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Evaluation of the Biodegradation of Starch and Cellulose Under Controlled Composting Conditions

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In order to verify the response of the controlled composting test method (i.e., the ISO/DIS 14855:1997, the ASTM D 5338-92, or the CEN counterpart) to starch at different concentrations, the maximum amount prescribed by the test method (100 g) and lower amounts (60 and 30 g), as if starch were a coingredient in a blend, were tested. After 44 days of incubation (at a constant temperature of 58°C) the biodegradation curves were in a plateau phase, displaying the following final values (referred to a nominal starch initial amount of 100 g): starch 100 g, 97.5%; starch 60 g, 63.7%; and starch 30 g, 32.5%. The data show a CO_2 evolution roughly equal, in each case, to the theoretical maximum, indicating a complete starch mineralization. We cannot discern whether the deviations found at lower concentrations are caused by a priming effect. In any case, the extent of the deviations is not high and is acceptable in biodegradation studies. The average biodegradation of cellulose, obtained gathering four independent experiments with 11 biodegradation curves, turned out to be 96.8 ± 6.7% (SD) after 47 ± 1 days. The data indicate that the controlled composting is a reliable test method also for starch and cellulose and, consequently, for starch-based and cellulose-based materials.

KEY WORDS: Starch; cellulose; biodegradation; ISO/DIS 14855:1997; ASTM D 5338-92; priming effect.

INTRODUCTION

The respirometric test methods used to assess biodegradability of a given substrate in solid state are based on the determination of the net CO_2 evolution, i.e., the CO_2 evolved from the mixture substrate–soil (or substrate–compost) minus the CO_2 evolved from the unamended soil (or compost) tested in a different reactor (the background CO_2 production). The net CO_2 evolution is ascribed to the mineralization of the substrate. The assumption of this approach is that the addition of the test substrate does not significantly alter the background CO_2 evolution of the solid matrix. It has been reported that glucose and its polymers can stimulate the mineralization of the organic matrices used in solid-phase biodegradation studies [1, 2]. This induction, called the "priming effect," has raised many concerns regarding the reliability of test methods such as the controlled composting (coco) test [3, 4] and the soil contact test [5]. The measurement of the biodegradability with the coco test is considered a very important procedure for the definition of the compostability of plastics and packaging. As a matter of fact, the coco test method has been standardized by several organizations, such as the ASTM (AMST D5338-92), the CEN (Cen Draft), and the ISO (ISO/DIS 14855), in order to be used within the respective compostability guidelines. The substantiation of the reliability of the coco test method is, therefore, an outstanding issue, because it is at the foundation of the compostability criteria under discussion.

In a biodegradation study under composting conditions with ^{14}C-labeled glucose and cellulose, a complete biodegradation of the two substrates could be established using the traditional respirometric approach, that is measuring the cold, net CO_2 evolution (about 95% for glucose and 100% for cellulose), while the simultaneous

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^{14}C-CO_2/^{14}C-glucose (or cellulose) measurements indicated only ≈50% biodegradation [6].

This means that only about 50% of the total net CO_2 produced by the mixture substrate–compost derived directly from the radiolabeled substrate. The remaining fraction derived from the (cold) organic carbon present in the compost before the addition of the radiolabeled substrate. This is indisputable proof that a substantial mineralization of the compost is occurring and it is considered as strong evidence toward the "priming effect" hypothesis. A major consequence of this explanation is that the reliability of the coco test method is drastically reduced, whenever a "priming" substrate is tested, because a significant overestimation of the real biodegradation can be expected. Still, the data do not indicate a failure of the coco measurements. In fact, in the same study it was proved, using radiolabeling techniques, that the original substrate (glucose and cellulose) was not recoverable from the matrix in the end of the experiments [7]. This is an indication that the coco method, even if based on the cold, net CO_2 measurements, gives an accurate representation of the biodegradation degree reached by the substrate. As a matter of fact, complete substrate removal was accompanied by ≈100% production of CO_2 (with both glucose and cellulose). The lower than expected evolution of ^{14}C-CO_2 strongly suggests that an exchange process between the ^{14}C deriving from the substrate and the organic carbon in the matrix (i.e., biomass, compost, humic acids) occurs. A possible hypothetical explanation is the following: metabolic turnover is triggered after substrate (glucose or its polymers) addition and, operating for a limited time, "traps" some ^{14}C within biomass (i.e., in cellular structures). However, an equivalent amount of carbon present in the biomass before the addition of the substrate (and, therefore, cold) is released by the turnover process and eventually respired in place of the radiolabeled carbon, so that the actual CO_2 evolved is equivalent to the CO_2 expected in the case of full mineralization of the test substance. It was not the aim of this work to clarify the fate of the radiolabeled starch (or cellulose) when biodegraded in the coco system. We rather wanted to give some further piece of information to verify the accuracy of the coco test method in measuring starch and cellulose biodegradation.

MATERIALS AND METHODS

Test Substances

Corn starch (GL 03405) was purchased by Cerestar. Cellulose microcrystalline (Avicel) was purchased from Merck. The carbon content was, respectively, 44.44 and 42.50% (determined by elemental analysis). The compost was obtained from a composting plant located in Alessandria (Italy), treating sewage sludge and bark at a 1 : 1 ratio. The percentage of volatile substances of this compost (substances evaporated at 550°C) was 59.27% (dry weight).

Apparatus

The controlled composting apparatus used in this work has been described previously [8]. Compressed air pressure is reduced by a pressure reduction device to about 0.5 atm. The flow is then split into 12 lines, corresponding to 12 composting reactors. The airflow rate of each line is measured by a rotameter and adjusted by a valve (Flow-Meter srl, Italy) and led to the reactor by means of natural rubber thick tubing (Ascenso, Italy). The air is fluxed upward in each reactor (3-L glass bottles) via a hollow shaft finishing with a "T." The reactors are heated in a thermostatic water bath. The exit gas is passed, before measurement, through a water-cooled glass coil, to trap the vapor. The airflow rate of each line is measured with a precision rotameter (Flow-Meter srl) and the CO_2 concentration is measured by an infrared CO_2 detector (Gas Monitor ADC 2000 Series, The Analytical Development Company, UK). The CO_2 evolution rate (CER) is calculated by multiplying the CO_2 concentration (g/L) by the airflow rate (L/h). The amount of CO_2 produced during the time interval within two measurements is estimated by multiplying the CER by the elapsed time from the last measurement. The mineralization percentage is the ratio between the total net CO_2 produced by the sample and the amount produced in the case of complete transformation of its carbon into CO_2.

Test Setup

A mixture of mature compost (600 g, dry weight) and test material (100 g or less) is introduced in each reactor and maintained under thermophilic (58°C) nd aerobic conditions for 45 or more days. Three replicates for test material and blank reactors are used (see Table I). The quantity of substances to be tested is determined by weighing with a precision balance (Sartorius MC1). At the beginning of the test, the water content is adjusted at 50%. The water content is then periodically controlled by weighing each reactor and adding water to restore the initial weight, if needed.

Table I. Test Setup

Reactor No.	Test material	Amount (g)	Compost (g)
1	Blank	0	600.0
2	Blank	0	600.0
3	Blank	0	600.0
4	Starch	30.00	600.0
5	Starch	30.00	600.0
6	Starch	30.00	600.0
7	Starch	60.00	600.0
8	Starch	60.00	600.0
9	Starch	60.00	600.0
10	Starch	100.00	600.0
11	Starch	100.00	600.0
12	Starch	100.00	600.0

Effect of Using Normal Air Instead of Decarbonated Air

The equipment and procedures described above are based on the CEN draft [9], with the following modification. Normal compressed air is used instead of decarbonated air. The CO_2 present in the compressed air is measured, subtracted, and not taken into account. To verify that this procedure does not introduce some disturbance to the test system, a preliminary experiment has bee performed. The CO_2 production of six identical samples of compost was measured using either decarbonated air (three reactors) or normal air, following our procedure (three reactors). The results, shown in Fig. 1, indicate that the use of normal air does not affect the CO_2 assessment.

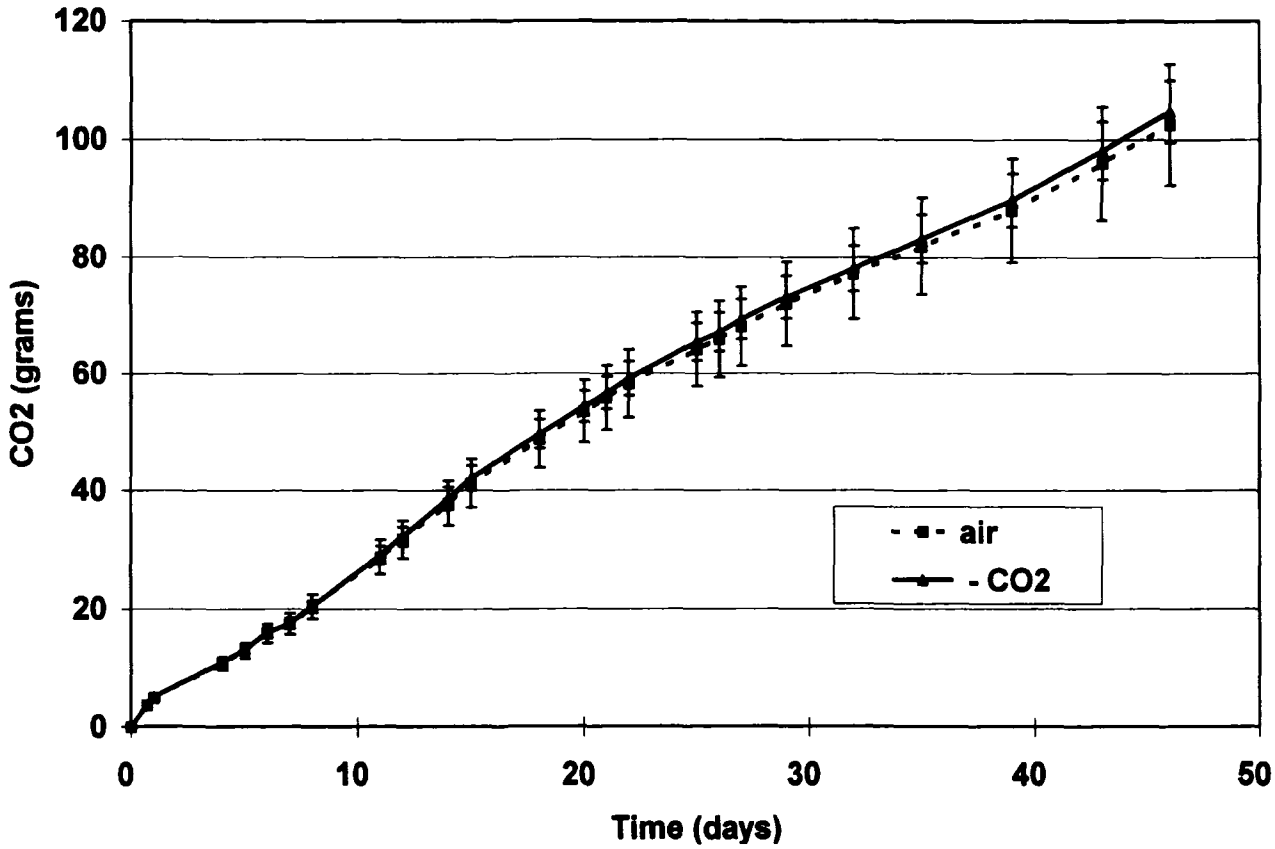

Fig. 1. The cumulative CO_2 productions of mature compost aerated with decarbonated air (-CO_2) as required by the standard test methods or with normal air (air) are shown. In the last case the CO_2 present in the inlet is measured and subtracted from the outlet measurement to get a net estimation of the CO_2 concentration.

RESULTS

Biodegradation of Starch Under Composting Conditions

To verify the capability of the coco test method to respond to different amounts of starch, we checked the maximum amount prescribed by the test method (100 g) and lower amounts (60 and 30 g), as if starch were a coingredient in a blend. In Table II the cumulative CO_2 production of each reactor is shown. In Table III the percentage of theoretical CO_2 after 44 days of incubation at 58°C is reported (average), along with the individual values of the three replicates for each group. The data are plotted in Fig. 2. Full mineralization of starch occurred at each concentration. In both the 30- and the 60-g series the average mineralization values after 44 days were higher than 100%, the theoretical maximum. The deviation is, in both cases, caused mainly by one replicate displaying an erratic behavior in comparison with the other two. In fact, two replicates are very similar and very close to 100% (102.7 and 103.5 for the 60-g set, 99.4 and 100.4 for the 30-g set), while the other is much more divergent (112.3 for the 60-g set, 125.9 for the 30-g set). In any case the average deviation is not high.

Table II. CO_2 Cumulative Production (Grams) of Each Reactor

	Reactor No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Material	Blank	Blank	Blank	Starch	Starch	Starch	Starch	Starch	Starch	Starch	Starch	Starch
Amount (g)	—	—	—	30	30	30	60	60	60	100	100	100
Time (days)												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	1.4	1.7	1.8	17.4	21.7	21.0	34.5	35.7	39.8	40.9	42.4	42.4
1	1.8	2.0	2.1	20.2	25.5	25.1	40.6	42.3	46.4	48.1	49.2	49.3
2	2.9	3.3	3.4	28.2	34.6	35.1	59.6	62.5	64.6	70.5	71.3	76.5
2	3.3	3.8	3.9	30.5	37.2	38.3	65.3	68.3	69.9	78.8	79.6	86.5
3	4.3	4.9	5.1	35.3	42.0	43.4	75.4	78.3	79.3	96.5	95.1	105.8
4	6.2	6.9	7.1	42.5	49.4	49.1	85.7	87.3	88.2	114.1	117.0	121.8
5	7.6	8.4	8.5	46.7	53.0	52.8	91.4	92.3	93.6	129.7	132.8	131.7
6	8.8	9.6	9.7	49.5	55.2	55.0	95.2	95.5	97.5	137.6	139.9	136.4
7	10.1	10.9	11.0	52.5	57.5	57.0	98.2	98.7	100.9	144.2	146.2	140.6
8	11.4	12.3	12.5	55.2	59.6	58.8	100.6	101.3	103.8	149.4	150.6	144.5
9	12.5	13.6	13.9	57.2	61.3	60.3	102.4	103.3	105.9	152.5	153.3	147.0
10	13.7	15.0	15.5	59.3	63.0	61.8	104.1	104.9	107.9	155.9	155.9	149.3
12	15.3	16.9	17.7	61.7	65.1	64.1	106.2	107.1	110.2	159.1	158.9	151.8
13	16.3	18.0	19.0	63.2	66.4	65.4	107.4	108.1	111.6	160.7	160.5	153.2
14	17.5	19.3	20.4	64.3	67.7	66.7	109.1	109.5	112.9	162.1	162.5	155.1
15	18.8	20.7	21.9	65.5	69.0	68.4	110.8	110.9	114.2	163.4	164.4	156.8
16	19.9	21.8	23.2	66.6	70.2	69.9	112.1	112.0	115.3	164.5	166.1	158.2
18	23.3	25.4	27.2	70.4	73.9	74.5	116.1	115.3	119.8	168.1	171.1	162.8
20	24.4	26.5	28.3	71.5	75.0	75.9	117.2	116.3	121.0	169.0	172.4	164.2
21	25.5	27.6	29.4	72.6	75.9	77.3	118.4	117.3	122.4	169.9	173.9	165.5
22	26.8	29.0	30.8	73.9	77.1	79.0	119.9	118.5	124.1	171.2	175.7	167.3
26	30.8	33.1	35.0	78.1	80.9	84.7	124.2	122.7	129.7	175.6	180.8	172.3
27	32.0	34.3	36.1	79.4	82.0	86.4	125.6	123.8	131.1	176.7	182.2	173.6
28	33.3	35.5	37.2	80.7	83.1	88.2	126.6	125.1	132.4	177.9	183.5	174.7
29	34.5	36.6	38.3	81.9	84.1	89.9	127.7	126.3	133.8	179.0	184.7	175.8
30	36.2	38.1	39.5	83.3	85.3	91.9	128.9	127.8	135.6	180.5	186.1	177.0
33	40.3	41.7	42.5	86.4	88.0	96.2	131.8	131.1	139.5	183.8	189.2	180.0
34	41.5	42.7	43.3	87.3	88.7	97.3	132.6	132.0	140.5	184.6	190.0	180.8
35	43.2	44.1	44.7	88.7	90.0	99.0	134.1	133.7	142.2	186.0	191.7	182.3
37	45.6	46.0	46.6	90.3	91.6	101.3	136.3	135.9	144.2	188.0	193.8	184.1
40	49.5	48.9	49.8	93.0	94.2	104.6	139.9	139.3	147.6	191.0	197.0	186.9
44	54.3	52.7	54.1	97.0	97.4	108.5	143.8	143.2	151.5	194.5	200.7	190.3

Table III. Percentage of Mineralization of Starch Under Controlled Composting Conditions After 44 Days at 58°C

Initial starch amount (g)	% of theoretical CO_2	% of theoretical CO_2 of single replicates		
100	97.5	97	101.3	94.1
60	106.2	103.5	102.7	112.3
30	108.6	99.4	100.4	125.9

Mineralization of Cellulose Under Composting Conditions

Microcristalline cellulose for chromatography (Avicel by Merck) is frequently used as a reference material in the controlled composting tests. To verify the reproducibility of the coco test method, we plotted the data of four independent trials with three replicates each (only two in one case) for a total of 11 biodegradation curves of cellulose. The data are shown in Fig. 3. The average mineralization value of cellulose, determined grouping the data collected after 47 ± 1 days, is 96.8 ± 6.7% (SD).

DISCUSSION

The data shown in this paper indicate that the coco test method is capable of responding to the starch present in the system with a variability which is inversely proportional to the initial starch concentration. Low at a high starch concentration, it increases at a lower concentration, still being remarkably satisfactory. We cannot discern whether the deviation found at lower concentrations is caused by a decrease in sensitivity at lower CO_2 productions or whether it is caused by a priming effect. In any case, the extent of the deviation is not high and is acceptable in biodegradation studies.

The data concerning the biodegradation of cellulose show that, in some cases, values higher than 100% can be found. The overcoming of the 100% limit (in one case a 109% value was reached after 50 days) could be considered as a clear sign that a priming effect is acting. However, these "over-100%" values could be seen as a consequence of the error of the measurement. As a matter of fact, when a sufficient number of replicates is taken into account, the average value turns out to be very close to,

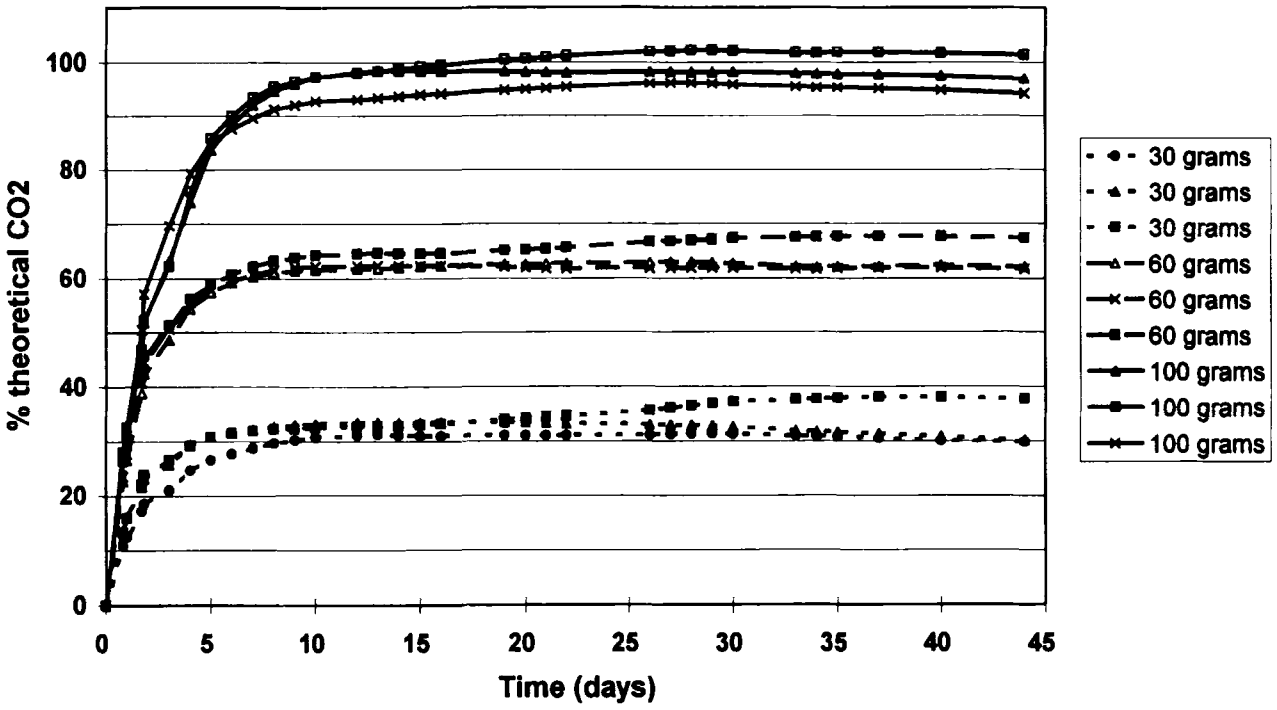

Fig. 2. The mineralization curves of starch tested under composting conditions in different amounts (30, 60, and 100 g). The mineralization values are referred to a nominal starch initial amount of 100 g.

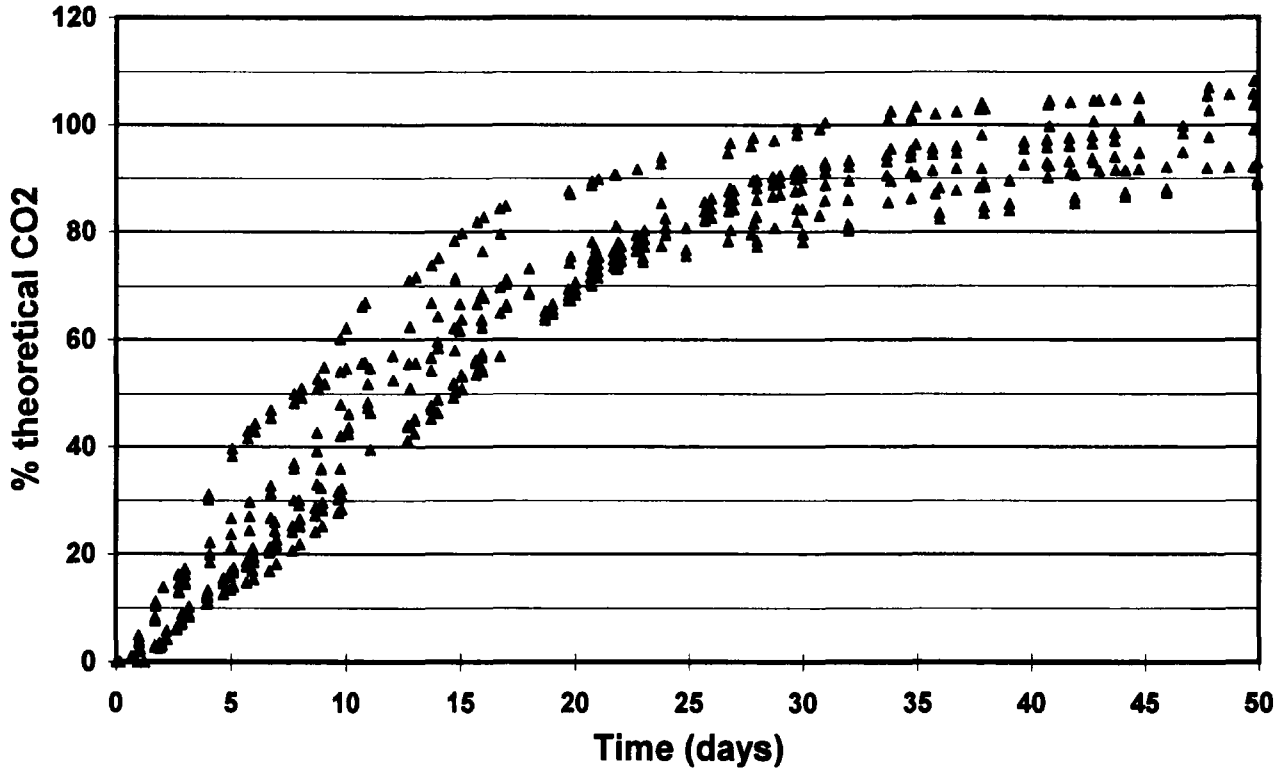

Fig. 3. Mineralization data of microcristalline cellulose (Avicel) obtained in four independent experiments of composting with a total of 11 replicates. The average mineralization value of cellulose, determined grouping the data collected after 47 ± 1 days, is 96.8 ± 6.7% (SD).

but below, 100%. Therefore, also for cellulose, as seen before for starch, the data indicate a full mineralization of the substrate after 45–50 days.

The variability of the test method is, in our opinion, acceptable considering that it is a biological system. The data reported here give support to the hypothesis that the coco test is a reliable system also for starch and cellulose and, consequently, for starch-based and cellulose-based materials.

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