although in method B it is recommended to use one of the methods for direct carbon measurement to determine the initial and final content of carbon in digested material, we applied COD determination to describe carbon content as it is recommended for method A, in order to diminish to the largest extent the differences between both methods that may be influenced.

Table 1. Botanical composition of grass biomass sample used to compare the method of Prof. Dohányos (A) and the Oxi Top Control AN 12 Merck method (B)

Grasses = 64%	Clover crops = 10%	Other herbs = 26%
Phleum pratense	*Lathyrus pratensis*	*Prunella vulgaris*
Lolium perenne	*Trifolium pratense*	*Plantago lanceolata*
Festuca rubra	*Trifolium repens*	*Plantago major*
Festuca pratensis	*Trifolium dubium*	*Alchemilla vulgaris*
Holcus lanatus		*Ranunculus repens*
Arrhenatherum elatius		*Ranunculus acris*
Alopecurus pratensis		*Taraxacum officinale*
Agrostis stolonifera		*Galium album*
Agrostis capillaris		*Galium mollugo*
Agropyron repens		
Dactylis glomerata		
Trisetum flavescens		

Table 2. Mean $\bar{a}$ of max. yield of biogas $Y_{BG, S}$ (l/g), methane $Y_{CH_4, S}$ (l/g), max. rate of production of biogas $r_{X, BG\ max}$ (l/g.h), methane $r_{X, CH_4\ max}$ (l/g.h), range R, standard deviations of range S_R, reliability interval of the mean $\bar{a}$ at significance level $\alpha = 0.05$ according to Dean and Dixon (Eckschlager et al. 1980), $L_{1, 2}$ for traditional method A and method B (Oxi Top Control AN 12 Merck)

	Method A				Method B			
	$\bar{a}_A$	R_A	$s_{R\ (A)}$	$L_{1, 2\ (A)}$	$\bar{a}_B$	R_B	$s_{R\ (B)}$	$L_{1, 2\ (B)}$
$Y_{BG, S}$	0.328	0.066	0.022	(0.345–0.311)	0.352	0.077	0.025	(0.372–0.332)
$Y_{CH_4, S}$	0.199	0.039	0.013	(0.209–0.189)	0.240	0.202	0.068	(0.293–0.187)
$r_{X, BG\ max}$	0.045	0.019	0.006	0.050–0.040	0.040	0.024	0.008	0.046–0.034
$r_{X, CH_4\ max}$	0.027	0.010	0.003	0.030–0.024	0.031	0.020	0.007	0.036–0.026

$s_R = k_n.R$ k_n for $n = 9$ determinations is 0.3367
$L_{1, 2} = \bar{a} \pm K_n.R$ K_n for $n = 9$ determinations and $\alpha = 0.05$ is 0.26

Table 2 documents that mathematical and statistical characteristics of method B are in fact worse than in the traditional method A. Table 3 illustrates that the correspondence between the means $\bar{a}$ of biogas and methane production in both methods is not quite evident.

Table 3. Testing of the consistence of the means $\bar{a}$ of method A and method B by Lord's test $u = |\bar{a}_A - \bar{a}_B| / (R_A + R_B)$ pro $\alpha = 0.05$ and number of parallel determinations by both methods $n = 9$ (critical value u_α for $n = 9$ and $\alpha = 0.05$ is 0.167)

$Y_{BG, S}$	$Y_{CH_4, S}$	$r_{X, BG\ max}$	$r_{X, CH_4\ max}$
u = 0.168	u = 0.170	0.116	0.133

Therefore we tried to answer a question to what extent the tested method B differed from the traditional method A: this is the reason why we carried out sequential statistical analysis in the form of so called sign test as described by Eckschlager et al. (1980). Table 4 shows the results of 18 pairs of determinations by methods A and B and their sign differences; a graph was constructed from these results (Figure 1). The ratio of the number of differences for a sign (+, –) to the total number of differences

$$p_{o+} = n^+/n \qquad p_{o-} = n^-/n$$

should theoretically be

$$p_o = p_{o+} = p_{o-}\ 0.5$$

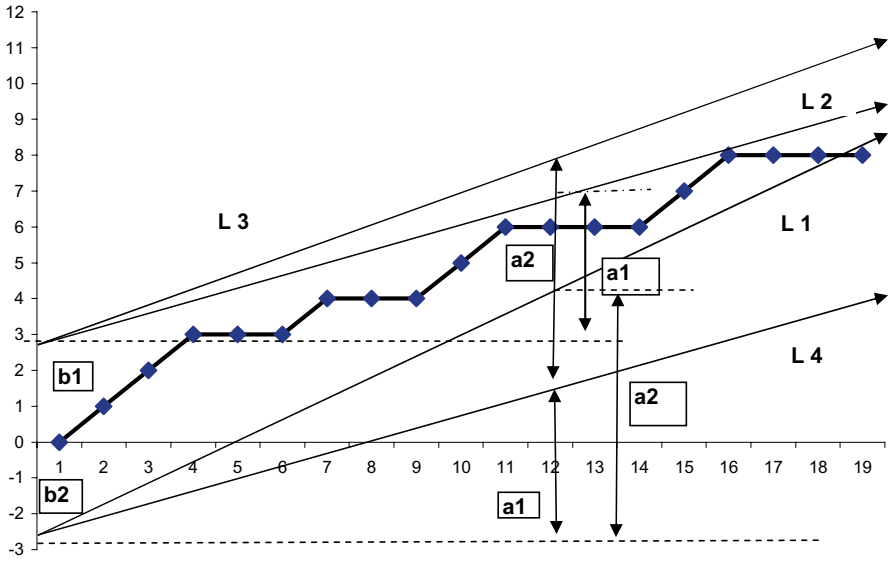

Figure 1. Sign test (Eckschlager et al. 1980) to determine the equivalence of method B and traditional method A

if method B was not biased with systematic error in relation to method A.

Because the pair of results is a random sample, we choose the upper and lower values p_A and p_B, mostly $p_A = 0.25$ and $p_B = 0.75$. The actual value of the ratio of the number of differences with sigh + or – should lie between them. For the chosen significance levels $\alpha = \beta = 0.05$ and the above chosen p_A and p_B in tables of sequential analysis we find sections b_1, b_2 for n pairs: 0 and a_1, a_2 for $n = 10$ ($a_1 = 3.7$; $a_2 = 6.3$; $b_1 = b_2 = 2.7$) for the construction of testing diagram. Because the broken line of the test did not touch line l_4 or l_3, but it crossed l_1 or l_2 it can be concluded that the number of pairs of results was sufficiently high for a reliable decision and the tested method B (Oxi Top Control) did not provide significantly lower or higher results than the traditional method A, from which it differs in worse reproducibility only; so we cannot recommend method B as a scientific method, only as a preliminary, project and operation method.

Many authors (Straka et al. 2003) believe that the advantages of pressure methods of measuring are also accuracy and correctness of the measurements. Unfortunately, we cannot identify ourselves with these opinions on the basis of conclusions presented in this paper.

Table 4. Results of determination of maximum production of CH_4 $Y_{CH_4, S}$ (l/g) in pairs, parallelly determined by method A and method B, and the sign difference of method B from traditional method A (higher = +, lower = –)

$Y_{CH_4, S (A)}$	$Y_{CH_4, S (B)}$	Difference
0.192	0.241	+
0.204	0.227	+
0.201	0.295	+
0.189	0.186	–
0.205	0.190	–
0.210	0.223	+
0.198	0.190	–
0.195	0.187	–
0.185	0.283	+
0.199	0.205	+
0.191	0.185	–
0.205	0.197	–
0.203	0.199	–
0.188	0.272	+
0.197	0.213	+
0.192	0.190	–
0.200	0.193	–
0.274	0.208	–

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ABSTRAKT

Metody k posouzení rozložitelnosti substrátu při anaerobní digesci a výrobě bioplynu

Byla stanovena maximální výtěžnost bioplynu a metanu a maximální rychlost produkce bioplynu a metanu na hmotnostní jednotku biomasy při použití substrátu, jehož základem byla travní biomasa, pufrovaná a obohacená makro- a mikroživinami, a to dvěma metodami: metodou prof. Dohányose a doc. Zábranské z VŠCHT Praha (A) a metodou Oxi Top Control AN 12 firmy MERCK (B). Statistické vyhodnocení ukázalo, že metoda Oxi Top Control nedává výsledky významně nižší či vyšší než druhá metoda, kterou považujeme za standardní. Přesto není standardní metodě rovnocenná, protože charakteristika statistického vyhodnocení výsledků, které dává, je horší. Navzdory tomu metodu Oxi Top Control AN 12 pro pohodlnost a rychlost práce doporučujeme nikoli jako vědeckou, ale jako projektovou a provozní metodu.

Klíčová slova: anaerobní digesce; bioplyn; substrát; rozložitelnost substrátu; metody stanovení

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