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Xylanase activity and thermostratification during the thermogenic phase of industrial composting in aerated trenches

Extracellular xylanase activity and thermostratification were monitored during the thermogenic phase of industrial composting (mainly garden waste) in aerated trenches. Xylanase activity was assayed at different temperatures and pH by incubation of clarified compost extracts with xylan. The highest xylanase activity and temperatures were measured during the summer (when waste was rich in freshly cut grass). Xylanase activity was higher in the peripheral layers of the trenches, at moderately high temperatures (48 to 68°C), than in the hottest central layers (70 to 78°C). Optimal temperature for the xylanases was between 60 and 80°C at pH 6, depending on the temperature of the sampling site. The thermostability of the xylanases from surface samples was high until 60°C, moderate at 70°C and weak at 90°C. Our results show a broad thermostratification of the compost mass. Frequent turnings of the compost stimulate xylan degradation by redistributing the substrates, the free enzymes and the microorganisms.

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Keywords – Compost; enzyme; temperature; thermostratification; xylanase

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Introduction

During the thermogenic phase of composting, easily degradable compounds (soluble sugars, amino and organic acids, proteins) are considered to be the principal sources of carbon and energy (De Bertoldi *et al.* 1983; Biddlestone *et al.* 1987). High temperatures (> 55 to 60°C) reduce the rate of degradation, by loss of the microbial diversity (MacGregor *et al.* 1981; MacKinley & Vestal 1984, 1985; Strom 1985a). Later, cellulose and hemicelluloses, which make up the main fraction of the waste to be composted (Lynch 1993), become the principal source of carbon and energy for less thermophilic, but more diversified, microorganisms (Finstein & Morris 1975; De Bertoldi *et al.* 1983). Xylan is the most abundant of the hemicelluloses (Gilbert & Hazlewood 1993). On account of its structure, lignin is recalcitrant to microbial degradation, particularly at high temperatures (Reid 1995). The covalent attachment of lignin to cell-wall polysaccharides limits the accessibility of degradative enzymes to both cellulose and hemicellulose (Lynch 1993).

The importance of thermophilic bacteria during composting, as well as their number and diversity, has been recently emphasized (Beffa *et al.* 1996a,b; Blanc *et al.* 1997; Blanc 1998) and the evolution of their populations is beginning to be described (Blanc *et al.* 1999). These results complete different studies on thermophilic bacterial populations (Waksman *et al.* 1939a,b; Finstein & Morris 1975; Strom 1985b; Hellmann *et al.* 1997; Herrmann & Shann 1997).

However, the role of these thermophiles in the degradation of biopolymers is poorly documented (Ethier *et al.* 1994; Holtz *et al.* 1991; Kleeberg *et al.* 1998; Sakai *et al.* 1998).

Several authors have shown the presence of extracellular lignocellulose-degrading enzymes in industrial and laboratory composts (Godden *et al.* 1983; Bono *et al.* 1992; Herrmann & Shann 1993; Shin & Jeong 1996). Cellulolytic activity was monitored by Stutzenberger *et al.* (1970) during windrow composting. Xylanase activity was characterized in extracts from spent mushroom-compost simulation (Ball & Jackson 1995).

High temperatures ($> 60°C$) are essential for effective thermohygienization, and do not affect the degradation of organic matter if they are coupled with frequent turning (Beffa *et al.* 1998; Lott Fischer 1998; Lott Fischer *et al.* 1998).

The goals of the present study were the detection, localization and thermal characterization of extracellular xylanase activity during intensive composting in aerated trenches. The role of the thermostratification on xylanase production and activity was monitored.

Material and methods

Composting site

The study was carried out in a closed compost hall. About 12 000 tonnes of organic waste are treated annually in eleven aerated trenches. In summer, the initial mixture consisted of garden waste (mainly freshly cut grass) (~70%), agricultural and kitchen waste (~10%) and shredded wood (~20%). In winter, it contained garden waste (mainly dead leaves) and shredded wood (~90%) mixed with agricultural and kitchen waste (~10%). Before its placing in the trenches, the moisture of the shredded waste was adjusted to 65% approximately.

Batches of the initial mixture (~14 m^3) transited in trenches (66 × 2 × 2 m) during 4 to 5 weeks (summer) and 5 to 6 weeks (winter). The compost mass was turned every working day in summer and 3 to 4 times per week in winter. With each turning, the compost was moved forward 3.6 m and a new batch could thus be added to the trench. Elevated moisture content (~55%) was maintained by periodical addition of water during turning.

Positive pressure aeration was delivered by pulsed cycles (~100 s aeration every 30 to 57 min, depending on the age of the compost batches). At the bottom of the trench, gravel and wood bark shavings assured an even distribution of the forced air (Fig. 1).

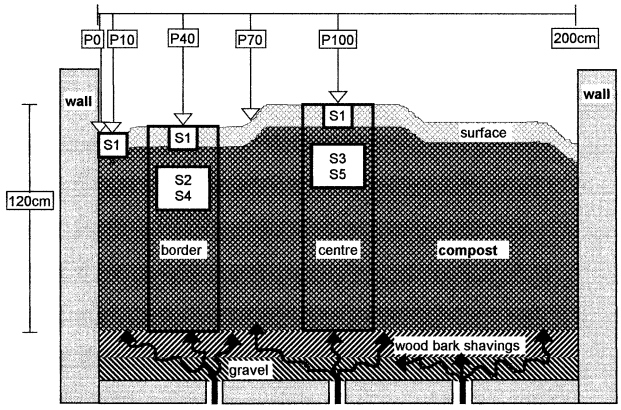

Fig. 1. Cross-section through a trench, filled with compost, showing the five different sampling points (before turning – S1 (surface), S2 (border), S3 (centre) and just after turning – S4 (border), S5 (centre). The positions of the five vertical temperature profiles are also indicated (P0 to P100).

Composting processes were monitored during 2 consecutive years.

Determination of the thermostratification and gas concentrations

For each compost batch, five vertical temperature profiles (P0 to P100) were measured (with standard temperature probes) from the surface to the bottom of the trench, at different distances from the trench wall (0, 10, 40, 70 and 100 cm), see Fig. 1.

As the vertical thermostratification was shown to be symmetrical for both sides of the compost batches, the temperature profiles were measured on one side only. The missing values were obtained by linear interpolation.

Oxygen, carbon dioxide, and methane were measured using electrochemical cells (O_2-CO_2-Ex-H_2S Multiwarn P detectors, Drägerwerk, Lübeck, Germany).

Temperature and gas concentrations were measured just before turning.

Samplings

Samples were taken from compost batches of various ages at different periods of the year.

For every compost batch, three different zones were defined (Fig. 1): the surface (0 to 10 cm of deep, across the entire batch), the border (30 to 50 cm from the wall, covering the whole depth) and the centre (90 to 110 cm from the wall, covering the whole depth).

Just before turning, three samples per batch were taken (Fig. 1): at the surface (S1: 0 to 10 cm of deep, right across the batch), at the border (S2: 40 cm from the wall, 30 cm deep) and in the centre (S3: 100 cm from the wall, 30 cm deep).

Just after turning, only two samples were collected per batch (Fig. 1): at the border (S4) and in the centre (S5) of the batch.

For samples S1, S4 and S5, the sample temperature was calculated as the mean temperature of the whole corresponding zone.

A sample consisted of approximately 1 kg of compost that was quickly cooled at 4°C then frozen (– 20°C). Controls showed that freezing for 3 to 4 weeks did not reduce the xylanase activity (XA).

Extraction of enzymes from compost samples

A 30-g sample of the well-mixed compost (fresh weight) was suspended in 270 ml of sterile potassium hydrogen phthalate-NaOH buffer (50 mM pH 6.0) and shaken at 150 r.p.m. for 30 min at room temperature. Shaking times of 10 and 60 min gave similar results. The pH of the suspension was adjusted to 6.0 with 6 N NaOH or 6 N HCl (unless specified). After pH adjustment, the compost suspension was left to settle at room temperature for 5 min. A 7-ml aliquot of the upper phase was centrifuged at about 8000 G for 10 min at 10°C and the supernatant was used for the XA assay. Compost suspensions were also sonicated (Sonifier Branson 450, 5 × 30 s: power 20 W, duty cycle 50%, with pauses of 30 s) before clarification, to check for a possible solubilization of enzymes fixed to the cell surfaces or particles.

The following sterile buffers were used for the determination of the optimal pH: 84 mM boric acid-NaOH for pH 9.0, 55 mM boric acid-NaOH and 50 mM potassium phosphate for pH 8.0, 50 mM citric acid-Na_2HPO_4 for pH 7.7, 50 mM potassium phosphate and 50 mM citric acid-Na_2HPO_4 for pH 7.0, 50 mM citric acid-Na_2HPO_4 and 50 mM potassium hydrogen phthalate-NaOH for pH 6.0, 50 mM citric acid-Na_2HPO_4 for pH 5.0 and 4.0. XA were also measured in samples suspended in sterile deionized water.

Assay of compost extracts for extracellular xylanase activity

The reaction mixture contained 250 µl of sterile suspended birchwood xylan (2% w/v, Sigma) and 250 µl of clarified compost extract. After incubation for 1 h at 70°C and pH 6.0 (unless specified), the reducing sugar concentration was determined by the dinitrosalicylic acid method (Miller 1959). Residual xylan was eliminated by centrifugation at about 13 000 G during 5 min at room temperature, before absorbance measurement at 600 nm. One unit (U) of XA was defined as the release of 1 µg of xylose equivalents per min and per g of dry weight compost (DWC).

Substrate analysis

Moisture, pH and organic matter content of the compost samples, as well as total carbon (C) and nitrogen (N), were determined as described earlier (Lott Fischer *et al.* 1998).

Analysis of Neutral Detergent Fibre (NDF) was carried out using the method of Van Soest & Wine (1967). Acid Detergent Fibre (ADF) was determined by the method of Van Soest (1965). The difference between NDF and ADF was taken as the amount of hemicellulose.

Results

Chemical and physical analysis

Under standard conditions of operation, the oxygen content (v/v) measured in the interstitial phase of the compost mass was always higher than 10% and no methane was detected.

Water content (w/w) decreased during composting, but remained between 46 and 59% during the summer and 51 and 67% during the winter. The wettest samples were always collected at the surface of the batches, whereas the samples collected at the border and in the centre of the batches showed few differences.

The shredded waste had a pH around 5. During composting, the pH increased first at the surface (pH 7 to 8 after 1 week), then at the border and in the centre of the compost batches (pH 7 to 8, after 2 to 3 weeks). At the end of the process, the pH was close to 8.5 in the entire compost mass.

Initial nitrogen content was higher during summer (C/N 21 to 25) than during the winter (C/N 35 to 40). At the end of the process, C/N ratio reached 12 to 13 in the summer and

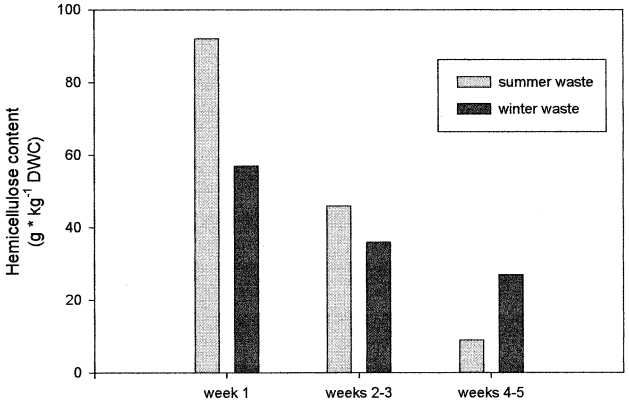

Fig. 2. Evolution of hemicellulose content during composting. Average of 3 to 4 independent samplings. On account of the heterogeneity of fresh waste, the hemicellulose content of samples taken at the same stage of the composting process, but from different batches, varied by 30 to 50%.

15 to 16 in the winter, respectively. These differences correspond to the change in the initial waste composition, mainly freshly cut grass in summer, substituted by dead leaves in winter.

Hemicellulose was more quickly and completely degraded in summer than in winter (Fig. 2).

Thermostratification

A vertical and a horizontal thermostratification of the compost mass were always observed, with substantial variations on account of the season (summer or winter) and the composting time (Fig. 3 and Table 1).

Temperature intervals were chosen in function of microbial population growth potential:

- 70 to 78°C for the *Thermus* strains (Beffa *et al.* 1996b);
- 62 to 69°C for the thermophilic *Bacillus* strains (Beffa *et al.* 1996a; Blanc 1998) and highly thermophilic actinomycetes (Waksman *et al.* 1939a; Holtz *et al.* 1991);
- 53 to 61°C for less thermophilic *Bacillus* (Strom 1985a,b), thermophilic actinomycetes and fungi (Waksman 1939a; Stutzenberger *et al.* 1970; Miller 1996);
- Below 53°C for moderately thermophilic, thermotolerant and mesophilic microorganisms (De Bertoldi *et al.* 1983; Finstein & Morris 1975).

In summer, temperatures rose quickly to high values (maximum 78°C), which were maintained during the whole process. During the first week, most of the compost batch reached temperatures over 61°C. Later, the temperatures decreased mainly from the border and inferior regions of the batches inwards, whereas the upper part of the central zone remained at high temperatures (> 69°C).

In winter, the temperatures (maximum 73°C) were systematically lower than in summer, and rose more slowly. The highest temperatures were reached during weeks 2 to 3 in the central zone of the compost batch. During weeks 4 to 5, most of the compost mass remained at temperatures below 62°C.

Immediately after turning of the compost, temperatures decreased by approximately 10°C. Thermostratification was restored after 16 to 20 h (data not shown).

Evolution and localization of extracellular xylanase activity

Xylanase activity was measured in all the samples collected during the composting process, but great variations were observed (Table 2). Xylanase activity was systematically 2 to 6 times higher in summer than in winter. The highest XA (250 to 392 U) was measured in samples taken at the surface and at the border of the compost batches. Xylanase activity was always very low (< 61 U) in the hottest central zone. Except in the centre, XA decreased as a function of the composting time. Samples collected after turning also gave higher XA at the border than in the centre of the compost batch, especially during the first week of composting.

A series of XA, measured in samples collected, just after turning, the same day but in different parts of the trench corresponding to different composting times, is given in Fig. 4. Highest XA were observed in the 2nd week of composting, but only if the mean temperature of the zone (border or centre) was below 60°C. Differences of XA between the border and centre seemed mainly correlated with the temperatures of the samples.

Characterization of extracellular xylanases

The optimal temperature for xylanases was linked to the localization of the samples in the compost batches (Fig. 5). Xylanases from the surface zone had lower optimal temperature (~60°C) than xylanases from the border (~70°C) and from the centre (~80°C). Samples taken just after turnings showed intermediate values, XA was very

Table 1. Percentage of compost mass at different temperature intervals

	Week 1		Weeks 2 to 3		Weeks 4 to 5	
Temperature interval (°C)	Summer	Winter	Summer	Winter	Summer	Winter
			Compost mass per batch (%)			
< 53°C	4%	39%	19%	30%	30%	63%
53 to 61°C	9%	25%	21%	25%	17%	21%
62 to 69°C	23%	36%	30%	28%	41%	16%
> 69°C	64%	0%	30%	17%	12%	0%
> 72°C	39%	0%	5%	3%	0%	0%

Percentages are average values from 3 to 8 independent measurements. Maximal temperatures measured in the compost mass were 78°C in summer and 73°C in winter, respectively.

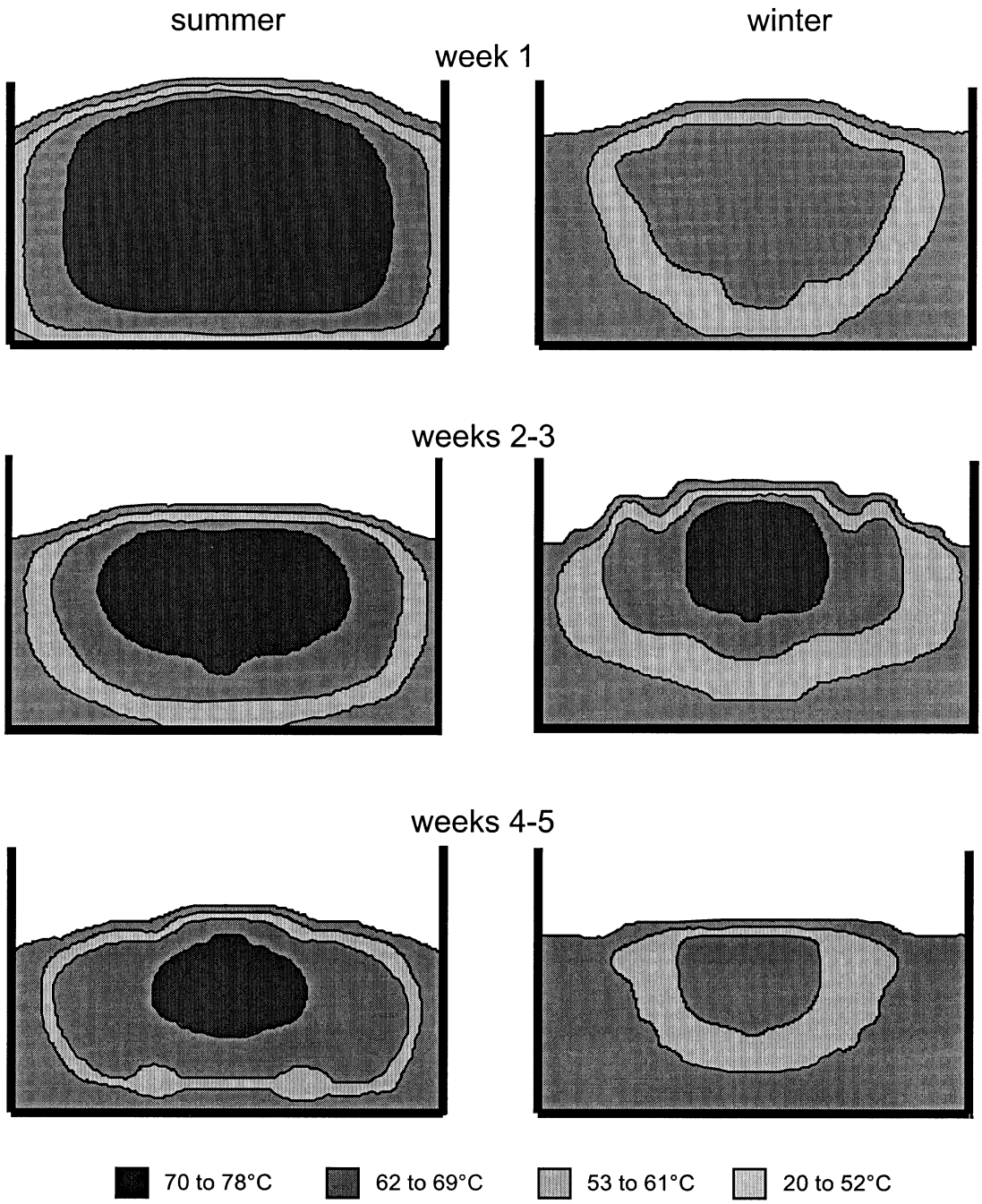

Fig. 3. Isotherms of three cross-sectional cuts showing the thermostratification evolution of summer and winter material. Average isotherms were obtained from 3 to 8 independent measurements.

similar at 50°C and at 80°C, with an optimum at 65°C for the border samples and 70°C for the centre samples, respectively.

Xylanase activity was maximal between pH 6 and 8, whatever the sampling localization (Fig. 6). Xylanase activity measured at pH 5 and 9 were approximately 40% lower. Xylanase activity were negligible at pH 4. The choice of the buffer influenced the XA, but potassium hydrogen phthalate-NaOH buffer was well adapted for XA measurement at pH 6. At pH 8, boric acid-NaOH buffer gave higher XA values than potassium phosphate buffer. Compost samples suspended in water (tested samples ranged between pH 7 and 9) gave slightly higher XA than the maximal values obtained with buffers (data not shown).

The thermostability of the xylanases from surface samples was assayed by pre-incubation at various temperatures for varying lengths of time (Fig. 7). Below 60°C activity loss was slight, but it increased at 70°C and was high at 90°C.

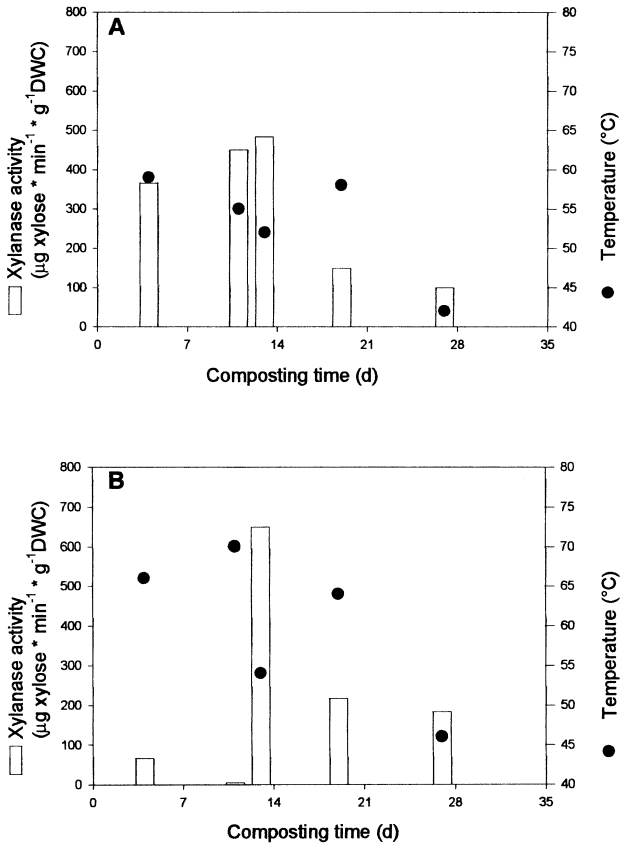

Fig. 4. Effect of composting time on xylanase activity at the border (A) and in the centre (B) of a trench. Compost samples were taken the same day, in different compost batches, just after turning of the trench. Xylanase activity was measured at 70°C and pH 6. ● mean temperature of the zone, □ xylanase activity. DWC – dry weight compost.

Discussion

Thermostratification and thermophilic bacteria

Heat generation in the compost mass is on account of the biological activity of the microorganisms (Finstein & Morris 1975). As previously described and reviewed by Miller (1992), both physical factors and the chemical composition of the initial material affect the intensity of metabolic heat generation. The slower and lower increase of temperature observed in winter, as seen against the one taking place in summer, might be a result of the fact that the degradation of dead leaves brings less energy than that of freshly cut grass. Previous studies have indicated that a minimal grass/dead leaves ratio is necessary to optimize the composting process and to obtain a well humidified end-product (Michel *et al.* 1993, 1996).

The initial C/N ratio was higher in winter (35 to 40) than in summer (21 to 25), but both were within the optimum range for composting (Gray *et al.* 1971).

The presence of thermophilic *Bacillus* spp. (maximal growth temperature: 65 to 72°C) and *Thermus thermophilus*

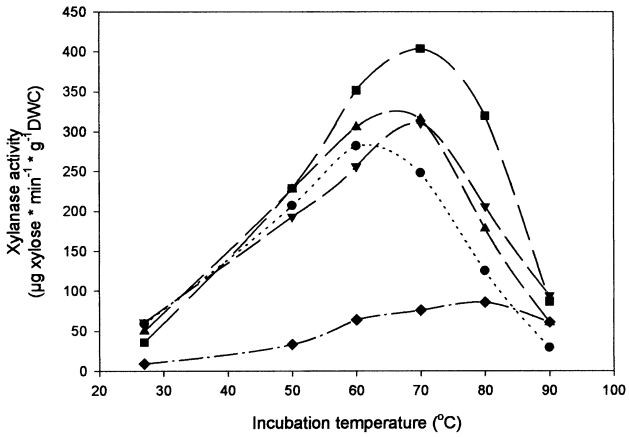

Fig. 5. Optimal temperature of xylanases in function of the localization of the compost samples in the trench. Xylanase activity measured at pH 6. Before turning: surface (·····), border (–■–) and centre (– – ◆ – –). Just after turning: border (–▲–) and ···●··· centre (–▼–). Average of 3 to 4 samples. DWC – dry weight compost.

(maximal growth temperature: 80 to 82°C) (Beffa *et al.* 1996b; Blanc *et al.* 1997; Blanc 1998) made it possible to maintain high temperatures in the core of the compost mass. The vertical air flow (from bottom to top) as well as heat losses at the surface and at the border of the trenches induced two thermal gradients (thermostratification). Except during the first week in summer, the mass of compost at temperatures below 62°C was large enough to ensure the development of less thermophilic microorganisms (bacteria, actinomycetes and fungi) able to produce a wide diversity of extracellular enzymes.

Optimal conditions for industrial composting have often been deduced from static reactor simulations, using a heating system with or without adiabatic chamber (Atkinson *et al.* 1996; Suler & Finstein 1977; Nakasaki *et al.* 1985; Hogan

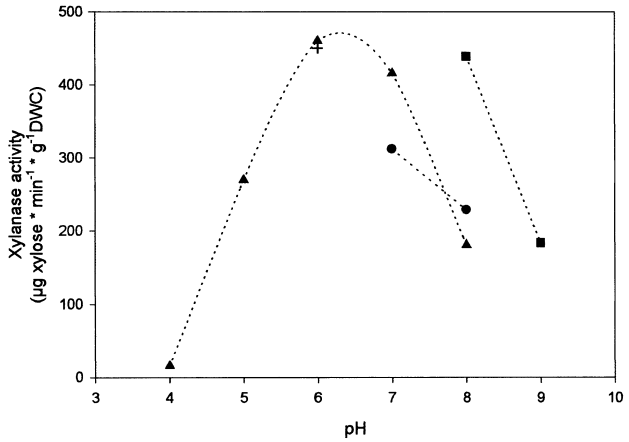

Fig. 6. Effect of pH and buffer on xylanase activity (measured at 70°C). Average of six samples from different locations in the compost mass. The following buffers were used: 50 mM citric acid-Na_2HPO_4 (···▲···), 50 mM potassium hydrogenophtalate-NaOH (+), 50 mM potassium phosphate (·····), 84 mM (pH 9) and 55 mM (pH 8) boric acid-NaOH (···■···). DWC – dry weight compost.

Table 2. Effect of composting time and sample localization on xylanase activity (XA)

Localization and sampling temperature	Week 1	Weeks 2 to 3	Weeks 4 to 5
XA before turning			
Surface			
(48 to 55°C)	392 U	308 U	230 U
Border			
(62 to 68°C)	317 U	250 U	125 U
Centre			
(70 to 71°C)	8 U	58 U	60 U
XA after turning			
Border			
(54 to 59°C)	367 U	375 U	183 U
Centre			
(61 to 68°C)	50 U	231 U	161 U
Winter			
Localization and sampling temperature	Week 1	Week 2 to 3	4 to 5
XA before turning			
Surface			
(39 to 50°C)	163 U	133 U	80 U
Border			
(45 to 56°C)	83 U	39 U	33 U
Centre			
(67 to 71°C)	21 U	6 U	21 U
XA after turning			
Border			
(47 to 53°C)	111 U	61 U	61 U
Centre			
(50 to 56°C)	28 U	50 U	94 U

XA are average values of 2 to 8 independent samplings. XA were measured at 70°C and pH 6. Unit – 1 U = 1 μg xylose min^{-1} g^{-1} DWC.

et al. 1989). In these cases, the temperatures of the compost mass were homogeneous and low degradation rates were measured at temperatures over 50 to 60°C. Experiments carried out by Schulze (1962) in a rotating drum showed that high temperatures (~70°C) and high rate of degradation could be maintained by a constant input of fresh waste. The activity of the most thermophilic bacteria seems to depend mainly on the presence of easily degradable compounds.

Xylanase activity and xylanolytic microorganisms

Given the high complexity of xylan, microorganisms have to produce a variety of main-chain- and side-chain-cleaving enzymes. Many combinations of enzymes have been reported to interact synergistically for the extensive hydrolysis of xylan (Coughlan & Hazlewood 1993). In our study, XA in compost samples was measured as the resulting activity of a mixture of endo- and exo-xylanases synthesized by different species of microorganisms.

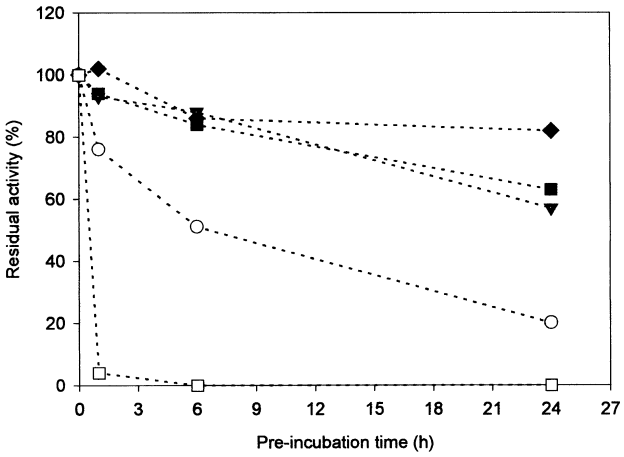

Fig. 7. Thermostability of xylanases after preincubation at different temperatures: 40°C (···◆···), 50°C (···■···), 60°C (···▼···), 70°C (···○···) and 90°C (···□···). Two samples were taken at the surface of the trench after 2 weeks of composting. Xylanase activity was measured at 70°C and pH 6.

The great difference of XA observed between summer (high activity) and winter (low activity), during the whole composting process, indicated that hemicellulose degradation was lower in dead leaves than in freshly cut grass.

High XA measured at the border of the trenches (62 to 68°C) showed that thermophilic bacteria were important for the degradation of xylan, as well as less thermophilic microorganisms present only at the periphery of the compost (48 to 55°C). As optimal temperature for xylanases varied in function of the localization of the sample within the compost batch, xylanases seemed to be synthesized simultaneously by different populations of xylanolytic microorganisms. The importance of each population for the maintenance of high xylan degradation seems mainly related to the thermostratification.

Samples collected after turning showed that high XA was only observed when a large percentage of the compost mass was at temperatures below 70°C. Activity of highly thermophilic xylanolytic bacteria seems limited.

The known optimal temperature of xylanases synthesized by different species of microorganisms are summarized as follows:

- above 80°C for *Thermus thermophilus* (Lyon *et al.* 1998) and other highly thermophilic but anaerobic bacteria (Shao & Wiegel 1992; Gibbs *et al.* 1995; Winterhalter & Liebl 1995)
- between 70 and 80°C for thermophilic actinomycetes (Berens *et al.* 1996; Holtz *et al.* 1991), *Clostridium* sp. (Bérenger *et al.* 1985), thermophilic and mesophilic *Bacillus* spp. (Nakamura *et al.* 1994; Gilead & Shoham 1995; Lyon *et al.* 1998) and some thermophilic fungi (Gomes *et al.* 1993; Tan *et al.* 1987; Saha & Bothast 1998)

- 60 to 70°C for thermophilic actinomycetes (Elegir *et al.* 1994) and thermophilic or mesophilic *Bacillus* spp. (Nanmori *et al.* 1990; Gessesse 1998)
- Below 60°C for thermophilic and mesophilic fungi (Duarte & Costa-Ferreira 1994; Chandra Raj & Chandra 1996) and mesophilic *Bacillus* sp. (Nakamura *et al.* 1993; Degrassi *et al.* 1998).

Xylanases synthesized by some mesophilic *Bacillus* spp. and thermophilic fungi have higher optimal temperature than those synthesized by thermophilic bacteria. Enzymes are often more thermostable than the organisms that synthesize them (Madigan *et al.* 1997). Frequent turning of the compost mass can increase the rate of the degradation process, by redistribution of thermostable enzymes in the hottest zones of the compost mass (> 69°C), zones where the growth of xylanolytic bacteria seems more limited. However, the redistribution of the xylanases from the surface to the centre seemed only able to lead to a transitory increase of xylan degradation (after turning), at temperatures over 60°C (Fig. 7).

Extracellular enzymes liberate mono- and di-mers, which can be assimilated by highly thermophilic bacteria that do not produce extracellular hydrolytic enzymes.

Xylanase activity measurements, carried out in the same compost sample before and after clarification by centrifugation, indicated that the clarification did not lead to a substantial loss of activity (~4%) in the supernatant. As xylanase can be both extracellular and cell-surface-located (Coughlan & Hazlewood 1993), it was determined if the sonication of the compost suspension led to the liberation of cell-surface-located xylanases or to the release of xylanases attached to compost particles. The results varied strongly from one sample to another, XA increases (18 to 59%) as well as XA decreases (down to 15%) were observed.

Turning and xylanase activity

After turning, large differences of XA were observed between the border and the centre of the compost batches (Table 2 and Fig. 4). This indicated that the design of the turning machine allowed good mixing vertically, but not horizontally. The lack of horizontal mixing may delay the degradation of organic matter at the beginning of the process (lower XA in the hottest central zones). But these differences seemed to be none existent by the end of the process, when XA became similar both at the border and in the centre of the compost batch.

Conclusion

This work showed that extracellular xylanases existed in the whole compost mass, although XA was high at sampling temperatures below 68 to 69°C. Xylanase activity decreased with composting time and was lower in winter, when the waste was richer in dead leaves and wood.

The different temperature optima for xylanases suggested that different microorganisms played a role for the degradation of the xylan. Thermophilic bacteria, growing at the border of the trenches until approximately 70°C, seemed highly active, as well as less thermophilic microorganisms present only at the periphery of the compost (20 to 52°C). In the hottest central zones (70 to 78°C), activity of xylanolytic bacteria seemed more limited. Further studies are required to clarify the role of thermophilic xylanolytic microorganisms, especially highly thermophilic bacteria.

An unexpectedly broad thermostratification of the compost mass, similar to the one observed in windrows, developed during composting in aerated trenches. As a large central zone of compost reaches temperatures above 70°C, a high turning frequency, which must be adapted to the waste composition, will increase xylan degradation. High degradative activities and a good thermohygienization can both be achieved by the redistribution of the substrates, the free enzymes and the microorganisms.

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