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ORIGINAL PAPER

Assessing Aerobic Biodegradability of Plastics in Aqueous Environment by GC-Analyzing Composition of Equilibrium Gaseous Phase

Pavel Dřímal · Josef Hrnčiřík · Jaromír Hoffmann

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Abstract Testing biodegradability of plastics under varied conditions of the environment as well as under laboratory conditions in accordance with valid international standards is very laborious, lengthy and often also economically demanding. For this reason, applicability was verified of gas chromatography to analyze gaseous phase when investigating the biodegradation course of plastics in an aqueous environment as an alternative to customary employed methods. A mathematical model of acid–basic CO_2 equilibrium in a gas–liquid system was worked out, enabling to determine quantity of produced CO_2 through chromatographic analysis of gaseous phase, in dependence on ratio of liquid and gas phase volumes (V_l/V_g) and on actual pH of liquid phase. Experimental conditions for organizing the tests were optimized. A ratio that proved suitable was $V_l/V_g \cong 0.1$ at pH ≈ 7.1 of liquid phase. Under these test conditions, biodegradability of model samples, PHB, Gellan gum and Xanthan gum, was explored; course of biodegradation was studied through produced CO_2 (values D_{CO_2}) determined by analyzing gaseous phase through gas chromatography on the one hand, and through customary ''titration'' procedure on the other. With water-soluble polymers, the decrement in dissolved organic carbon (values D_{DOC}) was also studied. Difference between values does not exceed 5%. The procedures in question are alternative ''substituting'' procedures for observing course of aerobic biodegradation of substances in an aqueous environment.

Keywords Gas chromatography · Aerobic biodegradation · Carbon dioxide balance · Plastics

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Introduction

Increasing manufacture and consumption of plastics and plastics packaging gives rise to the issue of their further fate after use. The enormous pace at which waste is produced is alarming.

Potential following processing or disposal of plastics should be already considered during their manufacture, with employment of appropriate ingredients supporting biodegradability of the end-product while, of course, retaining its properties for use. Thence arises the necessity of suitable and fast (screening) methods for testing plastics biodegradability beside well-established standardized procedures. There are several standardized methods for observing biodegradation course of various substances in different environmental conditions [1]. Technique frequently employed involves methods observing changes in gaseous phase in reaction environment, specifically biological oxygen demand or produced carbon dioxide, or possibly both components. Practical execution of these tests in accordance with valid standards [2, 3] mostly requires assembling quite complex testing apparatus with limited number of measuring positions mostly enabling to study merely one of the mentioned parameters.

Methods focused on observing CO_2 production may be divided into two groups: (a) manual methods that consist in retaining carbon dioxide in a solution of hydroxide with following titration determination (procedure for determining CO_2 recommended by standard [2, 3]), (b) automated methods, for example, direct analysis of gas from headspace by means of gas chromatography [4, 5] or infrared spectroscopy [6, 7]. Automated methods may be executed throughout a test continuously or discontinuously, only in suitable time intervals. Fully automated methods also include automatic analyzer Micro-Oxymax Respirometer by Columbus Co, U.S.A., which enables continuous observation of O_2 demand (paramagnetic O_2 analyzer) and simultaneously also produced CO_2 (IR analyzer) in headspace [8].

Found works [4, 5] on application of gas chromatography to evaluate course of aerobic biodegradation did not include in total balance of CO_2 the quantity of its ionic forms, because chromatographic analysis of gaseous phase was performed only after acidifying liquid phase and establishing equilibrium in the reaction environment. Under such conditions, quantity of CO_3^{2-} in coal was determined in work [9] by analyzing headspace through chromatography. After adding HCl to liquid phase and establishing equilibrium in enclosed system with tested sample, quantity of CO_2 was determined in the gaseous phase. Productivity of this method ranged within 96.3–102.5%.

In work [10] authors investigated saturation of freshwater bodies with carbon dioxide by two different methods: (a) direct chromatographic analysis of headspace, (b) calculation, knowing content of inorganic carbon (DIC) under conditions of acid–basic CO_2 equilibrium, and knowing content of Ca^{2+} ion. A comparison of both methods was performed by determining concentration of dissolved CO_2. With all tested samples, CO_2 concentration calculated from DIC content and pH was about 5–15% greater than CO_2 concentration determined through GC analysis of gaseous phase. Authors explain this difference by inaccurately determined Ca^{2+} concentration or by erroneously determined DIC and pH. Nevertheless, chromatographic data determining CO_2 content in gaseous phase were not corrected for acid–basic equilibrium of CO_2 in this system.

The presented work verified application of gas chromatography to analyze gaseous phase (carbon dioxide) in an enclosed two phase gas–liquid system when studying course of plastics biodegradation. This analytical end-point device potentially enables to determine simultaneously quantity of produced CO_2 as well as O_2 content in gaseous phase, is relatively fast and enables to analyze a larger quantity of samples (parallel tests). The method in question could be a procedure investigating the course of biodegradation of substances (plastics) as alternative to standardized procedures. The objective was to propose a suitable experimental arrangement of tests based on mathematical description and experimental verification.

The first part of the contribution gives a mathematical description of CO_2 acid–basic equilibrium in a real biological system for conditions of studying biodegradations in an aerobic aqueous environment. The next part, examining biodegradation of a readily degradable substrate (glucose), seeks convenient experimental conditions for arranging these tests, particularly a suitable ratio of liquid and gaseous phase volumes (V_l/V_g) and pH of liquid phase. The method is subsequently verified by studying biodegradation course of ''model'' plastics under ''optimized'' test conditions.

Mathematical Description of CO_2 Acid–Basic Equilibrium in Gas–Liquid System

With pH of liquid phase less than 4, originating CO_2 is dissolved in liquid only in virtually free form. Quantity of ionic forms under these conditions is negligible and total produced quantity of CO_2 could be easily calculated from Henry's law and partition coefficient K_{lg} in accordance with Eq. 1

$$n_{CO_2g+l} = V_g \times c_{CO_2g} + V_l \times c_{CO_2l} = (V_g + V_l \times K_{lg}) \times c_{CO_2g} \quad (1)$$

where V_l, V_g are volumes of liquid and gaseous phase, K_{lg} is formal limit partition coefficient (partition coefficient valid in an acid environment at pH $<$ 4) and c_{CO_2g} represents CO_2 in gaseous phase of an enclosed system (for example, determined by GC analysis).

Partition coefficient K_{lg} expresses the quotient of carbon dioxide concentration in solution c_l and gaseous phase c_g in accordance with relationship $K_{lg} = c_l/c_g$ including merely concentration of non-ionic forms of CO_2. The partition coefficient so specified is only a function of temperature, not of pressure or pH.

In real conditions of biodegradability tests of substances, pH of liquid phase approaches pK_1 of carbonic acid. This practically means that concentration of ion-bound CO_2, i.e. $c_{HCO_3^-}$ and $c_{CO_3^{2-}}$, becomes comparable to or even greater than concentration of ''free'' dissolved CO_2. It hence holds true that $c_{CO_2l} = c_{CO_2T} - c_{HCO_3^-} - c_{CO_3^{2-}}$, where c_{CO_2T} is total concentration of all forms of CO_2 present in solution. Ratio of concentrations

c_{CO_2l}/c_{CO_2T} (non-ionic forms of CO_2 present in solution) calculated from Henry's law and conditions of carbonic acid dissociation to first degree according to relationship (2) dependently on pH are shown in Fig. 1.

$$100 \times (c_{CO_2l}/c_{CO_2T}) = \frac{100}{1 + 10^{-pK_1'} \times 10^{pH}} \quad (2)$$

Here, c_{CO_2l} is concentration of CO_2 non-ionic form in liquid, c_{CO_2T} represents total concentration of all forms of CO_2 in liquid phase, $pK_1' = 6.35$.

Relative presence of various CO_2 forms (CO_2, H_2CO_3, HCO_3^-, CO_3^{2-}) in liquid phase follows from relationships for dissociation of carbonic acid to first and second degree employing activity of components sharing in equilibrium:

$$K_1 = \frac{a_{H^+} \times a_{HCO_3^-}}{a_{H_2CO_3}} \text{ or } K_1' = \frac{a_{H^+} \times a_{HCO_3^-}}{a_{CO_2l}} \quad (3,4)$$

$$K_2 = \frac{a_{H^+} \times a_{CO_3^{2-}}}{a_{HCO_3^-}} \quad (5)$$

In a system with dissolved carbon dioxide, valid conditions must act concurrently, i.e. Henry's law or an equivalent expression with partition coefficient, and both relationships for dissociation constants of carbonic acid, (3) or (4) and (5). In a usual regime of biodegradations, system pH is not too high and dissociation of carbonic acid to the second degree does not practically occur, implying that presence of carbon dioxide in the form of CO_3^{2-} is insignificant, representation of CO_2 as HCO_3^- is conversely significant.

Value of pH in biological tests can range from 6.8 to 7.2, meaning a region where quantity of CO_2 in the form of HCO_3^- (see Fig. 1) is dominant in the solution and has to be taken into account in total CO_2 balance. Ratio of CO_2 concentrations in liquid and gaseous phase, c_{CO_2T}/c_{CO_2g}, will be designated $K_{lg}' = \frac{c_{CO_2T}}{c_{CO_2g}} = \frac{c_{CO_2l} + c_{HCO_3^-}}{c_{CO_2g}}$, which under these conditions is formal effective partition coefficient (K_{lg}') of CO_2 between liquid and gaseous phase in a real system. Dependence of K_{lg}' according to relationship (6) on pH in biodegradation tests is shown in Fig. 2.

$$K_{lg}' = K_{lg} \times \left(1 + 10^{-pK_1'} \times 10^{pH}\right) \quad (6)$$

where K_{lg} is formal limit partition coefficient (i.e. partition coefficient valid in acid environment).

It is obvious in Fig. 2 that K_{lg}', whose value in acid environment at 25 °C approaches limit value $K_{lg} = 0.83$, increases with a growing pH and under real conditions of tests may attain values up to about 15 (for pH ≈ 7.5).

If pH level of liquid phase at the moment of GC analysis and volumes of gaseous and liquid phase are known, we may calculate the total quantity of CO_2 produced under real conditions of biodegradation tests in accordance with Eq. 7

$$n_{CO_2g+l} = \left(V_g + V_l \times K_{lg}'\right) \times c_{CO_2g} = \left(V_g + V_l \times K_{lg} \times (1 + 10^{-pK_1'} \times 10^{pH})\right) \times c_{CO_2g} \quad (7)$$

Important parameters of balance Eq. 7 are, beside pH, volumes of liquid and gaseous phase designated V_l and V_g. Reducing ratio V_l/V_g, we are able to increase the so-called "degree of CO_2 transfer from liquid to gaseous phase" (hereafter "CO_2-transfer") specified by expression $100 \times n_g/(n_g + n_l)$, expressing the proportion of carbon dioxide present at given pH in gaseous phase (determinable by GC analysis) out of the

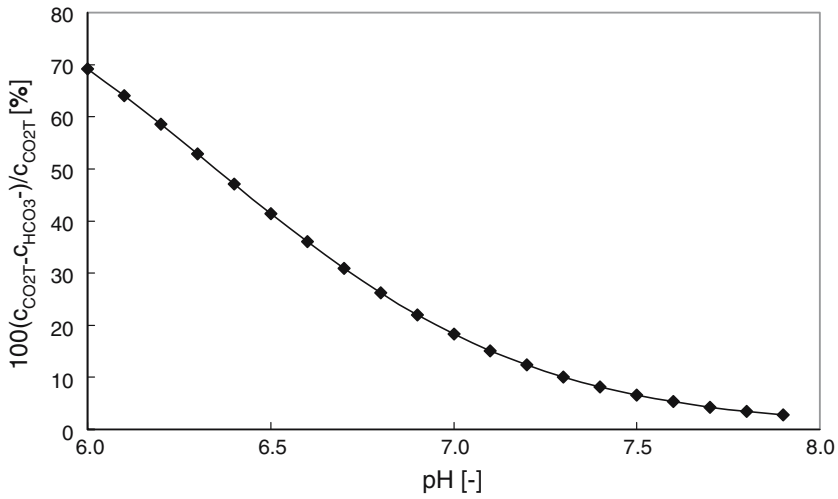

Fig. 1 Dependence of relative percentual presence of (non-ionic) form of CO_2 in liquid phase on pH at 25 °C

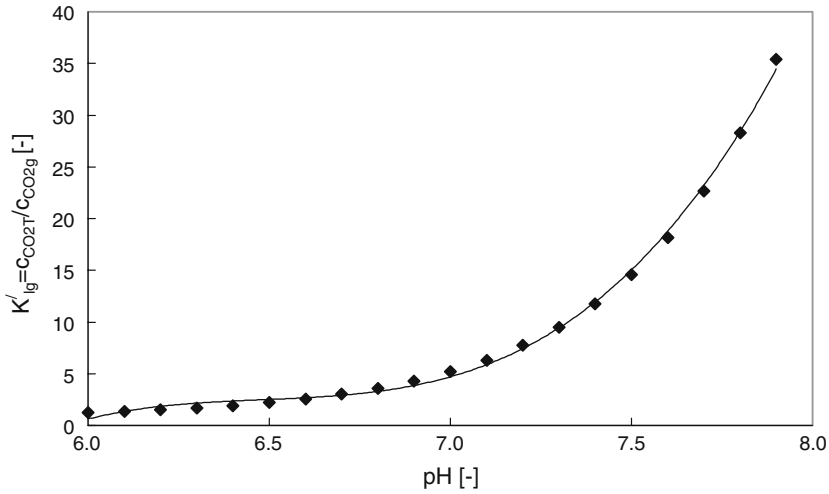

Fig. 2 Dependence of formal effective partition coefficient for CO_2 between liquid and gaseous phase on pH of real biological systems at 25 °C

total produced quantity of CO_2. Figure 3 indicates influence of pH on "CO_2-transfer" calculated according to relationship (8) for two systems differing in V_l/V_g (660/440 resp. 100/1040).

$$100 \times n_g/(n_g + n_l) = 100/\left(1 + K'_{lg} \times V_l/V_g\right) \qquad (8)$$

It is obvious in Fig. 3 that it is experimentally advantageous to work at a low V_l/V_g ratio. "CO_2-transfer" increases with a decreasing V_l/V_g ratio, and concentration changes of CO_2 in gaseous phase produced by potential changes of pH in liquid phase relatively decrease. On the other hand, CO_2 concentration in gaseous phase will decrease with a decreasing V_l/V_g ratio, which will make greater demands for sensitivity and accuracy of GC analysis. These conclusions are obvious in Fig. 4 describing dependence between quantity of CO_2 in gaseous phase (i.e. "CO_2-transfer") and quotient of volumes V_l/V_g at pH levels suitable for biodegradation processes (6.8; 7.0; 7.2).

It may be thus assumed that suitable conditions for balancing CO_2 by means of its GC analysis will be, taking into account what was mentioned above, a V_l/V_g ratio < 0.1 and pH of liquid phase stabilized with phosphate buffer system (this is a component in employed standard mineral medium, of pH approx. 7.1 [2, 3]).

Experimentally Verifying Theoretical Model

In order to shorten duration of experiments, glucose (readily degradable substrate) was used in the first part of these "verifying" tests. The main objective was to confirm a suitable ratio of liquid and gaseous phase with which high "CO_2-transfer" could be attained and at the same time low dependence of that on potential changes in pH of liquid phase during the test. Level of

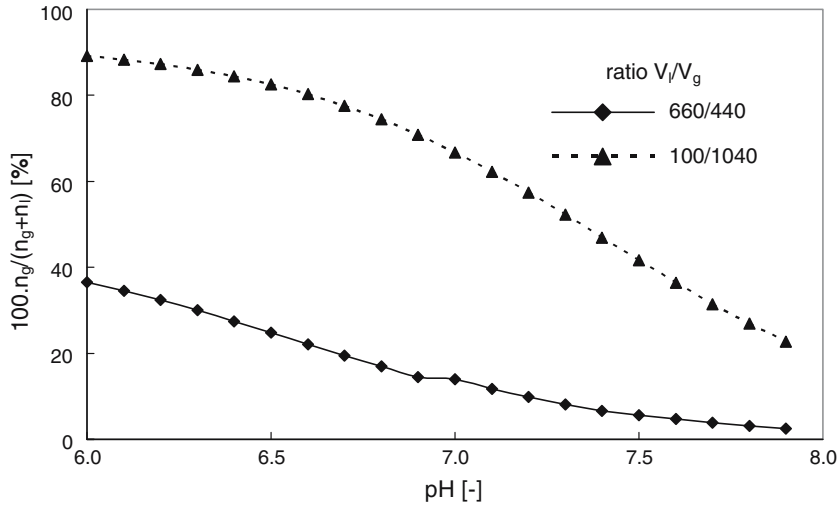

Fig. 3 Dependence of "CO_2-transfer" on pH for V_l/V_g ratios 660/440 and 100/1040. Calculated according to (8) at 25 °C

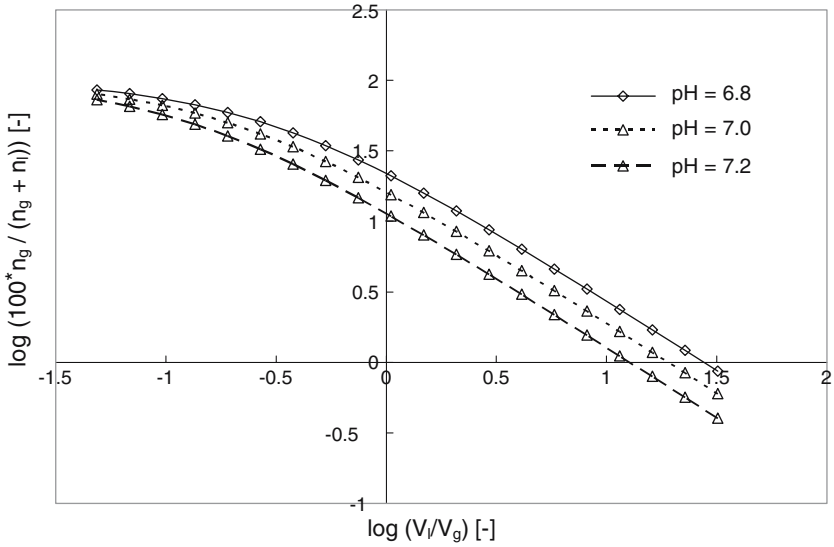

Fig. 4 Dependence of log "CO_2-transfer" on log (V_l/V_g) calculated according to (8) at 25 °C for pH levels in typical interval during biodegradations

pH ≈ 7.1 was maintained in liquid phase by phosphate buffer, which was a component of standardized mineral medium [2, 3].

Testing Apparatus

Biodegradability tests were performed in apparatus according to Fig. 5. The apparatus was designed according to standard ISO 14852; each reaction flask had a closure with gas-tight septum for withdrawing gaseous samples for GC analysis during testing.

Employed Biological Material

The soil leachate used for inoculation was prepared by mixing 20 g soil substrate (moisture content approx. 50%) with 100 ml solution of mineral medium (according to ISO 14852), the mixture was shaken for 2 h on shaking machine and filtered through pre-washed filter paper. This inoculum was aerated for 24 h before use. Inoculum dosage was such that 10^6 cells/1 ml reaction suspension were obtained (calculated per total quantity of cells [11]).

Experimental Conditions and Mode of Evaluating Results

In accord with above-mentioned mathematical description of CO_2 acid–basic equilibrium, two various ratios of liquid and gaseous phase volumes were selected: variant A and B, Table 1.

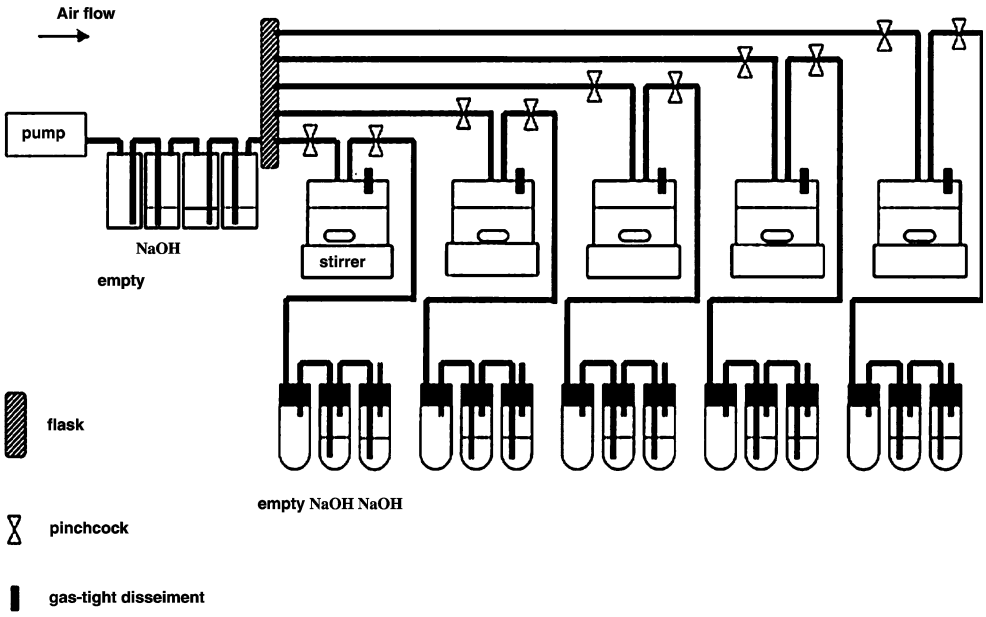

Fig. 5 Diagram of employed testing apparatus for testing biodegradations by analyzing gaseous phase

Table 1 Volume ratios in testing flasks

	Variant A	Variant B
Total volume of measuring flask (ml)	1140	1140
Volume of liquid phase (ml)	85	285
Volume of gaseous phase (ml)	1055	855
Ratio V_l/V_g	0.08	0.33

Determined quantity of CO_2 (through GC analysis) was corrected in accordance with conditions of CO_2 acid–basic equilibrium in a gas–liquid system according to Eq. 7, and related to theoretical quantity of CO_2 calculated for the theoretical case of complete degradation of tested substrate (D_{CO_2}-percentage of substrate removal evaluated by means of produced CO_2). DOC values measured at start and end of test allowed to calculate D_{DOC}-percentage of removal by the decrease in dissolved organic carbon. Obtained dependence $D_{CO_2} = f(t)$ was processed by regression employing first-order kinetics according to Eq. 9, and graphically shown in Fig. 6. Levels of liquid phase pH were measured at start and end of test.

$$D_{CO_2} = D_{CO_2max} \times \left(1 - e^{-k\times(t-t_{lag})}\right) \quad (9)$$

where D_{CO_2max} is regression coefficient expressing limit value in infinite time (%), k is value of rate constant (hour^{-1}), t_{lag} is shift on time axis expressing lag phase (hour).

where $pH_{initial}$ = pH of liquid phase at start of the test, pH_{final} = pH of liquid phase at end of the test, $\Delta(D_{CO_2max}/D_{DOC})$ = scatter between D_{CO_2max} and D_{DOC} calculated according to relationship (10).

$$\Delta(D_{CO_2max}/D_{DOC}) = \frac{100 \times (D_{CO_2max} - D_{DOC})}{(D_{CO_2max} + D_{DOC})/2} \quad (10)$$

At a ratio of V_l/V_g = 0.08, results of D_{CO_2} and D_{DOC} were in good agreement, difference between values under these conditions was about 4.0% (see Table 2). Changes in pH during test were quite small (±0.01) and the shift in acid–basic equilibrium they produced could lead to an approx. 0.6% change in CO_2 concentration in gaseous phase. This volume ratio of liquid and gaseous phase proved to be convenient for testing biodegradability by GC analysis of CO_2 in the gaseous phase. At a higher V_l/V_g = 0.33 (variant B), difference between values of D_{CO_2} and D_{DOC} was approx. 10% and probably caused by greater changes in pH of liquid phase during the test (± 0.05), which led to changes in CO_2 content in gaseous phase. The change in CO_2 concentration in gaseous phase caused by shift in pH during test was about 2.6% (calculated from Eq. 8).

Table 2 Measured and calculated values

	V_l/V_g = 0.08		V_l/V_g = 0.33	
	200 mg/l	300 mg/l	200 mg/l	300 mg/l
$pH_{initial}$ (–)	7.11	7.11	7.12	7.11
pH_{final} (–)	7.10	7.11	7.07	7.06
D_{CO_2max} (%)	92.7	92.9	85.8	86.9
D_{DOC} (%)	95.6	96.7	93.5	96.2
$\Delta(D_{CO_2max}/D_{DOC})$ (%)	3.08	4.00	8.59	10.16
k (h^{-1})	0.018	0.017	0.026	0.023
t_{lag} (h)	4.0	4.0	1.0	1.0

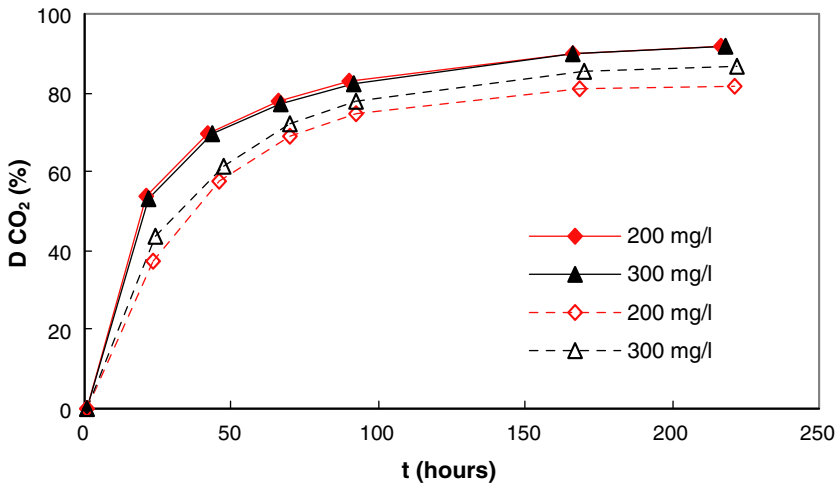

Fig. 6 Percentage of glucose removal by produced CO_2 processed according to Eq. 9. Thick line indicates V_l/V_g = 0.08, dashed line V_l/V_g = 0.33

Assessing Biodegradability of Polymers in Aerobic Aqueous Environment by GC Analysis of Gaseous Phase

Samples, Apparatus, Biological Material

Xanthan gum from Xanthomonas campestris

White powder, water-soluble, deacylated form, from Fluka Chemistry

Gellan gum

White powder, water-soluble, deacylated form, from Fluka Chemistry

PHB (*poly-β-hydroxybutyrate*)

Molecular mass 327,000 g mol^{-1}, powder form, produced by Biomer, Germany

Testing apparatus and biological material same as above (Chapter 3).

Testing Conditions and Mode of Evaluating Results

Based on preceding results (see above), volume ratio of liquid and gaseous phase selected for all following experiments V_l/V_g was 0.10, pH of liquid phase was maintained with phosphate buffer system (containing 66.70 mmol phosphorus/1000 ml mineral medium, pH ≈ 7.1).

Biodegradation degree was again evaluated on basis of produced CO_2 determined by gas chromatography, i.e. D_{CO_2}, and with water-soluble samples also by value D_{DOC}-decrease in dsissolved organic carbon at start and end of test. Obtained dependencies $D_{CO_2} = f(t)$ were processed by regression employing first-order kinetics in accordance with Eq. 9, obtained parameters are shown in Table 3.

Apart from described method of investigating biodegradation (GC analysis of gaseous phase), degradation course was studied on one sample (water-soluble Xanthan gum) by the standard procedure [2, 3] consisting in continuously stripping produced CO_2 from gaseous phase in a test flask into an absorber containing NaOH, with subsequent acidimetric determination. Results are shown in Table 3 and Fig. 7.

Figure 7 indicates aerobic biodegradation courses of tested samples (PHB, Gellan gum and Xanthan gum) determined by GC determinations of produced CO_2, and in the case of Xanthan gum also by standard procedure according to ISO 14852 [3]. Courses of Xanthan gum degradation prove possible mutual ''substitution'' of both test variants: calculated values D_{CO_2} after 450 h were 89.0% by GC analysis and 83.6% by standard CO_2 determination, D_{CO_2} determined after 700 h only for GC variant was 90.0% (calculated according to (9)). Values of kinetic constants (k) were 0.014 and 0.015 h^{-1} (Test 3, 4, Table 3). Differences between found values D_{CO_2} and D_{DOC} at end of tests with studied samples were less than 5%, hence within admissible limits from viewpoint of practical use.

We may thus state that individual procedures lead to comparable results and hence may essentially substitute each other. Certain differences between methods which consist in determining produced CO_2 could have been caused by less suitable conditions for acidimetric determination of CO_2 for which a higher V_l/V_g ratio would be more suitable, as opposed to GC analysis where low V_l/V_g is desirable.

Conclusion

A mathematical description is worked out for acid–basic equilibrium of CO_2 in a system of gas–liquid for conditions of observing biodegradations in an aqueous environment, which is expressed by balance Eq. 7; significance of symbols is given in previous text

$$n_{CO_2g+l} = \left(V_g + V_l \times K'_{lg}\right) \times c_{CO_2g} = \left(V_g + V_l \times K_{lg} \times (1 + 10^{-pK'_1} \times 10^{pH})\right) \times c_{CO_2g} E \quad (7)$$

From mathematical description follows:

- Change in pH during test will produce a change in ''degree of CO_2 transfer from liquid to gaseous phase'' (''CO_2-transfer''); magnitude of this change

Table 3 Measured and calculated values of both procedures for determining CO_2

No.	sample	D_{CO_2max} (%)	$(D_{DOC})_{700}$ (%)	$\Delta(D_{CO_2max}/D_{DOC})^b$ (%)	k (h^{-1})	t_{lag} (h)	Analysis of CO_2
1	PHB	71.0	–	–	0.006	78.6	GC analysis
2	Gellan gum	100.1	95.3	4.9	–	191.1	GC analysis (No. 2)
3	Xanthan gum	90.0	94.0	4.5	0.015	31.3	GC analysis (No. 3)
4	Xanthan gum	83.9[a]	81.5[a]	2.9	0.014	49.7	by [2]

$(D_{DOC})_{700}$—values measured after 700 h of the test

[a]—values measured after 450 h of the test

[b]—calculated by relationship (10)

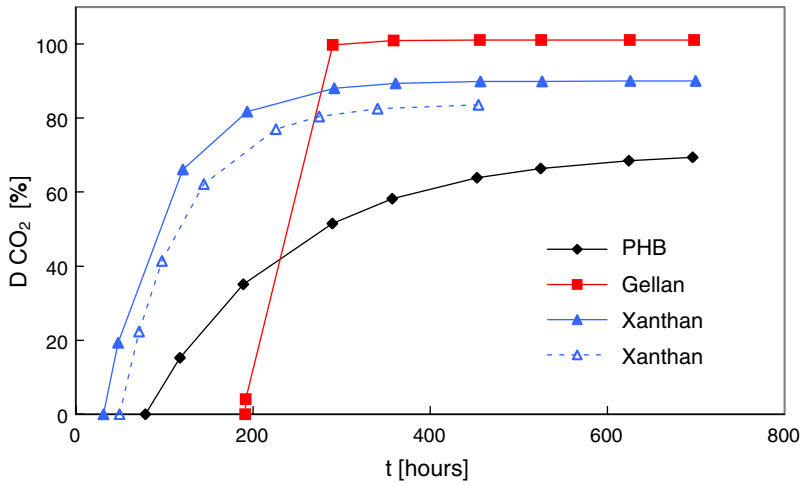

Fig. 7 Percentage of sample disposal evaluated by produced CO_2 through gas chromatography (bold lines), and through standard ''titration'' procedure (only Xanthan, dashed). Ratio $V_l/V_g = 0.10$, calculated according to (9)

depends on volume ratio V_l/V_g. Efficient compensation of potential changes in pH of liquid phase by a buffer of sufficient capacity (part of mineral medium) is also very essential for GC end-point of tests.

- Ratio of volumes V_l/V_g significantly affects CO_2 concentration in gaseous phase. A decreasing V_l/V_g ratio increases ''degree of CO_2 transfer from liquid to gaseous phase'' (''CO_2-transfer'') and relatively decreases concentration changes of CO_2 in gaseous phase produced by potential changes of pH in liquid phase during test. On the other hand, CO_2 concentration in gaseous phase decreases with a decreasing V_l/V_g ratio and demands for sensitivity and accuracy of GC analysis grow.
- Investigation of glucose biodegradation was experimentally verified by means of analyzing CO_2 in gaseous phase at two different ratios of V_l/V_g. Ratio $V_l/V_g \cong 0.1$ is suitable for studying course of biodegradations through GC analysis; pH is sufficiently stabilized throughout the test and thereby also ''CO_2-transfer''; concentration changes of CO_2 in gaseous phase during test can be well determined through GC analysis.
- Proposed experimental conditions are convenient for observing aerobic biodegradation of macromolecular substances (PHB, Gellan gum, Xanthan gum) through GC analysis of gaseous phase in accordance with balance Eq. 7. Results expressed as D_{CO_2} (percentage of CO_2 theoretical production determined through GC analysis as well as by standardized acidimetric procedure) and D_{DOC} (percentage of decrease in dissolved organic carbon) are very close, difference between values does not exceed 5%. The procedures in question are alternative substitutable procedures for investigating course of aerobic biodegradation of substances in an aqueous environment.
- Advantage of observing biodegradation on basis of observing produced CO_2 through gas chromatography is simplicity of apparatus (gas-tight flasks with outlets and septum), testing capacity (number of testing flasks essentially unlimited), variability of test conditions (for example, temperature, composition of inoculum, etc.).

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