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Continuous automated measurement of carbon dioxide produced by microorganisms in aerobic conditions: application to proteic film biodegradation

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Abstract

A new automated set-up based on the original Sturm test was developed for determining the biodegradability of proteic mulching films. This method is based on the measurement of released (respiratory metabolism of microorganisms) carbon dioxide to determine film biodegradability. Maintenance of constant aerobic conditions in thermoregulated bioreactors and continuous measurement of released CO_2 with an infrared differential gas analyser were the main advantages of this set-up. It was verified with a plasticised proteic film that soil extracts could be used as microbial sources instead of activated sludge that was usually used in laboratory biodegradation tests. ***To cite this article: S. Lefaux et al., C. R. Chimie 7 (2003)***.

Résumé

Un nouveau dispositif de mesure basé sur le principe du test de Sturm a été mis au point et adapté à la quantification de la biodégradation de films protéiques destinés au paillage agricole. Ce montage expérimental permet de déterminer la biodégradabilité potentielle d'un film polymère en mesurant le dioxyde de carbone respiratoire dégagé lors de la dégradation du matériau testé par des micro-organismes. Ce dispositif est équipé d'un analyseur différentiel à infrarouge, qui mesure en continu le CO_2 dégagé de six réacteurs thermorégulés. Les expériences réalisées en conditions aérobies sur un film protéique plastifié ont montré que la microflore du sol pouvait être retenue comme inoculum bactérien à la place des boues activées habituellement utilisées dans les tests de laboratoire. ***Pour citer cet article : S. Lefaux et al., C. R. Chimie 7 (2003)***.

Keywords: Proteic film; Biodegradation; Automated measurement; Carbon dioxide evolution

Mots clés : Film protéique ; Biodégradation ; Mesures automatisées ; Dioxyde de carbone

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1. Introduction

Knowledge of chemical compounds biodegradability is one of the most important aspects of their environmental behaviour. A biodegradable substance is expected to cause less ecological problems in the long term than a persistent one [1]. Polymer biodegradation test data are necessary before their marketing in order to know in which conditions they must be used. Degradation processes are constantly taking place on a large scale in the natural environment. The decomposers or 'biodegraders' are microorganisms, especially bacteria, and the decomposers implement several biochemical degradation pathways depending on natural substrates. In a laboratory, this natural degradation process has been simulated and facilitated by using liquid inorganic media in aerobic biodegradation tests. In this experimental set-up, biodegradation processes occurred under controlled conditions. Ultimate aerobic biodegradation consists in the breakdown of organic compounds by microorganisms into carbon dioxide, water and an increase of microbial biomass in the presence of oxygen.

The Organisation for Economic Cooperation and Development (OECD) is leading international efforts to standardize biodegradation test methods. The well-established OECD carbon dioxide (CO_2) method [2] based on Sturm's original test [3] is usually used for assessing biodegradability of more or less soluble organic chemicals. Evolution of carbon dioxide is considered by the OECD standard to be the only unequivocal proof of microbial activity [4]. The microbial inoculum may be extracted from a variety of sources: activated sludge or soils [5]. The aim of this work was to perfect a reliable method for testing the biodegradability of polymeric films under laboratory conditions.

The biodegradation test described here made allowance for recommendations of standards [2–9] and opted for a continuous-aerated system in which CO_2 released into the headspace of bioreactors was measured with an infrared CO_2 analyser. At defined regular intervals of time, a computer recorded instantaneous concentration of CO_2 existing between air intake and air exit (differential method) in bioreactors. Automated measurement of CO_2 is an advantage over the manual Sturm test [3]. This modified Sturm test procedure has been adapted to proteic mulching films using soil-extract microorganisms.

2. Experimental

2.1. Conditions of aerobic biodegradation test

All experiment vessels and mineral media [9] were sterilised by autoclaving at 121 °C and a pressure of 1 bar for 20 min. The water used in all the experiments was purified by filtration (MilliQ system®, Millipore, USA). All the reagents used were analytical grade.

The conditions of the biodegradation were based on standards for aerobic batch tests [1]. Bioreactors contained the tested film, the inorganic medium and soil microorganisms. So, the organic tested film was the only source of carbon and energy for the microorganisms. The standardized inorganic medium [9] had sufficient buffering capacity to maintain a pH value of about 7 throughout the biodegradation trial. According to standardized methods [5,9], the reaction medium initially contained about 10^6 colony-forming units (CFU) per millilitre. Test mixture thermostated at 23 °C was continuously aerated and mixed by stirring. Blank control bioreactor contained only inorganic medium [9] and microbial inoculum. For each trial, a set of six bioreactors (Fig. 1) was prepared as follows: (*i*) four bioreactors for the tested material, (*ii*) two blank bioreactors. Tested material and microbial inoculum were added into each bioreactor when CO_2 saturation of liquid inorganic medium was reached.

The biodegradation test normally runs for 28 days [5], but may be prolonged or stopped depending on the occurrence of the plateau phase. The CO_2 produced was measured in the exhaust air of each bioreactor at regular time intervals using a differential infrared CO_2 analyser (IRGA).

The microbial source was obtained from samples of soil (30 g) aseptically collected in a sterile vessel from the upper layer (0–20 cm) of a field planted with vegetables located at Parence (Sarthe, France). Soil analysis gave 1.39% (percentage in dry mass) of organic carbon less than 4% as recommended by standards [10,11] (Microanalyses ICSN–CNRS, Gif-sur-Yvette, France). Soil pH was determined in a supernatant of slurry (5 parts distilled water, 1 part soil) [12,13]. The soil water pH was between 7 and 8, in accordance with standards [11].

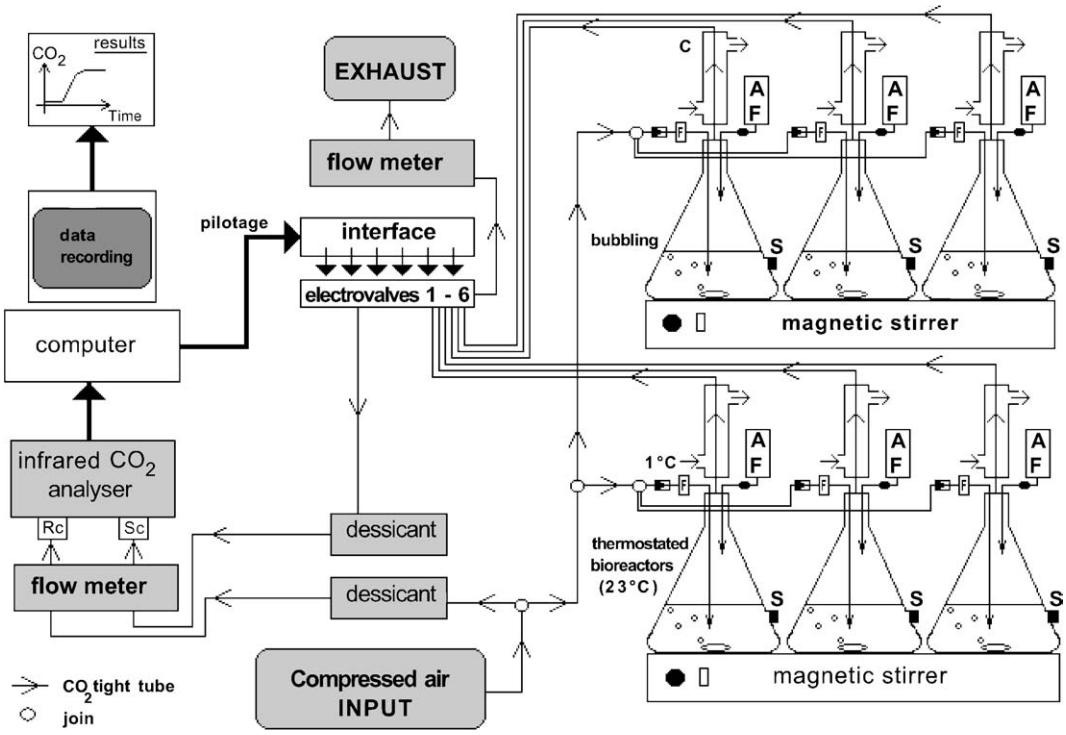

Fig. 1. Scheme of automated experimental set-up for measuring polymer biodegradation (R_C: reference cell, S_C: sample cell, AF: addition funnel, F: sterile filter, S: septum, C: condenser).

2.2. Description of the experimental biodegradation set-up

This set-up is shown in Fig. 1. The analytical system is composed of a differential infrared gas (CO_2) analyser (IRGA LI-6252, LI-COR®, Nebraska, USA). The analysis was based on the difference in absorption of infrared radiation passing through a reference cell (Rc) and a sampling cell (Sc). Tests were carried out using 3-l cylindrical thermoregulated (23 °C) bioreactors. All bioreactors were equipped with a check valve preventing reverse flow (VWR International SAS, Strasbourg-Cronenbourg, France), sterile filter (Ø = 0.22 μm, Nalgene®, USA) and magnetic stirring bar. Compressed air is flowed permanently into CO_2-tight tubes [8] connecting the six bioreactors. Before analysis by IRGA, the air was regulated by flow meters (0.5 l min^{-1}), dried through silica gel columns and sterilised by filtration. Airflow and stirring speed were adjusted at the beginning of the test. At regular intervals, the gaseous flow of each bioreactor was swept into the IRGA. The carbon dioxide given off is measured automatically and stored on a computer file. Biodegradation tests were monitored automatically by a homemade operating program which controlled opening and closing of each three-way electrovalve located downstream of each bioreactor. After the set period of analysis for one bioreactor, the computer switches on the electrovalves for another bioreactor. Moreover, during a biodegradation test, instantaneous concentration of CO_2 and test parameters were displayed on the computer screen for the bioreactor in process. The level of biodegradation is calculated by comparing the CO_2 given off with the theoretical amount ($ThCO_2$) and expressed in a percentage.

2.3. Calculations

The theoretical amount of CO_2 ($ThCO_2$, g) produced by total oxidation of the tested material was calculated using the following equation:

$$ThCO_2 = C_{TOT} \times (44/12) \times W \quad (1)$$

where C_{TOT} is the total organic carbon contained in the tested material (g g^{-1}), W is the weight of tested material sample used in each bioreactor (g), 44 and

12 are the molar mass of CO_2 and atomic mass of carbon (g mol^{-1}), respectively.

From the kinetic of the formation of CO_2 determined for each reactor, the cumulative amounts of carbon dioxide released were calculated in relation with time. The percentage of biodegradation (Pb) of the tested material for each measurement interval was expressed by:

$$Pb = [(CO_2)_T - (CO_2)_B / ThCO_2] \times 100 \quad (2)$$

where $(CO_2)_T$ is the cumulative amount of CO_2 evolved in each bioreactor containing tested material (g), $(CO_2)_B$ is the cumulative amount of CO_2 evolved in the blank bioreactor (g), $ThCO_2$ is the theoretical amount of CO_2 that can be produced by the tested material (g).

2.4. Example of application to proteic film biodegradation

The proteic film was prepared with sunflower (*Helianthus annuus* L.) oilcakes plasticised with glycerol (20%, w/w) (V. Monti, personal communication). From the measured data, percentage of biodegradation is calculated and a biodegradation curve is drawn. The test result is obtained by comparing the measured CO_2 with a theoretical value ($ThCO_2$), which is calculated from the molecular formula of the reference substance and from elementary analysis (ponderal percentages) of the tested polymeric film according to calculations of standard ISO 14852 [9].

Fig. 2 shows the biodegradation degree of two proteic film samples with two different amounts as a function of time. This curve is distinguished by a lag phase (four days), a short exponential phase (four days) and a nearly linear phase. The start of the plateau phase only began after 27 days. Previous tests using the same proteic film (data not shown) but with microorganisms extracted from activated sludges showed that the plateau of the mineralisation curve versus time was reached sooner than with soil microorganisms. The percentage of the biodegradation here reached only 25%. This low level of mineralisation may be explained by the fact that a part of available carbon is used by microorganisms for heterotrophic growth. This large biomass production was verified at the end of each biodegradation test by cell counting.

Whatever the pass level criteria are, good biodegradation [5,14] of a homogeneous test material is 60% in the case of tests using released CO_2 as an indirect measure of biodegradation. Moreover, aqueous biodegradation tests have a considerably lower degrada-

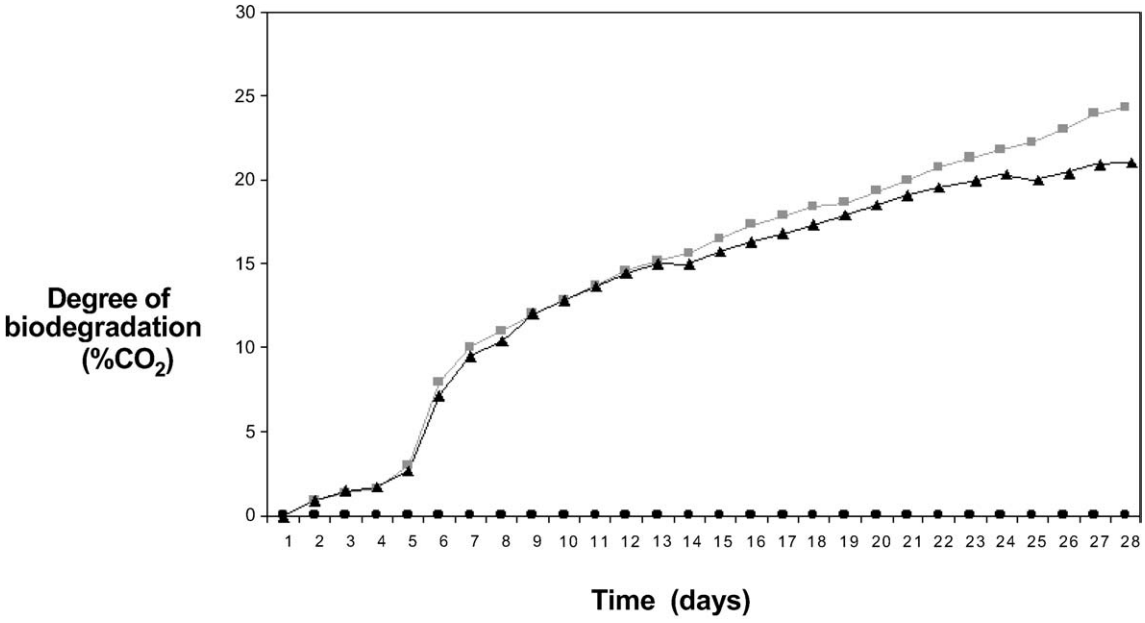

Fig. 2. An example of biodegradation curves (mineralisation) as a function of time for two quantities of proteic film (full circle: blank (only soil microorganisms), full triangle: 0.5 g of initial proteic film, full square: 1 g of initial proteic film).

tion potential compared to terrestrial tests, because fungi, which play an important part in polymer degradation, do not have optimum growth conditions in liquid medium.

3. Final remarks

For testing biodegradability on an appropriate laboratory-scale, this test method was available. The proteic film biodegradation by soil microorganisms was effective according to a molecular structure (spectrometric infrared analysis, data not shown). Therefore, in the case of new macromolecules, this aquatic test provides in the first step, basic information on potential biodegradation of such polymeric film. Moreover, biomolecular approach showed that the microbial community of soil extracts changed between the beginning and the end of the biodegradation test (data not shown). Consequently, there is a need for a clear identification of biodegradation actors that can be obtained with further biomolecular characterisation in order to normalise laboratory tests. Results of biodegradation tests are an important criterion for such material as polymeric mulching film, because the new European law [15] will classify them as environmentally hazardous or not.

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