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Calibration of Chemical and Biological Changes in Cocomposting of Biowastes Using Near-Infrared Spectroscopy

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Cocomposting of green wastes and sewage sludges is a complex process involving rapid biological and chemical changes. The objective of the study was to assess the usefulness of near-infrared reflectance spectroscopy (NIRS) to characterize these changes, as an alternative to standard procedures which are often time-consuming and laborious. Samples obtained during 146 days of composting were analyzed by 14 conventional methods and NIRS. Results from conventional methods demonstrated a noticeable separation into two distinct phases. An initial phase from 4 to 50−60 days was characterized by intensive degradation. A second phase up to 146 days was characterized by a decrease in all biological activities. NIRS calibrations allowed accurate predictions of nitrogen (N), carbon (C), C/N, humic acid (HA), pH, respiration, cellulase, phenoloxidase, and composting time successfully. Results were less accurate for organic matter (OM), protease, acid, and alkaline phosphatases and unsatisfactory for fulvic acid. NIRS calibration allows composting time/state of progress of maturation to be predicted accurately to within 10 days. A global index of composting evolution (GICE), resulting from the 14 parameters studied, is proposed. It is precisely predicted and shows that since NIRS is able to predict essential parameters of compost maturity, it could prove invaluable for monitoring biowastes cocomposting.

Introduction

Composting is a controlled biological decomposition of organic material, such as sewage sludge, animal manures, or green wastes, through a controlled oxidation process that results in the production of biologically mature, stable, and chemically complex organic compounds resembling soil humic substances (*1*). Composting presents many other advantages such as reducing the volume of biowastes, preventing soil erosion and nutrient runoff or reducing the need for fertilizers when applied as amendments.

Successful use of compost to maintain plant health and soil fertility requires consistent monitoring of various quality characteristics such as stability and maturity. Stability is associated with microbial activity, while maturity refers to the degree of humification of the material. The quality of compost is reflected by its effectiveness on plants as a fertilizer, its potential to minimize odor generation, or its prevention of pathogen regrowth (*2*). Physico-chemical parameters such as C/N ratio, ash content, cationic exchange capacity, or humic acid content (*3*−*5*) are commonly used to assess compost maturity.

Microbial activity, expressed by O_2 consumption, is widely used to measure compost stability (*6, 7*). Recently, enzyme activities were monitored to follow the state of progress of composting (*8*−*10*) because they vary over time as a consequence of a complex sequential intervention of various micro-organisms. Different microbial hydrolytic enzymes, including cellulases, hemicellulases, phenoloxidases, and proteases, are involved in the depolymerization of constituents of organic wastes. Mineralization of organic phosphorus is catalyzed by acid and alkaline phosphatases according to their pH-optima of activity. These enzymes are considered as the predominant phosphatases in most types of soil and litter (*11*).

However, all these approaches are very time-consuming and expensive when a large number of samples are required. Near-infrared reflectance spectroscopy (NIRS) is a physical very fast, precise, cost-effective, and nondestructive technique, making it possible to analyze a large number of samples in a practical and rapid manner. NIRS is used primarily for the rapid determination of organic constituents (*12*). Reflected light of the samples in the near-infrared (800−2500 nm) regions gives a unique signature with important chemical information about the character and number of covalent bonds between C−H, O−H, N−H, and S−H which are the primary constituents of the organic molecules. The absorption bands in the NIR arrive from combinations of fundamental vibrations (that appear mostly in the mid-infrared region 2500−25 000 nm), overtones of fundamental vibrations and combinations of them. Nevertheless, the quality of the signal (very high signal/noise ratio) allowed to unravel spectra and to calibrate the NIRS signature, i.e., to relate the spectra of samples to their laboratory reference values in foods, feed, pharmaceuticals, petrochemical products, and other commodities (*12*). Many authors have studied the chemical changes during litter decomposition using NIRS (*13*−*16*) but few reports are concerned with the use of NIRS to study the composting process (*17*−*19*)

The objectives of this work were (1) to determine biological and chemical changes during the composting process of green waste and sewage sludge and (2) to evaluate the usefulness of NIRS to accurately predict these changes.

Experimental Section

Experimental Materials. Composts obtained from local dewatered digested municipal sewage sludge, green wastes, and pine barks at a 1:1:1 v/v ratio (Company Biotechna, Ensues, Bouches du Rhône, France). Pine barks are incorporated into other biowastes to improve aeration during the process. The mixture was composted for 20 days in impervious boxes (100 m^3) with forced aeration and then stored in windrows (10 m long, 4 m high, and 5 m deep) on a composting platform for six months. The heaps were mixed several times during the process to promote organic matter humification. Approximately 1 kg of homogeneous compost

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TABLE 1. Chemical Parameter Changes during the Composting Process[A]

composting time day	pH	N %	C %	C/N	OM % DM	HA mg·kg^{-1} DM	FA mg·kg^{-1} DM	HA/FA
4	6.8 (0.1)	1.6 (0.1)	27.7 (1.6)	17.7 (1.0)	58.5 (3.0)	28.3 (7.5)	54.9 (6.6)	0.54 (0.22)
18	6.8 (0.1)	1.8 (0.1)	27.8 (0.3)	15.3 (0.9)	57.5 (3.6)	28.3 (1.9)	51.9 (9.1)	0.57 (0.15)
31	6.9 (0,1)	1.9 (0.2)	28.9 (0.8)	15.5 (1.7)	57.0 (3.0)	22.7 (5.8)	52.3 (5.2)	0.43 (0.10)
40	7.2 (0.1)	2.4 (0.1)	28.3 (1.6)	11.6 (0.7)	60.3 (3.0)	31.5 (5.4)	52.0 (6.3)	0.62 (0.16)
57	7.3 (0.1)	1.7 (0.2)	24.1 (2.5)	14.1 (0.5)	48.3 (2.1)	39.3 (1.8)	38.2 (4.5)	1.04 (0.08)
67	7.2 (0.1)	1.9 (0.1)	27.6 (1.9)	14.2 (1.4)	53.5 (5.9)	26.6 (8.0)	47.7 (6.7)	0.56 (0.19)
84	7.7 (0.1)	1.7 (0.1)	24.0 (3.0)	13.8 (1.2)	47.0 (4.6)	37.5 (9.2)	34.1 (6.6)	1.15 (0.40)
101	7.5 (0.1)	2.1 (0.1)	26.1 (1.4)	12.7 (0.5)	52.6 (2.7)	53.5 (4.7)	46.8 (5.1)	1.16 (0.21)
114	7.4 (0.1)	2.0 (0.1)	24.9 (1.2)	12.7 (0.1)	50.8 (4.1)	59.5 (7.7)	47.9 (4.4)	1.24 (0.09)
128	7.8 (0.1)	1.9 (0.2)	25.3 (1.7)	13.1 (0.6)	50.5 (4.3)	51.9 (4.6)	47.2 (4.2)	1.11 (0.15)
146	7.8 (0.1)	1.9 (0.1)	23.7 (1.0)	12.4 (0.3)	48.9 (2.2)	63.9 (4.0)	39.7 (0.8)	1.61 (0.08)

[A] Values in parentheses are standard error ($n = 4$).

TABLE 2. Biological Parameter Changes during the Composting Process[a]

composting time day	respiration mg O_2·h^{-1}·g^{-1} DM	cellulase U·g^{-1} DM	protease U·g^{-1} DM	alkaline phosphatase mU.g^{-1} DM	acid phosphatase mU·g^{-1}	phenoloxidase U·g^{-1} DM
4	38.0 (1.3)	2.50 (0.11)	0.26 (0.09)	82.1 (5.5)	26.4 (3.9)	3.68 (0.50)
18	24.0 (2.6)	3.98 (0.15)	0.24 (0.06)	121.5 (5.0)	40.0 (2.6)	29.53 (4.79)
31	24.2 (0.7)	3.52 (0.18)	0.21 (0.09)	111.7 (3.7)	37.0 (3.3)	12.79 (2.23)
40	8.3 (0.5)	2.23 (0.23)	0.16 (0.01)	70.5 (7.4)	23.4 (1.5)	3.58 (0.76)
57	2.7 (0.5)	2.24 (0.21)	0.22 (0.01)	128.6 (4.8)	39.6 (7.1)	4.23 (0.17)
67	12.2 (1.4)	0.79 (0.12)	0.10 (0.02)	62.7 (1.4)	12.4 (1.7)	4.01 (0.87)
84	4.2 (0.9)	1.18 (0.05)	0.12 (0.01)	73.9 (2.0)	20.8 (2.6)	6.07 (0.65)
101	3.3 (0.5)	1.71 (0.07)	0.06 (0.01)	61.5 (3.0)	20.0 (1.7)	2.07 (0.12)
114	12.3 (1.0)	1.31 (0.03)	0.05 (0.01)	53.1 (1.5)	21.1 (4.8)	1.28 (0.14)
128	10.6 (0.9)	1.91 (0.02)	0.07 (0.02)	58.5 (1.2)	20.4 (1.3)	0.78 (0.07)
146	0.6 (0.6)	1.08 (0.04)	0.06 (0.01)	44.8 (1.9)	11.3 (2.7)	0.61 (0.13)

[a] Values in parentheses are standard error ($n = 4$).

was collected, in autumn 2005, from each windrow at 11 different stages of composting (4, 18, 31, 40, 57, 67, 84, 101, 114, 128, and 146 days) with four replicates for 44 samples in total. Composts from 4 to 18 days came from boxes, whereas the others came from windrows. All samples were sieved (<20 mm mesh). For characterization of biological parameters, samples were stored at 4 °C. For chemical and spectral analyses, samples were freeze-dried, ground with a Cyclotec 1093 mill (FOSS) to 1 mm size, and stored at −20 °C.

O_2 Consumption Rate. Compost respiration was measured on samples ($n = 44$) using the Oxitop system (WTW). Compost samples (10 g) were humidified to 60% and incubated at 20 °C. Micro-organisms consume oxygen and form CO_2 which is absorbed by NaOH, creating a vacuum which can be read directly as a measured value in mg O_2·h^{-1}·g^{-1} of dry matter (DM).

Enzymatic Assays. Phenoloxidases were extracted from 5 g of compost mixed to 25 mL phosphate sodium buffer (50 mM, pH 6) stirred for 1 h and then centrifuged 10 min at 10 000 rpm and 4 °C. Phenoloxidase activity was measured following the oxidation of the 2,7-diaminofluorene (2,7-DAF) (*20*). The reaction mixture contained 1 mL enzymatic extract, 2 mL phosphate buffer (0.1 M, pH 6), 10 μL H_2O_2 (1.3 mM) and 10 μL 2,7-DAF (6.9 mM). Absorbance was measured with a spectrophotometer (Kontron, model Uvikon 860) at 600 nm. Results are expressed in U·g^{-1} DM, U corresponding to the number of μmole of oxidized 2,7-DAF released per min.

Cellulases were extracted from 3 g of compost mixed to 15 mL acetate buffer (50 mM, pH 5), stirred for 1 h and then centrifuged 10 min at 10 000 rpm and 4 °C (*21*). Cellulase activity was measured by mixing 0.5 mL of enzymatic extract and 0.5 mL of a solution of carboxylmethylcellulose (CMC) 1% in acetate buffer (50 mM, pH 6). The dinitrosalicylic acid (DNS) method (*21*) was used to quantify reducing sugars after 1 h of incubation at 50 °C. The cellulase activity was measured at 520 nm and was expressed as U·g^{-1} DM (U corresponding to the number of μmole of glucose released per min).

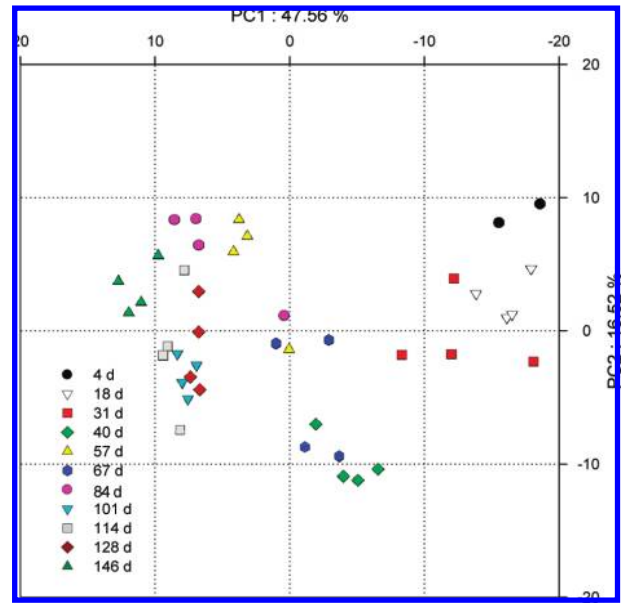

FIGURE 1. Results of PCA analysis calculated on second derivative and detrended VIS-NIRS (400−2500 nm) data with 42 composts of 11 stages of composting (d = day).

Acid phosphatase activities were measured by reacting 1 g of compost, mixed to 5 mL of acetate buffer (0.1 M, pH 5), with 10 mM p-nitrophenol phosphate at 50 °C, stirred for 1 h and then centrifuged 10 min at 10 000 rpm and 4 °C (*22*). Then, 1 mL $CaCl_2$ 0.5 M and 4 mL NaOH 0.5 M were added and absorbance measured at 412 nm. To measure alkaline

TABLE 3. NIRS Calibration and Validation Statistics for Nitrogen (N), Carbon (C), C/N, Humic Acid (HA), Fulvic Acid (FA), HA/FA, pH, Respiration, Cellulase, Protease, Phenoloxydase, Acid and Alkaline Phosphatase Activities, and Composting Time[a]

parameters	*n*	mean	SD	SEC	r^2	SECV	expl. var.	SD/SEC	SD/SECV	category	number of PLS components	math treatment
N (%)	42	1.91	0.24	0.08	0.89	0.11	78	3	2.1	A	4	02/10/2010
C (%)	42	26.2	2.6	0.93	0.87	1.08	83	2.8	2.4	A	3	02/10/2010
C/N	42	13.9	1.7	0.34	0.96	0.54	90	5.1	3.2	A	5	02/10/2010
OM (%)	41	52.8	5.9	2.66	0.8	3.54	64	2.2	1.7	B	3	02/10/2010
HA ($mg \cdot kg^{-1}$ DM)	41	39.9	14.7	5.6	0.86	6.7	79	2.6	2.2	A	2	02/10/2010
FA ($mg \cdot kg^{-1}$ DM)	42	46.1	8	3.5	0.8	5.6	53	2.3	1.4	C	5	02/10/2010
HA/FA	41	0.92	0.41	0.13	0.9	0.15	86	3.1	2.7	A	2	02/10/2010
pH	40	7.33	0.3	0.15	0.79	0.15	79	2.2	2.2	A	1	01/10/2010
respiration ($mg\ O_2 \cdot h^{-1} \cdot g^{-1}$ DM)	39	12.2	10	3	0.91	3.9	84	3.4	2.5	A	5	01/10/2010
cellulase ($U.g^{-1}$ DM)	41	1.98	0.97	0.27	0.92	0.41	82	3.6	2.4	A	4	02/10/2010
protease ($mU \cdot g^{-1}$ DM)	42	0.14	0.09	0.04	0.82	0.05	67	2.4	1.8	B	2	01/10/2010
phenoloxydase ($U \cdot g^{-1}$ DM)	39	5.72	7.21	1.59	0.95	2.04	92	4.5	3.5	A	4	02/10/2010
acid phosphatase ($mU \cdot g^{-1}$ DM)	42	24.7	10.3	6	0.65	6.6	59	1.7	1.6	B	2	02/10/2010
alkaline phosphatase ($mU \cdot g^{-1}$ DM)	42	78.9	28.6	12.9	0.8	16	68	2.2	1.8	B	3	02/10/2010
composting time (day)	40	74.8	43.3	6.6	0.98	8.5	96	6.6	5.1	A	5	02/10/2010

[a] *n* = number of samples, SD = standard error, SEC = standard error of calibration, SECV = standard error of cross validation, and expl. var. = percentage of variance explained. Math treatment indicates the mathematical transformation of spectral data: the first number is the order of the derivative function, the second is the segment length in data points over which the derivative was taken, and the third is the segment length over which the function was smoothed. The spectral range used in the calibration equations was 1100−2500 for protease activity and 400−2500 for all other parameters.

phosphatase activities, acetate buffer was replaced by NaOH-glycine (0.1 M, pH 9) buffer (*22*). Results for both acid and alkaline phosphatases were expressed in units defined as μmole of *p*-nitrophenol released $min^{-1}\ g^{-1}$ DM.

Proteases were extracted from 1 g of compost mixed to 15 mL of phosphate buffer (50 mM, pH 7.5), stirred for 1 h and then centrifuged 10 min at 10 000 rpm and 4 °C. Protease activities were measured by reacting 0.5 mL of this enzymatic extract and 0.5 mL of a solution of Azocoll (2.5 mg/mL) in a phosphate buffer (50 mM, pH 7.5). After one hour of incubation at 37 °C, 0.5 mL of (TCA (5% of trichloracetic acid in water) was added to stop proteolysis. The azo dye released by proteolytic activity was followed by measuring absorbance at 520 nm (*23*). Protease activity was expressed as $U \cdot g^{-1}$ DM, a unit corresponding to the number of μmole of amino acid released per min with Subtilisin from *Bacillus licheniformis* as standard.

Chemical Characteristics. Carbon (C) and nitrogen (N) content were analyzed using a Perkin-Elmer 2400 CHN elemental analyzer. pH was determined in a 1:10 water extract (w/v) after 1 h of agitation. Organic matter content (OM) was determined by combustion in an electric muffle at 550 °C for 16 h. Humic (HA) and fulvic acid (FA) fractions were analyzed using a precipitation method (*24*). Compost samples were diluted with NaOH (0.1 M) in a conical flask and shaken for 4 h. Then, samples were centrifuged (Sorval Super T21), to recover the supernatant fraction containing the humic material. The supernatant was acidified to pH 1.0 using HCl (6 M), and then centrifuged to separate the HA (precipitate) and FA (supernatant) fractions. Both fractions were dried (105 °C, 24 h) and weighed. HA and FA substances were expressed in $mg \cdot kg^{-1}$ DM.

NIRS Analyses. Before NIRS scanning, each sample (5 g) was dried in a fan oven for 48 h at 55 °C. Next, samples were packed into a quartz-glass cell and scanned using a NIR-Systems 6500 spectrophotometer (NIRSystems Inc., Silver Spring, MD) following procedure described in ref *15*.

Statistical Analyses. Spectral changes through time were evaluated using principal component analysis (PCA) with STATISTICA 6.0 software. Two series of PCA were calculated, the first on the entire spectrum (400−2500 nm) and the second on the near-infrared range (1100−2500 nm). PCA were done on second derivative and detrended spectral data.

Calibration equations, to derive spectrochemical models relating the spectra to their laboratory reference values, were developed for all measured parameters (C, N, C/N, HA, FA, pH, enzymatic activities, and respiration) using the ISI software system (*25*). The calibration equations were calculated using the cross validated partial least-squares regression (PLS) method (*26*). This method uses all the spectral information, unlike stepwise regression type methods which use only a small number of wavelengths (*27*). Two series of calibrations were produced, the first on the entire VIS-NIR region (400−2500 nm) and the second on the NIR region (1100−2500 nm). For each calibration, six mathematical treatments, corresponding to the first and second derivative and a gap of 5, 10, and 15 data points, were compared. After comparison of the results of various treatments, the calibration equation that gave the best results in terms of SECV was selected.

Results and Discussion

Chemical and Biological Changes during the Composting Process. All chemical and biological analyses results are presented in Tables 1 and 2 respectively.

Results show an obvious separation into two distinct phases during the composting process. An initial phase from 4 to 50−60 days was characterized by an intensive degradation. A deceleration of all activities was then noted up to 146 days, corresponding to a maturing phase. The pH increased during composting from 6.8 to 7.8 (Table 1) in accordance with other compost studies (*28, 29*). The acidity in the initial phase is due to the presence of fatty acids and short-chain organic acids, mainly lactic and acetic acid (*28*).

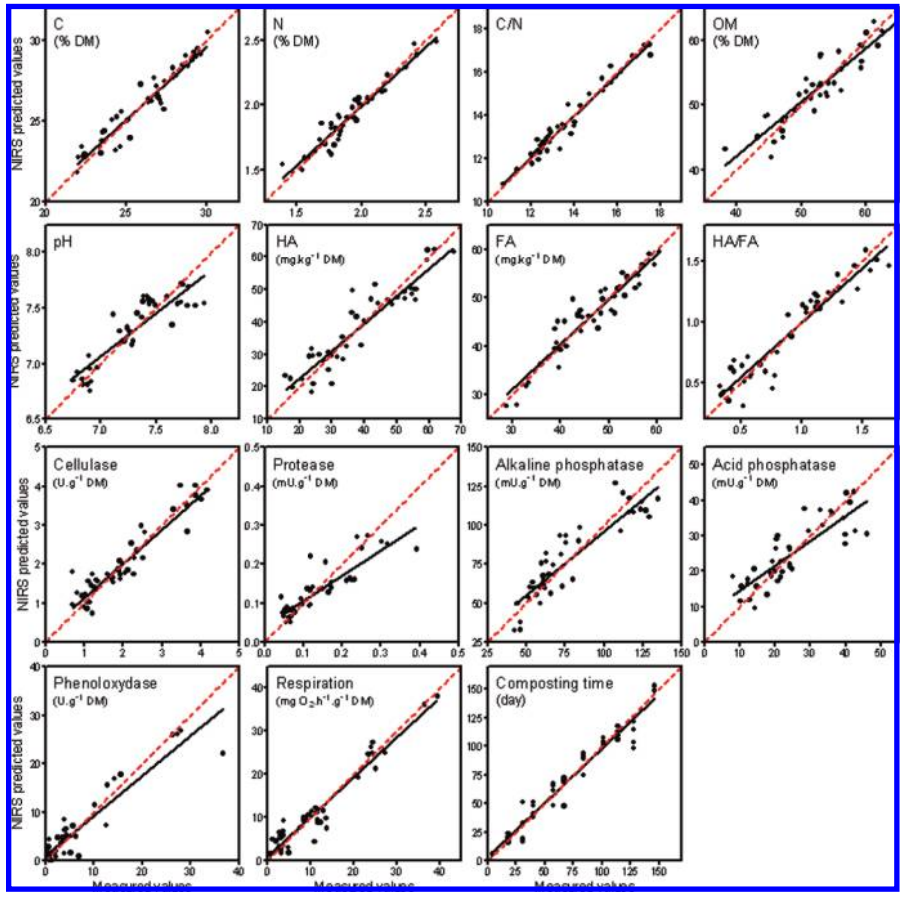

FIGURE 2. Relationship between NIRS predicted values and measured values for chemical and biological parameters, composting time. Calibrations are shown in Table 3.

Both C/N ratio and OM content reflect decomposition and stabilization during composting (*30, 31*). Here, N concentration remained relatively constant, with 1.6 and 1.9% at 4 and 146 days, while C decreased from 27.7 to 23.7%, reflecting the mineralization of carbon compounds. C/N ratio, which is widely used to assess compost maturation (*32*), decreased from an initial value of 17.7 to 12.4 after 146 days. Loss of C is related to loss of OM during process with values of 58.5 and 48.9%. OM and C/N showed two phases (*1*): days 0−57, with an intensive decomposition and mineralization (*2*); and days 57−146 with a low rate of decomposition and the beginning of the maturation stage.

Humic (HA) and fulvic acids (FA) presented opposite trends, with an increase of 126% in HA and a decrease of 30% in FA between 4 and 146 days. During composting, a large part of the original OM is mineralized or transformed into new organic materials, particularly "humic-like substances". Increase in HA is related to the humification process and indicates maturity of compost (*33*). The HA/FA ratio tripled from 0.54 to 1.61 between 4 and 146 days. This ratio appears to be one of the most sensitive ways to follow the humification process and has been proposed by numerous authors as an index of maturity (*34, 35*). This increase is explained by the formation of complex molecules (HA) as a result of polymerization of simple molecules (FA) or by a biodegradation of nonhumic or easily decomposable components of the FA fraction followed by the formation of more polycondensed humic structures (*36*).

Biological activities also reflected two distinct phases (Table 2). Respiration experienced a strong initial decrease from 38 to 2.7 mg $O_2 \cdot h^{-1} \cdot g^{-1}$ DM between 4 and 57 days, followed by a slower decrease to 0.6 mg $O_2 \cdot h^{-1} \cdot g^{-1}$ DM up to 146 days. The respirometric test being the best to measure biological stability in compost (*37*); the low respirations measured at the start of the second phase indicate biological stability and the extent to which easily biodegradable organic matter has been decomposed (*38*).

Cellulase and phenoloxidase activities presented a similar trend toward a strong increase during the first month of composting with 2.50 to 3.98 $U \cdot g^{-1}$ DM for cellulases and 3.68 to 29.53 $U \cdot g^{-1}$ DM for phenoloxidases (Table 2). After one month, both cellulase and phenoloxidase activities showed a simultaneous decrease then a stabilization until the end of the process with a final activity of 1.08 and 0.61 $U \cdot g^{-1}$ DM for cellulases and phenoloxidases, respectively. Cellulases hydrolyze the β−1,4-glucosidic linkages of cellulose, while phenoloxidases modify the lignin structure and allow its mineralization. However, cellulose fibers are embedded in a matrix of hemicelluloses and lignin and part of the cellulose or hemicelluloses is bound to lignin in the so-called ligno−cellulose or lignin−polysaccharide complex (*39*). This complex is supposed to be held together by hydrogen bonds and covalent (ester or ether) linkages. This structure of the ligno−cellulose complex might explain why cellulase and phenoloxidase activities are complementary.

Protease activities displayed a systematic decrease from 0.26 $U \cdot g^{-1}$ DM at 4 days to 0.06 $U \cdot g^{-1}$ DM at the end of composting (Table 2). High protease activity is ascribed to induction by proteins present in considerable amounts in sewage sludge (*40*). The high activity during the initial stage of composting reflects the large quantity of substrates suitable for microbial flora. When these substrates disappear over the period of composting, protease activity also declines.

Alkaline and acid phosphatases exhibited high activities between 4 and 57 days. Then, they declined up to the end of the process with, respectively, 82.1 to 44.8 $mU \cdot g^{-1}$ DM and 26.4 to 11.3 $mU \cdot g^{-1}$ DM between 4 and 146 days of composting. Both phosphatase activities displayed a strictly

TABLE 4. Wavelengths (nm) with High Coefficients in PLS Calibration Equations for N, C/N, HA and HA/FA and Their Development with Time during the Composting Process (↑ increase, ↓= decrease, → = steady)[a]

constituents	wavelengths (nm) with high coefficients in PLS calibration equations	assigned to	progression over time
N	1996	N—H stretch	↑
	2050	N—H stretch protein	↓
	2180	N—H bend and C—H stretch protein	↓
	2320	C—H stretch	↓
C/N	860−870	C—H cellulose	↑
	1190	C—H	↑
	1570	N—H stretch	→
	1690	C—H stretch	→
	2040−2050	C═O stretch and N—H stretch protein	→
	2060−2070	N—H bend protein and N—H_2 deformation	↓
	2320	C—H stretch and CH_2 deformation	↓
	2380−2390	C—H stretch	↓
HA and HA/FA	1170−1180	C—H and HC═CH	↑
	1540−1560	O—H stretch	→
	1620−1640	C—H stretch	→
	1680−1700	C—H stretch	→
	2190−2210	N—H bend, C—H stretch and C≡O	↓
	2380−2410	C—H stretch	↓

[a] The NIR Band Assignments Originated from refs *25* and *52*.

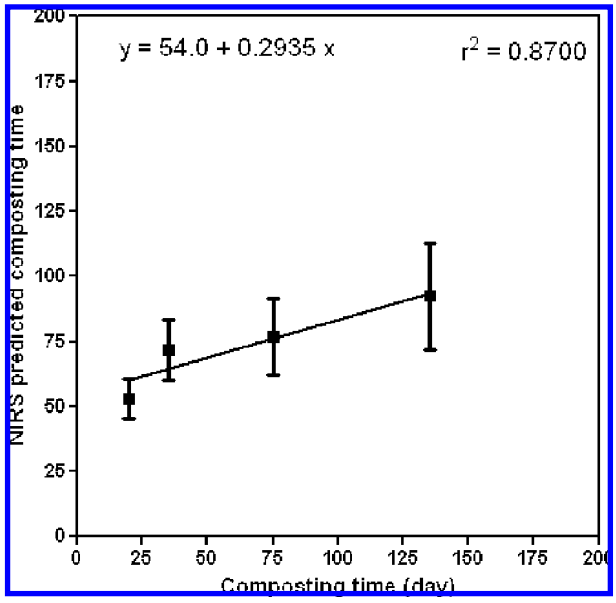

FIGURE 3. NIRS predicted composting time of 295 compost samples studied by ref *45* using the present equation based on 40 samples.

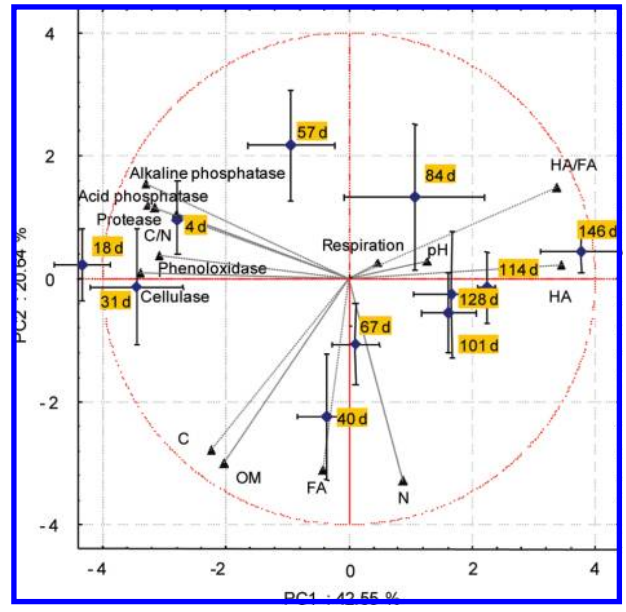

FIGURE 4. Results of PCA analysis on biological and chemical data with 44 composts of 11 stages of composting. (d = day)

parallel trend, with a ratio of 3 between alkaline and acid phosphatase activities. Several authors found the same trend, with a maximum of phosphatase activity at the beginning of composting followed by a decrease (*41*). The high organic matter content and large quantity of nutrients in original compost stimulate growth of total aerobic bacteria and subsequent phosphatase and peptidase synthesis (*42*). The decline in phosphatases activities during composting may be due to humic compounds which can form complexes with enzymes (*41*). As a result, enzymes are less active and bind less readily with the substrate. The decline in phosphatase activities may also be due to the feed-back on the synthesis of this enzyme. Plant roots constitute an important source of acid phosphatases in soils, but are devoided of alkaline phosphatases, which are ascribed to soil bacteria and fungi (*43*). In our study, alkaline phosphatase, which declined up to the end of the process as respiration, may provide an invaluable indication of microbial activity in compost (*44*).

Therefore, a main characteristic of the composting process is the succession of two distinct phases. Many parameters such as C, C/N, respiration, cellulase, or phosphatase activities displayed a decrease at the beginning of composting, followed by a stabilization phase.

Organic Matter Transformation during the Composting Process. Two PCA corresponding to VIS-NIR (400−2500 nm) and NIR (1100−2500 nm) regions were applied on second derivative and detrended NIRS data. The spectra of two initial samples were clearly separated from all the others with a high normalized Mahalanobis distances ($H > 3$) (*26*). Consequently, they were not considered in all subsequent analyses.

We present here results obtained with these 42 samples over the VIS-NIR (400−2500 nm) region as percent of explainable variance by the first two components was slightly increased when calculating PCA over the VIS-NIR region than for the NIR (1100−2500 nm) region. PC1 and PC2 accounted for 47.6 and 16.5% of the explainable variance, respectively.

The factorial map presented a strong differentiation between composts, with two groups corresponding to composts from 4 to 67 days on the right and older composts from 84 to 146 days on the left with a chronological distribution along the PC1 (Figure 1). The four samples corresponding to day 67 pertaining to the first group are clearly differentiated on PC3 (data not shown). So, as quoted by various authors (*17, 45*), NIRS−PCA strategy make it possible to distinguish between mature and young composts based on spectral changes.

Prediction of Chemical and Biological Parameters. NIRS calibration statistics based on the same 42 samples are presented in Table 3 and scatter plots of NIRS predicted vs actual values for chemical and biological analytical parameters in Figure 2. NIRS calibrations allowed accurate predictions of N, C, C/N, HA, HA/FA, respiration, cellulase, and phenoloxidase activity and composting time with r^2 higher than 0.85. Results were less accurate for organic matter, pH, protease, acid, and alkaline phosphatases and unsatisfactory for fulvic acid. The ratio of standard deviation and standard error in cross validation (SD/SECV) was used to test the accuracy of the calibration models (*46*). Values of SD/SECV > 2 were considered acceptable, and unreliable when valued were <1.4 (*47, 48*). These authors propose classifying the NIRS model according to three categories based on SD/SECV values. Category A (SD/SECV > 2) includes N, C, C/N, HA, HA/FA, pH, respiration, cellulase activity, phenoloxidase activity, and composting time. Category B (1.4 < SD/SECV < 2.0) includes OM, protease, acid, and alkaline phosphatase activities. Category C (r^2 < 0.50, SD/SECV < 1.4) includes only FA. According to refs *46* and *47*, predictions classified in category B could be improved by using different calibration strategies, but those in category C could not be reliably predicted using NIRS. It has already been shown that NIRS can predict the chemical composition of soil and litters (*13−16*), soil physical and chemical properties (*49*), and soil respiration (*50*).

The wavelength assignment could be as a guidance to interpret the spectra (*51*) Table 4 shows wavelengths with high coefficients in PLS calibration equations of N, C/N, HA, and HA/FA and their development during the composting process. In addition, Figure 2 shows two important results to confirm the result of the Figure 1. At first, NIR spectral information reflects distribution of the chemical and biological parameters of the compost. Second, maturation stage of the compost can be judged synthetically because the chemical and biological parameters of the compost could be predicted using all NIR spectral information even if the assignments of the spectral information of the organic components of the compost are not clear.

In our work, 10 chemical or biological parameters, as well as composting time, were satisfactorily predicted by NIRS (category A). Some of these are already used as index of compost maturity such as HA, HA/FA, C/N, and respiration. Others, such as cellulase and alkaline phosphatase activities, could be useful in monitoring the composting process. In our study, NIRS could accurately predict composting time and thus could determine the stage of composting of unknown compost from the same initial composition within 7 days. Composting time of different materials has already been predicted with an SEC of 12.38 days for grape marc compost and 3.06 days for manure compost (*17*). Nevertheless, including with the same initial material, temperature and humidity conditions prevailing during the compost process could change the biological and chemical status of the compost reflected in the spectral signature at the same age of composting. As a matter of fact, the predicted composting time of 295 samples studied in ref *45* with the present equation gave a significant correlation ($r^2 = 0.87$) but with a poor regression line ($y = 54 + 0.29x$) (Figure 3). This result could be explained by the small size of the database

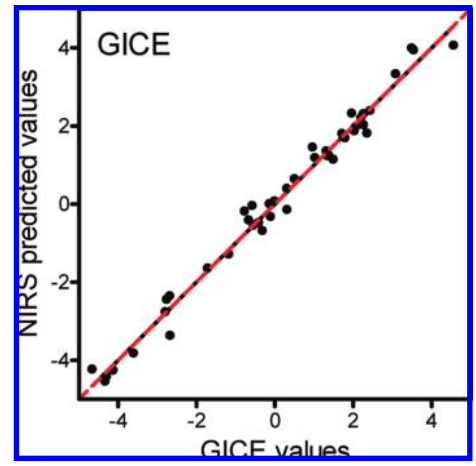

FIGURE 5. Relationship between NIRS predicted values and present values of global index of composting evolution (GICE). $n = 41$; mean = 0.09; SD = 2.39; SEC = 0.24; $r^2 = 0.99$; SECV = 0.62; 93% of variance explained; SD/SEC = 9.8; SD/SECV = 3.8.

under consideration in this study. We obviously had to increase the size of our database to capture the chemical diversity of municipal and green wastes and the distinct abiotic conditions of composting process. More than a simple prediction of composting time, characterization of various chemical and biological parameters seems useful to assess several aspects of compost maturity.

However, one single parameter cannot be taken as an index of compost maturity (*8*). Thus, we propose to calculate a global index of composting evolution (GICE) using all the biological and chemical studied parameters. PCA was done on biological and chemical parameters. The first component PC1 accounted for 42.55% of the total variance with 47.62% for chemical parameters and 52.38% for biological parameters. The main parameters influencing compost ordination on PC1 were pH, HA, HA/FA, and C/N for chemical parameters, cellulase, protease, phenoloxidase, protease, acid, and alkaline phosphatases activities for biological parameters between 7 and 11% of variance. PC1 allowed an ordination of compost samples according to their age with more mature compost on the right side of the axis (Figure 4). The second component, PC2, accounted for 20.6% of the total variance with C, N, and FA as main factors (80.54% for chemical parameters and 19.45% for biological parameters). To describe the dynamic of the composting process, we choose to assign the value of each sample on the PC1 as the value of GICE as this component was more related with biological processes than the second one. GICE was accurately predicted by NIRS (category A) with r^2 of 0.99, a high SD/SECV ratio of 4.32, and a model explaining 93% of the variance (Figure 5). It proves a valuable tool in monitoring the composting process.

Our results indicated a clear succession of two phases: a first phase from 4 to 50−60 days characterized by intensive degradation and a second phase up to 146 days with deceleration of all activities measured and characterized by processes of humification. Since an increasing of maturity corresponds to an increase of GICE, the state of progress of the process, i.e., its degree of maturity, can be identified using the GICE. These results clearly demonstrated that an NIRS-PCA strategy allows chemical changes during composting to be monitored. They also show that NIRS is well suited to prediction of a set of chemical and biological characteristics of compost. N, C, C/N, HA, HA/FA, pH, respiration, cellulase, and phenoloxidase activities and composting time provide satisfactory results. A practical daily use of these models will need new samples to be analyzed to increase prediction accuracy. Furthermore, GICE could

be completed by agronomical parameters such as yields of vegetable cultures after supply to the soils of composts characterized by NIRS.

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