



An automated test for measuring polymer biodegradation

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Abstract

The biodegradability of polymer materials as evaluated by the modified Sturm test is labor-intensive, cumbersome and costly and also tends to cumulate errors. An automated system for the measurement of carbon dioxide would overcome many of these disadvantages. We describe here a method in which CO₂ was determined by IR spectroscopy. We compared the results with those from trapping CO₂ in a solution of barium hydroxide (Ba(OH)₂) followed by manual titration. The automated system was more reproducible, less costly and more compact. The automated system could also be employed to measure the biodegradability of other substances such as oils and detergents. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The increasing problems posed by waste management of packaging materials (Directives 94/62/EC, 1994) have stimulated interest in biodegradable materials (Mayer and Kaplan, 1994; Chiellini and Solaro, 1996). However, methods are required to accurately assess degradation of materials in the environment. There are a wide variety of methods currently available for measuring the biodegradability of polymer materials. In general they are based on an indirect measure of degradation such as the consumption of oxygen, the amount of carbon dioxide produced, or the increase in biomass.

Most of the available tests for material degradation are based on determination of CO₂ produced. Standard methods include the modified Sturm test (OECD, 1992) for insoluble chemical compounds, and the American

Standard test (ASTM, 1993) for plastics. Determination of CO₂ production is also used to quantify the biodegradation of polymer materials (McCassie et al., 1992; Coma et al., 1994; Müller et al., 1994; Musmeci et al., 1994; Van Der Zee et al., 1994; Itävaara and Vikman, 1995; Spitzer et al., 1996).

Methods used to quantify carbon dioxide can be classified into two groups: (i) manual methods such as precipitation of carbon dioxide in a basic solution followed by titration (Sturm test), (ii) automated methods such as automatic titration or a direct sampling of head gas and measurement of carbon dioxide by gas phase chromatography or infrared spectroscopy.

The Sturm test (Sturm, 1973) (manual method) is commonly employed for evaluation of the biodegradability of polymer materials, although it is not without drawbacks. Not all the CO₂ may be trapped leading to underestimation and errors cumulate from daily determinations (Müller et al., 1992). The test is also time consuming (100 h of titration work for a test duration of 45 days) (Calmon, 1998). Furthermore, a considerable amount of equipment is required including three

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scrubbing flasks at the outlet of each reactor to trap the CO₂. Errors also stem from the large volumes of medium and the small amount of sample (50–100 mgC/l).

In view of these disadvantages, workers have attempted to develop automated systems that can be used to measure low concentrations (De Baere, 1992; Müller et al., 1992; Itävaara and Vikman, 1996; Calmon, 1998). In all these automated methods, the material under test is the sole source of usable carbon by the microorganisms.

The Sturm test cannot be readily automated due to difficulties in handling the barium carbonate (BaCO₃) precipitate, although semi-automatic or automatic techniques of titration have now been devised. Müller et al. (1994) replaced the solution of Ba(OH)₂ by a solution of sodium hydroxide (NaOH) to trap CO₂, while the pH (approximately 11.5) was maintained constant by an automatic titrator. Since the solubility of CO₂ is low, the trap is maintained 10°C and stirred continually. Itävaara and Vikman (1996) have described an automated Sturm test based on measurement of the change in electrical conductivity of a basic solution (0.1 M KOH). The conductivity is calibrated with known amounts of CO₂ and the results are recorded by computer. However, this system requires different measuring electrodes for each reactor.

In these two examples, CO₂ not detected by the automatic titration system is trapped in additional scrubbing flasks and determined by conventional titration at the end of the test. The error in determination of CO₂ is reduced as the scrubbing bottles are not disconnected avoiding entry of air, which contains CO₂, into the system.

Boatman et al. (1986) and Struijs and Stoltenkamp (1990) have described semi-automatic methods for determination of CO₂ by gas chromatography on soluble organic compounds. More recently, De Baere (1992) has described a multichannel system for automated measurement of CO₂ and O₂ by gas phase chromatography of samples of gas produced. This system has been patented for evaluation of the biodegradability of composted materials, but using a more sensitive detection system, it can also be applied to liquid media. The main disadvantage of gas phase chromatography is the requirement for a vector gas (generally nitrogen). This vector gas must pass through the whole system for the duration of the test (up to two months) and introduces additional cost.

High concentrations of gas produced from the degradation of xenobiotic compounds have been determined on-line by infrared spectroscopy (Buitron et al., 1993; Fiechter and Von Meyenburg, 1968; Saudeco-Castaneda and Trejo-Hernandez 1994; Spérandio and Paul, 1997). Derradji (1995) has developed an automated system based on the measurement of CO₂ (by IR spectroscopy) and O₂ (by paramagnetic spectroscopy) to

monitor the degradation of strong concentrations of insoluble polymers (10 g/l) in a series of 10 reactors. The degradation of compounds can now be evaluated from the O₂ consumed and the CO₂ produced using commercially available equipment (Micro-Oxymax Repirimeter, Columbus Instruments USA). However, this equipment originally designed for determination of high concentrations of CO₂ is rather costly (Czekajewski and Nennerfelt, 1990).

The automated system described here is based on the discontinuous measurement of CO₂ by infrared spectroscopy. It was designed to (1) measure automatically production of carbon dioxide over a 1 to 2 month period; (2) have an overall sensitivity not less than sensitivity of the detector; (3) measure low concentration of CO₂; (4) convert the measured variable directly into amount of carbon dioxide produced; (5) be convenient in use and cost-effective.

2. Materials and methods

2.1. Materials

NaHCO₃ (Aldrich) was employed to check the efficiency of CO₂ trapping. Sodium acetate (Aldrich), a water-soluble compound, was selected as the reference molecule to check the reproducibility of the different methods for measuring biodegradability. A Carlo Erba Model 1106 analyzer was used for the elemental analysis of this compound.

2.2. Modified Sturm test

The modified Sturm test was conducted according to the CEN standard (Draft Method, 1995). The inoculum was prepared as follows: for each test, an activated sludge sample was taken from the Ginestous water treatment plant (Toulouse, France). The activated sludge was mixed for 1 h. After a decantation for 30 min, the supernatant was extracted and characterized. Dry matter (DM) and volatile matter (VM) were determined by measurement of material in suspension in an oven heated to 100°C and 500°C, respectively. The concentration of viable cells (CFU) was counted on Petri dishes.

The compound to test (70 mg C/l) was introduced in the mineral medium (1.5 l) and inoculated with supernatant (15 ml). The gas produced in each reactor was trapped in a solution of barium hydroxide (Ba(OH)₂, 0.05 M). The quantity of CO₂ produced was determined by titration of the excess Ba(OH)₂ by a standard solution of hydrochloric acid (HCl, 0.05 N) using phenolphthalein as indicator.

The theoretical carbon dioxide content (mg ThCO₂) produced by total oxidation of the material was calculated from

$$\text{ThCO}_2 = \text{mgC} \times \frac{44}{12} \quad (1)$$

The degree of mineralization was expressed by

$$\text{Mineralization level} = \frac{(\text{CO}_2)_{\text{material}} - (\text{CO}_2)_{\text{blank}}}{\text{mThCO}_2} \quad (2)$$

where (CO₂)_{material} is the amount of CO₂ measured in the test vessel containing the material, and (CO₂)_{blank} in the blank control.

The reproducibility of the system was evaluated on 9 reactors (8 containing sodium acetate and 1 control) from the standard deviation of the CO₂ production after 28 days. This experiment was replicated twice.

2.3. Automated system

2.3.1. Overall description of the set-up

A diagram of the experimental set-up is presented in Fig. 1. The analytical system was an infrared CO₂ analyzer (BINOS 1004; range 0–1000 ppm, Rosemount). The method was based on a photoacoustic measurement of the selective absorption wavelength of CO₂.

Tests were conducted using 3 l cylindrical reactors (Verres Wagner, Toulouse, France), containing 1.5 l of liquid and thermostated at 23°C. The liquid phase was homogenized by a magnetic stirrer (MS) and by circu-

lation of gas through the liquid medium. The air from a compressor was filtered and regulated by a flow meter. It was then passed through solutions of 5 M KOH and Raschig rings to remove CO₂. The air was then dried in a coalescing filter. The purified air containing a low and constant concentration of CO₂ (<0.3 ppm) is introduced into the reactors.

At the beginning of each series of tests, each reactor (FM1 to FM5) is fed with CO₂-free air for 24 h to clear residual CO₂ from the liquid medium and gas volumes. All reactors are then isolated by closing electrovalves (EVA and EVB). The liquid and gas phases in each reactor are continuously mixed by pumps (P1 to P5).

From time to time, the head space of each reactor is swept into the gas analyzer by a stream of CO₂-free air. This gas mixture passes through a coalescing filter and the gas flow is measured accurately. The carbon dioxide level is measured automatically and stored on computer file. At the end of the analysis period (renewal of the head space with CO₂-free air and return to the base line), the reactor is isolated by closing the electrovalves.

The electrovalves are under computer control. The IR analyzer and flow meter are connected to the same computer via RS232 serial interfaces. The computer continuously monitors the gas concentration and integrates the area under the curve from the volume of CO₂ produced and the concentrations of CO₂ over the duration of the analysis. In common with the modified Sturm test, levels of mineralization (Eq. (2)) are plotted as a function of time.

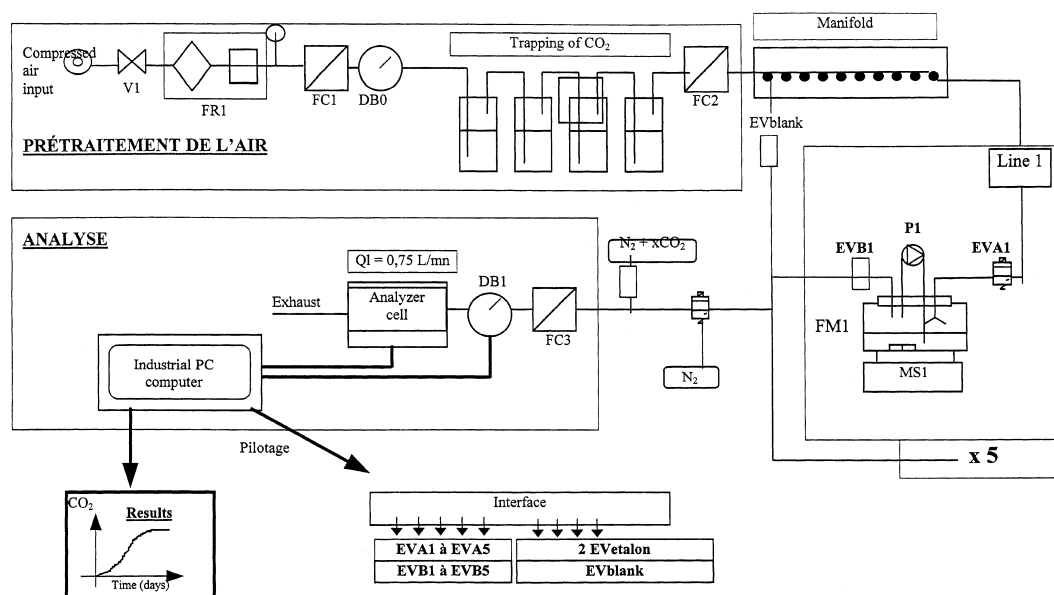


Fig. 1. View of automated test set-up for evaluation of biodegradability (*Air pretreatment*: V1 valve, FR1 regulator filter, FC1 coalescing filter, DB0 flow meter, CO₂ traps KOH with Raschig rings, *Reactor* (FM1) MS Magnetic stirrer, EVA & EVB electrovalves, P pump for gas circulation (option), *Analysis*: FC3 coalescing filter DB1 flow meter (weight) Analyzer).

2.3.2. Experimental conditions

For all studies on the automated system, the input air flow rate was fixed at 0.75 l/min. The temperature of the liquid medium was kept constant at $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$. We employed the same reaction medium, and the same methods for preparing the inoculum and general procedures as with the manual method (modified Sturm test). The tests with microorganisms were conducted with a supernatant of an inoculum from activated sludges from the aeration pool at the Castelnau le Lez waste-water treatment plant (Hérault, France). An identical procedure of preparation as for the Sturm test was employed. Prior to seeding, the concentration in Colony Forming Units was determined (mean value of 5×10^5 CFU/ml) according to Sharpe et al. (1972). At the end of the test, 1 ml of concentrated HCl was added and the amount of dissolved inorganic carbon (DIC) was determined from the amount of CO_2 liberated.

2.3.3. Performance tests

A carbon balance throughout the system was conducted to check for leaks. An accurate quantity of CO_2 was produced by addition of a known amount of NaHCO_3 in a phosphate buffer solution. The reaction medium consisted of 125 ml of phosphate buffer and 1125 ml of deionized water (Raghavan et al., 1993). The medium was stirred with a magnetic bar and the pump, and all residual CO_2 was driven out in a stream of CO_2 -free air for 24 h. Seventy five milligrams of NaHCO_3 were then added to the reactor. During dissolution, some carbon dioxide is liberated immediately, while the other part, in the form of dissolved CO_2 was driven out in a stream of CO_2 -free air. The efficiency of the system to measure the CO_2 content was defined as follows:

$$\text{efficiency}(\%) = [(\text{CO}_2)_{\text{measured}} / (\text{CO}_2)_{\text{theoretical}}] \times 100.$$

The error was evaluated from the standard deviation, and the test was repeated three times on three different reactors.

2.3.4. Optimization

Two modes of production of CO_2 were evaluated in this automated system (Fig. 2.):

1. A conventional mode based on a closed still reactor; the reaction mixture was stirred with a magnetic bar, and at 6 h intervals the head volume gas was swept into the gas analyzer.
2. An optimized mode base with circulation in a closed loop within the closed reactor; the reaction mixture was stirred by a magnetic bar and churned round by the pump to enhance contact between the head space gas and the aqueous medium.

Two tests on five reactors were carried out to monitor the degradation of sodium acetate in the two modes of operation.

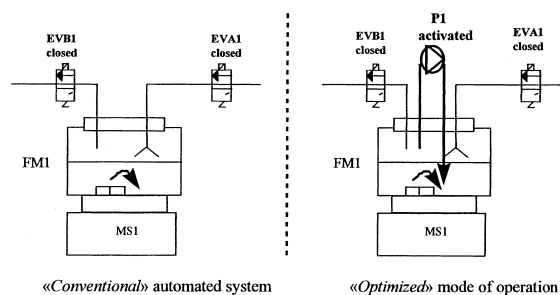


Fig. 2. Diagram of the two modes of production of CO_2 in the automated system.

2.4. Comparison between the two methods

For each method, the mean standard deviation (σ) was calculated for all points (p) of the plot of mineralization, and for the n tests from relationship:

$$\sigma = \sqrt{\frac{\sum_1^n \left(\sum_1^p (x - \bar{x})^2 \right)}{N - 1}} \quad (3)$$

with $N = p \times n$ $\sum_1^p (x - \bar{x})^2$ corresponds to the sum of squares of the deviations of each measurement of CO_2 (x) at a time t compared to the mean (\bar{x}) of the p points at this same time t .

The overall error from lack of repeatability of n tests was thus calculated for each mode of operation.

3. Results and discussion

3.1. Efficiency of the measurement system

We found an efficiency of $97 \pm 3\%$, for a flow rate of 0.75 l/min. It took around 5 h to obtain this level of conversion from NaHCO_3 .

3.2. Modified Sturm test

Fig. 3 shows the rate of conversion of carbon dioxide as a function of time after improvement of the manual method.

The final ratio of mineralization of sodium acetate was 76% with a mean standard deviation between two series of experiments of 7%.

Production of biomass and intermediate products can account for the difference between 76% and 100% conversion of CO_2 . Although CO_2 is the final catabolite of the oxidation of the polymer, some of this CO_2 will be used to synthesize new microbial cells.

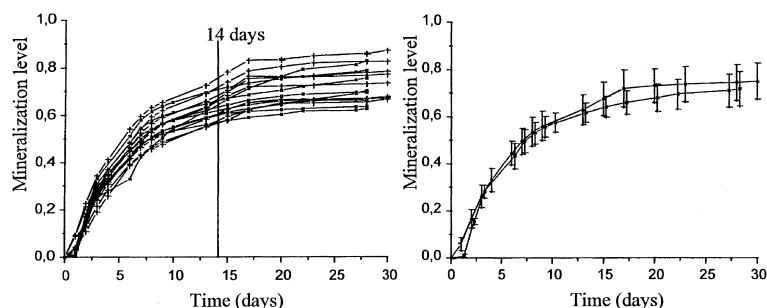


Fig. 3. Plot of the degree of mineralization of sodium acetate (155 mg) against time: series of 16 experiments (left), average of each series with standard deviation (right).

3.3. Optimization

It can be seen from Fig. 4 that the automatic system produced a larger amount of CO_2 . In the conventional automatic system much inorganic carbon remains dissolved in the liquid medium in the form of carbonates and bicarbonates.

It can be seen from Fig. 5 that the automated system gave more reproducible results than the manual method. The mean standard deviation, calculated for all points, was significantly less with the optimized system (3.1% vs 4.9% and 4.5% for the manual method and the conventional automated system, respectively).

The curve for the degree of conversion of CO_2 in the conventional automated system was below the two other curves, indicative of an accumulation of CO_2 in the liquid phase. Indeed, a considerable amount of CO_2 was liberated on acidification of the medium at the end of the experiments. The plateau in the plot of the mineralization of sodium acetate was reached sooner with the optimized method than with the manual method.

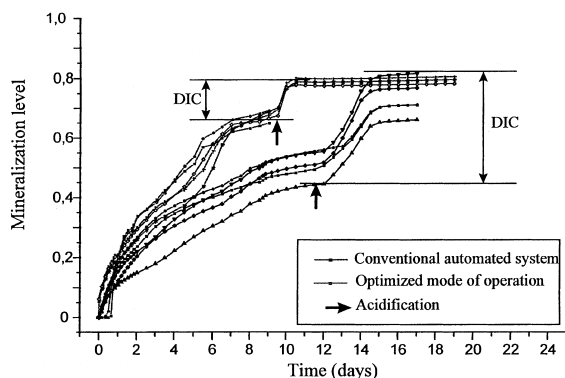


Fig. 4. Comparison of the degradation of sodium acetate between the conventional and optimized modes of operation: reproducibility plots (arrows indicate the time of acidification, which liberated dissolved inorganic carbon (DIC) into the liquid medium).

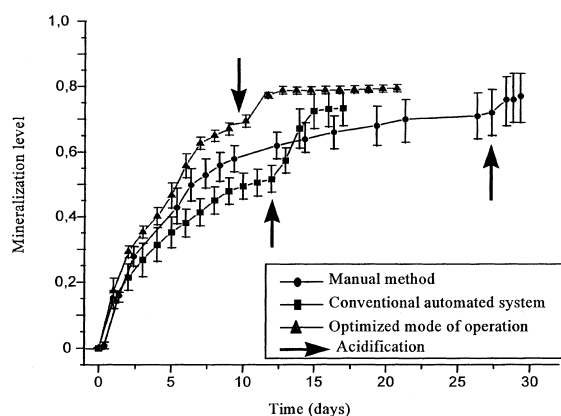


Fig. 5. Comparison of the degradation of sodium acetate between the manual and the two automated methods.

The only difference between the conditions used for the manual and the two automated methods was in the origin of the inoculum. For the manual method, samples were taken from the waste water treatment plant in Toulouse, whereas for the automated method, samples were taken from the Castelnau le Lez plant in another region of France. Both were activated sludges harboring a diverse population of microorganisms. The studies of Dias and Bhat (1964) and Thomazeau (1982) have shown that different stations do harbor different strains, which can affect the reproducibility of biodegradability tests (Nylholm and Kristensen, 1992). This can account for the difference in production of CO_2 between the manual and optimized automatic methods.

4. Conclusion

We selected the infrared spectroscopic method for determination of CO_2 rather than titration or gas phase chromatography in an attempt to simplify the measurement system. Conductivity methods necessitate electrodes in each reactor (Itävaara and Vikman, 1996).

The infrared method is simpler to implement as the measuring probe is continuously swept by the gas at constant pressure and flow rate. The gas (room air) is supplied by a pump or a compressor and is purified by passing it through CO₂ absorbents.

However, conventional infrared probes are only suited for determination of high concentration of CO₂, and in the CEN draft standard, little CO₂ is produced as samples are small (50 mgC/l). We thus developed a method of concentrating CO₂ in the head space by circulation in a closed loop within a closed reactor. The reaction medium was stirred by a magnetic bar, and contact between the liquid and gas media was enhanced by pumping. At regular intervals, the head space gas was swept through the CO₂ detector. Repeatability was evaluated on an automated chain of five reactors.

Experiments were also conducted using the manual method (determination of CO₂ trapped in a solution of barium hydroxide by titration). The optimized automated system was found to give more reproducible results than the manual method. It was also less labor-intensive, more compact and cost-effective. The system could also be employed to evaluate the biodegradability of other chemicals such as oils and detergents.

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