Bioresource Technology 100 (2009) 3604–3612

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Changes in enzymatic activity in composts containing chicken feathers

Justyna Bohacz *, Teresa Korniłłowicz-Kowalska

Chair of Agricultural Microbiology, Laboratory of Mycology, University of Life Sciences, ul. Leszczyńskiego 7, 20-069 Lublin, Poland

ARTICLE INFO

Article history:
Received 4 December 2008
Received in revised form 20 February 2009
Accepted 23 February 2009
Available online 25 March 2009

Keywords:
Composting
Enzyme activities
Waste feathers
Lignocellulosic wastes

ABSTRACT

Enzymatic activity, i.e. respiratory activity, dehydrogenase activity, phosphatase activity, caseinian protease activity, BAA protease activity and urease activity, was determined to investigate the process of biochemical transformations and to select enzymatic indices of maturity of composts prepared from feathers and lignocellulose wastes (bark, straw). Composting was conducted for 7 months, with periodic determinations of activity of the enzymes. The study revealed significant differences in the enzymatic activity, related with the duration of composting and with the substrate composition of the composts. Generally, composts enriched with straw were characterised by higher enzymatic activity than composts without any addition of straw. It was found that the activity of such enzymes as cellulase and protease, towards the end of the period of composting decreased and stabilised. The enzymes enumerated can be taken into consideration in estimation of the maturity of composts prepared from feathers and lignocellulose wastes.

1. Introduction

For several years the preferred method of utilisation of class 3 animal wastes, such as chicken feathers and other keratin wastes, has been recycling through composting (resolution (WE) No. 1774/2002 of European Parliament and the Council of Europe dated 3rd October, 2002). Studies on biodegradation of chicken feathers by keratinolytic fungi in pure cultures (Korniłłowicz-Kowalska, 1997a,b) and in the process of composting of such wastes with lignocellulose material (Bohacz and Korniłłowicz-Kowalska, 2007) indicate intensive mineralisation and transformation of nitrogen and sulphur of keratin from feathers to forms easily available for plants, i.e. N-NH_4, N-NO_3 and S-SO_4. The composting process as well as the fertilising value of the compost is affected by the chemical composition of waste materials used for composting, their moisture content, availability of oxygen and biochemical activity of microbial groups inhabiting the composted mass. Garcia et al. (1992, 1993) and Vourinen (1999, 2000) report that extracellular enzymes play the main role in biodegradation of organic matter during the process of composting. Those enzymes include phosphatases, that detach phosphate groups from organic complexes of phosphorus (Nannipieri et al., 1990), dehydrogenases, constituting an indicator of oxidation of simple organic sources of carbon and of respiratory activity of microorganisms (Aguilera et al., 1988; Mersi von and Schinner, 1991), as well as aminohydrolases and ureases that participate in mineralisation of nitrogen (Mondini et al., 2004; Garcia et al., 1992). In the course of composting of various organic materials the activity of the enzymes mentioned above decreases and stabilises, which results from bonding by humus that is formed in the process (Diaz-Burgos et al., 1993; Garcia et al., 1993; Mathur et al., 1993; Mondini et al., 2004). A specific group of enzymes cooperating with one another are those that catalyse the degradation of hard-decomposing polymers present in lignocellulose wastes. Decomposition of those polymers, i.e. cellulose, hemicelluloses and lignins, by microorganisms leads to the formation of compounds partially transformed into humus (Perez et al., 2002), which has a significant effect on the maturation of composts. Studies performed so far on the activity of degradation and respiratory enzymes during the process of composting of poultry wastes are scarce (Mondini et al., 2004; Tiquia, 2002). In particular, there is a lack of information concerning the variability of those parameters in the process of composting of waste feathers.

The present paper presents a part of a comprehensive study on the composting of feathers – a waste product of the poultry industry. Its objective was to determine the changes in the activity of selected enzymes – dehydrogenases, phosphatase, protease (caseinian and BAA) and urease – that take place during the composting of those wastes with lignocellulose wastes, and to attempt the selection of enzymatic indices of maturity of the composts produced.

2. Methods

2.1. Composted materials

The material used for composting comprised chicken (broiler) feathers obtained from the Zakłady Drobiarskie "Indykpol" poultry

* Corresponding author. Tel.: +48 81 5248104; fax: +48 81 5248106.
E-mail address: justyna.bohacz@up.lublin.pl (J. Bohacz).

0960-8524/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2009.02.042

processing company in Lublin, with the following composition [g kg^{-1} dry matter (d.m.)]: Corg. (organic carbon) – 490.9, N total – 147.2, S total – 36.7, C/N = 3.33, pine bark purchased from a gardening wholesaler in Lublin, containing (g kg^{-1} d.m.): Corg. – 438.7, N total – 4.8, S total – 0.5, C/N = 91.39, and rye straw (g kg^{-1} d.m.): Corg. – 464.0, N total – 4.3, C/N = 107.90, originating from the Czesławice Experimental Farm of the University of Life Sciences in Lublin (Poland).

2.2. Preparation of composts

The study on composting chicken feathers was conducted under laboratory conditions, setting up a model experiment in two variants – with and without the addition of straw. For each of the variants two treatments were prepared, differing in the C/N ratio.

The particular experimental treatments were as follows:

(I) chicken feathers 12% + pine bark 88%; C:N = 25 (FB-I),
(II) chicken feathers 6.6% + pine bark 93.4%; C:N = 35 (FB-II),
(III) chicken feathers 12.36% + pine bark 43.82% + rye straw 43.82%; C:N = 25 (FBS-III),
(IV) chicken feathers 6.92 + pine bark 35.81% + rye straw 57.27%; C:N = 35 (FBS-IV).

For the composting, composting bins were used, with a volume of 12 dcm^3 and with double walls with granulated styrofoam filling, with perforated bottom, equipped with a set of temperature sensors coupled to a digital indicator.

The composting was conducted for 7 months, maintaining moisture at the level 60% of total water holding capacity, controlling the temperature and mixing the composted mass many times in order to maintain good aeration and to achieve homogeneity of the mass. Two replications were prepared for each experimental treatment.

2.3. Analytical methods

Periodically, i.e. after 18 h (time 0) from setting up the experiment, and after 1, 2, 3, 4, 6, 10, 15, 20 and 30 weeks of composting, biochemical analyses were performed that comprised the following determinations:

- respiratory activity, on the basis of measurement of emitted CO_2 with the method of Rühling and Tyler (1973),
- dehydrogenase activity, with the method of Casida et al. (1964) with 2,3,5-triphenyltetrazole chloride (TTC) as substrate,
- cellulases (endo-glucanase), with carboxymethylcellulose (CMC) as substrate acc. to Pancholy and Rice (1973),
- phosphatases, with *p*-nitrophenol (PNP) used as substrate acc. to Garcia et al. (1992),
- ureases, with the method of Zantua and Bremner (1975) as modified by Furczak et al. (1991),
- proteases, with sodium caseinian as substrate, with the method of Ladd and Butler (1972),
- BAA proteases, with *N*-α-benzyl-argininamide (BAA) as substrate acc. to Garcia et al. (1993).

Other determinations included:

- dry matter content, with the gravimetric method at 105 °C,
- organic matter content, with the gravimetric method – roasting losses at 550 °C,
- pH with the potentiometric method.

2.4. Evaluation of results

All determinations of enzymatic activity were made in three parallel replications, with 2 measurements per replication.

The results were presented as average values from three repetitions. The results were analysed by the statistical method counting standard deviations, by the statistical method of variance analysis with Tukey confidence intervals defined in our work as lowest significant difference (LSD) and correlation coefficients (r) between dehydrogenase and respiratory capacity, dehydrogenase with urease activity and with a time of composting.

Statistical processing of the results was performed with the use of the Stat Graphix v. 5.0 software package.

3. Results

The performed analyses of variance showed that the values of the biochemical parameters under study were affected both by

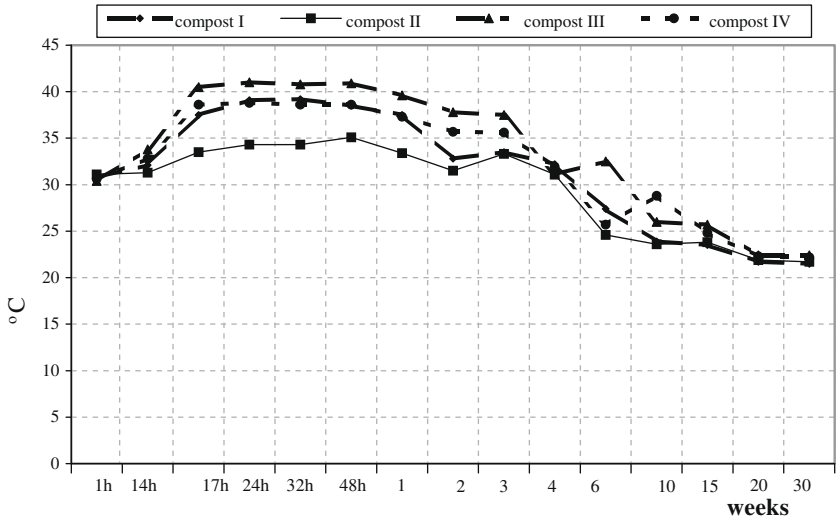

Fig. 1. Temperature changes (°C) in composts containing keratin wastes. Explanations: (I) chicken feathers 12% + pine bark 88%; C:N = 25 (referred to as compost FB-I), (II) chicken feathers 6.6% + pine bark 93.4%; C:N = 35 (compost FB-II), (III) chicken feathers 12.36% + pine bark 43.82% + rye straw 43.82%; C:N = 25 (compost FBS-III), (IV) chicken feathers 6.92 + pine bark 35.81% + rye straw 57.27%; C:N = 35 (compost FBS-IV).

the duration of composting and by the chemical composition of the composts studied – experimental treatments ($\alpha > 0{,}001$). The role of time of composting in the variability of activity of caseinian protease and cellulase was greater than that of the experimental treatments applied, while in the variability of activity of dehydrogenases, urease and phosphatase the effect of experimental treatments was greater than that of the duration of the experiment.

3.1. Temperature changes

It was demonstrated that after 18 h from mixing feathers with pine bark (compost FB-I and II) or with bark and straw (compost FBS-III and IV) a gradual though mild increase in temperature occurred and persisted for 48 h. The increase in temperature was stronger and started somewhat earlier in the composts with straw (38.8 °C and 41.0 °C) than in the composts without any addition of straw (35.1 °C and 39.2 °C). In subsequent times of analyses a systematic decrease in temperature was observed, down to the ambient temperature at the end of the composting period (Fig. 1).

3.2. Respiratory and dehydrogenase activity

It was found that the respiratory activity, as measured by the amount of emitted CO_2, and dehydrogenase activity (mean values) of microbial groups inhabiting the composted mass were the highest in the initial 3 weeks of the experiment (Figs. 2 and 3). The highest increase in mean values of CO_2 emission occurred during the first week of composting. The highest mean value of dehydrogenase activity was recorded after 18 h from setting up the experiment, following which the value of that index was gradually decreasing. From the 2nd month (6th week) of composting of feathers with bark and straw, there appeared a notable decrease in the rate of emission of CO_2 which continued until the end of the experiment (Figs. 2 and 3). Towards the end of the experiment the mean values of emitted CO_2 and of dehydrogenase activity were 3–6-fold lower than during the initial 2 weeks.

Dehydrogenase activity and respiratory activity in the composts enriched with straw (FBS-III and FBS-IV) were significantly higher (mean values) than in the composts without the addition of straw (FB-I and FB-II). The level of activity of those enzymes was not affected by the C/N ratio, which is evident from the lack of significant differences between treatments FB-I and II, and FBS-III and IV (Figs. 2 and 3).

3.3. Phosphatase activity

Over the whole period of composting, phosphatase activity remained at a high level. All measurement results were higher in the first week, but after that they decreased and then increased until the end of the experiment, depending on the chemical composition of the composts and on the time of composting (Fig. 4). Phosphates activity was higher in composts FBS-III and FBS-IV than in FB-I and FB-II.

3.4. Cellulase activity

The composts studied were characterised by relatively high activity of cellulases of the type of endo-glucanase (carboxymethylcellulase) throughout the whole period of the experiment. Increase in the activity of carboxymethylcellulase was initially observed in the 1st (FBS-III and FBS-IV) and 2nd week (FB-II), and then in the 6th week of processing the wastes under study (all experimental treatments). Repeated and, at the same time, the strongest increase in the activity of those enzymes took place in the 10th (treatments FB-I, FBS-III), 15th and 20th weeks (treatments IV and II, respectively) (Fig. 5). On the final date of analysis, a decrease was observed in the activity of enzymes decomposing cellulose with relation to their activity recorded after 10–20 weeks (Fig. 5).

It was found that cellulolytic activity of the composts was dependent primarily on the value of the C/N ratio. Composts with a lower C/N ratio (25), i.e. FB-I and FBS-III, were characterised by higher activity of cellulases than composts with a higher C/N ratio (35): FB-II and FBS-IV, while no differentiation was found in the le-

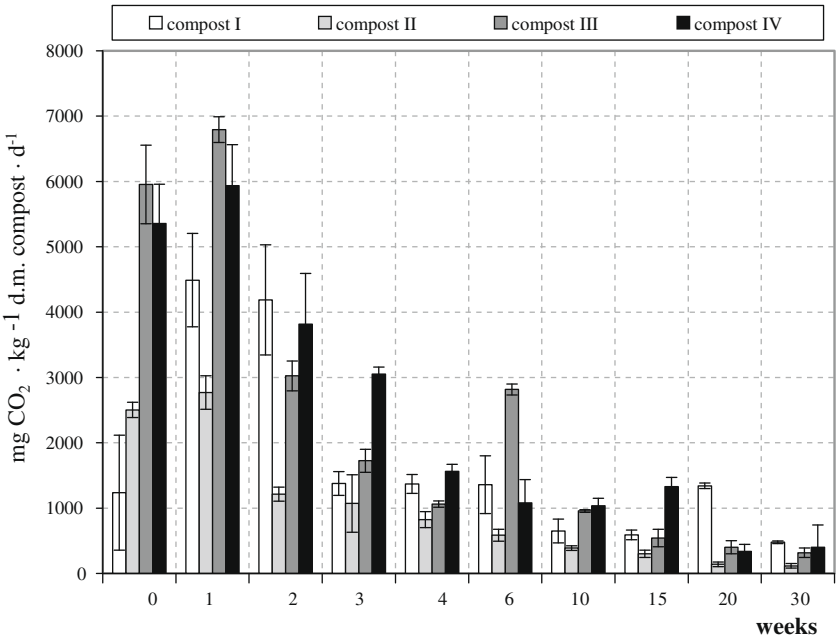

Fig. 2. Changes in the amount of emitted CO_2 in composts containing keratin wastes. $LSD_{0.05}$ for time 460.35; $LSD_{0.05}$ for experimental object 234.74. Values means of three replicates ± standard deviation. Explanations as in Fig. 1.

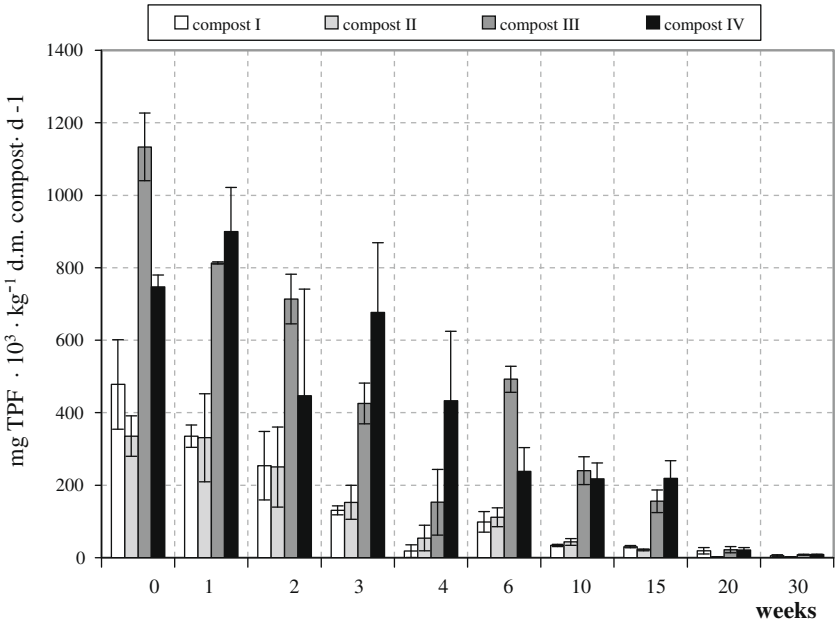

Fig. 3. Changes in dehydrogenase activity in composts containing keratin wastes. $LSD_{0.05}$ for time 110.20; $LSD_{0.05}$ for experimental object 56.20. Values means of three replicates ± standard deviation. Explanations as in Fig. 1.

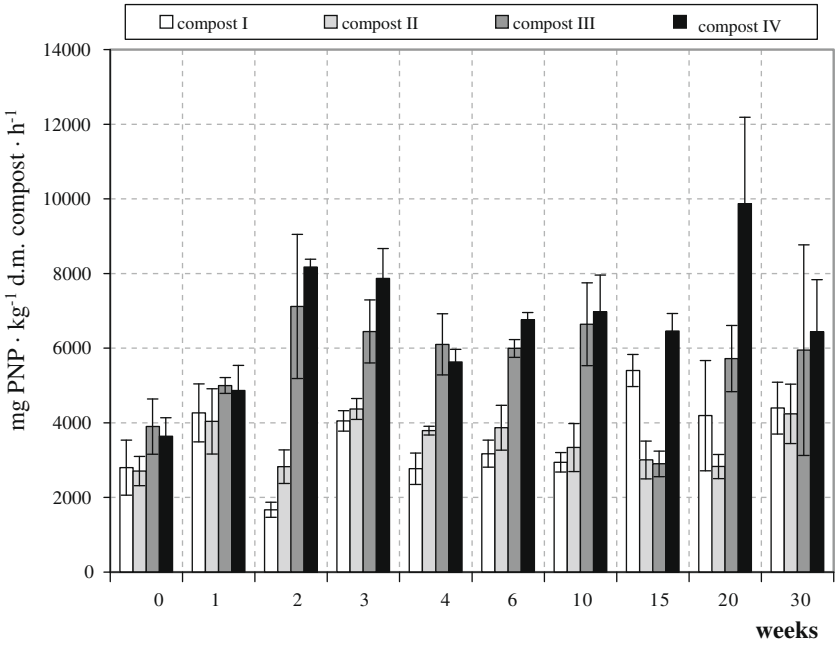

Fig. 4. Changes in phosphatase activity in composts containing keratin wastes. $LSD_{0,05}$ for time 1213,90; $LSD_{0,05}$ for experimental object 619,00. Values means of three replicates ± standard deviation. Explanations as in Fig. 1.

vel of activity of those enzymes within treatments with the same C/N ratio (Fig. 5).

3.5. Protease and urease activity

Caseinolytic activity of the composts was adopted as an index of activity of enzymes hydrolysing peptide bonds (proteinases = endopeptidases) of proteins. Generally it was observed that synthesis of proteases with caseinolytic activity was determined by the amount of protein wastes (feathers) on the one hand, and by the amount of straw added on the other.

Data presented in Fig. 6 indicate that the mean values of caseinolytic activity were the highest in the keratin-bark compost with the higher content of feathers (FB-I). The mean activity of the proteases in the presence of study in both composts enriched with straw was at a similar level. A significant increase in the activity of caseinolytic protease in the FB-I and FBS-IV composts, was observed in the 2nd week of the process of composting, followed by a decline in that activity. In some of the treatments there appeared a repeated, weaker stimulation of the activity of caseinian protease – in weeks 6 and 10 (compost FBS-III) and in weeks 15 and 20 of composting (FBS-IV and FB-I), respectively (Fig. 6).

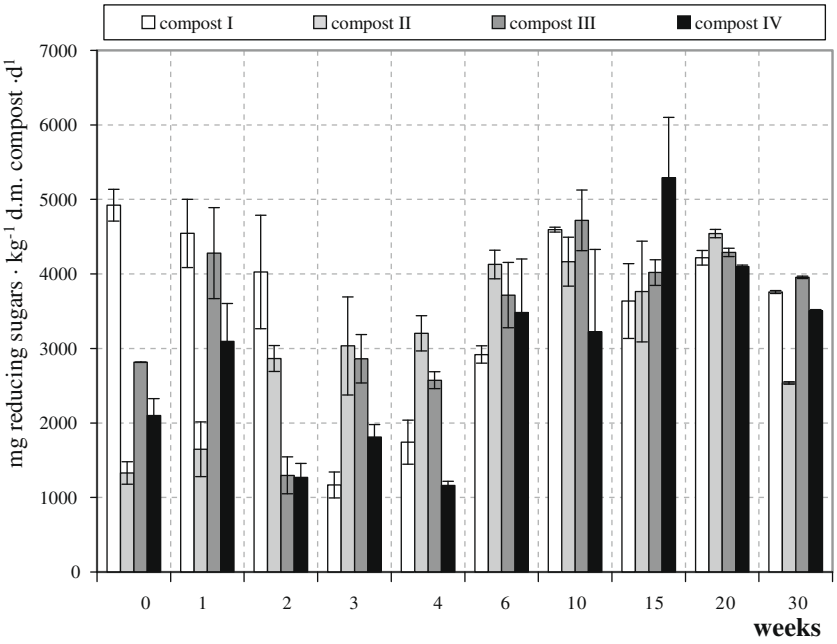

Fig. 5. Changes in cellulase activity in composts containing keratin wastes. $LSD_{0.05}$ for time 524.51; $LSD_{0.05}$ for experimental object 267.46. Values means of three replicates ± standard deviation. Explanations as in Fig. 1.

The obtained data indicate a weakening of the proteolytic activity of microorganisms inhabiting the composted mass in the presence of more easily available sources of carbon (straw), that effect being intensified by the presence of a larger amount of bark.

Enzymes active towards the synthetic peptide BAA are represented by proteases using peptides as substrate (Garcia et al., 1992). In composts FB, BAA protease was detected already at time "0", while in composts FBS on the 7th day of the experiment (Fig. 7). The activity of that enzyme was low throughout the period of the study. It was noted, however, that in the later months of composting there occurred a slight increase in its activity, usually preceded by an increase in the activity of caseinian protease (Fig. 6).

The performed study demonstrated that changes in the activity of urease in the course of composting waste feathers with pine bark and rye straw depended on the chemical composition and the C/N ratio of the composts. It was found (Fig. 8) that the urolytic activity of composts enriched with straw (FBS-III and IV) was generally higher than that of composts without the addition of straw (FB-I and II).

In all the experimental treatments the highest level of urease activity was observed in the initial period. At later times of analy-

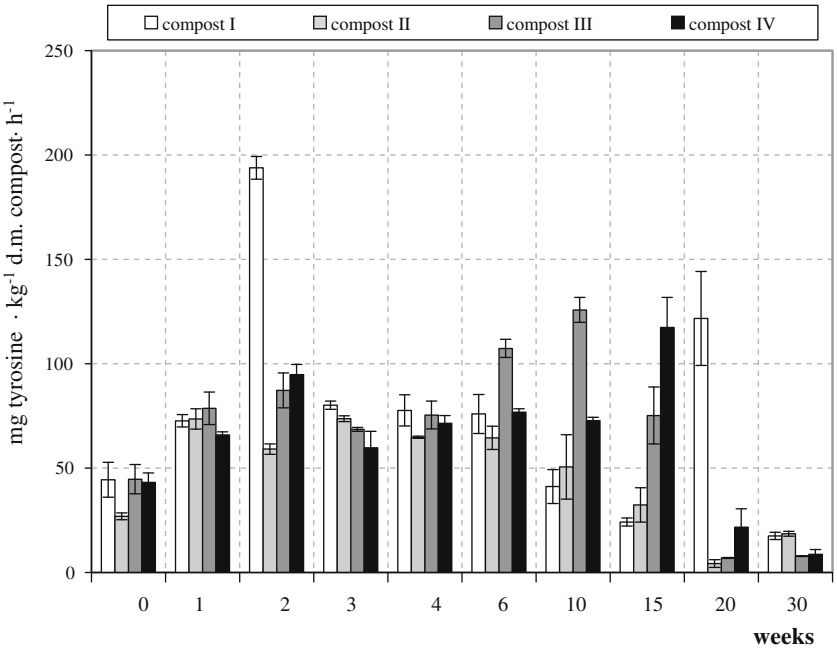

Fig. 6. Changes in caseinolytic activity in composts containing keratin wastes. $LSD_{0.05}$ for time 9.61; $LSD_{0.05}$ for experimental object 4.09. Values means of three replicates ± standard deviation. Explanations as in Fig. 1.

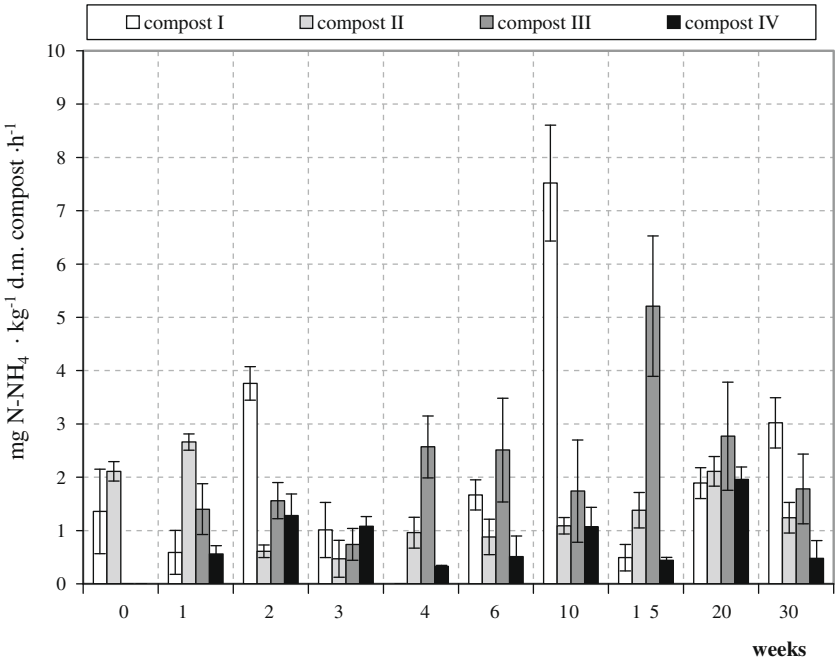

Fig. 7. Changes in BAA-protease activity in composts containing keratin wastes. $LSD_{0.05}$ for time 2.02; $LSD_{0.05}$ for experimental object 1.03. Values means of three replicates ± standard deviation. Explanations as in Fig. 1.

ses the activity of that enzyme revealed a decreasing tendency, with the exception of week 20 in which an increase in its activity was noted in composts with lower C/N ratio (FB-I and FBS-III).

3.6. Physicochemical properties of the composts

Dry matter content in the composts varied in the course of the process of composting. A significant increase in dry matter content occurred in week 10 of composting (~60%) in all studied composts, and remained at a similar level until the end of the experiment (Table 1). Organic matter content decreased during the process of composting. This was evident primarily in the initial phase of the transformations, when the reduction of organic matter content varied from 3% in composts II and III to 8% in compost IV. A notable decrease in organic matter content in the composts without straw addition (FB-I and II) took place after 3 weeks of composting. In the composts with straw (FBS-III and IV) a similar phenomenon was noted in weeks 4 and 6.

The study showed that the reaction of the composted mass (Table 1) decreased gradually in all the composts studied. Only in

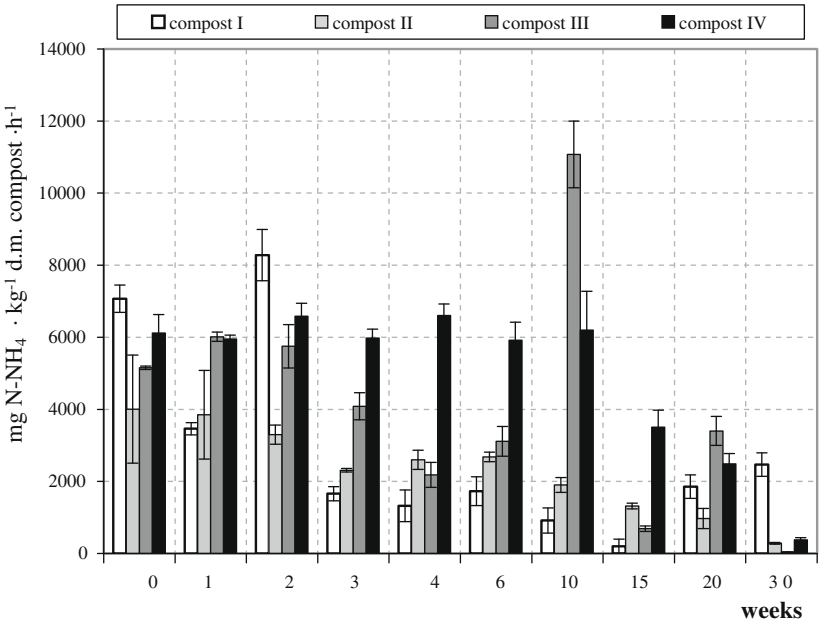

Fig. 8. Changes in urease activity in composts containing keratin wastes. $LSD_{0.05}$ for time 665.13; $LSD_{0.05}$ for experimental object 334.06. Values means of three replicates ± standard deviation. Explanations as in Fig. 1.

Table 1
Changes of some physicochemical parameters of composts containing keratin wastes. Explanations as in Fig. 1.

Terms (weeks)	Dry matter (%)				Organic matter (%)				pH			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
0	34.94	35.64	37.64	38.00	78.43	69.50	81.74	78.84	6.28	6.02	6.23	6.12
1	32.68	33.63	34.57	37.27	75.04	67.44	79.46	72.46	7.99	7.14	7.98	7.07
2	34.35	32.54	34.76	36.08	72.55	71.44	78.05	77.50	8.31	6.78	7.84	6.94
3	34.23	33.06	35.39	36.50	69.72	70.18	77.51	77.50	7.85	5.21	7.06	6.81
4	34.23	31.93	34.17	35.36	72.68	70.33	77.12	76.51	6.78	5.26	6.17	6.18
6	33.41	31.76	33.51	35.23	75.22	70.44	78.92	74.97	4.82	5.57	7.16	5.82
10	94.27	95.60	95.03	94.94	75.32	71.71	77.01	75.25	4.45	4.78	7.17	5.27
15	93.58	95.88	95.47	94.33	75.89	71.46	77.24	75.45	4.07	4.33	6.97	5.37
20	93.04	94.58	94.34	94.45	75.02	71.72	76.61	78.51	6.36	4.20	4.19	4.56
30	89.67	91.17	90.85	83.34	77.78	72.26	77.87	76.10	4.24	4.15	4.25	4.08

the initial 3 weeks there occurred an increase in pH value, especially in composts with the lower C/N ratio (FB-I and FB-III). In the final phase of composting (weeks 20–30), pH of the composts assumed values of 4.08–4.25 (Table 1).

4. Discussion

In the course of composting feathers with bark and straw, the temperature increased to a maximum of 41 °C (Fig. 1). The lack of a distinct thermophilous phase in the course of the biodegradation of the composted mass was caused by the low content of easily available sources of organic carbon and by the relatively small volume of the composted mass (4 kg).

A significant variation was observed in the biochemical activity of the composts. Respiratory activity, as well as numerous enzymatic activities, was higher in the composts with straw. The exceptions were caseinolytic protease and cellulase of the type of endoglucanase that had higher activity in the compost without straw and with the lower C/N ratio (FB-I).

Dehydrogenase activity is a basic index of intensity of oxidation processes conducted by microorganisms and it is positively correlated with their respiratory activity as measured by the amount of emitted CO_2 (Aguilera et al., 1988; Camiña et al., 1998). The very rapid increase in dehydrogenase activity in the initial period of composting was caused by oxidation of simple carbon substrates catalysed by those enzymes. Similar observations were made by Tiquia (2002) in the course of composting poultry wastes with plant material, and by Benito et al. (2003) during composting of plant waste materials and by Castaldi et al. (2008) during composting of municipal solid wastes with plant waste (leaves, grass clippings and shredded bark). Decrease in the respiratory activity and in dehydrogenase activity observed in most of the treatments in the 4th week of composting indicated depletion of easily available sources of carbon and energy for microorganisms. The performed analyses of correlation showed that all of the enzymatic activities mentioned revealed a significant negative correlation with the duration of composting (dehydrogenase activity $r = -0.630^{***}$, respiratory activity $r = -0.625^{*}$, urease activity $r = -0.546^{***}$).

Taking into account all the compost treatments, significant correlation was obtained between the respiratory activity and dehydrogenase activity ($r = 0.875^{***}$). Dehydrogenase activity was also significantly positively correlated with urease activity ($r = 0.620^{***}$).

Jimenez and Garcia (1989) report that the biosynthesis of the hydrolytic enzymes begins in the initial phase of composting, those enzymes begin responsible for transformations of complex compounds of carbon, nitrogen and organic phosphorus in composts. Garcia et al. (1993) demonstrated that hydrolases, due to their inductive character, are a good indicator of qualitative and quantitative changes in the content of particular organic polymers in the process of composting. Castaldi et al. (2008) reported that the activity of hydrolases, such as protease, urease, cellulase, B-glycosidase, as well as the activity of dehydrogenases, increased significantly during the initial two weeks of composting of municipal solid wastes (MSWs) with plant waste, and proposed the evolution of enzymatic activities during composting as indicators of the state and evolution of the organic matter but only for this kind of waste.

The polysaccharides such as cellulose, and also proteins, probably with a structure less complex then that of keratin, started to be hydrolased already after 7–14 days from mixing chicken feathers with lignocellulose wastes. This was evidenced by increase in the activity of cellulase of the type of endo-glucanase, breaking bonds within cellulose molecules, and of caseinian protease, hydrolysing peptide bonds of "standard proteins". The results of the study permit also the conclusion that proteases, active in the initial 3 weeks of composting, were synthesized mainly by bacteria. This appears to be supported also by the results of studies by Tiquia (2002) who reports that the process of composting of a mixture of chicken offal, feathers, residues of fodders and wood shavings is initiated by proteolytic bacteria. A rapid growth of bacterial populations during 3-week composting of municipal solid wastes was also reported by Raut et al. (2008). Our own studies indicate that decomposition of native keratin intensified at a later period, i.e. in months 2–5 of composting feathers, which results from the participation of keratinolytic fungi in the decomposition of the native keratin of feathers (Bohacz and Korniłłowicz-Kowalska, 2007).

Protease activity was the higher especially between 2nd and 6th weeks) in the keratin-bark compost with lower C/N ratio (compost FB-I) than in the remaining composts. It was determined by high content of proteins with simultaneous deficit of more easily available sources of carbon and energy. This indicates a higher intensity of processes of organic nitrogen mineralisation compared to the other composts. Interaction between mineralisation of protein nitrogen and high caseinolytic protease activity in composts has been reported by Garcia et al. (1993). The lower values of caseinolytic protease activity observed in our study in both composts with straw (FBS-III and FBS-IV) than in compost FB-I should be attributed to katabolic inhibition of synthesis of those enzymes under the effect of simple carbon complexes, released during hydrolysis of polysaccharides contained in straw. That phenomenon has been discovered by Meevootison and Niedepruem (1979) in the course of decomposition of hair by keratinolytic fungi.

Davis et al. (1992) demonstrated that microorganisms isolated in various stages of composting bark actively synthesise carboxymethylcellulase breaking intramolecular bonds in cellulose (endoglucanase). Our own study showed that in the course of composting native keratin of feathers with lignocellulose wastes the activity of that endo-glucanase increased the most rapidly in composts containing straw, which should be attributed to better availability of that polysaccharide in the chemical structure of straw compared

to bark (Ljundahl and Eriksson, 1985). Observation of the dynamics of changes in the activity of cellulases in the composts under study shows that in weeks 3–4 of composting there occurred an inhibition of the activity of those enzymes. That effect could have been caused by katabolic inhibition of their biosynthesis under the effect of products of decomposition of cellulose. Studies by Emtiazi et al. (2001) demonstrated that the presence of simple sources of carbon can cause an inhibition of cellulase activity (of the type of endoglucanase) by as much as 50%. Ljundahl and Eriksson (1985) report that katabolic inhibition is a fundamental mechanism of control of synthesis of extracellular cellulases – enzymes with an inductive character. A repeated, later and much higher increase in the activity of extracellular cellulases indicates biodegradation of less easily available fractions of cellulose. Different results were obtained by Castaldi et al. (2008) who observed a decrease in cellulase activity throughout the process of composting of municipal solid waste with plant wastes.

It can also be supposed that, in a longer time-frame, biodegradation of cellulose (as well as of other, even less easily available polymers, e.g. native keratin) was largely dependent on the influx of available forms of phosphorus. High correlation of the growth of cellulolytic fungi with the level of orthophosphates in the soil was reported by Korniłowicz-Kowalska et al. (2003).

The results obtained in this study may indicate successive influx of available forms of phosphorus in the course of composting of the studied organic materials, as – with the exception of the final stage of composting – the activity of phosphatases, enzymes responsible for releasing inorganic phosphates from organic complexes, remained at a fairly high level. This concerned mainly composts enriched with straw, with the higher content of that component. The higher activity of phosphatases in the composts with straw (FBS-III and FBS-IV) than in the composts without straw (FB-I, FB-II) should be attributed to stronger growth of microorganisms. This is in agreement with the results obtained by Garcia et al. (1993), who report that the level of activity of those enzymes in composts is correlated with a high content of microbial biomass.

Among enzymes breaking up small-molecular nitrogen complexes, in the composts under study urease continued at a high level and BAA-protease, an enzyme responsible for the decomposition of peptide substances, at a low level.

The low – compared to caseinian protease – activity of BAA protease may indicate a low content of peptides in the composted mass, although Garcia et al. (1993) suppose that low activity of BAA-protease in composts is rather the result of the low stability of that enzyme. Our own study indicates that a certain increase in the activity of BAA protease became apparent in a later period and was related with biodegradation of keratin. The distinct shift in time of the maximum of activity of that enzyme in composts with a higher C/N ratio (FB-II and FBS-IV) with relation to composts with lower C/N ratio (FB-I and FBS-III) indicates extension in time of the processes of nitrogen mineralisation in those composts, caused by their higher content of organic carbon complexes that slow down the synthesis of those enzymes.

Urease participates in the final stages of degradation of organic nitrogen compounds that, with a very high level of probability, can be arranged in the following series: proteins – polypeptides-(urea)- ammonia (Nannipieri et al., 1990). The activity of urease – an enzyme with very narrow substrate spectrum – generally tended to decrease in the course of the process of composting, which corresponds to reports on the subject published to date (Garcia et al., 1993; Diaz-Burgos et al., 1993; Castaldi et al., 2008). Diaz-Burgos et al. (1993), Garcia et al. (1992) and Castaldi et al. (2008) demonstrated a decrease in the activity of urease with progressing time of composting, which was related with decrease in microbial biomass due to depletion of available nitrogen compounds. The results of this study seem, however, to indicate that another important cause for the lowering urease activity may be accumulation of $N\text{-}NH_4$, an inhibitor of biosynthesis of that enzyme, as has been reported in an earlier study (Bohacz and Korniłowicz-Kowalska, 2007).

In the present study only slight losses of organic matter were noted. However, there were changes in its quality composition, as evidenced by a lowering of the C/N ratio, and by the appearance of humus compounds, as expressed by the value of Q_4:Q_6 oscillating at the level of 6.72–9.38, which indicates stabilisation of organic matter and maturity of the composts under study (unpublished data). In the course of the experiments a notable decrease was observed in the pH value of the composts, that coincided with the period of intensification of transformations of mineral forms of nitrogen and sulphur. The decrease in pH of the composts was caused by an increase in the concentration of $N\text{-}NH_4$ and $S\text{-}SO_4$ ions, which indicates the formation of physiologically acidic ammonium sulphate, as discussed in the paper by Bohacz and Korniłowicz-Kowalska (2007).

5. Conclusion

To sum up the study presented above, one can conclude that enzymatic activity can be taken into consideration in the evaluation of transformations of organic matter in composts prepared from feathers and lignocellulose wastes, and can be used for the estimation of their maturity. Changes in the level of activity of particular enzyme classes may indicate transformations of C, N, S and P, and - in consequence – the appearance of forms of elements available to plants. The performed study revealed significant differences in enzymatic activity, related to the substrate composition of composts. In general, composts enriched with straw were characterised by a higher level of activity of the enzymes under study than composts without straw. It was demonstrated that the respiratory activity and dehydrogenase activity, indicators of transformation of easily available organic matter, were high only in the initial period of composting. Whereas, cellulases, proteases and ureases participated in the transformations of the less easily available organic matter (the lignocellulose complex, keratin) which was dominant in the study. The decrease in the activity of those enzymes in the final weeks of composting indicates stability of organic matter and, consequently, attainment of maturity by the composts.

References

Aguilera, M., Borie, G., Rokov, P., Peirano, P., 1988. Bioquimica de derivados de cenizas volcánicas VII Determinación de deshidrogenasas. Agricultura Técnica (Chile) 48, 147–151.

Benito, M., Masaguer, A., Moliner, A., Arigo, N., Palma, R.M., 2003. Chemical and microbiological parameters for the characterization of the stability and maturity of pruning waste compost. Biol. Fertil. Soils 37, 184–189.

Bohacz, J., Korniłowicz-Kowalska, T., 2007. Choice of maturity indexes for feather-plant material composts on a base of selected microbiological and chemical parameters-preliminary research. Pol. J. Environ. Stud. 16 (2A), 719–725.

Camiña, F., Trasar-Cepeda, C., Gil-Sotres, F., Leirrós, C., 1998. Measurement of dehydrogenase activity in acid soils rich in organic matter. Soil Biol. Biochem. 30, 1005–1011.

Casida Jr., L.E., Klein, D.A., Santoro, T., 1964. Soil dehydrogenase activity. Soil Sci. 98, 371–376.

Castaldi, P., Garau, G., Melis, P., 2008. Maturity assessment of compost from municipal solid waste through the study of enzyme activities and water-soluble fractions. Waste Manage. 28, 534–540.

Davis, C.L., Hinnch, S.A., Donkin, C.J., Germishuizen, P., 1992. Changes in microbial population numbers during the composting of pine bark. Bioresour. Technol. 39, 85–92.

Diaz-Burgos, M.A., Ceccanti, B., Polo, A., 1993. Monitoring biochemical activity during sewage sludge composting. Biol. Fertil. Soils 16, 145–150.

Emtiazi, G., Noghavi, N., Bardbar, A., 2001. Biodegradation of lignocellulosic waste by *Aspergillus terreus*. Biodegradation 12, 259–263.

Furczak, J., Szember, A., Bielińska, J., 1991. Enzymatic activity of littoral zone Piaseczno and Głebokie lakes (Łęczna-Włodawa Lake District) with variant tropic level. Studia Ośr. Dok. Fizjogr. PAN 19, 307–325.

Garcia, C., Hernández, T., Costa, C., Ceccanti, B., Ciardi, C., 1992. Changes in ATP content activity and inorganic nitrogen species during composting of organic wastes. Can. J. Soil Sci. 72, 243–253.
Garcia, C., Hernández, T., Costa, C., Ceccanti, B., Masciandaro, G., Ciardi, C., 1993. A study of biochemical parameters of composted and fresh municipal wasted. Bioresour. Technol. 44, 17–23.
Jimenez, E.I., Garcia, V.P., 1989. Evaluation of city refuse compost maturity: a review. Biol. Wastes 27, 115–142.
Korniłłowicz-Kowalska, T., 1997a. Studies on the decomposition of keratin wastes by saprotrophic microfungi. P. I. Criteria for evaluating keratinolytic activity. Acta Mycol. 32, 51–79.
Korniłłowicz-Kowalska, T., 1997b. Studies on the decomposition of keratin wastes by saprotrophic microfungi. P. II. Sulphur and nitrogen balance. Acta Mycol. 32, 81–93.
Korniłłowicz-Kowalska, T., Iglik, H., Wojdyło, B., 2003. Correlation between the abundance of cellulolytic fungi and selected soil properties. Acta Mycol. 38, 157–168.
Ladd, J.N., Butler, J.A.H., 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol. Biochem. 4, 19–30.
Ljundahl, L.G., Eriksson, K.E., 1985. Ecology of microbial cellulose degradation. Adv. Microbiol. Ecol. 8, 237–299.
Mathur, S.P., Owen, G., Dinel, H., Schnitzer, M., 1993. Determination of compost biomaturity. I. Literature review. Biol. Agric. Hortic. 10, 65–85.
Meevootison, V., Niedepruem, D.J., 1979. Control of exocellular proteases in dermatophytes and especially *Trichophyton rubrum*. Sabouraudia 17, 91–106.
Mersi von, W., Schinner, F., 1991. An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. Biol. Fertil. Soils 11, 216–220.
Mondini, C., Fornasier, F., Sinicco, T., 2004. Enzymatic activity as a parameter for the characterization of the composting process. Biochem. Soil Biol. 36, 1587–1594.
Nannipieri, P., Greco, S., Ceccanti, B., 1990. Ecological significance of biological activity in soil. In: Bollag, J.M., Stotzky, G. (Eds.), Soil Biochem.. Mercel and Dekker, New York, pp. 293–355.
Pancholy, K.S., Rice, L.E., 1973. Soil enzymes in relation to old field succession: amylase, cellulase, invertase, dehydrogenase, urease. Soil Sci. Soc. Amer. Proc. 37, 47–50.
Perez, J., Munoz-Dorado, J., de la Rubia, T., Martinez, J., 2002. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. Inter. Microbiol. 5, 53–63.
Raut, M.P., Prince William, S.P.M., Bhattacharyya, J.K., Chakrabarti, T., Devotta, S., 2008. Microbial dynamics and enzyme activities during rapid composting of municipal solid waste-A compost maturity analysis perspective. Bioresour. Technol. 99, 6512–6519.
Rühling, A., Tyler, G., 1973. Heavy metal pollutions and decomposition of spruce needle litter. Oikos 24, 402–415.
Tiquia, S.M., 2002. Evolution of extracellular activities during manure composting. J. Appl. Microbiol. 92, 764–770.
Vourinen, A.H., 1999. Phosphatases in horse and chicken manure composts. Comp. Sci. Util. 7, 47–54.
Vourinen, A.H., 2000. Effect of bulking agent on acid and alkaline phosphomonoesterase and–D-glucosidase activities during manure composting. Bioresour. Technol. 75, 113–138.
Zantua, M.J., Bremner, J.M., 1975. Comparison of methods of assaying urease activity in soils. Soil Biol. Biochem. 7, 291–295.